An investigation of breeding methods applicable to Tasmanian-grown pyrethrum

by

Kristin Groom B.Sc. (Hons.), Tas.

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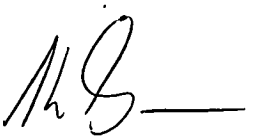

Kristin Groom
University of Tasmania, Hobart
August 2003

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Kristin Groom
University of Tasmania, Hobart
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Summary

Pyrethrum is a perennial daisy that is predominantly out-crossed. It is grown commercially in Tasmania for pyrethrins, which are extracted from the flowers and are used in the preparation of insecticides. A pyrethrum breeding program was conducted by the University of Tasmania from 1978 to 1998, with the aim of developing varieties suitable for Tasmanian growing and production systems. The primary selection character was pyrethrins yield, and the program consisted of population improvement through recurrent selection for two indirect selection characters; visually-estimated flower yield and the UV-assay of pyrethrins at a specific maturity stage (open but not over-blown). Varieties, consisting of either clones or single crosses between two clones, were selected from each successive recurrent selection generation.

This study was undertaken to evaluate the selection methods used for the University's breeding program from the period of 1985 to 1998, and to assess the impact on breeding methods of the change in the mid-1990's from the estblishment of the crop by clonal splits planted at a contant spacing (0.5 m) to establishment by direct-drilling at a somewhat higher average plant density. Data records for the two main selection characters were analysed in order to identify genetic changes in the breeding population. This indicated that there had been a genetic gain in UV-assays but not in flower yield.

The efficiency of direct selection for first year yields in the Univeristy's base population was compared with indices of the indirect selection characters and yield components. A trial was established to obtain estimates of genetic parameters to construct selection indices and predict selection responses. Estimated heritability was moderate for pyrethrins yeld (0.26-0.39) and for the yield components of pyrethrins content (0.24-0.34) and flower yield (0.17-0.30), and low for percent dry matter content (0.00-0.15). Estimates were also obtained

for two product quality characters, both derived from the ratio of the six different esters that form the active product in the pyrethrum extract. Heritability for these character was moderate to high. The effect of planting density on selection for pyrethrins yield was also investigated.

The index of the two indirect selection characters was the most efficient method for single-plant selection in the planting densities used commercially from 1980 to 1995. However, an index of component characters was more efficient for the densities currently used in commercial areas. There was evidence for a genotype-density interaction for flower and pyrethrins yield but not for percentage pyrethrins or the ratios of pyrethrins esters.

The potential to reduce the length of the period of obligate vegetative growth through breeding was also assessed. There was evidence for significant level of additive genetic control for this character and it was concluded that the vegetative period could be reduced by recurrent selection. The performance of new synthetic varieties were evaluated and the relative merits of varieties derived from a single cross of two clones and polycross of several clones were discussed.

1 Introduction

Pyrethrum is a perennial daisy grown commercially for the insecticide that is extracted from its flowers. The crop was introduced to Tasmania in the early 1980's and since then, the area under cultivation has grown to more than 2000 ha and Tasmania has become the world's second largest producer of pyrethrum.

Early development of the industry in Tasmania was based on two clonal varieties, *CIG 3* and *CIG 11* - both produced by a breeding program at the University of Tasmania. Following the release of these clonal varieties, the University's breeding program concentrated on the development of new varieties that could be propagated by seed, in contrast to the tissue-culture and hand-splitting propagation methods required for clones. Six new seedling varieties, each a cross between two clonal lines, were released to industry in the mid-1990's. These proved to have the advantages of reduced establishment costs compared to clones and, as a consequence of the lower cost per propagule, the ability to be planted at higher densities that results in increased yields per hectare. The seedling varieties have now superseded the clones in Tasmanian commercial areas.

The main aim of the University's pyrethrum breeding program has been to increase yields of pyrethrins - the insecticidally active component of the plant. Pyrethrins are concentrated in the flowers and the program has aimed to increase both flower yields per plant and the levels of pyrethrins within the flowers. From 1985 to 1997, the program has consisted of recurrent selection to improve the average yield of the breeding population. Selection of the parents for each breeding cycle has been on the basis of two characters related to pyrethrins yield - a visual estimate of flower yield and the percent pyrethrins content of flowers at a specific maturity stage (fully-open but not overblown) evaluated using a relatively inexact assay method. Both selection traits have the advantage of a

low cost per plant compared to more precise evaluation methods. Parents were chosen using a Smith-Hazel type selection index that combined data from individual plants with the family means of their full-sib families for each trait. Potential varieties consisted of clones and biclonal crosses (full-sib families), and each recurrent selection generation was screened for both variety types. Parent selection was made solely on the basis of pyrethrins yield. However, mating design incorporated a second trait - the ratio of the two different types of pyrethrins esters (Pyrethrins I/II) - and parents with a high Pyrethrins I/II were crossed to parents with a low Pyrethrins I/II in order to produce potential varieties with values within the intermediate range required in commercial extracts.

Selection trials from 1980 to 1997 were established at an intraplant spacing of 0.5 m, which corresponds to the agricultural standard of the 1980's and early 1990's. However, with the change to crop establishment by direct-drilled seed, agricultural plant densities have increased.

The main aim of this study is to evaluate the breeding methods and material used by the University of Tasmania's pyrethrum breeding program from 1985 to 1997 in order to;

- determine whether the breeding goals of the program have been met,
- determine whether index selection for correlated or component characters is an effective method for increasing pyrethrins yield,
- determine whether there is sufficient variation in the University's base population to allow improvement of characters of importance,
- assess the effect of the change in planting density on selection for pyrethrins yield,
- to recommend appropriate goals and methods for future pyrethrum breeding in Tasmania,
- determine whether selection characters other than yield should be utilised,
- identify priorities for future research.

2 Literature Review

2.1 Introduction

2.1.1 Pyrethrum

The common name "pyrethrum" is used for a number of plant species, including the Dalmatian pyrethrum (*Tanacetum cinerariifolium* (Trev.) Schulz-Bip. syn. *Chrysanthemum cinerariifolium* (Trev.) Vis.) and Persian pyrethrum (*Tanacetum coccineum* syn. *Chrysanthemum coccineum* Willd.). "Pyrethrum" is also used to refer to the botanical insecticide produced from the flowers of *T. cinerariifolium* and *T. coccineum,* which can be in the form of a solvent-based extract or a powder made from the dried flowers. The insecticidally active component of the pyrethrum extract consists of six closely related esters collectively referred to as the "pyrethrins".

The cultivation of *T. coccineum* pre-dates the discovery of the insecticidal properties of *T. cinerariifolium* in the 1840's. Both species were used for the commercial production of pyrethrum powder in the last century but the superior pyrethrins levels in the flowers of *T. cinerariifolium* led to a decline in the use of *T. coccineum. T. cinerariifolium* has been the sole commercial source of commercial pyrethrins since the 1930's. Consequently, references to the crop plant "pyrethrum" after 1930 almost exclusively apply to this species. Accordingly, the agricultural pyrethrum grown in Tasmania, which is the subject of this study, is *T. cinerariifolium.* In this thesis, the term "pyrethrum" will applied exclusively to the Dalmatian species - *T. cinerariifolium* - and to the commercial insecticide produced from this plant.

Pyrethrum has a similar appearance to the common chrysanthemum daisy, with deeply-divided blue-green leaves and a rosette growth habit, growing in the form of a bush of up to 90 cm diameter. The flowers are

produced on elongated stems, typically 45 to 90 cm long. The capitulum, although it has the appearance of a single flower with white petals and a yellow centre, is a composite structure consisting of approximately 100 small flowers, which are densely packed on a flattened receptacle. Generally, the pyrethrum capitulum is referred to as the "flower" and the true flowers are distinguished by the term "florets", a convention which will be followed in this thesis.

There are two types of florets in pyrethrum; the "disc" florets, which are tubular in shape, yellow and hermaphrodite, and the "ray" florets, which occupy a single whorl on the perimeter of the flower, are female and bear one white, ligulate petal. The florets have a single, inferior ovary, surrounded by a ribbed outer wall, which is covered with surface glandular trichomes (oil-glands) and has internal secretory lacunae (secretory ducts). The fruit, technically an achene, is small (5 mm long), hard, dry and consists of a single seed together with the ovary wall. In pyrethrum, the term "achene" is also applied to the immature fruit prior to seed maturation (Brewer, 1973). The achenes of the ray and disc florets are not morphologically distinct although there are small differences in the size and shape of achenes from different parts of the floral disc (Chandler, 1951).

Pyrethrum is a native of the Adriatic coastal mountains of Croatia, Bosnia and Herzegovina and has been grown as a crop in Europe, Asia, Africa, New Guinea and S. America. It has been particularly successful in East Africa, especially in Kenya, which has dominated the world market since the 1940's (Mkawale, 2001). Commercial production of pyrethrum began in Australia in the early 1980's, when the crop was established in Tasmania from varieties developed by the University of Tasmania (Bhat and Menary, 1984b; MacDonald, 1995). Since then, there has been a rapid expansion of the area under pyrethrum cultivation, and Tasmania has become the second largest world producer of pyrethrum (MacDonald, 1995), supplying as much as 30% of the total world market (Macatta, 2001).

A pyrethrum breeding program began at the University of Tasmania in 1979 with the importation of material from a breeding program in Kashmir. The Kashmir material was chosen because:

- It was available.
- There had been some selection and improvement.
- Growing conditions in the selection environment in Kashmir are similar to Tasmania.

The breeding program in both Kashmir and Tasmania consisted of population improvement with selection of varieties from successive generations. Selected varieties consisted of clones in the 1980's and single crosses of two clones in the 1990's, which are propagated from seed.

Tasmania is an island to the south of the Australian continent. It lies between 41 and 44° south and has a cool temperate climate. Temperatures in the main pyrethrum growing region of the NW coast lie between an average summer maximum of 21°C and winter minimum of 4°C with an average monthly rainfall of 40mm (January) to 105mm (July)[1]. Currently, pyrethrum is established in Tasmania by direct seeding in August to December and the first crop is usually harvested 15 to 18 months after sowing (NRE, 1998). Harvesting takes place in late December to January using cutter-rowers that slash the flower stems at a few centimetres above ground-level and form them into windrows for field-drying. Once the windrows are dry, harvesters separate the achenes from other material and return the trash to the field (Macatta, 2001). Tasmanian pyrethrum is generally cropped for four annual harvests, although up to eight harvests are possible (NRE, 1998).

The success of commercial development of pyrethrum in Tasmania has been attributed to the selection of high yielding varieties, development of mechanical harvesting and cost-effective establishment and cultural practices

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[1] 16 year average, Commonwealth Bureau of Meteorology data for Devonport, Tasmania.

(Chudleigh and Bond, 1994). Currently, Tasmania is the only place in the world where pyrethrum is fully mechanically harvested.

2.1.2 Pyrethrum taxonomy

Pyrethrins appear to be restricted to a small number of species of *Tanacetum* L. Flowers of *T. coccineum* contain the same six pyrethrin esters as *T. cinerariifolium* (Fujii and Shimizu, 1990; Hogstad *et al.*, 1984), although generally at a lower concentration (Gnadinger, 1936). *T. corymbosum* (L.) Schulz-Bip. also contains low concentrations of pyrethrins (Chandler, 1951; Chandler, 1954) and *T. macrophyllum* Walst. & Kit. and *T. tamrutense* Schulz-Bip. are reported to be toxic (Contant, 1976). A survey of some 100 species of *Tanacetum* and closely related genera found no other species with a detectable insecticidal activity (Contant, 1963b). Claims that various *Tagetes* species contain pyrethrins (Kamal and Mangla, 1987) have been refuted (Crombie, 1995; Hogstad *et al*, 1984). Similarly, the report that peonies (*Paeonia albiflora*) contain pyrethrins (Chmielewska and Kasprzyk, 1962) was shown to be erroneous (Godin *et al.*, 1967).

Tanacetum is part of the Asteraceae family. In the past, *T. cinerariifolium* has been classified in three different genera; *Pyrethrum* Zinn., *Chrysanthemum* L. and *Tanacetum*. These genera form part of the *Chrysanthemum* complex; a group with a complicated taxonomy that has been revised a number of times.

Pyrethrum was established as a genus by Haler in 1742 and included all the species generally accepted as producing pyrethrins. Subsequent classifications either recognised *Pyrethrum* as a separate genus or included it as part of *Chrysanthemum*. *Chrysanthemum* was considered to be a large genus by the majority of taxonomic systems prior to 1900, containing a number of taxa now regarded as separate, including *Dendranthema* DC., *Pyrethrum* and, less commonly, *Tanacetum* (Heywood, 1954). However, Heywood (1954) argued that there is no clear generic distinction between *Pyrethrum* and *Tanacetum*. The

species included in *Pyrethrum* have epidermal leaf glands and homomorphic achenes, both features of *Tanacetum*. Additionally, *T. cinerariifolium* and *T. alpinium* have similar secretory lacunae (ducts) in the achenes. In contrast, Heywood (1954) considered *Chrysanthemum* to form a small, natural genus if it is restricted to species that produce morphologically distinct achenes from ligulate and tubular florets and lack secretory vallecular canals.

Subsequent taxonomists, (Davis, 1975; Tutin *et al.*, 1976), followed the classification system for the *Chrysanthemum* complex proposed by Heywood (1954) and the inclusion of pyrethrum species in *Tanacetum* is now widely accepted by taxonomists. However, horticulturists have been slow to recognise these changes (Anderson, 1987). This is particularly true in the pyrethrum literature and the use of *Chrysanthemum* as the genus name for commercial pyrethrum persisted through the 1990's (for example, see Casida and Quinstad, 1995b). An alternative spelling of the species name as *cinerariaefolium* is also common (for example, Casida and Quinstad, 1995b; Gnadinger, 1936).

2.1.3 Pyrethrins

The pyrethrins (Figure 2.1) are six naturally occurring esters that are toxic to insects. As esters, they can regarded as being formed from an acid and a ketone, specifically chrysanthemic or pyrethric acid, and one of three ketones; pyrethrolone, jasmolone or cinerolone. Individually, each pyrethrin takes its name from the ketone moiety and are classified as either Pyrethrins I or Pyrethrins II on the basis of the acid component. The Pyrethrins I (pyrethrin I, jasmolin I and cinerin I) are formed from chrysanthemic acid, and the Pyrethrins II (pyrethrin II, jasmolin II and cinerin II) from pyrethric acid.

All six pyrethrins esters are found in *T. coccineum* (Hogstad *et al*, 1984), as well as *T. cinerariifolium*, and it appears that they always occur together, as there are no published reports of natural extracts that lack any one or more of the six. However, the proportions of the esters can vary considerably. The relative

concentration of the Pyrethrins I and II (PyI/II) can range from 0.5 to 4 (Bhat, 1995; Head, 1967; Parlevliet, 1975). The relative concentrations of the jasmolins, cinerins and pyrethrin I and II also vary. However, the ratio of pyrethrin:jasmolin:cinerin is approximately the same in the Pyrethrins I as in the Pyrethrins II, and the concentration of pyrethrin I and II is always greater than the corresponding jasmolin or cinerin (Head, 1967).

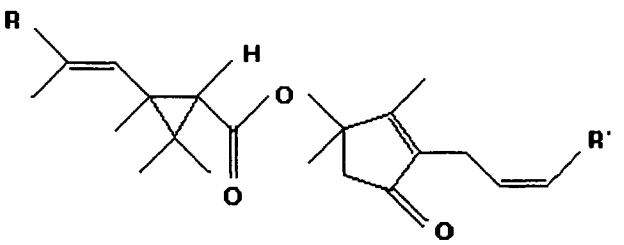

Figure 2.1: Generalised structure of the pyrethrins.

Pyrethrins	R	R'
pyrethrin I	CH_3	$CH{=}CH_2$
cinerin I	CH_3	CH_3
jasmolin I	CH_3	CH_2CH_3
pyrethrin II	CH_2COOH	$CH{=}CH_2$
cinerin II	CH_2COOH	CH_3
jasmolin II	CH_2COOH	CH_2CH_3

The pyrethrins are unstable biodegradable insecticides. They are toxic to insects and fish (Bridges and Cope, 1965) but have very low order acute toxicity to mammals (Schoenig, 1995). In insects they cause paralysis (knock-down) which is followed by recovery or death, depending on dose. It appears that the intact esters are toxic rather than any metabolic products derived from them (Elliot, 1969) and the pyrethrin molecules bind to specific sites on the nerve fibre, that are also the active sites for the pyrethroids and DDT analogues (Soderlund, 1995). This means that insect populations can develop cross-resistance, in which tolerance to one type of insecticide confers resistance to another type (Cochran, 1995).

Pyrethrins are metabolised quite rapidly by insects and mammals. Death occurs when the insecticide is absorbed more rapidly than it is detoxified (Casida and Quinstad, 1995a). The pyrethrins are relatively non-toxic to mammals due to a low rate of absorbence compared to the rate of detoxification (Casida and Quinstad, 1995a). Detoxification in insects is inhibited by pyrethrum synergists such as sesamin (Haller *et al.*, 1942), a natural component of pyrethrum flowers (Doskotch and El-Feraly, 1969) and piperonyl butoxide (Wachs, 1947), which is commonly added to commercial pyrethrins preparations.

The low mammalian toxicity and rapid degradability of the pyrethins is their main commercial attraction and they are considered safe for use in food handing areas and human and animal pesticide applications (NRE, 1998). They are also used in mosquito coils.

Little is known about the biosynthesis of the pyrethrins. Chrysanthemic and pyrethric acid are classified as monoterpenes as they are formed by the condensation of two isoprene units derived from mevalonic acid (Crowley *et al.*, 1961). However, they belong to a small sub-class known as irregular monoterpenes, which are formed by a 'middle-to-tail' condensation of the isoprene units (Crowley *et al*, 1961), in contrast to the majority of monoterpenes,

which are derived from a 'head-to-tail' condensation (Epstein and Poulter, 1973). Chrysanthemic and pyrethric acid appear to share a common biosynthetic pathway (Crowley *et al.*, 1962), possibly with chrysanthemic acid acting as a precursor of pyrethric acid (Abou Donia *et al.*, 1973).

The ketone moiety of the Pyrethrins I esters is not derived from mevalonic acid and may be, at least partly, synthesised from acetate (Crowley *et al*, 1962). Crombie (1995) suggested that these molecules could originate from fatty acid metabolism. Drawing from biosynthetic pathways established in other plant, fungi and bacteria species, he speculated that jasmonic acid may be synthesised from linolenic acid and is the precursor of jasmolone, pyrethrolone and cinerolone.

Pyrethrins occur throughout the pyrethrum plant, at levels ranging from <0.4% in the roots and mature leaves, to over 1% in the flowers and unexpanded leaves (Head, 1966b; Zito *et al.*, 1983). Within the flowers, most (94%) of the floral pyrethrins are found in the achenes (Gnadinger and Corl, 1930; Head, 1966b; Martin, 1934). Excised florets are able to synthesise pyrethrins from radioactively labelled isoprene precursors and it seems likely that the floral pyrethrins are synthesised *in situ*, and not translocated from some other part of the plant (Crowley *et al*, 1961). However, the site(s) of pyrethrins synthesis have not been investigated in any detail, and it is not certain whether pyrethrins are synthesised throughout the plant or in specific organs or tissue-types and then translocated to other parts of the plant.

Commercial harvesting usually consists of collecting flowers or achenes, and pyrethrins are generally extracted from dried material by a non-polar hydrocarbon solvent. The extract is filtered and the solvent removed to form an oleo-resin of approximately 30% pyrethrins. The oleo-resin is refined using a solvent extraction method in Kenya and the USA and by liquid carbon-dioxide in Tasmania (Carlson, 1995).

2.2 Reproductive physiology and crop propagation

2.2.1 Pollination

Pyrethrum is a predominantly out-crossing species and is usually unable to produce seed in the absence of cross-pollination (Kroll, 1958; Thorpe, 1948). Self-fertilisation is prevented by system of sporophytic incompatibility, with compatibility between the stigma and pollen grain determined by the diploid genotype from which the pollen is derived rather than the haploid genotype of the pollen. Self-pollination is prevented by inhibition of the pollen-tube, preventing penetration of the stigma surface (Brewer, 1974). Pollination among clones and individuals that share incompatibility alleles is also inhibited, and crosses between close relatives are often infertile (Contant, 1963a). Incompatibility in pyrethrum appears to be determined by a single multi-allelic locus with dominance effects (Brewer, 1974). This means that compatibility is not necessarily consistent between reciprocal crosses (Brewer and Parlevliet, 1969). Although self-incompatibility can be over-come by bud pollination in some species (Marrewijk, 1989), this is not the case in pyrethrum (Singh and Sharma, 1989).

Pyrethrum is not exclusively self-sterile. Some clones can produce self-pollinated seed (Delhaye, 1956), including *CIG 3*, a clonal variety grown extensively in Tasmania (Potts and Menary, 1988). However, self-fertility generally appears to be uncommon. A survey of Kenyan pyrethrum found that less than 2% of the clones tested were self-fertile (Brewer and Parlevliet, 1969), and seed-set following self-pollination is usually either low or absent (Beckley *et al.*, 1938; Kroll, 1958; Singh and Sharma, 1989; Thorpe, 1948). However, Delhaye (1956) reported relatively high seed-set rates in one clone, out of four that were tested.

It is not uncommon to have both self-compatible and incompatible genotypes within a species, and self-fertility within a predominantly self-incompatible species can be divided into two main types (Levin, 1996). "True" self-fertility occurs when one or more alleles at the incompatibility locus confers self-fertility. This is characterised by similar seed-set rates following self- and cross-pollination and a self seed-set rate that is insensitive to environmental influence. "Pseudo" self-fertility occurs when a gene or polygenes, separate from the main incompatibility locus, have a modifying effect. This type of self-fertility is characterised by a higher seed-set rate with cross-pollination than self-pollination, and more rapid growth of the pollen-tube of compatible pollen. As a result, there is a preference for cross-pollination and self-pollination only occurs in the absence of compatible foreign pollen (Levin, 1996).

Plants that are self-fertile need to be emasculated to ensure cross-pollination, and Delhaye (1968) recommended emasculation in order to ensure cross-pollination of pyrethrum. However, most pyrethrum breeders consider emasculation to be unnecessary (Bhat, 1995; Parlevliet and Contant, 1970; Singh and Sharma, 1989). The percentage seed-set for each of the clones tested by Delhaye (1956) was higher following cross-pollination than for self-pollination. This is consistent with the interpretation that florets only accept their own pollen in the absence of compatible foreign pollen (Brewer, 1968). Therefore, it is likely that the incidence of self-pollination is negligible when pyrethrum is pollinated with compatible foreign pollen and that it is not necessary to emasculate flowers to ensure out-crossing.

In general, observations of pyrethrum are consistent with it being predominantly out-crossing. The progeny of crosses between two clones are highly heterogeneous, as would be expected for parents with a high degree of heterozygosity (Parlevliet, 1974), typical of plants that are predominantly out-crossing (Borojevic, 1990). Similarly, there is far greater phenotypic variation among individuals of a biclonal cross than individuals of a clonal variety

(Glynne Jones, 1968), which is also consistent with the assumption that the parents are predominantly heterozygous. If the parent plants of a hybrid cross, are highly homozygous, then progeny would be as genetically homogeneous (Welsh, 1981), and would show comparable levels of phenotypic variation as clones. In addition, inbreeding depression is observed in small populations of pyrethrum (Dalgety, 1975), which is also typical of predominantly out-crossed species (Liedl and Anderson, 1993; Welsh, 1981).

2.2.2 Variety types

Pyrethrum is easily propagated vegetatively and by seed, and both methods are used for crop establishment (Anon., 2001b; Kroll, 1958; Macatta, 2001; Tattersfield, 1948). Vegetative propagation is by cuttings (Brown, 1965; Dalgety, 1975), tissue culture (Karki and Rajbhandary, 1984; Roest, 1976) or by dividing mature plants into 'splits' (Beckley *et al*, 1938). The ease of vegetative propagation allows production of clonal varieties which have been used extensively in Africa (Delhaye, 1968; Parlevliet, 1975) and Tasmania (Macatta, 2001).

Varieties that are propagated by seed (seedling varieties) are typically produced by crossing two or more clones (Dalgety, 1975; Kroll, 1958; Parlevliet and Contant, 1970). Seed is easily produced in the field and pyrethrum is insect pollinated, mainly by species of Coleoptera and Diptera (Purseglove, 1968).

The production of pure-lines is not usually considered possible in pyrethrum (Thorpe, 1948). However, as there are self-fertile genotypes, selection for self-fertility would probably allow the development of pure-line varieties. Nevertheless, there is no commercial advantage to uniformity for most breeding characters in pyrethrum (Parlevliet and Contant, 1970) and development of pure-line varieties would not have the same advantages as, for example, horticultural crops, where homogeneity of product is important (Welsh, 1981). Naturally out-crossed species generally carry a high genetic load of deleterious

recessive alleles. Deleterious recessives can be purged through inbreeding, but this typically takes between five and 20 generations. Consequently, in a naturally out-crossed species, any advantages that result from the development of selfed lines, do not necessarily justify the development costs (Williams and Savolainen, 1996). Therefore, while pure-lines may be possible in pyrethrum, inbreeding is probably not an efficient breeding method for this crop.

As commercial pyrethrum can be propagated by clones or seed, a choice of variety type needs be made in any pyrethrum breeding program. There are advantages to both propagation methods.

In pyrethrum there is a period of exclusively vegetative growth prior to first flowering and this is shorter for splits than for seedlings. In Africa, splits produce their first flowers in a minimum of four months after planting, while seedlings do not flower until they are at least six to eight months old (Beckley *et al*, 1938; Marr, 1964b). In Tasmania, splits planted in the autumn produce a commercial crop in their first summer, six to eight months after planting (MacDonald, 1995), while seedlings do not produce a commercial crop in their first summer, even when planted early in the year (Fulton, 1998). Another potential advantage of clonal varieties is that they are less variable than seedling crosses (Glynne Jones, 1968), although, homogeneity is not considered to be a particular advantage for most breeding characters in pyrethrum (Parlevliet and Contant, 1970).

There are also a number of benefits to propagation of pyrethrum by seed. While a single plant can produce sufficient seed for some hundreds of seedlings, it can rarely be divided into more than 15 splits (personal observation). In Tasmania, all aspects of seed harvesting, cleaning and sowing are fully mechanised. In contrast, the production of clones by splits, tissue culture or cuttings, is always labour intensive and the planting of splits or established plants cannot be fully mechanised (Pylines, 1999). As a result, in Tasmania, establishment of crops by clonal varieties is more expensive than crop

establishment by seed, with an establishment cost of $400/ha for direct drilled seed and $2500/ha for splits (Macatta, 2001).

Direct-drilled seedlings are substantially cheaper to produce than splits, so it is economical to sow seedlings at relatively high planting densities. In Tasmania, seedlings are established at a rate of 16 to 30 plants/m^2, compared to only 4 plants/m^2 for clones. Flower and pyrethrins yields per hectare increase with increasing plant densities from 4 to 16 plants/m^2 (Fulton, 1998). The increased yields at higher planting densities compensate for the lack of any first year crop in seedlings. Fulton (1998) estimated that the accumulated three-year yield of a seedling crop at 16 plants/m^2 is 29% higher than a clonal crop at 4 plants/m^2, even though the clones produce three commercial harvests, in this period, and the seedlings only two (Table 2.2.1).

Table 2.2.1. Relative yields of pyrethrum established by splits and seed.

	Plants /m^2	Planting date	Dry flower yield (kg/ha)			
			First summer	Second summer	Third summer	Total
Splits	4.5	May	1600	3000	2700	7300
Seedlings	4	October	0	3491	2745	6236
Seedlings	16	October	0	4807	4589	9396

Source: Table 2.1, Fulton, 1998.

Note: In this study, pyrethins content were not affected by plant density, and the pyrethrins yields of the seedlings are proportional to flower yields.

An additional disadvantage of splits, in Tasmania, is that planting is restricted to autumn, while seed can be sown from February to November. The short planting window and high labour requirement for clones, limits the area of

new pyrethrum that can be established by splits in any year, and a rapid expansion of the crop area is only possible by seed (Macatta, 2001; Pylines, 1999).

Seed based production does not have the same marked cost advantage in Africa, as seedlings are transplanted by hand from nurseries to commercial areas (Anon., 2001b). Clonal propagation by splits has the disadvantage of distributing root-knot nematodes, a problem absent in seedlings provided nursery beds are nematode-free. Clonal varieties usually suffer some degree of yield reduction due to nematode damage (Parlevliet, 1975). A mix of clonal and seedling varieties have been used in the past (Parlevliet, 1975) but currently seedlings appear to be more common in Kenya (Anon., 2001b). In Tasmania, a direct-drilled seed-based production system clearly has advantages over clonal propagation in both increased yields and reduced costs. Yields of clones must be substantially greater than their seedling progeny, in order to compensate for the 30% yield reduction that is the result of lower planting densities and the $2100 increase in establishment costs. Therefore, clonal varieties are unlikely to have any place in the local production system in the foreseeable future, and the principal aim of Tasmanian pyrethrum breeding will be to produce direct-seeded varieties that are superior to those in current use.

2.2.3 Flowering physiology

Pyrethrum produces a single main flush of flowers each summer in its native, temperate environment (Tattersfield, 1931), and has an annual vernalisation requirement, producing few or no flowers unless exposed to low temperatures each year (Martin, 1934). Conditions reported as meeting the vernalisation requirement include 5 weeks of 12° C nights (Brown and Menary, 1994) and 10 days of continuous 15.5° C (Glover, 1955), with genetic variation in the threshold temperature required to promote flowering (Kroll, 1964; Roest, 1976). The responses to vernalisation are quantitative and both the number of

flowers initiated and the rate of flower development are increased as the period of low temperature is extended (Brown and Menary, 1994; Roest, 1976) and with decreasing temperature (Brown and Menary, 1994; Glover, 1955; Muturi *et al.*, 1969; Roest, 1976).

The first microscopic signs of flower bud development can be observed approximately four weeks after the onset of vernalising conditions (Brown and Menary, 1994). The first macromorphic sign of flower bud development is elongation of the internodes, and the flowers are born on extended stems, in contrast to the rosette growth form of the vegetative plant (Brown, 1992).

Pyrethrum is perennial and continues to grow vegetatively after flowering. Brown (1992) suggested that a proportion of the buds are not vernalised, and speculated that, like *Geum urbanum* (Chouard, 1960), individual buds are only capable of responding to vernalisation for a specific period during their development. Buds which are at the appropriate maturity stage at the time of chilling will develop as flowers, while buds that are either too young or too old will continue to grow vegetatively.

Pyrethrum does not appear to have any obligate photoperiod requirements. More flowers were initiated, in vernalising temperatures, in short-days than long-days, in a pyrethrum clone tested by Roest (1976). However, the effects of daylength in non-vernalising temperatures were not tested, and it is not possible to determine if short-days can replace the low temperature requirement in this genotype. In contrast, Brown and Menary (1994) found no evidence for any photoperiodic effect in another clone (*CIG 3*). This suggests that the species contains both day-neutral and short-day genotypes.

Brown and Menary (1994) found that long days promote flower development, an effect that appears to be the result of increased photosynthesis rather than a true photoperiodic effect. Similarly, the number of flowers that develop is increased by conditions that enhance plant growth; these include high photon flux density (Brown and Menary, 1994) and high rainfall or irrigation in

field crops (Chung *et al.*, 1990; Kroll, 1963; Muturi *et al*, 1969). Flower initiation is inhibited by low photon flux densities (Brown and Menary, 1994) and high daytime temperatures (Brown and Menary, 1994; Glover, 1955). The presence of mature flowers also appears to inhibit initiation of new flower buds (Kroll, 1962).

As a consequence of the vernalisation requirement, pyrethrum production is limited to temperate regions, or highland areas (>1800 m) of the tropics, that experience cold nights (Anon., 2001b). In highland tropical conditions, pyrethrum can flower continuously, although flowering generally ceases when conditions are dry or hot (Beckley *et al*, 1938; Marr, 1964b).

2.3 Breeding methods used for pyrethrum improvement

The majority of commercially important plant characters, including pyrethrins yield, are quantitative, displaying continuous variation. Selection outcomes for quantitative characters are determined by three main factors; the genetic variance of the selection population; the intensity of selection and selection accuracy (Dekkers, 1992). Selection accuracy corresponds to the correlation between the measured phenotypic value and the true genetic value (Dekkers, 1992; Falconer, 1989), and can be quantified by estimating heritability.

There are a number of different types of heritability which are applicable to different types of selection units. For example, broad-sense heritability is defined as the ratio of genetic variance to the total phenotypic variance and applies to selection of clonal varieties (Welsh, 1981). Phenotypic resemblance between parents and offspring is determined by additive rather than dominant and pleiotropic genes, so the breeding value of an individual is determined by the additive genes it carries. The correlation between breeding value and phenotype is estimated by narrow-sense heritability which is defined as the ratio of additive genetic variance to total phenotypic variance (Falconer, 1989).

Selection may also affect characters other than the primary selection character if they are genetically correlated (Falconer, 1989). Genetic correlations are caused by pleiotropic genes or linkage between genes (Gallais, 1984; Liu *et al.*, 1997). Estimates of genetic correlations can be used to determine the effect of selection for one character on other economically important characters. They are also used to predict the effect of indirect selection, which occurs when selection for a secondary character is used for the improvement of another trait (Searle, 1965).

The effectiveness of a selection method can be assessed by determining the magnitude of the response to selection, which is defined as the difference between the mean phenotypic value of the selected population and the whole

parent generation. Response can be measured by determining the average improvement following selection (realised response). Alternatively, it can predicted prior to selection, from heritability, selection intensity and the variance of the selection population (Falconer, 1989). Predicted and realised responses can also be used to compare the effectiveness of different selection methods (Searle, 1965; Searle, 1978). Alternatively, methods may be compared by estimating their efficiency which is the response per unit cost (Hallauer and Miranda, 1981). Generally the time factor is also important and it is usually desirable to compare the response per unit time when the length of the selection cycle varies among alternate methods (Yano *et al.*, 2000; Yonezawa *et al.*, 2000).

Selection in pyrethrum breeding is aimed at the development of either clonal or seedling varieties. Clonal varieties are selected by multistage selection trials (Bhat, 1995). Typically, these consist of selection of single plants from a genetically variable seedling population (single plant selection), followed by evaluation in unreplicated clonal plots (single line selection). Selected clones will then be assessed in one or more site replicated trials, with as many as five selection stages in total (Bhat, 1995; Parlevliet, 1975). The breeding program conducted by the University of Tasmania used a three-stage method for clone selection, consisting of single plant and single line selection followed by evaluation in block-replicated trials, at two (Potts and Menary, 1988) or three locations (Bhat, 1982; Bhat and Menary, 1984b; Bhat and Menary, 1984c).

Seedling varieties are formed by crossing two or more clones and can be selected by direct evaluation of individual crosses, evaluation of potential parent clones, or both. Parents can be selected by phenotype, as assessed for the individual plant or in clone trials; or by progeny testing, which consists of evaluating the seedling progeny of potential parents. Seedlings for progeny testing can be produced by crossing each potential parent to a test variety or a polycross of all potential parents (Bhat, 1995). Progeny testing of polycross families has been used in Kenya to select parents for seedling varieties

(Parlevliet, 1975). In Tasmania, seedling varieties were selected by evaluation of individual crosses. Seedling trials typically contained some 150 to 200 full-sib families, each produced by crossing two clones. Family means were used to select biclonal crosses for use as varieties (Potts and Menary, 1988).

Population improvement is another technique that has been applied to pyrethrum breeding (Bhat, 1995). This consists of maintaining a genetically variable breeding population which is improved by one or more cycles of parent selection and mating. New varieties are then selected from successive generations of the improved breeding population. Parents for each selection cycle can be selected by single plant phenotype, clonal evaluation or progeny testing (Parlevliet, 1975). Single plant selection has been used for population improvement in Kashmir (Bhat *et al.*, 1985) and progeny testing has been used in Kenya (Parlevliet, 1975). The University of Tasmania used a method of clonal evaluation consisting of single plant and single line selection to choose parents for population improvement (Bhat *et al*, 1985; Potts and Menary, 1988).

In most breeding programs, the aim is to improve more than one character. Methods for multiple character selection include tandem selection, in which one character is improved until its reaches an ecceptable level, then the second and subsequent characters; independent culling, in which individuals that fail to meet a minimum standard in all characters are rejected; and index selection, in which some kind of score is generated to measure net merit (Lerner, 1958). Independent culling is typically used for the final stages of clone selection as varieties finally selected need to display a range of desirable characteristics (Bhat, 1995), and is also commonly used for population improvement. For example, in the population improvement program conducted by the University of Tasmania in the early 1980's, plants were initially selected on the basis of morphological characters such as form. Selected plants were then evaluated for pyrethrins content (Bhat *et al*, 1985). It is also common, in multistage variety selection trials, to assess different characters at different stages. For example, in Tasmania in the

early 1980's, pyrethrins content and morphological characters were assessed at the single plant selection stage and flower yield was not evaluated until the single line trial stage (Bhat *et al*, 1985). In contrast, pyrethrins content was not evaluated until the more advanced selection trials in Kenya in the 1970's (Parlevliet, 1975). Index selection was used for both population improvement and variety selection at the University of Tasmania from 1985 to 1997.

The optimum breeding method is determined by a number of factors; including the reproductive physiology of the crop, the type of variety produced, the economic value of different selection characters, the inheritance of selection characters, and the costs of alternate methods (Borojevic, 1990; Ranalli and Cubero, 1997; Welsh, 1981). The choice of selection characters and breeding priorities will vary among growing areas (Welsh, 1981), as these are determined by the economics of the crop, commercial deficiencies of current varieties and genetic factors (Hallauer and Miranda, 1981). To some extent, genetic parameters are determined by the inheritance and physiology of characters. However, they can vary among populations and growing conditions (Falconer, 1989). Hence, the optimum breeding method needs to be determined for each individual breeding program (Annicchiarico and Pecetti, 1995).

2.4 Breeding for improved pyrethrins yields

2.4.1 Introduction

Like most crop breeding programs (Welsh, 1981), the main aim of pyrethrum breeding programs is typically to increase yield of commercially saleable product (Bhat, 1995; Contant, 1963a; Kroll, 1958; McDaniel, 1990; Parlevliet, 1975; Thorpe, 1948; Tuikong, 1984). Pyrethrins yields are determined by a range of factors, including crop maturity and environmental factors, and these will be reviewed in Sections 2.4.2 to 2.4.4. Yield assessments may also be affected by assessment methods, which will be discussed in Sections 2.4.5 to 2.4.7. A number of different selection characters have been used or proposed for pyrethrins yield improvement. These will be reviewed in Section 2.4.8.

2.4.2 The relationship between flower maturity and pyrethrins yields

The compound flower of pyrethrum opens in stages. First, the ray petals unfold and then the florets open sequentially from the margin to the centre, with a single whorl of florets opening each day (Chandler, 1951). Once the disc florets are open, flower colour begins to fade and seed-set takes place if the florets have been fertilised (Head, 1966b). Flower development was divided into eight maturity stages by Head (1966b) (Table 2.4.1), a system modified by Potts and Menary (1987) to the flower maturity stage definitions shown on Table 2.4.2. The classification system shown on Table 2.4.2 is used commercially in Tasmania and subsequent references to numerical flower maturity stages will refer to this system.

Table 2.4.1. Flower maturity stages as defined by Head (1966a), with pyrethrins levels (mg per flower) and pyrethrins contents (percent of flower dry weight) for clone 1708 as assessed by Gas Liquid Chromotography.

Stage number	Description	Pyrethrins / flower (mg)	Pyrethrins content (%)
1	Well developed closed buds	0.40	0.76
2	Ray florets emerged from bud	1.78	1.49
3	First row of disc florets open	2.48	1.97
4	Three rows of disc florets open	3.45	2.10
5	All disc florets open (mature)	3.89	2.00
6	Disc floret colour fading, ray florets intact	3.92	1.55
7	Ray florets dry, disc florets intact but with little colour (late over-blown)	4.02	1.16
8	Petals of disc florets fallen, stems dry 1 cm below flower, seed mature	3.78	not recorded

Pyrethrins accumulate in the flowers as they mature, typically ranging from 0.4 mg/flower in closed buds to 4 mg in mature flowers (Beckley *et al*, 1938; Head, 1966b; Martin and Tattersfield, 1931; Parlevliet, 1970b)[2]. A decline in pyrethrins is generally observed in the final stage of flower maturity (Head, 1966b; Ikahu and Ngugi, 1988a; Martin and Tattersfield, 1931). Sampling variation has not been reported by any of the published studies of pyrethrins

[2] Ikahu and Ngugi (1988b) reported that pyrethrum buds contained only 0.05 mg pyrethrins and mature flowers 0.4 mg. However, these values are not consistent with the pyrethrins contents and flower weights presented for the same samples (Table 1, Ikaku and Ngugi, 1988b), and it seems likely that the reported values represent centigrams of pyrethrins / flower.

accumulation, so true differences between flower maturity stages cannot be distinguished from random sampling variation. However, the pattern of development is consistent among all published studies and typically, there is a 10-fold increase in pyrethrins as the flower opens, and a decline in mature (stage 8) flowers.

Table 2.4.2. Flower maturity stages as defined by Potts and Menary (1987) which are used commercially in Tasmania.

Stage number	Description
1	Well developed closed buds
2	Ray florets emerged from bud
3	1/3 of diameter of disc with florets open
4	1/3 to 2/3 of diameter of the disc with florets open
5	Between 2/3 and all of the disc with florets open
6	All florets open and up to half the disc area brown
7	Ray florets dry, disc florets intact but with little colour (late over-blown)
8	Petals of disc florets fallen, stems dry 1 cm below flower, seed mature

There are no published investigations of the causes of the decline of pyrethrins in the final stage of flower development. It is common for levels of monoterpenes and other plant secondary products to decrease in mature leaves, and this is generally attributed to active catabolism of the molecules, rather than

translocation to other parts of the plant (Burott and Loomis, 1969; Croteau, 1991; Croteau *et al.*, 1987; Daddona *et al.*, 1976; Funk and Croteau, 1993; Mihaliak and Lincoln, 1989). Therefore, it is possible that pyrethrins in maturing flowers are actively metabolised, and the amount of pyrethrins present might depend on the balance between the rates of synthesis and catabolism.

Pyrethrins levels are often reported as pyrethrins content, which is defined as the percentage pyrethrins per unit of dry matter. Pyrethrins content typically peaks at flower maturity stage 5 and then declines as the flower matures (Bhat, 1995). This decline in the pyrethrins content is partially due to the decrease in total pyrethrins. In addition, reduced pyrethrins content is also caused by a more rapid accumulation of dry matter than pyrethrins in the later stages of flower development (Head, 1966a; Ikahu and Ngugi, 1988a).

2.4.3 Crop development

In Tasmania and other temperate growing regions, flowers are initiated during the winter (Brown, unpublished data) and by late spring, mature pyrethrum plants have produced a mass of well developed flower buds. The flower buds tend to open in a single co-ordinated flush during the first weeks of summer, and generally, no new buds are initiated as the flowers develop and mature (Faber, 1980; Tattersfield, 1931). Initially (late spring), the amount of pyrethrins present in the crop are low, at less than 50 mg/plant. Total pyrethrins rise during the first weeks of summer, peaking at over 500 mg/plant (Faber, 1980; Tattersfield, 1931). Pyrethrins yields are then stable for a period of two to six weeks. After this, yields decline as the flowers reach maturity stage 8 (Faber, 1980).

In Tasmania, the flower maturity index (FMI) is considered to be the best indicator of optimum harvest time (Boevink, 1991; Chung *et al.*, 1994; Faber, 1980). FMI is calculated as the mean flower maturity stage using the classification system shown on Table 2.4.2. The FMI of a temperate crop

typically increases steadily from late spring to late-summer when the entire crop consists of stage 8 flowers (Faber, 1980; Tattersfield, 1931). Pyrethrins yield is generally at a maximum at an FMI of 4.5 to 6.5, and this is the usual harvest criterion for pyrethrum crops in Tasmania, with harvesting taking place in late December to early January. Pyrethrum also produces a single annual flush of flowers in northern India (Gulati *et al.*, 1982), the USA (McDaniel, 1990) and European countries (Gnadinger, 1936; Tattersfield, 1931).

In Africa, pyrethrum is grown at high altitudes (1800 m above sea level) (Anon., 2001b), where the night temperatures meet the vernalisation requirement of the plant. Typically, pyrethrum flowers continuously throughout the year (Dalgety, 1975), although flowers may develop in distinct cyclic flushes, with maximum flower initiation occurring in the wet seasons (Parlevliet, 1970c). Flowering may also cease in the hot, dry parts of the year (Glover, 1955). Flowers are harvested by hand-picking of open flowers at two to three weekly intervals (Kroll, 1962; Parlevliet, 1974).

Therefore, there are substantial differences between pyrethrum crops in Tasmania and in the main growing areas in Africa, in both climate and crop development. In particular, pyrethrum yield in Tasmania consists of a single annual crop that is mechanically recovered, so crops consist of a range of flower maturity stages. In Africa, the total yield each year is the accumulation of multiple harvests and consists of flowers at a specific maturity stage selected by the pickers, and corresponds to maturity stages 3 to 5, using the Tasmanian classification system (Table 2.4.2).

2.4.4 Environmental factors affecting pyrethrins yields

Pyrethrins yields are known to be affected by a number of different environmental factors. Flower initiation is promoted by low temperature and this response is quantitative. Fewer flower buds are initiated in sub-optimal conditions, and the number initiated increases with decreasing temperature and

increasing time of exposure to chilling (Brown and Menary, 1994; Glover, 1955; Muturi *et al*, 1969; Roest, 1976). Pyrethrins content is also affected by temperature, and lower temperatures are conducive to high pyrethrins content (Kroll, 1964; Parlevliet, 1970c). High rainfall and irrigation increase both flower yield and pyrethrins content (Chung *et al*, 1990; Muturi *et al*, 1969; Parlevliet, 1970c), and, in Tasmania, the crop needs to be irrigated during the period of flower development (NRE, 1998).

Sowing time affects yield in temperate growing conditions, and plants that are immature at harvest-time have substantially lower flower yields than mature plants (Bhat, 1995; Fulton, 1998). In Tasmania, pyrethrum seedlings need to be 14 months old before they produce sufficient quantities of flowers for harvesting to be economical (NRE, 1998). Therefore, first year yield evaluations need to be made for mature plants (Bhat, 1995). Sowing time has no noticeable effect on second year yields (Bhat, 1995; Fulton, 1998).

At one time, pyrethrum growers believed that the crop needed to be pollinated for maximum achene development and yield, and that clonal plantations needed to include pollinator clones (Brown, 1965; Thorpe, 1948). Seed-set in pyrethrum can range from 5% (Delhaye, 1956) to 70% (Pandita, 1983) and the fertile achenes within a seedlot tend to be heavier and have a higher pyrethrins content than the 'hollow' achenes taken from the same flower (Chandler, 1955). This may indicate that pollination increases pyrethrins yields (Kroll, 1961). However, this would only be the case if flowers that contain a proportion of fertilised achenes also produce more pyrethrins overall. It is also possible that pollination affects the partitioning of resources and pyrethrins production among the individual achenes, without altering the total resources available to, and the total pyrethrins produced by, the entire flower.

Tests of the effect of pollination on pyrethrins yields of partially-open flowers (maturity stage 4 to 6), suggest that pollination has little effect on pyrethrins yields for these flower maturity stages (Kroll, 1961; Potts and Menary,

1987). This is to be expected as partially-open flowers consist of a proportion of unopened, unpollinated florets and newly pollinated florets. It seems unlikely that any effect of fertilisation on pyrethrins production would be expressed at this early stage. Therefore, in Africa, pollination is unlikely to affect commercial pyrethrins yields, as commercial crops consist exclusively of partially-open flowers (Kroll, 1961). Temperate crops typically contain some late-stage flowers (stage 7 to 8) when harvested (Faber, 1980), and currently, it is not clear whether pollination has any effect on commercial yields in mechanically-harvested temperate crops.

2.4.5 Factors affecting determination of pyrethrins in plant material

Assay methods for pyrethrins determination

Pyrethrins can be quantified by a number of different methods. However, the method of the Association of Official Analytical Chemists (AOAC) is the only one currently accepted by industry and government regulators. The AOAC method involves the hydrolysis of the esters to produce chrysanthemic acid (CA) and chrysanthemum dicarboxylic acid (CDA), which is derived from pyrethric acid. CA and CDA are separated and quantified by titration following reduction with mercury (CA) or titration against a standardised base solution (CDA). This determines the concentrations of the Pyrethrins I and Pyrethrins II but not of the individual esters (Moore, 1975; Shah, 1970).

The AOAC method is quite time consuming and complex (Shukis *et al.*, 1951) and results can vary between laboratories (Head, 1973; Shah, 1970). Consequently, a number of simpler and more easily reproducible methods have been developed. The most widely used are assay by UV-spectroscopy, Gas Liquid Chromatography and High Performance Liquid Chromatography. These three techniques are all comparative procedures, which need to be calibrated by a standard that has been quantified by chemical analysis, usually the AOAC

method (Carlson, 1995).

Beckley's (1949; 1950) UV method consists of an extraction and purification procedure with spectrophotometric determination of the percentage pyrethrins from absorbence at a fixed wavelength (λ = 220 – 230nm), utilising the strong absorbence of UV-light that is characteristic of pyrethrins (Crombie and Elliot, 1961). This method has some disadvantages. It only estimates total pyrethrins and cannot determine the levels of the individual esters. Further, it is not specific for pyrethrins. Any substance that absorbs UV light at 220 - 230nm will be detected and hence, the method is inaccurate when the sample contains unusually high levels of such substances (Head, 1973). The UV method is also less accurate when the sample and the standard differ in the ratio of PyI/II or pyrethrin:cinerin:jasmolin (Head, 1973; Ward and Newham, 1962). However, fairly close agreement between the UV and AOAC methods has been reported (Beckley, 1950; Shukis *et al*, 1951).

Gas Liquid Chromatography (GLC) and High Pressure Liquid Chromatography (HPLC) allow separation of the six esters. These are quantified using electron-capture or flame ionization, in the case of GLC (Head, 1973), or UV absorbence, in the case of HPLC (McEldowney and Menary, 1988; Otieno *et al.*, 1983). GLC has been used quite extensively for pyrethrins assays, but has the disadvantage of operating at high temperatures that can cause degradation of pyrethrins, particularly pyrethrin II (Mourot *et al.*, 1978). HPLC does not cause pyrethrins degradation, as it operates at room temperature and HPLC methods for pyrethrins assays have generally superseded the use of GLC.

Like the UV method, inaccuracies in both GLC and HPLC-assay are expected if the pyrethrin:cinerin:jasmolin ratio of the samples are not the same as the standard (Carlson, 1995), although differences in PyI/II ratio should not be a problem if separate calibration curves are prepared for the Pyrethrins I and Pyrethrins II. GLC-assays of clones with disparate proportions of component esters agreed quite well with AOAC-assays (Head, 1967), with a correlation

coefficient of 0.89[3]. Assays of single samples report errors of 0.6% (Wang *et al.*, 1997) to 1.3% (Otieno *et al*, 1983) for the HPLC method compared to AOAC evaluations, and Kasaj *et al* (1999) reported a correlation of 0.95 between HPLC and AOAC assays of three natural extracts with PyI/II values from 1.1 to 2.6. In general, the HPLC method is considered to be both precise and reproducible (Kasaj *et al*, 1999).

The complexity of the AOAC method and length of time required for each assay means that it is not particularly practicable for screening large numbers of plants and, consequently, the AOAC method has limited application in pyrethrum breeding programs. Most published accounts of breeding programs report the use of the UV-method for pyrethrins assessments (see for example Bhat *et al*, 1985; Ikahu and Ngugi, 1988b; Parlevliet, 1974; Parlevliet and Contant, 1970).

The University of Tasmania's breeding program used a modified version of Beckley's UV method, that lacks the sample purification steps specified by Beckley (1949). Bhat and Menary (1986c) reported HPLC and UV-assay values for a number of clones, replicated at three sites. Analysis of this data (Appendix 1.1) indicates that the University's UV-method overestimates pyrethrins content by 0.39 percentage units, on average (Table 1, Appendix 1.1), assuming that the HPLC-assay values are accurate. The magnitude of the difference between the UV and HPLC assay varies between samples, with a correlation of 0.60 between the two methods (Appendix 1.1). This suggests that the University's UV-method is considerably less accurate than Beckley's UV-method (Beckley, 1950; Shukis *et al*, 1951) and GLC analysis (Head, 1967). However, the University's UV-method is also considerably less labour intensive and many more samples can be evaluated per unit cost when Beckley's more complex sample purification steps are omitted.

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[3] Pearson's product-moment coefficient calculated from data presented in Table 1, Head, 1967.

Stability of pyrethrins in plant material and extracts

Pyrethrins are unstable in the presence of heat or light, and assay values will be inaccurate if samples are dried or stored in inappropriate conditions. Drying plant material at temperatures above 40°C causes a loss of pyrethrins which is proportional to the temperature and the length of the drying time (Githinji, 1973; Ngugi and Ikahu, 1990a). Once plant samples are dried, the pyrethrins are relatively stable, and can be stored at low temperature (-2° C) for six months with no significant degradation (Gnadinger, 1936). However, the rate of pyrethrins degradation can vary, and appears to be affected by flower maturity (Boevink, 1991; Gnadinger, 1936), and genotype (Bhat and Menary, 1984c). Solutions of pyrethrins are extremely unstable in light at room temperature, although they can be stored in the dark for two months or more with no detectable decline in concentration (Dickinson, 1987).

Pyrethrins are degraded by a number of different reactions. The type of reaction is determined by physical conditions, such as the presence of light and oxygen, and the type of chemical solution containing the pyrethrins. Pure solutions of individual esters will produce a single photoproduct, which results from the isomerisation of the side chain of the alcohol portion of the molecule (Bullivant and Pattenden, 1976; Dickinson, 1987; Kawano *et al.*, 1980). However, thin films of purified esters, with the solvent removed by evaporation, produce 11 or more different photoproducts, which are the result of a number of different reactions affecting the alcoholic portion (Brooke, 1967; Chen and Casida, 1969; Glynne Jones, 1960). Changes to the chrysanthemate moiety occur in unpurified extracts and these appear to be catalysed either by chlorophyll or another substance associated with the pigment fraction (Brown and Phipers, 1955; Head *et al.*, 1968). There is a further light-independent process, which is limited to highly concentrated solutions, involving polymerisation of chrysanthemic and pyrethric acid to form a resin (Campbell and Mitchell, 1950).

Therefore, the following conditions are required for reliable pyrethrins

assays of plant samples:

1. Plant material must be dried soon after harvesting at temperatures of 40°C or below.
2. Dried samples should be stored at low temperature and assayed within 6 months of collection.
3. Extracts should be stored in the dark and unpurified extracts should be assayed within a short time of extraction.

2.4.6 Experimental yield evaluation methods for temperate crops

Pyrethrins yields change as the crop matures in temperate growing regions, so the measured value of trial yields will vary between sample-dates. One method used to determine pyrethrins yields in Tasmania is serial sampling, which typically consists of harvesting at weekly intervals over a period of two months (Faber, 1980). Although this provides a detailed assessment of yield development, serial sampling is a relatively inefficient assessment method, as data collected on sample dates that fall outside the usual harvest criterion (FMI 4.5 to 6.5) are not relevant to commercial conditions. Serial sampling also requires replicated trial material and cannot be used for evaluations of single plants. Consequently, this type of harvesting is usually restricted to optimum harvest-time trials, in the final stages of variety assessment.

An alternative sampling method is to choose one or more harvest dates that fall within the commercial harvest period (Fulton, 1998; Salardini *et al.*, 1994). This method has the advantage of reduced sampling cost. However, the date of harvest maturity time can vary among sites and seasons (Boevink, 1989; Chung, 1995; Faber, 1980), so individual trials need to be monitored to ensure that the harvest-date(s) coincide with crop maturity.

Fixed-date sampling may be inappropriate when the treatment effect alters the rate at which the crop matures. Assessments will be biased in favour of

treatments that are at harvest-maturity at the time of assessment, and yields for early- and late-maturing treatments may be under-estimated. There appears to be genetic variation in the time of harvest maturity of temperate crops (Bhat, 1995), so fixed-date sampling is inappropriate for Tasmanian breeding trials. It is preferable to harvest sample units independently at a consistent FMI, a method used by Bhat and Menary (1986b).

Another sampling method used to assess pyrethrins content in temperate crops is to restrict samples to a single flower maturity stage, specifically either stage 5 (Bhat and Menary, 1984b) or stage 6 (Potts and Menary, 1988). This has the advantage of comparing all sampling units at a consistent flower maturity and, in some circumstances, sampling costs can be reduced. For example, in the University's breeding program, small samples of 15 stage 6 flowers were picked for assay (Potts and Menary, 1989). This method was substantially less time-consuming and expensive than the alternative of stripping all flowers, particularly for plot assessments, as Tasmanian-grown pyrethrum plants typically produce some 300 flowers each (Bhat and Menary, 1986b).

However, the pyrethrins assay of a single flower stage is not necessarily a reliable indicator of the pyrethrins content of the entire crop. Mechanically-harvested temperate crops consist of a range of flower maturity stages (Faber, 1980). There is considerable variation in the pattern of pyrethrins accumulation as flowers mature (Bhat, 1995; Bhat and Menary, 1984a; Ikahu and Ngugi, 1988a; Parlevliet, 1970b), as well as in the synchronicity of flowering (Bhat, 1995; Bhat *et al*, 1985). Therefore, there will not be a perfect correlation between the pyrethrins content of stage 5 (or 6) flowers and the entire multiple flower stage crop. The relationship between the pyrethrins content of a specific maturity stage and the entire crop has not yet been investigated, so, currently, it is not certain that treatment rankings based on assay of a single maturity stage accurately reflect crop pyrethrins levels. It has been suggested that there is genetic variation in the pattern of pyrethrins accumulation (Bhat, 1995; Bhat and Menary, 1984a; Ikahu

and Ngugi, 1988a; Parlevliet, 1970b) and flowering synchronicity (Bhat, 1995). Consequently, assays of a specific flower maturity stage may be inappropriate for variety evaluation trials.

2.4.7 Factors that may affect genotypic rankings in breeding trials

The rankings of genotypes in breeding trials may vary between test environments. Inconsistent genotypic rankings occur when there is an interaction between genotypes and environments. The relative size of the interactions between clonal varieties and test location were investigated by Bhat and Menary (1986a) and between clones and environment (location and harvest season) by Parlevliet (1969). Both studies reported the presence of clone-environment interactions for pyrethrins yield and its component characters (Table 2.4.3.). However, in all cases, the proportion of the total phenotypic variance attributable to the interaction was substantially smaller than for genetic effects.

Table. 2.4.3. Genotype-environment interactions for clonal varieties tested in Tasmania (Bhat and Menary, 1986a) and Kenya (Parlevliet, 1969). Figures represent the percent of total phenotypic variance attributable to genotype-environment interactions and genotype.

Character	Bhat and Menary (86)		Parlevliet (69)	
	Clone	Clone x location	Clone	Clone x season-location
Pyrethrins yield	77	19	-	-
Pyrethrins content	92	5	87	5
Flower yield	73	22	60	30
Flower number	85	10	-	-

Pyrethrins yields vary with plant density, and in Tasmanian growing conditions, yield per unit area is at a maximum over a broad range of densities, spanning 16 to 40 plants/m^2 (Fulton, 1998). Commercial areas in Tasmania are currently established by direct drilling of seed. Germination rates of pyrethrum seed can be highly variable and currently it is not possible to establish seedling areas at a precise density by mechanical sowing (Chung and Bourke, 1997; Chung *et al*, 1994; Fulton, 1998; Greenhill, 1997). Consequently, plant densities are highly variable both between and within commercial areas.

Genotype rankings have been shown to vary between different plant densities in other crop plants (for example, Annicchiarico and Piano, 2000; Mallikarjun and Khanure, 1998; Sekhon *et al.*, 1996). So it is possible that genotype rankings in pyrethrum may not be consistent across the full range of densities that occur in commercial areas in Tasmania. If this is the case, variety assessments may be affected by the establishment density of the assessment trial. An investigation of the effect of plant density on yield assessment in Kenyan-grown pyrethrum (Parlevliet, 1968) found no evidence of any genotype-density interaction in flower yields of three clones. However, the range of densities tested in this trial (< 6 plants/m^2) were substantially lower than the target density for Tasmanian commercial areas (>9 plants/m^2). The only study of the effect of plant density on pyrethrins yield in Tasmanian-grown pyrethrum (Fulton, 1998) was restricted to a single variety, and the existence of a genotype-density interaction has not yet been investigated for Tasmanian growing conditions.

It has been suggested that each pyrethrum genotype has a characteristic pattern of pyrethrins net accumulation (Bhat, 1995; Bhat and Menary, 1984a; Ikahu and Ngugi, 1990). If this is the case, then genotype rankings will be affected by the harvest-maturity criterion used for yield evaluations and the optimum harvest stage needs to be individually determined for each clone (Bhat, 1995; Bhat and Menary, 1984a; Ikahu and Ngugi, 1990).

Evidence of genetic variation in the pattern of pyrethrins accumulation was presented by Parlevliet (1970b) in the form of graphs of the pyrethrins content of flowers sampled from 2 to 21 days after opening, with flowers collected from four clones, replicated at two sites. While there was considerable variation in the shape of the pyrethrins accumulation curve, the patterns of pyrethrins accumulation for each clone were similar at the two sites. This was particularly the case for two of clones, although the similarities are less striking for the other two. These observations suggest that the variation between dry matter and pyrethrins is a characteristic of each clone (Parlevliet, 1970b).

Parlevliet's (1970b) conclusions are supported by data collected from a replicated sampling trial in Tasmania (Faber, 1980). Flower samples were taken over a 10 to 14 week period from two clonal varieties, *CIG 3* and *CIG 11*, replicated at two commercial sites. Pyrethrins contents for each flower maturity stage are shown on Figure 2.2. The shapes of the curves were reasonably consistent for the two clones, although they were not identical at each site. This variation could indicate a site effect on the pattern of pyrethrins accumulation. However, samples were not replicated in this experiment, and a true site effect cannot be distinguished from random sampling variation. Nevertheless, the presence of variatiability within clones shows that there is an environmental component to variation in the pattern of pyrethrins accumulation as well as a genetic effect.

Bhat and Menary (1984a) and Ikaku and Ngugi (1990) also presented data that show there is considerable phenotypic variation in patterns of pyrethrins accumulation. However, in both these studies flowers were collected from unreplicated clonal plots, so it is not possible to distinguish genetic and environmental effects.

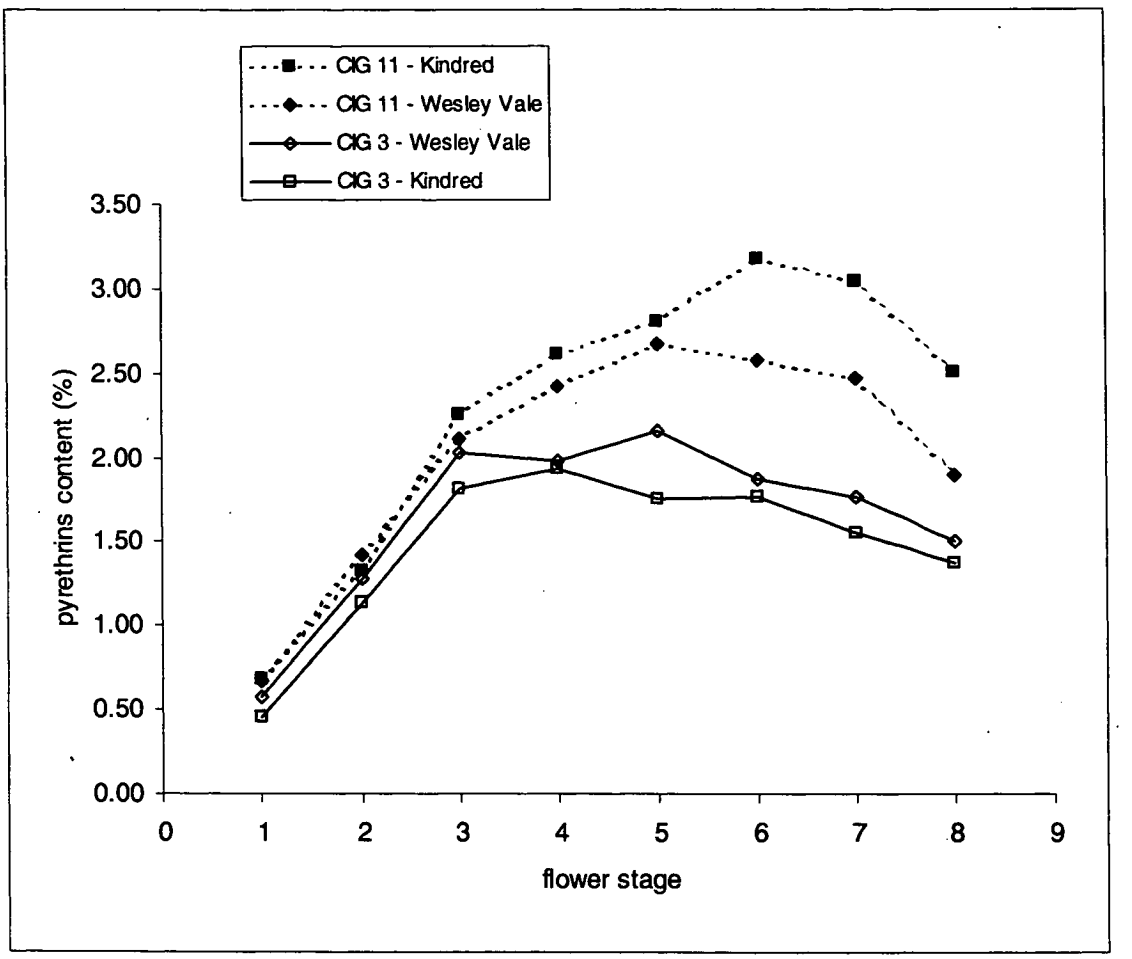

Figure 2.2. Pyrethrins content for individual flower maturity stages for two clonal varieties (*CIG 3* and *CIG 11*), tested at two sites (Kindred and Wesely Vale). Data adapted from Faber (1980) and flower maturity stages are as defined on Table 2.4.2.

Although the variation in the pattern of pyrethrins accumulation appears to have a genetic component, currently there is no evidence for genetic variation in the optimum harvest time of temperate crops. Reports of serial sampling trials

conducted in Tasmania have concluded that there are no differences in the optimum harvest time between the clonal varieties, *CIG 3* and *CIG 11* (Boevink, 1989; Faber, 1980), or seedling varieties (Chung, 1995). This is probably because pyrethrins yields are not solely a function of flower maturity. A range of environmental factors affect yield (Section 2.4.4) and micro-climate variation, both within a site and over time, may mask any genetic pattern of pyrethrins accumulation at the individual flower level.

To summarise, there is clearly considerable phenotypic variation in the pattern of pyrethrins accumulation. This variation appears to be determined by both genetic and environmental effects and the relative importance of each has not yet been investigated. Currently, there is no evidence that different varieties have different optimum harvest times in Tasmanian growing conditions. It is possible that genetic differences in the patterns of pyrethrins accumulation do not have any observable effect on optimum harvest time because they are obscured by environmental variation.

2.4.8 Selection criteria used for yield improvement in pyrethrum

Introduction

Pyrethrins yields can be improved by direct selection for yield. However, a range of alternative selection characters have also been used for yield improvement in pyrethrum (Bhat, 1995). These include yield components, such as flower yield, pyrethrins content and flower number; and a range of related characters, such as flowering synchronicity and the number of oil-glands on the surface of the florets.

The technique of yield improvement through selection of correlated or component characters has been applied to a number of crop plants, including cotton (Manning, 1958) and maize (Weyrich *et al.*, 1988). The effectiveness of any indirect selection character depends on it having a high genetic correlation with

the primary character (yield) and the relative values of their heritabilities (Searle, 1965). Indirect selection for a single character is rarely more effective than direct selection (Gallais, 1984). However, indirect selection characters can be more efficient when they are less expensive to evaluate than the target character (Wright, 1984) or when two or more secondary characters are used (Banziger and Laffite, 1997; Dolan *et al.*, 1996; Johnson *et al.*, 1955; Manning, 1958). Secondary characters that are suitable selection traits are characters with a high genetic correlation with yield, high heritability, low genotype-environment interaction or low assessment cost (Annicchiarico and Pecetti, 1998).

Direct selection for pyrethrins yield

The only reported value for broad-sense heritability of pyrethrins yield is relatively high at 0.77 (Bhat and Menary, 1986a), and the narrow-sense heritability for this character appears to be moderate, at 0.29[4]. Parlevliet (1969) reported that direct selection of clones tested in Kenya (block replicated trial, 120 plants/clone) resulted in a 34% increase in pyrethrins yield. However, most of this improvement was due to a 31% increase in flower yield and there was only a 2% increase in the average pyrethrins content of the selections, compared to the selection population. This was considered to be a poor selection outcome (Parlevliet, 1969), as any increase in flower yield also results in an increase in picking, transport and extraction costs. In contrast, an increase in pyrethrins content increases the yield of active product without affecting production costs.

The proportionally higher increase in flower yield was attributed to the greater phenotypic variation in this character in the selection population (Parlevliet, 1969). This appears to be typical in pyrethrum and a higher level of variability has been observed in flower yield than for pyrethrins content in other pyrethrum populations (Bhat and Menary, 1986b; Bhat and Pandita, 1982).

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[4] Calculated from data presented in Table 1 (Parlevliet and Contant, 1970)

Therefore, it is possible that direct selection for pyrethrins yield may often result in proportionally higher gains in flower yield than pyrethrins content.

Although pyrethrum is mechanically harvested in Tasmania, an increase in flower yield would be expected to increase post-harvest costs (Potts and Menary, 1989). Therefore, the net economic gain will be greatest when the improvement is solely through increased pyrethrins content, as there will be no change in processing costs. However, currently the economic difference between flower yield and pyrethrins content is not considered to be important by Botanical Resources Australia, the main pyrethrum industry group in Tasmania (Brian Chung, pers comm). Similarly, Tasmanian growers are paid by weight of active product (NRE, 1998), so an increase in flower yield provides the same return to the grower as a comparable increase in pyrethrins content. Therefore, while direct selection for pyrethrins yield may typically favour flower yield at the expense of pyrethrins content, this may not have the same economic disadvantage in Tasmania as in other growing regions.

Selection for yield components

Pyrethrum yields are never directly evaluated by extracting and weighing the total pyrethrins. Rather, yield is determined from its components; fresh flower weight, the dry matter content of the fresh flowers, and pyrethrins content of the dried flowers (Bhat, 1995; Parlevliet and Contant, 1970), with the dry matter and pyrethrins contents typically determined from sub-samples of the total flowers harvested. Consequently, the evaluation costs for direct selection and selection by these component characters are identical. Direct selection for yield is not usual in pyrethrum breeding programs and selection is typically for yield components (Bhat, 1995; Contant, 1963a; Kroll, 1958; McDaniel, 1990; Parlevliet, 1975; Thorpe, 1948; Tuikong, 1984).

Parlevliet and Contant (1970) found that the genetic variation in dry matter content was negligible for a seedling population assessed in Kenya.

content was negligible for a seedling population assessed in Kenya. Consequently, dry matter content is generally not used as a selection character and is usually amalgamated with fresh flower weight into a single trait (dry flower yield) (Bhat, 1995; Contant, 1963a; Parlevliet, 1975; Singh *et al.*, 1988).

Published estimates of broad-sense heritability for dry flower yield and pyrethrins content are high for both characters when clones are evaluated in replicated plot trials (Table 2.4.4). Similarly, published estimates of narrow-sense heritability for these characters are moderate to high (Table 2.4.5), indicating that an improvement would be expected from selection of either of these characters.

Table 2.4.4 Broad-sense heritability estimates for pyrethrins yield and its components.

Character	Bhat and Menary (86)	Parlevliet (69)	Parlevliet (75)	Singh *et al* (87)	Pandita and Bhan (89)*	
					1st yr	2nd yr
Pyrethrins yield	0.77	-	-	-	-	-
Pyrethrins content	0.92	0.87	-	0.83	0.73	0.45
Flower yield	0.73	0.60	0.07*	0.39	0.73	0.69
Visual estimate of flower yield	-	-	0.15*	-	-	-
Flower number	0.85	-	-	-	0.76	0.62
Growing region	Tasmania	Kenya	Kenya	S. India	Kashmir	
Estimation method	Variance component	Variance component	Realised heritability	Variance component	Variance component	
Selection trial design	Site and block replicated, 120 plants /clone	Single-site, block replicated, 120 plants /clone	Unreplicated seedling trial	Single-site, block replicated	Single-site, block replicated, 40 plants/clone	

* Calculated from data presented on Table IV (Parlevliet, 1975)

Table 2.4.5. Narrow-sense heritability estimates for pyrethrum, calculated from parent-offspring regression method (P-O) and from single crosses (SC) for plants assessed in Kenya (Parlevliet and Contant, 1970) or as realised heritability (Bhat and Menary, 1984a) for plants assessed in Tasmania.

Character	Parlevliet and Contant (1970)		Bhat and Menary (1984)
	P-O	SC	
Pyrethrins yield	0.29*		
Pyrethrins content	0.70	0.64	0.13 - 0.26***
Flower yield	0.31	0.56	
Dry matter content	0.00**		

* Value calculated from Parlevliet and Contant's (1970) data, Table 1.

** Parlevliet and Contant reported that there was no correlation between parent clones and offspring for this character and did not estimate heritability from data.

*** Estimate determined by values used in calculations (see Appendix 1.2 for details).

The only published study of genetic correlations between pyrethrins yield and the component characters (Bhat and Menary, 1986a) found that the correlations were positive and relatively high (Table 2.4.6), indicating that selection for either flower yield or pyrethrins content should result in higher pyrethrins yields.

Early work in Kenya suggested that there was a negative association between flower yield and pyrethrins content, indicating that an increase in one character would result in a decrease in the other (Contant, 1976). A negative genetic correlation between flower yield and pyrethrins content was also observed for second year yields of pyrethrum in Kashmir (Pandita and Bhan, 1989). However, other estimates of correlations between the two characters are low ($<|0.1|$) and positive (Table 2.4.6), and later evaluations of Kenyan clones found no evidence of any association between these traits (Parlevliet, 1974). The negative association reported by Contant (1976) may be due to linkage

effects, as correlations due to linkage are unstable and will change as the result of breeding (Gallais, 1984).

Table 2.4.6. Genetic (r_g) and phenotypic (r_p) correlations between breeding characters in pyrethrum, estimated for clones in Tasmania (Bhat and Menary, 1986a), Southern India (Singh *et al.*, 1987) and Kashmir (Pandita and Bhan, 1989).

Characters	Bhat and Menary (86)		Singh *et al* (87)		Pandita and Bhan (89)	
	r_p	r_g	r_p	r_g	r_p	r_g
Pyrethrins yield - pyrethrins content	0.69	0.66	-	-	-	-
Pyrethrins yield - flower yield	0.75	0.79	-	-	-	-
Pyrethrins yield - flower number	0.38	0.43	-	-	-	-
Pyrethrins content - flower yield	0.06	0.09	0.08	0.04	0.12^{+} -0.3^{++}	0.15^{+} -0.4^{++}
Number of test locations	3		1		1	

$^{+}$ first year data $^{++}$ second year data

As there appears to be no evidence of a consistent negative association between these flower yield and pyrethrins content, an improvement in both characters should be possible. This conclusion is supported by selection outcomes reported by Bhat and Menary (1984b), who found substantial increases in both the pyrethrins content and flower yields of selected clones (Table 2.4.7). Similarly, clones selected in Kenya had substantially greater flower yields and pyrethrins content than unselected material collected in Yugoslavia (Parlevliet, 1970a). The outcomes reported for a population improvement program conducted in Kashmir (Bhat *et al*, 1985) also suggest that a negative correlation between flower yield and pyrethrins content is unlikely. In this breeding program, selection for pyrethrins content (Bhat, 1995; Bhat *et al*, 1985) did not

result in the reduction in flower yields (Bhat *et al*, 1985) which would be expected if there was a negative genetic association between these characters.

Table 2.4.7. Realised response to clonal selection experiments conducted in Kenya (Parlevliet, 1969; Parlevliet, 1975), Kashmir and Tasmania (Bhat and Menary, 1984b). Figures represent the percent increase for each character.

Selection method	Direct selection for pyrethrins yield	Selection for flower yield		Pyrethrins content and flower yield	
		By weight	By eye	Kashmir selections	Tasmanian selections
Pyrethrins yield	34	-	-	34[+]	45[+]
Pyrethrins content	2	-	-	10[+]	15[+]
Flower yield	31	18	26	22[+]	27[+]
Selection criteria	Pyrethrins yield	Flower weight	Visual yield	Not specified - probably independent selection for pyrethrins content and flower yield	
Proportion selected	34%	10%	10%	Not specified	
Selection trial	block replicated, single site, 120 plants/ clone	seedling trial (unreplicated, single plants)		Not specified	
source	Parlevliet (69)	Parlevliet (75)		Bhat and Menary (84)	

[+]Clones tested in site-replicated trials in Tasmania. Response calculated as difference between the mean of the selected clones and the SP seedling population (Table 2.6).

Clones selected from a breeding population developed in Kashmir (Bhat and Menary, 1984b; Bhat and Menary, 1986a) had a proportionally higher increase in pyrethrins content (10-15%) than clones selected in Kenya (Parlevliet, 1975) by direct selection for pyrethrins yield (Table 2.4.7). The selection methods used by Bhat *et al* were not specified (Bhat and Menary, 1984b; Bhat and Menary,

1986a). However, it is likely that selection was on the basis of the component characters because all published data for the Kashmir population consists of yield components rather than yield *per se* (Bhat, 1995; Bhat and Menary, 1986a; Bhat and Menary, 1986b; Bhat and Pandita, 1982). This suggests that component character selection does not favour flower yield to the same extent as direct selection, although comparisons between different populations and growing areas are not necessarily valid.

The number of flowers/plant has also been proposed as a selection character (Bhat and Menary, 1986a). Flower number per plant should be an effective selection character as it has a moderate (0.4) genetic correlation with pyrethrins yield (Table 2.4.6) and slightly higher broad-sense heritability (Bhat and Menary, 1986a). However, pyrethrum plants grown in Tasmania produce some 300 flowers/plant (Bhat and Menary, 1986a), so counting flowers is substantially more labour-intensive and expensive than weighing them. Therefore, any advantage from the use of this character is likely to be out-weighed by assessment cost, at least in Tasmania where labour costs are high.

The breeding program at the University of Tasmania has used the pyrethrins assay of a specific flower maturity stage as a primary selection character; assessing stage 5 flowers from 1980 to 1985, and stage 6 flowers from 1985 to 1997. Mechanically-harvested temperate crops consist of a range of flower maturity stages, including stage 5 and 6 (Faber, 1980), so the assay of stage 5 (or 6) flowers will not be identical to the assay of the entire multiple stage flower crop. Nevertheless, restricting assay sample to a single flower stage has a number of potential advantages. Harvest costs may be reduced, and the use of a single maturity stage ensures that comparisons are made at a consistent flower maturity stage. However, this is potentially a disadvantage, as there is genetic variation in the patterns of pyrethrins accumulation (Parlevliet, 1970b), so there will be bias in favour of genotypes which have maximum pyrethrins at stage 5 (or 6). Nevertheless, this may also be the case when comparisons are made of

entire flower crops harvested at a constant FMI.

It is possible that stage 5 (or 6) assay could be a more efficient selection criterion than the pyrethrins assay of the entire crop. This will be the case if the heritability of stage 5 (or 6) pyrethrins is higher than heritability of crop pyrethrins and there is a high positive genetic correlation between the two characters (Gallais, 1984; Hansel, 1984; Searle, 1965). To date, there has been no investigation of the relative heritabilities of the pyrethrins content of stage 5 (or 6) flowers and the entire crop, or the genetic correlation between them. A positive genetic correlation between these characters is likely because stage 5 and 6 flowers form a part of the entire crop. However, the correlation will be less than 1.0 because pyrethrins content of the entire crop is determined by a number of other variable factors, such as flowering synchronicity (Bhat, 1995; Bhat *et al*, 1985) and in the pattern of pyrethrins accumulation as flowers mature (Bhat and Menary, 1984a; Ikahu and Ngugi, 1988a; Parlevliet, 1970b).

If there is a substantial random, environmental component of variability in pyrethrins accumulation or synchronicity, then the proportion of non-genetic variation in the measured value for stage 5 (or 6) assay will be smaller than for the entire crop assay, and the stage 5 (or 6) assay could be the more effective selection character (Dickerson, 1969). However, the converse might also apply. There may be a genetic component of the variation in the patterns of pyrethrins accumulation (Parlevliet, 1970b) and synchronicity of flowering (Bhat, 1995). If there is significant genetic variation in these characters, stage 5 (or 6) assay may be the less effective selection character as it will have a low genetic correlation with the pyrethrins content of the entire crop.

To date there have been no published investigations of the genetic relationship between stage 5 (or 6) pyrethrins content and the pyrethrins content of the entire flower crop. Published data on the response to selection for stage 5 assay (Bhat, 1982; Bhat and Menary, 1984b; Bhat and Menary, 1984c; Bhat and Menary, 1985; Bhat and Menary, 1986c; Bhat *et al*, 1985) have been restricted to

assessments of the selection character and crop pyrethrins were not evaluated (Bhat *et al*, 1985). Consequently, while it seems likely that stage 5 (or 6) assay is an effective method for improving crop yields, currently there are no published data that confirm this. Similarly, there are no published data that can be used to compare the efficiency of stage 5 (or 6) assay with crop pyrethrins content as selection characters.

The use of approximate evaluation methods to reduce evaluation costs

For a constant selection intensity, the response to selection will be lower for a relatively inaccurate method of character evaluation. This is because a reduction in the accuracy of character evaluation will have the effect of reducing selection accuracy and heritability (Dekkers, 1992; Falconer, 1989). However, if an approximate evaluation method is less expensive than the alternative, it is possible to increase the number of plants assessed for the same overall cost (Yonezawa, 1983). This means that the selection intensity can be increased. Therefore, the selection response per unit cost might be greater for a less accurate evaluation method if the decrease in selection accuracy is off-set by an increase in selection intensity (Yonezawa, 1983).

Visual yield evaluation is commonly used in plant breeding to reduce yield assessment costs. The University of Tasmania's breeding program used visual yield evaluations for single plant, family and clonal selection (Potts and Menary, 1988), from 1985 to 1997. Parlevliet (1975) compared visual yield evaluation with assessment by harvesting and weighing flowers for selection of single plants from seedling trials for use as clones, and found that the response in flower yield was greater for visual selection (Table 2.4.7). Possibly, this is because visual assessment can take additional factors into account, such as plant health (Parlevliet, 1975). Alternatively, the apparent superiority of visual selection may be due to the sampling variation of the realised response (Baker and Curnow, 1969; Hill, 1974; Hill, 1978; Sen and Robertson, 1964). Nevertheless, visual

evaluation was clearly an effective selection method in this trial, and the additional cost of harvesting and weighing flowers did not result in any increased gain.

In general, the effectiveness of visual yield evaluation increases with the variability of the selection material (Briggs and Shebeski, 1970). Typically, it is an effective method for selecting material from highly variable seedling trials, but less useful in more advanced trials (Manning, 1958). Therefore, while visual assessment may be an effective selection method in pyrethrum seedling trials, it is not necessarily effective in advanced variety trials.

Plant height, plant diameter and stem number have also been proposed as approximate selection characters for flower yield in pyrethrum (Pandita and Bhat, 1984; Singh *et al*, 1987). These characters tend to have fairly high phenotypic correlations with yield in seedling populations. For example a correlation of 0.62 was reported for plant height and yield and 0.77 for stem number (Pandita and Bhat, 1984). In contrast, the correlations reported for clonal trials were substantially lower, at 0.1 for plant height and 0.2 for bush diameter (Singh *et al*, 1987). It appears that, like visual assessment, bush size and stem number are likely to be more effective selection characters in highly variable seedling populations.

There is variation in the accuracy of alternate assay methods used to determine pyrethrins content (Section 2.4.5). The UV method used by the University of Tasmania is relatively non-specific for pyrethrins and appears to be relatively inaccurate compared to assay by AOAC, HPLC and Beckley's UV methods (Section 2.4.5). Nevertheless, the University's UV-method is the least expensive, so selection by UV-assay may be more efficient in terms of response per unit cost. However, the relative efficiency of different assay methods have not yet been evaluated.

In addition to the assay method, the accuracy of pyrethrins determinations will also be affected by sampling procedures. Typically, pyrethrins assays are of

a small sub-sample(s) of the total flower material produced by each experimental unit. For example, the standard method used at the University of Tasmania specifies a 0.3 g powder sample for extraction. Therefore, assay values will include an error component due to sub-sampling variation and the number and size of the assay sub-samples will affect the precision of pyrethrins assessments. Sampling procedures will vary among breeding programs and this needs to be taken into account when comparing heritability estimates from different programs.

The accuracy of pyrethrins determinations will increase if two or more sub-samples are taken for each experimental unit. However, this will also increase the assay costs. Again, the most efficient number of sub-samples is determined by the relative assessment cost and selection accuracy of alternative sampling protocols. As yet, there have been no published comparisons of the relative selection efficiency of different sampling methods.

The use of characters related to yield

A number of other characters have been used for yield improvement in pyrethrum, including flowering synchronicity and plant form.

Synchronicity of flowering has been recommended as a selection criterion for mechanically harvested crops, as crops that include buds and over-blown flowers are likely to have lower yields than crops consisting mainly of stage 4 - 7 flowers (Bhat, 1995). Synchronicity was used as a selection character for a population improvement program initiated in Kashmir and continued in Tasmania (Bhat *et al*, 1985). However, currently there are no published data on flowering synchronicity and it is not clear whether the control of this character has a significant genetic component or if it an effective character for pyrethrins yield improvement.

In practice, synchronicity may be a difficult character to assess. The variability of flower maturity stages changes as a crop matures (Figure 2.3).

Therefore, synchronicity scores will be partially determined by the time of assessment. Also, while there is some relationship between FMI and synchronicity (Figure 2.3), it is not known whether this varies among genotypes or environments. Therefore, synchronicity scores made for a similar FMI or assessment date may not necessarily be comparable. Consequently, repeated assessments of synchronicity may be needed to accurately compare genotypes, and meaningful assessments of this character may be difficult for single plants in seedling trials.

Nottcut (1955) reported a phenotypic correlation between pyrethrins content and the number of oil-glands on the achene surface and suggested that oil-gland number could be a used as a selection character. Oil-gland counts have the advantage of not requiring an specialist assay equipment or techniques, and could be used by growers. However, two independent studies failed to find any correlation between oil-gland counts and pyrethrins contents (Notcutt, 1955) and this method does not appear to have been utilised by any other growers or pyrethrum breeders.

Phenotypic correlations have also been reported between pyrethrins content and the width of the central floret (Pandita and Bhat, 1986). Bhat and Pandita (1982) also suggested that flower diameter and depth would provide an estimate of total pyrethrins per flower head. Again, the effectiveness of these selection characters has not been confirmed in any published reports. In general, a positive phenotypic correlation does not necessarily demonstrate that there is also a positive genetic association between characters (Falconer, 1989). The effectiveness of secondary selection characters, such as oil-gland number and flower dimensions, depends on there being a genetic association, and this has not yet been demonstrated in any published reports.

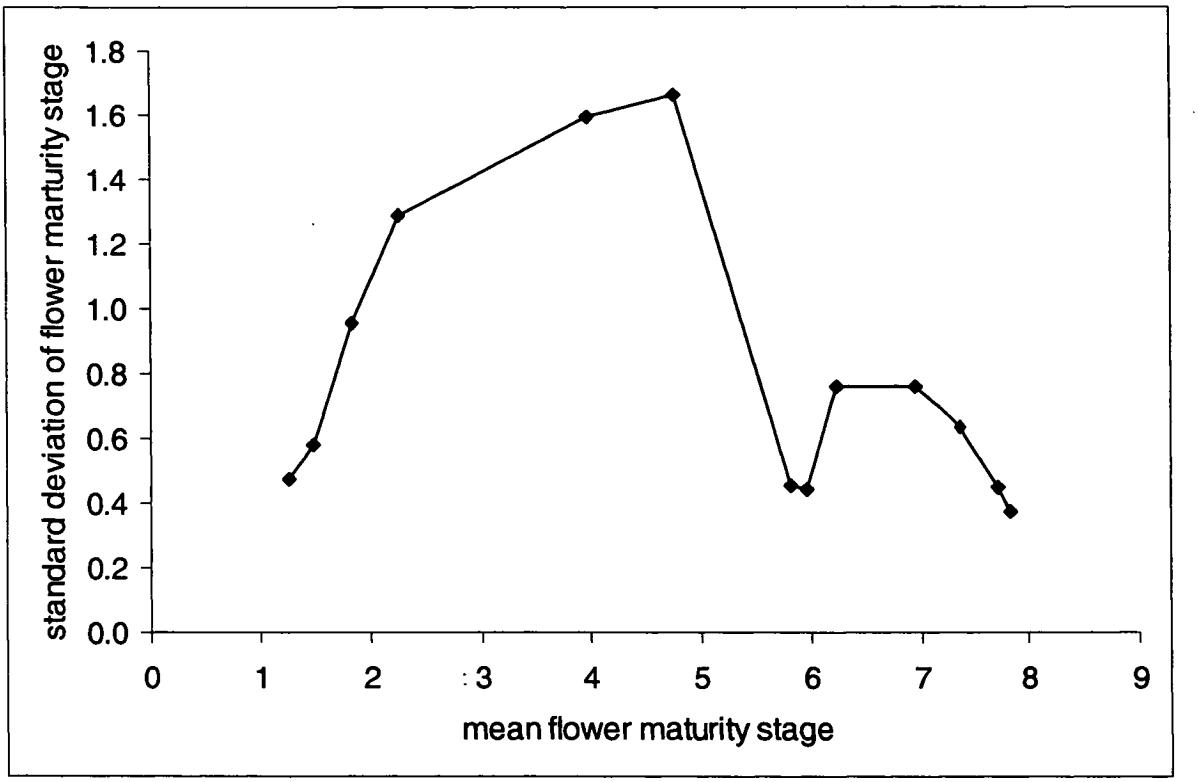

Figure 2.3. Mean and standard deviation of flower maturity stages for samples harvested at weekly intervals from November 22nd. Mean flower maturity increases with sample date. Data adapted from Faber (1980), and flower maturity stages are defined on Table 2.4.2.

2.4.9 Conclusions

Selection for pyrethrins content and flower yield appears to be an effective method for pyrethrins yield improvement. Selection for these component characters may be preferable to direct selection when there is an appreciable economic advantage to pyrethrins content over flower yield. However, the relative efficiency of direct and component selection has not yet been determined, either for Tasmanian-grown pyrethrum or in any other region. So, currently it is not possible to conclude that component character selection is the

superior method for pyrethrins yield improvement in Tasmanian-grown pyrethrum.

Selection for UV-assay of stage 5 (or 6) assay is potentially more effective than selection for pyrethrins content of the entire crop, in terms of response per unit cost. However, this is not necessarily the case and it is also possible that stage 5 (or 6) assay is the less efficient selection character. Similarly, visual yield evaluation could be more efficient than evaluation by harvesting and weighing flowers. Again, there are currently no published data that would allow these alternate characters to be compared.

2.5 Other characters used in pyrethrum breeding

2.5.1 Pests and disease resistance

The main fungal diseases that cause economic damage to Tasmanian pyrethrum are caused by *Sclerotinia* spp. Sclerotinia is capable of surviving in the soil for extended periods in the form of small bodies called sclerotia. These germinate when conditions are favourable to produce either mycelia, that can infect the roots or basal stems of plants, or apothecia, that produce the wind-born ascospores that can infect the leaves and flowers (Hanlin, 1990). In Tasmania, pyrethrum is affected by two species of Sclerotinia, *S. sclerotiorum* and *S. minor*. The primary infection site for *S. sclerotiorum* is the flowers and this disease can cause considerable economic damage through reduced flower yields and the plant mortality that follows secondary infection of the leaves and stems (MacDonald, 1989). This disease in now effectively controlled by spraying of fungicides during the flowering season (NRE, 1998). *S. minor* infects the roots of pyrethrum by mycelium that grow through the soil, and is more difficult to control (Wong, 1994).

Resistance to *Sclerotinia* spp has been identified in a number of crop plants and can be classified into three different types; mechanical disease avoidance strategies (Lu and Fu, 1990; Newton and Sequeira, 1972; Schwartz *et al.*, 1978), production of antifungal substances (Castano *et al.*, 1992; Hemery *et al.*, 1987; Picman *et al.*, 1990) and resistance or tolerance to pathogenic fungal metabolites (Blanchette and Auld, 1979; Huang and Dorrell, 1978). Resistance appears to be under polygenic control in cauliflowers (Baswana *et al.*, 1991) and a number of bean *(Phaseolus)* species (Fuller *et al.*, 1984; Lyons *et al.*, 1987), although a single dominant gene has also been identified in *Phaseolus coccineus* (Abawi *et al.*, 1978). In sunflowers, *S. sclerotiorum* infects the flowers and leaves by ascospores (Sedun and Brown, 1987; Tourvieille *et al.*, 1984), and the roots and basal stems by

mycelia (Huang and Hoes, 1980). There appears to be polygenic resistance to sclerotinia in sunflowers with different genes conferring resistance in different organs of the plant (Robert *et al.*, 1987; Tourvieille *et al*, 1984). In addition, the susceptibility of the flower to ascospore establishment and resistance of the flower tissue to mycelial growth appear to be controlled by two different genetic systems, possibly indicating two independent physiological mechanisms underlying these processes (Vear *et al.*, 1984). Resistance to *S. minor* and *S. sclerotiorum* in sunflowers, appears to be controlled by similar genes (Sedun and Brown, 1989).

Field officers employed by CIG-pyrethrum reported that *CIG 11* appeared to be more susceptible to infection by *S. sclerotiorum* than *CIG 3* (MacDonald, 1989). However, there have been no field trials to verify this observation. Campbell (1990) attempted to develop a screening method for identifying resistant genotypes for the University's pyrethrum breeding program. She carried out three experiments on the infection rates of detached flowers of *CIG 3* and *CIG 11* exposed to *S. sclerotiorum* ascospores. In two of these, the time when the first symptoms were observed was the same in both clones, but the infection developed more slowly in *CIG 3*. In the third experiment, symptoms were observed later in *CIG 3*, but the infection developed at similar rates in both clones. Eight other clones were tested in a fourth experiment, and there also appeared to be variation in the rate that the symptoms developed among these clones. The causes of the variation between the first three experiments is not clear. However, the results of these types of screening tests can be affected by temperature (Schwartz *et al*, 1978), water potential (Grogan and Abawi, 1975), nutrients and ascospore concentration (Leone and Tonneijck, 1990), and conditions must be carefully controlled for results to be reproducible. The two different types of response variation between *CIG 3* and *CIG 11*, may indicate the existence of more than one resistance mechanism, as in sunflowers. However, the results of laboratory tests using detached plant parts do not always correlate

with resistance of intact plants in the field (Nelson *et al.*, 1991). Screening tests would appear to have little value unless it has been demonstrated that they can identify varieties that are resistant in field conditions.

It is difficult to judge whether resistance to sclerotinia should be included as a breeding criterion for Tasmanian pyrethrum. Although anecdotal evidence and Campbell's data suggest that there may be genetic variation in susceptibility to the disease, there is currently no conclusive evidence of this, and field testing is required to determine whether there is any true genetic variation in resistance. *S. sclerotiorum* is currently controlled quite effectively by fungicides (NRE, 1998). So, a sclerotinia resistant variety would have little benefit unless it suffers minimal economic loss in the absence of fungicide application, allowing a reduction in spraying costs. *S. minor* is more difficult to control in the field and any resistance to this species should provide an economic advantage. However, there is currently no evidence of genetic variation in susceptibility to root infection by sclerotinia in pyrethrum. The value of breeding for sclerotinia resistance cannot be determined without first screening the current breeding material to determine whether there is genetic variation in this character.

Recent studies (Pethybridge and Hay, 2001) have associated the fungal pathogen, *Phoma ligulicola,* with poor spring growth in pyrethrum crops approaching their first harvest, and reduced first year yields. Although *P. ligulicola* appears to be ubiquitous to pyrethrum growing areas, significant yield reduction only occurs at cooler, wetter growing sites (Pethybridge and Hay, 2001). To date, there has been no assessment to determine if there is any genetic variation in susceptibility to *P.ligulicola.*

Seedling crops in Tasmania sometimes exhibit 'pyrethrum regrowth decline' after the first harvest, when plants die or fail to grow vigorously. Root-lesion nematodes (*Pratylenchus* spp) are common in the soils of pyrethrum growing areas and it is believed that they may be associated with regrowth decline in pyrethrum. Initial studies have shown that these nematodes can cause

poor growth and yield in pyrethrum, implying that they may either cause or contribute to regrowth decline (Anon., 2001a). Root nematodes (*Meloidogyne* spp) are also associated with reduced pyrethrum yields in Africa. There appears to be genetic variation in the tolerance of pyrethrum to nematodes (Delhaye, 1968; Parlevliet, 1971), although nematodes cause yield decline even in tolerant clones (Parlevliet, 1975). Tasmanian pyrethrum has not yet been screened for nematode tolerance, and it is probably too soon to determine the magnitude of any economic damage that is caused by nematodes in Tasmania.

To date, pest and disease resistance have not been included as specific selection criteria in the University's breeding program. However, pests or diseases may cause yield reduction, so genes that confer tolerance or resistance will be favoured in selection for yield. There is also some natural selection for disease and pest resistance as plants cannot be used as parents unless they survive in the field, although this will not be the case for *Sclerotinia sclerotiorum* as this fungus is controlled by fungicides. Screening for resistance or tolerance to specific diseases and pests will increase the overall cost of a breeding program. Also, reliable assessment of disease resistance can require several test seasons (Guimaraes *et al.*, 1998), so selection for resistance will also increase the length of the breeding cycle.

2.5.2 Toxicity of the individual pyrethrins esters and Pyrethrins I/II ratio

The six pyrethrins esters vary in the dose required to cause paralysis (knock-down) and death, and the degree that they are synergised (Sawicki, 1962). Pyrethrin I and II are generally agreed to be more toxic (for knock-down and death) than their corresponding cinerin (Table 2.5.1) or jasmolin (Sheppard and Swedlund, 2000), although the relative toxicity of the jasmolins is highly species specific (Godin *et al.*, 1966; Godin *et al.*, 1965). However, investigations on the comparative toxicity of pyrethrin I and II have produced conflicting data (Table 2.5.1). Most investigations concluded that pyrethrin I is more toxic than

pyrethrin II to a range of insect species (Table 2.5.1). However, a series of studies by Sawacki and co-workers consistently found pyrethrin II to be more toxic to the common housefly, for both knock-down and mortality (Table 2.5.1). In contrast, while Broadbent and Hagarty (1969) also found pyrethrin II to have the greater knock-down effect in house-flies, they found lower doses of pyrethrin I to be fatal to houseflies as well as three other species. In an additional inconsistency, Winney and Webley (1969) found that pyrethrin I had greater knock-down effect in mosquito coils, although they did not test mortality rates.

Table 2.5.1. Relative toxicity for paralysis (KD) and fatality (kill) of individual pyrethrins esters; pyrethrin I (PI), pyrethrin II (PII), cinerin I (CI) and cinerin II (CII), with pyrethrin I set at 10.

Insect	Effect	Relative activity				Source
		PI	PII	CI	CII	
mustard beetle	kill	10	3	4	2	(Crombie and Elliot, 1961)
mustard beetle	kill	10	4	5	2	(Ward, 1953)
mustard beetle	kill	10	1	1	½	(Elliot *et al.*, 1969)
cockroach	kill	10	2	2	-	(Broadbent and Hagarty, 1969)
housefly	kill	10	3	7	2	(Gersdorff, 1947)
housefly	kill	10	6	2-3	2-3	(Chang and Kearns, 1962)
housefly	kill	10	3	2	3	(Sheppard and Swedlund, 2000)
house-fly	kill	10	15	4	7	Sawicki *et al* *
	KD	10	26	8	10	(Sawicki and Thain, 1962)
house-fly	kill	10	6	6	-	(Broadbent and Hagarty, 1969)
	KD	10	25	3	-	
mosquito	KD	10	3	2.5	2.5	(Winney and Webley, 1969)

*(Sawicki and Elliot, 1965; Sawicki *et al.*, 1962; Sawicki and Thain, 1961)

Sawicki and Elliot (1965) investigated possible causes of the conflicting findings and discounted earlier suggestions (Crombie and Elliot, 1961) that the method and medium of insecticide application could account for relative differences in toxicity. They found that pyrethrin II was less stable in light at room temperature and suggested that some of the ester preparation methods used in early studies could result in degradation of pyrethrin II. This would result in a lower pyrethrin II concentration than the one reported and, consequently, a lower apparent toxicity. However, they felt that this problem was unlikely in the study by Chang and Kearns (1962), who reported pyrethrin I to be more toxic, and concluded that they could not account for the discrepancy between Chang and Kearns' findings and their own.

The latest study of relative pyrethrins toxicity (Sheppard and Swedlund, 2000) supported the majority finding that pyrethrin I is more toxic than pyrethrin II, although they did not speculate on the reasons for the discrepancy with the results of Sawicki *et al* (Sawicki, 1962; Sawicki and Elliot, 1965; Sawicki *et al*, 1962; Sawicki and Thain, 1961). Similarly, neither of the published reviews of pyrethrin toxicity has suggested possible explanations for the conflicting data (Maciver, 1995; Moore, 1966). Therefore, although the balance of evidence suggests that pyrethrin I is the more toxic molecule (Table 2.5.1), it is difficult to be certain of this in the absence of any explanation of the conflicting findings of Sawicki *et al.*

The physical properties of the molecule required for optimum knock-down and optimum kill are likely to be different. This is because the rate of the initial paralysis is determined by the rate that the pyrethrin molecule diffuses into the insect and attaches to the active site, while mortality is determined by the resistance of the molecule to degradation (Elliot, 1969). Consequently, rankings of the relative efficacy of the different pyrethrins esters are not necessarily the

same for knock-down as for kill. However, there are also some inconsistencies between different studies of the relative effectiveness of pyrethrin I and II for knock-down. While, Broadbent and Hagarty (1969) found that pyrethrin II had the greater knock-down effect than pyrethrin I in pure solutions, natural extracts with a PyI/II ratio of close to 0.6 were more effective for knock-down than extracts with a high concentration of pyrethrin II. They suggested that there is an analogue potential between the two esters in fly knock-down, an effect that appears to be absent in kill (Sawicki *et al*, 1962). In contrast, the knock-down effect of mosquito coils is mainly determined by the amount of pyrethrin I in the coil, and pyrethrin II appears to have little effect (Winney and Webley, 1969), although this finding may result from the relative instability of pyrethrin II at high temperature (Mourot *et al*, 1978) rather than any physiological differences in molecular activity. Therefore, while reported findings are consistent with pure solutions of pyrethrin II having greater knock-down effect than pure solutions of pyrethrin I (Broadbent and Hagarty, 1969; Sawicki and Thain, 1962), the knock-down activity of each ester within mixtures does not necessarily follow their activity in pure solutions.

Bhat (1995) includes the relative composition of the six esters as a selection criterion for pyrethrum breeding, although he does not specify the combination that he considers to be most desirable. Although the relative toxicity of pyrethrin I and pyrethrin II is not clear, these esters are generally more effective than their corresponding cinerin or jasmolin (Godin *et al*, 1966; Godin *et al*, 1965; Sheppard and Swedlund, 2000). So, extracts with a high ratio of pyrethrin to (cinerin+jasmolin) would be likely to be the most effective for both knock-down and kill. As the ratios of pyrethrin:cinerin:jasmolin appear to be similar in the Pyrethrins I and II fractions (Head, 1967), selection for a high pyrethrin to (cinerin+jasmolin) could lead to a more toxic extract. Parlevliet (1975) reported that the proportions of component esters are highly heritable, although it is unclear whether he was referring to the proportions of all six esters or the PyI/II

only. There is no other published data on the inheritance of the ratio of pyrethrin to (cinerin+jasmolin). Therefore, while this ratio may have some value as a breeding character, there is currently no published evidence of any genetic component of the reported phenotypic variation.

The PyI/II is also considered to be a character that should be included in pyrethrum selection (Bhat, 1995). The USA Environmental Protection Agency specifies that the PyI/II of pyrethrum extracts must be between 0.8 and 2.8 (Anon., 1992; Maciver, 1995). The distribution of this character is highly skewed, with an average of 1.0 in Kenyan pyrethrum (Parlevliet, 1974), with a range of 0.4 to more than 4 (Bhat, 1995; Head, 1967; Parlevliet, 1975). The inheritance of PyI/II appears to be predominantly additive (Parlevliet, 1974), although, currently, there are no published heritability estimates for this character.

Generally, it is desirable to avoid the selection of varieties with a PyI/II that falls outside the commercially acceptable range. However, commercial extracts are made by combining crops from a number of growers. In Tasmania, the commercial extract is currently formed from six individual varieties. In these circumstances, it is not necessary to reject a cultivar with an unsuitable PyI/II, if the PyI/II of the combined crop is within the commercially acceptable range.

A high PyI/II is optimum when the aim is to produce pyrethrum for use in mosquito coils (Parlevliet, 1975), as the knock-down activity of mosquito coils increases with increasing PyI/II (Winney and Webley, 1969). Selection for high PyI/II is recommended when the product is intended for this use (Parlevliet, 1975). Otherwise, the relative importance of PyI/II as a breeding character will vary among breeding programs: it will be more important when the aim is to develop a single variety for an entire growing region, as an inappropriate PyI/II cannot be corrected by combining varieties. Similarly, it may be less important when a high proportion of breeding material falls within the commercially acceptable range.

PyI/II was not used as a selection character for the population

improvement program conducted by the University of Tasmania. From 1985 to 1997, it was used to choose parent combinations in the crossing programs, crossing high PyI/II clones with low PyI/II clones, as an intermediate PyI/II is the ideal.

2.5.3 Characters related to flowering

Some individual plants fail to flower in African growing conditions (Marr, 1964a; Parlevliet, 1970a) and this is likely to be due to genetic variation in the threshold temperature for vernalisation (Kroll, 1964; Roest, 1976). Clones selected at high altitudes are often unsuitable for low altitude sites due to high numbers of non-flowering ('blind') plants (Ikahu *et al.*, 1994). Individuals with a higher threshold temperature of vernalisation have been selected for in Kenya through both deliberate culling (Dalgety, 1975) and selection (Parlevliet, 1975), as well as automatic selection of flowering, seed producing individuals. As a result, pyrethrum selected in Kenya contains a substantially lower proportion of blind plants in Kenyan-growing conditions than unselected Yugoslavian accessions (Parlevliet, 1970a). In contrast, it is extremely rare to find vegetative individuals in mature pyrethrum in Tasmania in the flowering season (personal observation) and the ability to flower does not appear to be a necessary selection character in Tasmania.

Pyrethrum has an extended juvenile period of some 6 – 7 months, in which floral evocation cannot take place (Brown, 1992). Seedlings grow slowly so the plant is still relatively small at the end of the juvenile phase, with only 6 – 10 fully expanded leaves (Brown, 1992). The obligate juvenile phase leads to an extended period of vegetative growth before the first flowers can be harvested. In Kenya, picking generally does not begin until seedlings are one year old (Anon., 2001b). In Tasmania, where flower production is restricted to the summer, seedlings must be at least 13 months old in order to produce commercial quantities of flowers (Fulton, 1998). Hence, pyrethrum grown in

Tasmania does not produce any commercial crop in the first summer following sowing. Young seedlings (<13 months old) generally fail to flower in their first summer after sowing or, otherwise, produce low numbers of flowers compared to mature (13+ month old) plants (Fulton, 1998).

Phenotypic variation in the length of the initial vegetative period has been reported for African-grown pyrethrum (Marr, 1964a), although there are no published accounts of any use or evaluation of this as a selection character. Contant (1963b) investigated the possible use of intra-specific hybridisation to improve early growth rates, in addition to a number of other traits. However, no progress was reported from this breeding method. There is also phenotypic variation in the flowering ability of young seedlings in Tasmanian growing conditions. Plots sown between January and March will contain a mix of flowering and vegetative plants in their first summer (Fulton, 1998). The causes of this variation are yet to be investigated and it is not known if there is any significant genetic component.

2.5.4 Other breeding characters

Plant form is considered to be an important breeding character in Africa, as lodging of flower stems makes harvesting and weeding difficult (Parlevliet, 1974). Form may also be important when the flowers are mechanically harvested (Bhat, 1995). However, this depends on the harvesting method. In Tasmania, the stems are slashed at ground level, and all above-ground plant material is harvested, including any lodged stems (personal observation). The tendency to lodge is reduced when plants are closely spaced (Parlevliet, 1968), and lodging is uncommon in Tasmanian seedling crops, as the stems support one another in the high planting densities currently used (personal observation). Plant form, therefore, does not appear to be an appropriate breeding character for Tasmanian production systems.

Flower size is also sometimes included as a selection character in Africa.

Larger flowers reduce picking costs (Parlevliet, 1974), although they may increase drying times and increase lodging of flower stems (Bhat, 1995). In Tasmania, the crop is dried in the field, in windrows made up of all the above-ground plant material present at harvest. The flowers remain attached to the stems, in contrast to the African system of drying detached flowers in the sun or in ovens. The effect of flower size on drying times in the Tasmanian system has not been investigated. However, it seems unlikely that flower size would have an impact in the economics of Tasmanian pyrethrum processing.

Characters relating to the rate and the timing of flower development are also considered to be useful (Bhat, 1995). In African production systems, the period between the opening of the first and last disc florets, determines the picking interval, and this has been recommended as a breeding character (Parlevliet, 1974). In temperate production systems there is variation in the time of crop maturity, with relatively early or late maturing clones (Bhat, 1982; Bhat, 1995). Planting early and late maturing varieties has the advantage of increasing the harvest window for the crop (Bhat, 1995). *CIG 11*, one of the main clones grown in Tasmania, usually reached harvest maturity approximately one week before *CIG 3* crops in similar areas (Faber, 1980). There are no published investigations of the inheritance of this trait and the economic value of an increased harvest window has not been evaluated.

Characters associated with propagation may also be useful breeding characters. 'Splittability' and capacity to establish after splitting are considered to be important characters for clonal varieties. Genetic variation is believed to exist in both these characters although they are considered difficult to assess (Bhat, 1995; Parlevliet, 1974). In general, clones that form few splits or have difficulty in recovering from splitting, are removed automatically from breeding programs, as they cannot be propagated in sufficient numbers to reach the final stages of selection programs (Bhat, 1995; Parlevliet, 1974).

Characters associated with seed propagation have not been investigated in

any detail. In general, as high flower yield is the main selection criterion, parents of seedling varieties would be expected to have high seed yields. However, seed yield is the product of achene yield and percent seed-set, and considerable phenotypic variation has been reported in seed set rates (Delhaye, 1956; Pandita, 1983). Seed production is currently a relatively small component of total costs in Tasmania, as crop establishment costs $400/ha, an amount that includes sowing costs. Genetic and phenotypic variation in seed-set rates have not yet been investigated and it is not clear whether there is sufficient variation in seed-set for this factor to have an impact on seed yields or production costs.

There is also variation in the germination success of fertile achenes. A study of the progeny of a biclonal cross (Fulton and Clark, 1997) found that virtually all fertile achenes germinate at constant temperatures of 15 to 20°C, and germination is rapid and uniform in this range. However, this is not the case when temperatures are higher or lower than the optimal range, and up to 75% of seeds may fail to germinate in non-optimum temperatures. Further, germination is less uniform at non-optimal temperatures. Failure to germinate was caused by two factors; dormancy at high and low temperature, and embryo death at higher temperatures (Fulton and Clark, 1997). The impact of dormancy and seed death on crop establishment in field conditions has not yet been assessed, although there is considerable phenotypic variation in crop establishment rates (Chung and Bourke, 1997). To date there has not been any investigation of the genetic variation in these characters and whether they have an impact on establishment costs or crop establishment failure.

Pyrethrum extracts contain a number of substances in addition to pyrethrins (Maciver, 1995). However, the only product quality factor considered to be important by pyrethrum breeders is the relative proportions of the pyrethrins esters (Bhat, 1995).

2.5.5 Conclusions

There are a number of potentially advantageous selection characters, in addition to pyrethrins yield. Some breeding characters considered to be important in other countries are not likely to be useful in Tasmanian conditions, for example flower size and plant form. Other characters, which are yet to be assessed in pyrethrum, may be of value in Tasmania. For example, characters associated with seed production and germination, crop maturity time and the length of the obligate vegetative period. Consideration also needs to be given to PyI/II to ensure that the final product meets market requirements.

Selection will be more efficient when the number of selection characters is small (Wright, 1976), so the inclusion of additional selection characters may reduce the progress in yield improvement. Therefore, it is important to ensure that each breeding character has a significant benefit before it is included as a selection character.

2.6 Conclusions

The primary aim of pyrethrum breeding programs is to increase pyrethrins yields. Direct selection for pyrethrins yield appears to be uncommon (Bhat, 1995; Parlevliet, 1975; Tuikong, 1984), and different selection characters have been used or proposed for pyrethrins yield improvement. Alternate characters vary in their selection accuracy and costs, and, consequently, in their efficiency (response per unit cost). The choice of sampling and assay method will also affect response and plant evaluation costs. In addition, plant densities in Tasmanian growing areas are extremely variable and it is possible that the establishment density of selection trials could affect genotype rankings.

The pyrethrum breeding program at the University of Tasmania used UV-assay of stage 5 or 6 flowers, morphological characters and visually estimated flower yield as selection characters to improve pyrethrins yields. UV-assay of stage 5 or 6 flowers can be regarded as an indirect selection character because the UV-method used by the University is not specific for pyrethrins, and the pyrethrins content of stage 5 (or 6) flowers is not identical to that of the multiple flower stage crop produced in Tasmanian production systems. Similarly, the other selection characters (synchronicity, form and estimated flower yield) can also be regarded as indirect selection characters for pyrethrins yield improvement.

Indirect selection can be more efficient than direct selection for the primary character (Annicchiarico and Pecetti, 1998; Gallais, 1984; Searle, 1965). However, as yet, there have been no investigations of the relative efficiency of any indirect selection methods in pyrethrum, with the exception of direct and visual selection for flower yield in Kenyan-grown seedlings. Nevertheless, visual yield selection has not yet been evaluated in Tasmanian-grown pyrethrum. Alternate selection methods can be compared by measuring selection outcomes or by estimating predicted response from genetic parameters such as heritability and genetic

correlations between characters. However, currently there are no published estimates of genetic parameters for Tasmanian-grown pyrethrum that would allow comparisons between direct and indirect or component character selection. Consequently, it is currently not clear whether the selection characters used in the Tasmanian breeding program are more efficient than direct selection for pyrethrins yield.

3 Standard methods used for pyrethrins assays

3.1.1 Introduction

The following are the standard assay methods used for pyrethrins determinations at the University of Tasmania.

3.1.2 Sample Preparation and Extraction

Flowers were dried at 50°C for 48h and then stored at -10°C for up to six months prior to analysis. Flowers were ground to a fine powder using a hammer mill and stored at 4°C overnight.

Approximately 0.3g powder was weighed into a 25 ml volumetric flask. Sample weight (SW) was recorded. Samples were extracted in a redistilled mix of petroleum-ether-hexane (90:10). The flasks were placed in the dark for 2 h, with manual shaking three times during the extraction process.

The percentage dry matter content (DMC) of the samples was determined by drying approximately 2g of powder at 110°C for 24h, recording sample weight before drying and immediately after removal from oven. DMC was used to adjust SW values in order to calculate pyrethrins per unit dry flower material.

3.1.3 Spectrophotometric (UV) analysis

Pet-ether extracts were diluted in ethanol (AR grade 100), at a rate of 1 ml extract in 50 ml of ethanol. Sample absorbence, at 227 nm, was measured relative to a blank of 2% pet-ether in ethanol, using a Shimadzu spectrophotometer.

The concentrations of extracts were calculated from absorbence using a formula derived from a standard curve. Standard curves were determined prior to each annual analysis program, using two serial dilutions of the commercial standard 'pyrocide'. Pyrocide constituent concentrations were determined by the

AOAC method (BRA laboratory, Ulverstone).

3.1.4 HPLC Analysis

Pet-ether extracts were decanted from the volumetric flasks and stored in 5ml vials at 4°C for 2 to 5 days to allow suspended solids to settle. Analyses were performed using a Waters HPLC system, using a Waters 8 mm, 10 μm silica Rad-Pak cartridge and 10 μm silica Waters RCSS pre-column in a Waters RCM 100. The mobile phase was HPLC grade hexane-tetrahydrofuran (96:4). A 20 μl volume of each sample was analysed with a flow rate of 1 ml/min for 20 minutes. Sample absorbence was measured at 229 nm using a Waters model 440 detector with extended wave-length module. Samples were delivered to the HPLC using an automatic sampling system (Waters intelligent sample processor) that processed 52 samples per run. Each batch of samples included 3 standards. Standards consisted of two sets of serial dilutions of the commercial pyrethrum standard 'pyrocide' in pet-ether. Pyrocide pyrethrins concentrations and PyI/II were determined by the AOAC method (BRA laboratories, Ulverstone).

Chromatographs were assessed visually, comparing sample chromatographs with those of standards to determine which peaks corresponded to the six pyrethrins esters. Peak areas were calculated by a Hewlett-Packard Sigma-10 station and the six peak areas corresponding to the pyrethrins were manually transferred to an Microsoft Excel spreadsheet.

Peak areas of standards were regressed on the known volumes of Pyrethrins I and II, providing a separate regression formula for each. Concentrations of Pyrethrins I and II were then calculated independently from the sum of the three peak areas corresponding to each and were used to calculate PyI/II and the total pyrethrins concentration of the extract.

The concentrations of the individual pyrethrins esters cannot be quantified by the AOAC-assay method, so it is not possible to derive an regression formula

for the individual esters. An approximate value of the ratio of the pyrethrins (pyrethrin I and II) to jasmolins and cinerins (p/jc) was calculated from the peak area for each ester as;

p/jc = [area(pyrethrin I) + area(pyrethrin II)] / [area(jasmolin I) + area(jasmolin II) + area(cinerin I) + area (cinerin II].

Data from sample weight spreadsheets and HPLC analysis were combined using Microsoft Access. Percentage pyrethrins was calculated from SW, DMC and extract concentration.

4 The population improvement program conducted by the University of Tasmania from 1985 to 1997

4.1 Introduction

A pyrethrum breeding program commenced at the University of Tasmania in 1978. One component of this was a recurrent selection program aimed at improving pyrethrins yield. Recurrent selection is a breeding technique that is commonly, although not exclusively, applied to cross-pollinated species (Welsh, 1981), such as forage grasses (Hopkins *et al.*, 1993) and tree crops (Namkoong *et al.*, 1988), and is equivalent to the herd or flock improvement methods used by animal breeders (Yonezawa *et al.*, 1999). Typically, repeated cycles of selection and crossing produce a sequential increase in the mean for the breeding population for as long as genetic variability exists within the population (Borojevic, 1990) and sometimes the mean of the improved population can exceed the extrema of the base population after relatively few generations (Namkoong *et al*, 1988). In some breeding programs the entire improved population is released as a new variety (Vogel and Pedersen, 1993), while in others, including the University's breeding program, varieties are selected from the improved population (Borojevic, 1990).

In the University's recurrent selection program, parents for each breeding cycle were selected by individual phenotype. From 1985 to 1997, phenotype was evaluated initially in seedling trials and secondly, in unreplicated clonal trials. In the initial selection stage (seedling trial), a selection index was used to combine the phenotypic values of the two main selection characters; the UV-assay of stage 6[5] flowers and visually-estimated flower yield.

In index selection, the phenotypic values for each trait are combined into a

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[5] All florets open and less than 1/2 of disc surface brown

single index value *(I)* for each plant (or variety) by;

$$I = b_1P_1 + b_2P_2 + b_3P_3 + \ldots b_iP_i$$

where P_i represents the phenotypic value for trait i and b_i is the corresponding trait coefficient. There are a number of different types of indices, including the base index, constructed from the relative economic value of each character (Williams, 1962); the heritability index, constructed from trait heritabilities (Geidel *et al.*, 2000); and the type used by the University's breeding program, the Smith-Hazel index (Hazel, 1943; Smith, 1936).

The *b* coefficients for the Smith-Hazel index are calculated using the heritability and economic value for each character and the genetic and phenotypic covariances between them. The coefficients are calculated to maximise the average genetic worth of the selected population (Hazel *et al.*, 1994) and the character with the greatest heritability and economic value will be assigned the highest coefficient (Cotterill and Dean, 1990). In general, index selection is either as effective or more effective than independent culling or tandem selection for multi-trait selection (Hazel *et al*, 1994; Young, 1961), and the Smith-Hazel index is more effective than alternative types of index, provided that it is constructed using reliable estimates of genetic parameters (Williams, 1962).

In this chapter, the methods that were used for the population improvement program are outlined in Section 4.2. The predicted responses for each selection cycle are collated in Section 4.3, and an analysis of the genetic changes in the breeding population is presented in Section 4.4.

4.2 Methods used for population improvement between 1985 and 1997

4.2.1 Development of base population

The main base population for the University's breeding program originated from a breeding program conducted in Kashmir. Pyrethrum has been grown in Kashmir since 1931, from seed introduced from a number of countries, including France, Japan and Kenya (Pandita and Bhat, 1984). The crop was propagated as an open-pollinated population with no organised selection until 1971, when a population improvement program started with the collection of seed from farms throughout the region (Gulati *et al*, 1982). Some 1500 seedlings were evaluated in an unreplicated trial at Srinagar (Pandita and Bhat, 1984) and 30 parents were selected, using the selection criteria of a pyrethrins content of over 1.5% (UV-assay of flower maturity stage 5[6]) and morphological traits such as erect plant form and synchronous flowering. Maturity was also included as a selection criteria, although it was not specified whether selection was for early or late maturing plants (Bhat *et al*, 1985). Although flower yields were assessed (Bhat and Pandita, 1982; Pandita and Bhat, 1984), yield was not used as a selection criterion (Bhat, 1995; Bhat *et al*, 1985).

Seed produced from a polycross of the selected plants (the SP population) was imported to Tasmania in 1978 (Bhat *et al*, 1985) and evaluated in large unreplicated trials in the south of the state (Bhat and Menary, 1984b). Single plants were selected from these trials using morphological characters (flowering synchronicity, erectness and resistance to lodging) and UV-assay of stage 5 flowers (Bhat *et al*, 1985). Twenty-seven selected clones (SP-clones) were planted at the Horticultural Research Centre (HRC) in Hobart (southern Tasmania) and these form the main base population for the University's breeding program.

A second population was acquired in 1983. This consisted of 35 clones

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[6] 2/3 to all disc florets open

selected in a breeding program conducted by Glaxo Australia (Glaxo-clones). The base population for Glaxo's breeding program consisted of eight clones initially selected by the University, seed from the SP population and additional material from Yugoslavia, India, New Guinea and France. The Glaxo-clones were selected for high pyrethrins yield with emphasis on pyrethrins content over flower weight, erect flower stems, synchronicity of flowering; a narrow vertical band of flowers for harvest, maturity time, and PyI/II. Detailed pedigree data were not provided by Glaxo. However, available records indicate that at least 12 of the Glaxo-clones were selections or descendants of the SP population[7].

The SP and Glaxo clones are referred to as the RS0 generation of the University's breeding program (Potts and Menary, 1987). The recurrent selection program continued in the early 1980's to produce RS1 and RS2 generations. A variety of selection characters were used, including assay of stage 5 or 6 flowers, flower yield, pickability and plant form, and all plants were grown and assessed at the HRC (Bhat and Menary, 1986b; Bhat *et al*, 1985; Potts and Menary, 1987).

4.2.2 Methods used for recurrent selection from 1985 to 1997

In the mid-1980's, the University's recurrent selection program developed a problem with poor seed-set for crosses between selections from the RS1. This problem was attributed to a high incidence of incompatibility reactions caused by the small population size, as only five parent clones were used to produce the RS1 generation in the early 1980's (Bhat *et al*, 1985). To combat this, between 1985 and 1988, parents were selected from the RS0 and RS1, as well as the RS2 generations. In particular, a increased number of RS0 clones were used as parents, in order increase the genetic base for the breeding population (Potts and Menary, 1987). Consequently, after 1985, the recurrent selection population has

[7] Records of the Glaxo breeding program consist of correspondence between the University and Glaxo and notes made by the Dr Bhat, who was the University's pyrethrum breeder in 1983.

consisted of a mix of generations.

After 1986, plants were evaluated in large seedling trials established in commercial areas. A number of different mating designs and crossing methods were used between 1985 and 1997, including open-pollination, field-based polycross, and controlled (hand) crossing in full-, half-diallel and factorial mating designs. Trial sites, mating designs and generation number are listed in Table 4.2.1, with generation referring to the majority of the seedlings in each trial. Plants selected from seedling trials were reassessed in clone trials, which were established next to each seedling trial that was planted after 1987.

Seedling trials were laid out in block-replicated family plots, with families consisting of full- or half-sibs depending on the crossing method. Family plots contained between three and 15 plants, with plot size adjusted to account for the variation in family size caused by the highly variable incidence of seed-set in pyrethrum. Seedlings were planted in beds containing three rows of plants, with a constant spacing of 0.5 m between plants (within and between rows), and 1.0 m between beds, which reflects the layout of commercial areas of clonal pyrethrum between 1980 and 1995. Four of the 10 annual trials were site replicated, and the others were restricted to a single site. The clone trials consisted of unreplicated plots of 15 plants per clone.

The family plots of seedling trials were scored visually and sampled for pyrethrins content assay. In addition, one or two individuals were scored from each plot. Individual plants were sometimes randomly selected for assessment, but usually they were the individuals judged by eye to have the greatest flower yield in each plot (Potts and Menary, 1989; Potts and Menary, 1990).

Table 4.2.1 Names, mating designs and locations of the seedling evaluation trials. Year refers to the time the seedlings were planted. Parent selection and crossing was conducted in the preceding year.

Year	Mating design	Trial site	Location	Trial code	Generation
86	31 open pollinated families	Cawood Marsh	Ouze, Derwent Valley	CM	RS2
87	84 open-pollinated families, 18 full-sib, 92 polycross families from 4 polycrosses (32♀, 19♀, 21♀, 20♀, 52 clones total)	Cawood Point Kindred	Ouze, Derwent Valley Inland, NW Coast	CP K	RS2
88	13 x 13 diallel	Ellesmere	Derwent Valley	E	RS2
89	14 x 14 diallel	Kindred Norley	NW Coast Hamilton, Derwent Valley	M N	RS2
90	11 x11 diallel	Fenton Forest	Bushy Park, Derwent Valley	FF	RS2,3
91	49 parents - dissassortive 15 parent polycross	Glenelg	New Norfolk, Derwent Valley	G	RS3
92	12x12 half-diallel 38 parents - disassortive, ~5 crossed per clone	Cawood Omeo	Ouze, Derwent Valley Devonport, NW coast	C D	RS3
93	12x12 half-diallel 38 parents - disassortive, ~5 crosses per clone	Killoran	Hamilton, Derwent Valley	J	RS3
94	10(♀)x8(♂) factorial 32 parents - disassortive, ~5 crosses per clone	Bellingers	Ulverstone, NW Coast	B	RS3,4
95	7(♀)x10(♂) factorial 32 parents - disassortive, ~5 crosses per clone	Omeo Bellingers	Devonport, NW Coast Ulverstone, NW Coast	O1 O2	RS4

Single plant and plot assessments were made using the following methods:

1. *Visual assessments* -

Visual assessments were made in late November or early December. Plant form was scored on a five point scale. Flower yield was scored on a scale of 1 to 6 that included decimal points (Potts and Menary, 1989). Yield scores were converted to a value of dry flower yield (g/plant) using a regression equation that was the same for each season and location, so a score of, for example 3.0, was assumed to correspond to the same mass of flowers in all trials and all seasons. Plot yield scores corresponded to the average yield per plant.

2. *Pyrethrins assays* -

Pyrethrins assays were made of stage 6 flowers (Potts and Menary, 1989), using the University's UV-method (Section 3.1.3). Flowers were generally harvested in late December. Fifteen flowers were sampled from individual plants and clonal plots, and eight from seedling family plots. Family samples from all four trial blocks were pooled to form a single assay sample of 24 flowers for each family.

Trials were usually assessed for two seasons. Family plots were scored in both seasons, but individual plant assessments were organised so that plants scored in the first harvest were not re-evaluated in the second.

Individuals were selected from seedling trials using a Smith-Hazel (Hazel, 1943; Smith, 1936) type of selection index that combined individual scores with family averages (Cotterill and Dean, 1990). The selection index (*SI*) was constructed from stage 6 pyrethrins content and the visual estimate of flower yield;

$$SI = b_1 PP_{ind} + b_2 PP_{fam} + b_3 dry_{ind} + b_4 dry_{fam}$$

Where PP_{ind} is the UV-assay of stage 6 flowers for the individual plant, PP_{fam}

the UV-stage 6 assay for its family, dry_{ind} individual (estimated) dry flower yield and dry_{fam}, the family value (mean of all replicate plots). The *b* coefficients were calculated by RESI (Jackson *et al.*, 1987) from estimates of the genetic and phenotypic variance and economic value of each trait and the genetic correlation between them (Potts and Menary, 1988).

Genetic parameters, *b* coefficients and family averages were estimated separately for each site and harvest season. Selected plants were re-evaluated in unreplicated clonal plot trials. Parents for each recurrent selection generation were selected using clone trial data. In the first harvest season, clones were selected solely on the basis of UV-stage 6 assay. A second set of clones was selected in the next season by estimated flower yield and UV-stage 6 assay, using an independent culling method, selecting clones with high values for both characters.

Clones with high UV-assays were re-assessed by the HPLC-method (Section 3.1.4). HPLC data was used to calculate PyI/II, which was used to choose cross combinations.

4.3 Previously reported estimates of heritability and response for the main selection characters

The University's recurrent selection program was evaluated each year by estimating the predicted response for the next selection cycle, where response is defined as the change in the population mean for each selection character (Falconer, 1989). Response was calculated from estimates of genetic parameters made each harvest season. Heritability was estimated by the sib-analysis method (Falconer, 1989), and estimates were specific to each site and harvest season. Estimates of the heritability of the two selection characters have been collated from the annual reports of the University's breeding program, and are listed on Table 4.3.1, with the estimates of the genetic correlation between them. Predicted responses were also collated from reports and are listed on Table 4.3.2. Response was calculated by RESI (Jackson *et al*, 1987), the program used to calculate *b* coefficients for selection indices.

In general, the estimates of heritability for UV-stage 6 pyrethrins were moderate to high, ranging from 0.22 to 0.94 (Table 4.3.1). There was an overall trend of decreasing heritability with time for this character, as would be expected because selection causes a decrease in genetic variance of the selection character (Falconer, 1989; Goddard, 2001; Hospital and Chevalet, 1993; Lerner, 1958).

The estimated heritability for flower yield was generally low to moderate (0.05 to 0.48) and usually lower than the estimate for UV-stage 6 assay in each environment (Table 4.3.1). In contrast to the UV-stage 6 assay, there was no trend of declining heritability for flower yield. The selection index gives greater emphasis to characters with higher heritability and economic weight (Cotterill and Dean, 1990), so the selection pressure for the UV-stage 6 assay would have been greater than for flower yield due the higher heritability and economic weight assigned to the latter character (Potts and Menary, 1989). Consequently, there was relatively low selection pressure for flower yield and this would have

resulted in a relatively small change in genetic variance for this character.

Table 4.3.1 Estimates of heritability of pyrethrins content and flower yield and genetic correlation (r_g) between the two traits. Estimates are specific to locations and harvest seasons unless otherwise indicated.

Trial	Generation	Year	Heritability		r_g
			UV-stage 6 assay	Flower yield	
CP-K	2	88+	0.66	0.15	0.05
E	2	88	na	0.06	0.00
N	2	89	0.53	0.05	-0.62
E	2	89	0.94	0.10	-0.19
K	2	89	0.47	0.26	-0.22
CP	2	89	0.68	0.23	0.20
FF	2,3	90	0.28	0.20	-0.15
N	2	90	0.30	0.10	-0.02
K	2	90	0.32	0.30	0.13
G	2,3	91	0.53	0.17	-0.47
K	2	91	0.53	0.28	-0.29
C	3	92	0.32	0.19	
D	3	92	0.42	0.30	n.a.
G	2,3	92	0.40	0.48	
C	3	93	0.32	0.19	
D	3	93	0.42	0.30	n.a.
J	3	93	0.40	0.48	
J	3	94	0.22	0.11	
B	3,4	94	0.34	0.13	-0.12
B	3,4	94&95+	0.27	0.18	-0.41
O	3,4	96++	0.27	n.a.	n.a.

na - not available

+ Estimated from data combined from two harvest seasons (1994 and1995)

++ Estimated from data combined from two locations

Source: (Groom and Menary, 1994; Groom and Menary, 1995; Groom and Menary, 1996; Potts and Menary, 1989; Potts and Menary, 1990; Potts and Menary, 1991; Potts and Menary, 1993a; Potts and Menary, 1993b)

Table 4.3.2. Predicted responses (percentage increase in population mean) for the two selection characters, assuming that parents are selected by selection indices constructed using the genetic parameters shown on Table 4.3.1 and that the percentage of individuals selected corresponds to *p*.

trial	year	Predicted response		*p*(%)
		UV-stage 6 assay	Flower yield	
N	89	24	2	10
E	89	38	5	10
K	89	27	2	10
CP	89	30	5	10
FF	90	15	9	2.9
N	90	17	2	1.5
K	90	13	2	6.1
G	91	34	-1	1.6
K	91	32	12	4.8
C	92	29	1	1.3
D	92	24	9	2.3
G	92	24	23	2.6
C	93	23	6	3.8
D	93	1	41	6.1
J	93	13	21	3.3
B	94	23	3	5.0
B	94&95	26	-1	4.2

Source: (Groom and Menary, 1994; Groom and Menary, 1995; Groom and Menary, 1996; Potts and Menary, 1989; Potts and Menary, 1990; Potts and Menary, 1991; Potts and Menary, 1993a; Potts and Menary, 1993b)

The lower selection intensity applied to flower yield is reflected in the low estimates of predicted response in this character compared to UV-stage 6 assay (Table 4.3.2.). Predicted responses were typically 15 to 30% for UV-stage 6 assay

and <10% for flower yield.

The estimation of predicted response is a useful tool for assessing selection outcomes prior to selection and crossing (Falconer, 1989; Goddard, 2001; Mackay, 1996). However, predictions of response are not always accurate and need to be checked by retrospective assessment of the realised response (Gebre-Mariam and Larter, 1996; Moll *et al.*, 1975; Pringle and Shaw, 1998).

Most of the predicted values shown on Table 4.3.2 were calculated from genetic parameter estimates that were specific to locations and seasons, and consequently, they may over-estimate response. This is because each commercial area and trial site is subject to a number of continuous, random variables; including temperature, light, nutrition and moisture. Therefore, each growing season and site is effectively a unique environment, and, potentially there may be interactions between genotypes and environments (Burdon, 1977; Hill, 1975). As a result, when genetic parameters are estimated from data collected in a single test environment, the total variance will be under-estimated if there is any non-trivial genotype-environment interaction. This will cause heritability and predicted responses to be over-estimated (Byrne *et al.*, 1987; Cotterill and Zed, 1980; Geidel *et al*, 2000; Namkoong *et al.*, 1966; Weyrich *et al*, 1988).

In addition, the use of inaccurate estimates of genetic parameters for index construction will reduce the effectiveness of the selection index (Baker, 1986; Williams, 1962). Again, this will cause response to be over-estimated (Eagles and Frey, 1974; Elgin *et al.*, 1970; Geidel *et al*, 2000). Plant breeders using a Smith-Hazel selection index must choose between genetic parameter estimates that are specific to each selection environment and general estimates generated from multiple growing environments. There are advantages to both types of estimate (Manning, 1958). The true heritability of a character may vary among test environments and is lower at a highly variable site (Falconer, 1989; Hodge and White, 1992). Therefore, the use of specific parameter estimates has the potential advantage of providing the most effective index for each individual selection site

(Manning, 1958). However, estimates of genetic parameters have large standard errors, and variation among estimates for different environments is more likely to reflect sampling error than differences in the true value for the parameter (Koots and Gibson, 1996). Pooling data from multiple sites has the advantage of increasing the precision of estimates by increasing the size of the population sampled (Manning, 1958), allowing the genotype-environment interaction to be taken in account (Cotterill and Zed, 1980; Namkoong *et al*, 1966) and providing a realistic estimate of heritability across the range of conditions experienced by the crop (Ceccarelli and Grando, 1991; Cotterill and Dean, 1990).

To summarise, data generated by the University's breeding program indicate that an increase could be expected for each selection cycle of some 15 to 30% in the mean UV-stage 6 assay of the breeding population and approximately 5% for flower yield. However, response will have been over-estimated if there is a non-trivial genotype-environment interaction in either of the selection characters. Therefore, predicted responses should be checked by retrospective analysis of selection outcomes.

4.4 Quantification of genetic changes in the breeding population

4.4.1 Introduction

The goal of recurrent selection is to produce a genetic improvement in the selection population, so genetic gain needs to be quantified in order to determine whether this goal has been achieved (Gall *et al.*, 1993). It is possible to estimate genetic trends from breeding program records (Hill, 1972b). However, comparisons between the phenotype records of the base population in early trials and the selected population in later trials will only be meaningful when there is no environmental variation between trials (Gall *et al*, 1993). This is rarely the case (Burdon, 1977) because both growing environment and management methods vary. Consequently, measurements of genetic trends will be confounded by environmental effects unless the phenotypic variation can be partitioned into its genetic and environmental components. One method to achieve this is to use genetically stable control varieties and to calculate the difference between the mean of the selected and control populations (Dickerson, 1969; Gall *et al*, 1993; Hill, 1972a).

The University's breeding program has archived data records covering the period from 1988 to 1997. These consist of phenotypes for the two selection characters; estimated flower yield and UV-assay of stage 6 flowers. All trials contained replicated clonal plots of *CIG 3* and *CIG 11*. The mean of these two varieties can be used as a control value to estimate environmental variation, which can be removed from phenotypic values in order to estimate genetic effects. The aim of the analysis presented in this section was to quantify genetic changes in the breeding population for the two selection characters, by using the two control clones, *CIG 3* and *CIG 11*, to control for environmental variation among trial locations and harvest seasons.

4.4.2 Methods

UV-assays of stage 6 flowers and visual estimates of flower yields assessed from 1988 to 1997 were stored in Microsoft Excel files on floppy discs, with separate files for each trial site and harvest season. There were also some hard copies of data records in the annual reports of the program. These take the form of trial means and lists of values for *CIG 3* and *CIG 11* and individuals with high selection index values. Assessment and selection methods, and trial designs and locations are outlined in Section 4.2.

The stored data were combined into a single Excel file. Data files were cross referenced with tables in the annual reports when trial locations or harvest years were unclear, and a consistent clone numbering system was developed.

Individuals selected from seedling trials were re-evaluated in clone trials, so some genotypes (clones) have multiple records at more than one trial site. Data were recorded for a total of 8651 individual clones, with records of stage 6 UV-assays for 10081 entries. Only 7758 entries have a record for estimated dry flower yield, as yields were not assessed (or saved) for all locations and seasons.

Individual entries consist of single-plant data collected from the seedling trials (Table 4.2.1) and plot data collected from clonal plot trials. Plot yields were estimated as average flower yield per plant, so they are equivalent in scale to single-plant data. The combined data file also records the identity of the mother clone. Paternal parents were also recorded when seedlings originated from controlled crosses. Pedigree records were used to assign a generation number to each clone using Potts and Menary's (1987) notation of RS0 for the SP and Glaxo-clones, RS1 for the progeny of RS0 clones, etc.

The mean of the control clones (y_C), *CIG 3* and *CIG 11*, was calculated for each site-season combination. The deviation (y_D) of each recorded value (y) from y_C was calculated as;

$$y_D = y - y_C$$

The average genetic value of generation (g_i) was calculated as the mean of y_D for

all individuals in generation *i*, and corresponds to the genetic value of the seedling population relative to the clones. Genetic response (R) was estimated as

$$R_i = g_i - g_{i-1}$$

Realised heritability was calculated for each generation from the selection intensity, genetic response and standard deviation of that generation. The selection intensity is the standard selection differential (Appendix Table A, Falconer, 1989) for the proportion of parents selected from each generation (assuming a normal distribution for each selection character).

4.4.3 Results

The combined data file was used to generate the number of clones with data records in each generation (Table 4.4.1), the number of individuals selected from each trial (Table 4.4.2) and the list of parent clones for each trial (Appendix 2). The selection intensity varied among breeding trials and ranged from 1 to 6%. The total percentage selected from the RS3 was 5.3% and from the RS4 was 1.5%.

Examination of pedigree records shows that 17 of the 27 SP-clones were recorded as parents in the recurrent selection program. However, some seedlots were produced by open-pollination, so the other ten clones may have descendants in the RS1. In addition to the 27 selected SP-clones, there were another 11 SP clones used as parents in the 1986 seedling trial (Appendix 2). However, none of the offspring of these 11 clones were selected as parents for subsequent trials, so these clones did not make any genetic contribution to the current breeding population.

Table 4.4.1. Number of individual clones that have surviving data records for at least one of the main selection criteria.

Generation	Number of clones with data record	Number selected as parents
RS0	34	32
RS1	144	155
RS2	2690	142
RS3	4398	67
RS4	1385	-
total	8651	

Table 4.4.2. Number of plants assessed and selected from seedling trials and populations.

Trial or population	Number assessed	Number selected	
		n	percent
CM	45	2	4
CP-K	739	46	6
E	540	35	6
M-N	1311	48	4
FF	700	19	3
G	1024	43	4
C-D	1278	11	1
J	841	-	-
B	1449	-	-

Seedling trials codes (CM, CP etc) are listed on Table 4.2.1.

Fifteen of the Glaxo-clones were used as parents for the RS1 (Appendix 2). However, only three descendants of Glaxo-clones were selected as parents for the RS2 generation. The pedigrees for these clones are listed on Table 4.4.3 and all are progeny of crosses between a Glaxo clone (#98 or 109) and either a SP-

clone or its descendent. Records indicate that Glaxo-clones #98 and 109 both originate from a population of open-pollinated seedlings from Kashmir clones, which is probably the SP population from which the SP-clones were selected.

Table 4.4.3. Descendants of Glaxo clones chosen as parents from seedling trials.

Glaxo descendant	parents	
224241	Clone # 98	*CIG 3*
634721 634828	Clone # 109	Clone # 78 (open-pollinated progeny of *CIG 11*)

There are data records for only 35 of the RS0 generation and 144 for the RS1. The surviving records are almost exclusively for clones that were selected as parents. Consequently, response and realised heritability have not been estimated for the RS1 and RS2 generations, as there are no data available for a representative sample of the entire RS0 and RS1 generations.

Generation means for pyrethrins content (UV-stage 6 assay) and (estimated) flower yield are shown on Table 4.4.4. The mean pyrethrins content is negative for the RS0, RS1 and RS2, indicating that the pyrethrins assays of the early generations are lower, on average, than the two control clones. In contrast, generation means for the RS3 and RS4 are positive, with a trend for increasing pyrethrins content from the RS2 to RS4 generations. The realised heritability for the RS2 and RS3 generations was 0.24 and 0.20, respectively (Table 4.4.5).

Table 4.4.4. Average genetic value for each recurrent selection generation of the breeding population. Values shown are the generation mean and standard deviation.

Generation	Pyrethrins content	Dry flower yield
RS0	-0.08 ± 0.42	-4 ± 21
RS1	-0.14 ± 0.36	-5 ± 22
RS2	-0.06 ± 0.37	4 ± 26
RS3	0.28 ± 0.48	5 ± 23
RS4	0.59 ± 0.57	-7 ± 16

Table 4.4.5. Estimation of heritability for UV-assay of stage 6 flowers for the RS2 and RS3 generations.

Generation	p (%)	i	R_i	h^2
RS2	5.3	2.043	0.34	0.24
RS3	1.5	2.520	0.31	0.20

There does not appear to be any consistent genetic trend in estimated flower yields. Generation means for estimated flower yields were negative for the RS0 and RS1 clones. They were positive for the RS2 and RS3 generations. However, the generation mean for the RS4 was negative and lower than for the RS0.

4.4.4 Discussion

Population size affects the selection outcomes of recurrent selection programs. After the first generation, it is one of the factors that determines the

magnitude of the selection response (Campo and Turrado, 1997) and, ultimately, the selection limit is also determined, in part, by population size (Aggery *et al.*, 1995). Both the magnitude and the limit of the response are reduced in small populations because genetic variance usually declines more rapidly, due to the effects of genetic drift and inbreeding depression (Goddard, 2001). Out-crossed species, such as pyrethrum, are usually less tolerant of inbreeding than self-fertile crops (Ranalli and Cubero, 1997). Potts and Menary (1987) reported poor seed-set for crosses among selections from the first RS1 generation that was produced in the early 1980's by crossing five parents (Bhat *et al*, 1985). Poor seed-set could be caused by a high incidence of incompatibility interactions among crosses between close relations (Potts and Menary, 1987). It could also be, at least partially, due to inbreeding depression, as this can be expressed as embryo abortion and poor germination (Husband and Gurney, 1998).

The second RS1 generation, produced in the late 1980's, may have been produced from as few as 19 clones, that is 17 SP-clones and two Glaxo-clones. Possibly, some of the other SP-clones acted as parents through open-pollination, so, at most, the current breeding population originated from 29 parents (all 27 SP-clones and two Glaxo-clones). This is substantially fewer than the population size of 100 recommended by Robinson (1966), so it may be advantageous to add new clones to the current breeding population that are not descended from any of the original RS0 parents. Potential sources of new breeding material currently available at the University are the SP- and Glaxo-clones that were not previously used as parents and material that was collected from Yugoslavia in 1987 (Potts and Menary, 1988).

The analysis of genetic trends in the recurrent selection population indicated a genetic improvement in the RS3 and RS4 generations in the UV-assay of stage 6 flowers. It was not possible to estimate the genetic improvement for the RS1 and RS2 generations as the surviving data records samples are not a representative sample of the entire RS0 and RS1 generations. Therefore, the data

are consistent with a genetic improvement in the UV-assay of stage 6 flowers when this was used as a selection character (1986 to 1997) for the generations that were produced by use of this character (RS3 and 4).

Estimates for realised heritability were 0.24 and 0.20. These are comparable to the estimates of realised heritability for UV- stage 5 assays of 0.13 to 0.26, calculated from Bhat and Menary's (1984) data (Appendix 1.2). However, they are somewhat lower than most of the estimates reported in the Annual Reports, which ranged from 0.28 to 0.94 for the RS2, and 0.22 to 0.42 for the RS3 (Table 2.3.2), even though both sets of estimates were made from the same data. Most of the heritability estimates listed on Table 4.3.1 were generated from a single environment, and, as such, they over-estimate heritability if there is a non-trivial genotype-environment interaction (Byrne *et al*, 1987; Cotterill and Zed, 1980; Geidel *et al*, 2000; Namkoong *et al*, 1966; Weyrich *et al*, 1988). The estimates of realised heritability (Table 4.4.5) made from the pooled data were lower, indicating that there is a genotype-environment interaction, a conclusion consistent with previous investigations of clone-environment interactions for UV-stage 5 assay (Bhat and Menary, 1986a) and pyrethrins content of open flowers (Parlevliet, 1969). The realised heritability estimates made from the pooled data (Table 4.4.5) are likely to be the more reliable predictors of response; firstly, because they include the genotype-environment interaction variance component, and secondly, because they are derived from a substantially larger sample.

In contrast to pyrethrins content, there was no evidence for a consistent improvement in flower yield in the RS3 and RS4 recurrent selection generations. There could be a number of reasons for the apparent lack of progress for this character. Firstly, flower yield was usually given a lower weight in the selection index due to its lower estimated heritability (Table 4.3.2) and the lower economic weight assigned to flower yield (Potts and Menary, 1989). Hence, the selection pressure on this character was relatively low. Secondly, the predicted response

for this character may be also inflated due to the use of genetic parameters generated from a single test environment. Finally, there may have been a small but positive genetic trend in flower yields that was not detected due to the imprecision of the visual yield assessment method.

Alternatively, visual yield selection is not always effective (Briggs and Shebeski, 1970), and this may be the case in Tasmanian-grown pyrethrum. Although visual selection of single-plants was highly effective in Kenyan-grown pyrethrum (Parlevliet, 1975), the methods used in Tasmania and Kenya are not necessarily comparable. The University's breeding program assigned a single annual flower yield score. In contrast, it is possible that the Kenyan breeding program used multiple scores, as the flowering extends for most of the year in Kenya (Parlevliet, 1970c). Repeated scores would be expected to increase the reliability of visual selection.

The data suggest that there may have been a decline in flower yield in the RS4 generation (Table 4.4.4). Possibly, yields have decreased due to reduced fitness resulting from inbreeding depression. Alternatively, variation among generation means may represent random sampling error, a conclusion consistent with the large standard errors of the estimates of the means.

The value of the conclusions on genetic trends in the breeding population could be limited by the use of a very small (n=2) population of clonal varieties as control for a seedling population. Interactions between genotype and environment can bias the differences between the controls and the selected population (Gall *et al*, 1993). There may be interactions because the control population is very small and the controls cannot be raised under identical conditions to the seedling population due to the different propagation methods. There may also be variation in early establishment between seedlings and splits or tissue-culture explants. Therefore, there may be interaction between the environment and propagation method (Kawano *et al.*, 1982). However, such interactions will only bias the estimate of genetic trends if there is an interaction

between the environmental trend (as measured by the controls) and the genetic trend in the breeding population (Dickerson, 1969; Hill, 1972a). Interactions between y_D and random environmental fluctuations will not bias estimates of genetic trends as they are also random effects. As such, they form part of the non-cumulative random error (Dickerson, 1969; Hill, 1972a), and the positive deviation of some trials will be counterbalanced by negative deviations of others. Therefore, while the use of a small population of clones is a potential weakness of the analysis, there is no compelling reason to suggest that there is, in fact, any bias in the method used to measure environmental trend.

To summarise, the data collected by the University's breeding program indicates that there has been a genetic improvement in one of the main recurrent selection characters; the UV-assay of stage 6 flowers. However, estimates of heritability made from data pooled from multiple test environments were lower than previous estimates made from data collected in a single environment. This indicates that heritability estimates that are specific to a single test environment are not appropriate for Tasmanian-grown pyrethrum.

5 1998 Seedling Trial

5.1 General aims and methods

5.1.1 Aims

A seedling trial was established in 1998 in order to test the following:

- Whether there is a significant component of additive genetic variance to the phenotypic variation in the ability of immature (<13 month old) plants to flower.
- Whether genotype rankings are likely to vary among the different plant densities found in commercial areas.
- Whether the activities of the breeding program between 1985 and 1997 have produced seedling varieties with higher pyrethrins yields than the original base population (RS0 generation) from which they were developed.
- Whether the recurrent selection characters used from 1985 to 1997 are a more efficient method for increasing pyrethrins yield than direct selection.

General materials and methods for the trial are outlined in Section 5.1.2 and a preliminary analysis of trial data is presented in Section 5.2. An investigation of the genetic control of the ability of immature to plants to flower is presented in Section 5.3, the effect of plant density in Section 5.4, and realised selection response in Section 5.5. Genetic parameter estimates for pyrethrins yield are presented in Section 5.6 and the relative efficiency of alternate selection criteria are evaluated in Section 5.7.

5.1.2 Materials and methods

Genetic material

The 1998 trial consisted of seedlings from four different groups:

1. Yugoslavian population. The Yugoslavian population originated from seed collected from wild plants growing at 19 sites along the Dalmatian coast (Potts and Menary, 1988). Some 460 plants raised from this seed were planted at the University Farm (southern Tasmania), in 1989, and the open-pollinated seed which was collected in 1993, forms the Yugoslavian seedlot for the 1998 trial.
2. Base population. The base population (RS0 generation) for the University's recurrent selection program consisted of 27 'SP-clones' and 35 'Glaxo-clones'. Twenty-three of the SP-clones and 23 of the Glaxo-clones are currently surviving. The base population for the 1998 trial is a polycross of these 46 clones.
3. Current commercial varieties. Six biclonal crosses are currently grown commercially in Tasmania. These consist of five crosses selected from the recurrent selection population in 1992 and the cross between the two clones which were grown commercially from 1980 to 1995 (*CIG 3* and *CIG 11*).
4. New selections. These are the 12 biclonal crosses that have been selected from the recurrent selection population after 1992.

Parent clones for the base population, commercial varieties and new selections are listed in Appendix 3.1.

Seed production methods

Seed for the Yugoslavian population was produced by a field polycross. All other seed for the trial was produced by hand-crossing in 1997, using the standard crossing methods used by the University for pyrethrum seed

production which are as follows:

Parent plants were lifted from outdoor plots in May, 1997. Plants were cleaned by removing soil from the roots, dead leaves and any flower stems, and planted into 30 cm pots, in the standard Horticultural Research Centre (HRC) potting mix of 70% pine bark, 20% sand and 10% peat with Osmocote Plus (300 g/50 l), micro-nutrients (20 g Micromax, 25 g $FeSO_4$/50 l) and dolomite (300 g/50 l). Additional nutrients were provided by weekly applications of normal Hoagland's solution (Hoagland and Arnon, 1950).

Plants were initially placed in a shadehouse with bottom heat (~18°C) for 2 weeks to encourage root development and then moved to the glasshouse (17 to 26°C). Glasshouse plants were watered with drippers and kept in natural light and photoperiods. Natural light intensities were augmented by overhead lights. Plants were vernalised by Autumn conditions in outdoor plots and flowers generally developed within six weeks of transfer to the glasshouse.

The florets of the composite flowers open sequentially, from the ray florets to the centre of the disc, over a period of 2 to 3 weeks. Pollen is exuded from the floret by the stigma as the floret opens and 1 whorl of florets opens each day. In the glasshouse, florets open synchronously a little after midday, on sunny days, and somewhat later when conditions are overcast. Pollen was collected daily, early in each afternoon, soon after the florets had opened, using gelatine capsules which were also served as storage containers. Pollen was not collected from flowers used for seed production in order to ensure that the stigmas were not damaged by pollen collection.

Flowers were pollinated by pollen that was freshly collected or that had been stored. Pollen was stored over a desiccant (copper sulphate) at 4°C for 24 to 72 h. For more long-term storage, pollen that had been dried at 4°C was stored in the freezer (-18°C), in screw-top containers containing desiccant. Desiccated pyrethrum pollen retains its viability for some months when stored at low temperatures (Potts and Menary, 1987).

Flowers that were used for seed production were pollinated three times a week, starting when the ray florets first opened. The flower's own pollen was removed prior to crossing by blowing it from the surface of the flower. Out-crossed pollen was applied from the collection capsules using a camel-hair brush. In the case of biclonal crosses, a separate brush was used for each flower to ensure pollen from different clones was not mixed. Brushes were cleaned in 80% ethanol and allowed to dry after use. Cross-pollinated flowers were enclosed in a paper-bag during the period of pollination, so that flowers requiring pollination could be identified easily and to eliminate any uncontrolled pollination. The stigmas of the disc florets shrink back into the florets approximately 3h after pollen application (personal observation) and pollen application continued until all the stigmas of the disc florets had emerged and then shrunk.

Paper bags were removed from flowers at the completion of pollination and the seeds allowed to mature on the mother plant. Flowers were harvested when the petals of the disc florets were dry and the top 10 mm of the flower stem was brown. Harvested flowers were stored in paper bags for 1 to 2 weeks to dry and then the achenes were separated from the rest of the flower material by hand.

The base population clones were crossed in a polycross, made by combining pollen from all 46 plants into a single polymix. Between four and six flowers were pollinated for each clone. The seedlings produced by each mother clone were combined to form 46 maternal half-sib families.

The current varieties and new selections consisted of biclonal crosses, which were produced by pollination of each mother by pollen from a single clone. Seedlots for each biclonal cross consisted of six pollinated flowers with three flowers from each reciprocal cross, with the exception of one of the new selections, which has a male-sterile parent. In total, the current varieties and new selections consisted of 18 full-sib families.

Fungicides and pesticides used during seed production are listed on Table

5.1.1.

Table 5.1.1. Fungicides and pesticides used for plants in the 1997 crossing program.

Date	Preparation
16/6/97	Thiram (2g/l)
1/7/97	Ronilan (1g/l)
4/7/97	Pyrethrum (25ml/l) Benlate (1g/l)
11/7/97	Malathion (1ml/l) Previcur (1.5ml/l)
25/7/97	Pirimor 0.5g/l)
8/8/97	Rogor (75ml/100l)
15/8/97	Benlate (1g/l)
18/8/97	Fongarid (2g/l)
21/8/97	Calibre 100 (0.25ml/l) Pyranica (1g/l) Pirimor (0.5g/l)
29/8/97	Malathion (125ml/100l) White oil (20ml/l)
2/10/97	Rogor (75ml/100l)

Trial establishment

The seedlots from individual flowers consisted of 100, or more, achenes, which were divided into two groups for sowing. The first, consisting of 40 achenes, was reserved for sowing in March 1998, and the residual was sown in the first week of December 1997. The December sowing date is consistent with the usual practice of the breeding program of sowing seed in late-November to early December, and these seedlings were mature (13 months old) at the time of the first harvest season (late December, 1998 to early January, 1999). The March-sown were immature in the first harvest-season.

Achenes were sown into flat trays containing the standard HRC potting mix and allowed to germinate in the glasshouse. Seedlings were pricked out into 200 cell trays after emergence and raised in the glasshouse with over-head watering and weekly applications of normal Hoaglands solution.

The incidence of seedling emergence was highly variable among single-flower seedlots and among families. In the December-sown seed, seedling counts conducted 70d after sowing showed a range of 0-60% in seedling emergence among the 283 single flower seedlots. The number of seedlings produced for each full-sib or half-sib family ranged from 5 to >200.

Both the incidence and rate of germination in the December-sown seed was lower than for previous years and the March-sown seed. This may have been caused by an electrical power failure in the week after the seed was sown. Glasshouse temperatures exceeded 30°C, on the two days without power, in contrast to the usual daily maximum of 26°C. Fulton and Clark (1997) reported that high incubation temperatures (>30°) can cause secondary dormancy and embryo mortality in pyrethrum, and this could account for inhibition of germination in the December-sown seed.

The trial was replicated at three sites:

- Omeo, at East Devonport, which is a coastal site on the NW coast of Tasmania.
- Kindred, an inland site on the NW coast.
- The University farm, at Cambridge, an inland site in southern Tasmania.

Omeo and Kindred are in the main pyrethrum growing region of the state and the trials at these sites were adjacent to commercial pyrethrum crops. Site locations and the times that seedlings were transplanted are listed on Table 5.1.2.

Two density treatments were used in the trial

- 4 plants/m^2, with a square design and 0.5 m between plants.
- 16 plants/m^2, with a square design and 0.25 m between plants.

Table 5.1.2. Sowing and transplanting dates for seedlings in the 1998 seedling trial.

Site	Elevation	December-sown		March-sown	
		Sown	transplanted	Sown	transplanted
University farm	60m	4/12/97	20/2/98	27/3/98	22/5/98
Omeo	46m	4/12/97	17/2/98	27/3/98	4/6/98
Kindred	200m	4/12/97	17/2/98	27/3/98	4/6/98

The 16 plants/m^2 treatment was chosen because it falls within the target density range currently used in commercial areas. However, previous experience with a breeding trial established at this planting density, in 1996, showed that some individual seedlings were difficult to separate and identify. Although this was partly due to disturbance by stock shortly after the seedlings were transplanted, it was unclear at the beginning of 1998 whether single plants could be identified at this spacing. Therefore, the 0.5 m spacing was chosen because this has used in breeding trials planted prior to 1996, and individual plants could be always be easily separated and identified in these trials.

Each site was laid out in a randomised split-plot design, with five blocks at each site. Each block was divided into three main plots which were randomly assigned to one of three treatments;

A: December-sown seedlings, 0.5 m between plants

B: December-sown seedlings, 0.25 m between plants

C: March-sown seedlings, 0.25 m between plants.

Each main-plot consisted of 56 single-plant plots, arranged in 8 rows of 7 plants. The sub-plot factor was full-sib or half-sib family. Families were fully randomised within main-plots. Families were grouped together in common plots in previous breeding trials. However, a fully randomised design was

chosen for the 1998 trial because it has the advantage of eliminating family-environment covariance caused by grouping sibs in common family plots (Magnussen, 1993).

Twenty-three of the 46 half-sib families and five of the 18 full-sib families produced fewer than 30 seedlings. So the trial was a randomised incomplete block design, with small families (n<30) randomly assigned to sites, densities and blocks.

A buffer row was planted around each plot to eliminate edge effects. Plants in the buffer rows were not assessed and were comprised of a randomised selection of excess seedlings from the larger families.

Plant assessments

The following plant assessments were made:

- *Plant survival.* This was assessed in December 1998, with plants scored as missing (dead) or alive. No attempt was made to determine cause or time of death.
- *Flowering.* Plants were scored for flowering in mid-December 1998. Individuals were recorded as 'flowering' if they had produced one or more elongated stems. That is, any observable lengthening of internodes, as this corresponds with the first microscopic changes in bud morphology that occur in flower initiation (Brown 1992). Plants that exhibited exclusively rosette growth form were scored as 'vegetative'.
- *Visual estimation of yield.* This was assessed in mid-December for mature (December-sown) plants at Omeo and the University farm, using the following method: At each site, a plant judged by eye to be one of the highest yielding individuals in the trial (MAX) was assigned a score of 10. Other plants were scored by estimating flower yield relative to MAX, so 10% of MAX corresponds to a score of 1 and 50% to a score of 5. Yield scores were converted to an estimated flower yield (g/plant) using regression equations

(Section 5.2). Plants were not scored for visual yield at Kindred due to insufficient time.

- *Pyrethrins yield at maturity.* This was assessed for all mature (December-sown) plants that had produced open flowers by the end of January 1999, using the following method: Plants were checked for harvest-maturity from mid-December to late-January, using the commercial harvest criterion of average flower maturity stage of 4.5 to 6.5. This typically occurs when approximately 20% of flowers have reached stage 6 (fully-open, < half disc surface brown) (Faber, 1980), and plants were harvested when they were judged by eye to have 20% of flowers at stage 6. The flower stems were tied together and cut at a few centimetres above ground level. The flowers were stripped from the stem by a metal comb and weighed to determine fresh flower yield. A 100g sub-sample was taken for drying (or all flowers when total fresh weight was less than 100g). Flowers were dried at 50°C for 48h, recording fresh weight and dry weight. The HPLC-method (Section 3.1.4) was used to assess pyrethrins content, the ratio of Pyrethrins I to pyrethrins II and the ratio of pyrethrins to (jasmolins + cinerins). The percentage dry matter content of the fresh flowers was determined from the percentage moisture lost during the first stage of the drying process (50°C for 48h) and the percentage moisture lost during the second stage (110°C for 48h).
- *UV-assay of stage 6 flowers.* UV-assay of stage 6 flowers was assessed for mature (December-sown) seedlings in the low density plots, using the following method: The total yield of flowers were divided into two portions. One was used for the HPLC-assay sample, as described in the previous paragraph. The stage 6 flowers were selected from the other portion and dried at 50°C for 48h and assessed for pyrethrins content by the UV-method (Section 3.1.3). Plants in the high density plots were not assessed for UV-stage 6 pyrethrins because they generally produced fewer flowers than the plants in the low density plots and there was insufficient material for two assay samples.

Table 5.1.3. List of characters assessed for the mature (December-sown) seedlings in the 1998 trial.

Character		Description
Fresh flower yield	FFY	Fresh weight of flowers (g/plant)
Percent dry matter	DM	Percentage dry matter content of the fresh flowers (%)
Dry flower yield	DFY	Calculated as the product of DM/100 and FFY (g/plant)
HPLC-assay of pyrethrins content	PP_H	Crop pyrethrins content assessed by HPLC (% pyrethrins/unit dry material)
Pyrethrins I/II	PyI/II	Ratio of Pyrethrins I to Pyrethrins II
Pyrethrins to (jasmolins+cinerins)	p/jc	Ratio of (pyrethrin I + pyrethrin II) to (jasmolins + cinerins)
Pyrethrins yield	PyY	Calculated as the product of DFY and PP_H /100 (g/plant)
Yield score	Y	Visual yield score (on a 10-point scale)
Estimated dry flower yield	DFY_{est}	Flower yield calculated from Y (g/plant)
UV-stage 6 assay	PP_{U6}	Pyrethrins content of stage 6 flowers as determined by UV-assay (% pyrethrins/unit dry material)

5.1.3 Data analysis methods

Data analysis was conducted using SAS (SAS Institute, 1996), Version 6.12 and 8.

Combining data from different sites

Combining data from different sites can be problematic when there is considerable variation in the error variance among sites (Yates and Cochrane, 1938). The procedures recommended by Williams and Matherson (1994) for analysis of multi-site breeding data were used. That is, data were initially analysed on a single site basis, in order to check for homogeneity of site error variances. Data from different sites was then pooled provided that the difference between the highest and lowest site error variance was less than 10-fold (Patterson and Silvey, 1980).

Estimation of variance components

Variance components can be estimated by ANOVA and equation of mean squares to their expectations or by maximum likelihood (ML) or restricted maximum likelihood (REML) methods. ML and REML methods are usually more efficient than ANOVA when data are unbalanced (Corbeil and Searle, 1976; Shaw, 1987) and are recommended in preference to ANOVA for estimation of variance components from breeding data (Harville, 1977; Hofer, 1998). An additional advantage of REML or ML is that variance estimates and their standard errors are computed directly by SAS procedures MIXED and VARCOMP. In contrast, analysis of variance procedures in SAS, such as GLM, only provide mean squares, so the variance components need to be calculated by hand. In this thesis, variance components were estimated using the REML option of the MIXED procedure.

Estimation of correlations between pairs of characters

The phenotypic correlation (r_p) between two breeding characters corresponds to the Pearson product-moment correlation (Roff, 1995). Genetic correlations (r_g) are the correlation between the breeding values for two characters (Roff, 1995) and are calculated as

$$r_g = \sigma_{G(12)} / \sigma_{G(1)} \cdot \sigma_{G(2)}$$

where $\sigma_{G(12)}$ is the genetic covariance between character 1 and 2, and $\sigma_{G(1)}$ and $\sigma_{G(2)}$ are the square roots of the genetic variance for character 1 and 2.

Genetic covariances are estimated in a similar manner to genetic variances by partitioning the total covariance into family and other components (Falconer, 1989). Covariance components can be calculated by calculating the cross-products between character and equating mean cross products to their expected covariances. REML or ML methods can also be used and, while they are more precise, their use is limited by the lack of commercial multivariate software (Liu *et al*, 1997). In this thesis, genetic correlations were estimated from cross products calculated using the multivariate analysis (MANOVA) option of the GLM procedure of SAS.

As phenotypic correlations correspond to Pearson's product-moment correlation it is possible to estimate approximate standard errors, confidence limits and test whether they differ significantly from zero (Roff, 1995; Sokal and Rohlf, 1995). These tests can be generated by SAS procedures such as CORR or GLM. However, the sampling variance for the genetic correlation is substantially greater than for the phenotypic correlation and more difficult to quantify (Roff, 1995). Approximate methods have been developed to estimate standard errors of the genetic correlation (Robertson, 1959b; Scheinberg, 1966) which have been used by plant breeders to test whether genetic correlations differ significantly from zero (for example, Herbert *et al.*, 1994; Thurling, 1974). However, methods of significance testing are unreliable (Windig, 1997; Wu *et al.*, 1997; Xie and

Mosjdis, 1999), as the distribution of the variance of the genetic correlation is not known (Reeve, 1955) and conventional formulae (Falconer, 1989) tend to under-estimate variance (Koots and Gibson, 1996; Roff and Preziosi, 1994). For these reasons it is not uncommon for plant breeders to test significance for phenotypic but not genetic correlations (for example, Byth *et al.*, 1969b; Lagunes-Espinoza *et al.*, 1999; Santalla *et al.*, 2001) or to present estimates of correlations with no tests of significance (for example, Miller *et al.*, 1978; Richards and Thurling, 1979; Rosielle and Hamblin, 1981; St Martin *et al.*, 1982).

As well as testing if a genetic correlation differs from zero, it is desirable to test if it differs from one. The latter test requires jackknife or bootstrap procedures, which are not directly available in commercial statistics programs (Windig, 1997).

In this thesis, phenotypic correlations will be tested to determine if they differ significantly from zero, as this test is well accepted and simple to perform. Significance tests will not be performed for genetic correlations.

Methods to estimate genotype-environment interactions

In conventional ANOVA, there will be a statistically significant genotype-environment interaction when differences between genotypes are not consistent between environments. A statistically significant interaction arises from change in the rank-order of genotypes between environments or variation in the magnitude of differences between genotypes (Gail and Simon, 1985). However, interactions are of consequence to selection only if there is a change in rank-order (Baker, 1988; Dickerson, 1962; Gregorius and Namkoong, 1986). Therefore, plant breeders need to distinguish between cross-over interactions that refer to variation in variety ranks, and non-crossover interactions that occur when true differences between genotypes vary in magnitude but not in direction (Baker, 1988).

A number of methods can be used to test for crossover interactions. Baker

(1988) suggested that the Azzalini-Cox test for changes in rank order or the Gail-Simon test for crossover interactions could be applied to plant breeding. However, the Azzalini-Cox test is a relatively conservative test that is limited to two test environments and trials with homogeneous error variances. The Gail-Simon test can be applied when error variances are heterogeneous but is most appropriate for analysis of the differences between two genotypes tested in a series of environments (Baker, 1988).

A more common approach in plant breeding is to regard a selection character in two environments as two separate characters (Falconer, 1952), and to analyse the genetic correlation between the phenotypes of the same genetic groups in the two environments (Dickerson, 1962; Falconer, 1952; Yamada, 1962). This approach has the advantage that it is fairly robust when there is heterogeneous site variation (Haapanen, 1996), and can be used to estimate the relative efficiency of selection when the test and the target environment are not identical (Falconer, 1952).

The genetic correlation can be estimated as the product-moment coefficient calculated from the genetic components of the variance and covariance for a pair of environments (Falconer, 1952). However, when there are more than two environments it is more convenient to estimate the average genetic correlation (Dickerson, 1962). This can be estimated from

$$r_g = \sigma^2_G /(\sigma^2_G + \sigma^2_{GE})$$

where σ^2_G is the variance of genotypes across environments and σ^2_{GE} is the interaction. However, σ^2_{GE} contains variation due to crossover and non-crossover effects, and the use of σ^2_G will result in the under-estimation of the genetic correlation. This can be corrected by

$$\sigma^2_{GE'} = \sigma^2_{GE} - V(\sigma_{Gi})$$

where $\sigma^2_{GE'}$ is the corrected value of the interaction variance, and $V(\sigma_{Gi})$ is the variance of the genetic scales, that is the variance of the square root of the genetic variance within each environment *i*. The corrected genetic correlation

(r_g') can then be calculated as

$$r_g' = \sigma^2_G / (\sigma^2_G + \sigma^2_{GE'})$$

In this thesis, Dickerson's (1962) method will be used to estimate the average genetic correlation when there are more than two test environments; and the product-moment method (Roff, 1995) will be used when there are only two test environments.

5.2 Preliminary data analysis

5.2.1 Regression analysis of visual yield scores

Plants were visually scored for yield in the low density plots at the University farm and in the low and high density plots at Omeo. Visual yield scores (Y) were compared to dry flower yields (DFY) by regressing the DFY onto the Y score for each plant, using the REG procedure of SAS. Regression formulae and correlation coefficients are presented on Table 5.2.1, and regression analysis tables are in Appendix 3.2.1.

All the regressions between DFY and Y were statistically significant ($P<0.001$), with r^2 values of 0.56 to 0.76.

Table 5.2.1. Relationship between visual yield (Y) and DFY. DFY = (slope).(Y) + intercept.

Site	Parameter estimate ± standard error		r^2
	Slope	Intercept	
Uni farm (low density)	7.9 ± 0.3	4.1 ± 1.6	0.72
Omeo (low density)	11.5 ± 0.7	10.4 ± 2.8	0.56
Omeo (high density)	10.1 ± 0.4	-2.3 ± 1.2	0.76

All regressions are statistically significant ($P<0.001$)

5.2.2 Regression analysis of HPLC standards

Standards were made by serial dilution of two stock solutions of "pyrocide" (Section 3.1.4) to produce 10 standard solutions. Standards were included in HPLC assays at a rate of one standard solution per twenty samples. Standard curves were derived for the Pyrethrins I and Pyrethrins II by regression of peak

areas on concentrations of standard solutions. Regression analysis was conducted by the REG procedure of SAS, using the NOINT option to set the intercept at zero. Regression formulae and correlation coefficients are shown on Table 5.2.2. Regression analysis tables are in Appendix 3.2.2. Formulae shown on Table 5.2.2 were used to calculate sample concentration for HPLC-assays.

Table 5.2.2. Relationship between peak area and concentration of extract (conc). conc = area/s.

Fraction	s ± s.e.	r^2
Pyrethrins I	598925 ± 13283	0.92
Pyrethrins II	605168 ± 9127	0.96

All regressions are statistically significant (P<0.001), intercepts set at zero.

5.2.3 Data adjustments

Selection accuracy can be increased by the use of block- or site-corrected data (Cotterill, 1987; Loo-Dinkins *et al.*, 1990; White and Hodge, 1989). Accordingly, the data analysed in this chapter consists of;

- values that have been standardised (Atlin and Frey, 1989; Hodge and White, 1992) to adjust for site and density treatment effects,
- values that have been adjusted for block effects (Cotterill, 1987; Loo-Dinkins *et al*, 1990), for characters which showed significant block affects,
- and raw values for each character.

Block-adjustments

Yield data collected from the 1998 trial was initially analysed to test for significant block effects in order to determine whether block-adjustment was appropriate.

One-way ANOVA was conducted for the entire seedling population for

each individual site and density treatment. Individual plant values were analysed by the model;

$$Y_{ij} = \mu + B_i + \varepsilon_{ij}$$

Where Y_{ij} is the jth plant in the ith block, μ is the overall mean, B_i is the effect of the ith block and ε_{ij} is the residual. The results of this analysis are summarised on Table 5.2.3.

Block effects were generally not significant (P>0.05), with the exception of HPLC-assay (PP_H) in the low density plots and dry matter content (DM) in three site-density combinations (Table 5.2.3).

Table 5.2.3. Results of analysis for block effects.

Site	Density	PyY	DM	DFY	FFY	PP_H	lPyI/II
1	Low	**	ns	ns	ns	***	ns
2	Low	ns	ns	ns	ns	***	ns
3	Low	ns	**	ns	ns	*	ns
1	High	ns	ns	ns	ns	ns	ns
2	High	ns	*	ns	ns	ns	ns
3	High	ns	*	ns	ns	ns	ns

Character abreviations shown in Table 5.1.3.

PP_H values for plants in low density plots and DM values were adjusted for block effects by;

$$Y_{adj} = Y_{ij} + (Y.. - Y_{i\cdot})$$

Where Y_{ij} is the raw data value, and $Y.. - Y_{i\cdot}$ is the difference between the block mean and the mean of the appropriate site and density. Block means and adjustment values are in Appendix 3.2.3. Block-adjusted values are indicated by subscript b, (PP_{Hb}, DM_b and PyY_b). Block-adjusted values for pyrethrins yield were calculated from PP_{Hb} and dry flower yield.

Standardised scores

Raw data can be standardised to adjust for variation among sites (Hodge and White, 1992) or treatment effects (Atlin and Frey, 1989). Standardised values (z_{ijk}) were calculated using the method of Atlin and Frey (1989), by;

$$z_{ijk} = (y_{ijk} - y_{.jk}) / \sigma_{.jk}$$

where y_{ijk} is the phenotype of the ith plant at site j and density k, $y_{.jk}$ is the mean for density k of site j, and $\sigma_{.jk}$ is the corresponding standard deviation. Standardised values are indicated by the subscript s, for example PyY_s.

5.2.4 Distribution of ratios of pyrethrins esters

The distribution of the ratio of pyrethrins I/II (PyI/II) is shown in Figure 5.2.1. The PyI/II for plants in this trial ranged from 0.2 to 41, a phenotypic range that is greater than the range of 0.4 to 4 previously reported (Head, 1967; Parlevliet, 1975). The distribution was skewed, which is consistent with previous observations for Kenyan-grown pyrethrum (Parlevliet, 1974). The mode of the data was 0.8 (Figure 5.2.1).

Approximately 64% of the plants assessed had PyI/II values within the commercially acceptable range of 0.8 to 2.8 (Anon., 1992; Maciver, 1995). Some 32% were below this range, and 4% had PyI/II of greater than 2.8 (Table 5.2.4).

Table 5.2.4. Distribution of PyI/II for plants in the 1998 seedling trial in relationship to the commercially accepted range of 0.8 to 2.8.

PyI/II	Percent of plants assessed
< 0.8	32
0.8 - 2.8	64
> 2.8	4

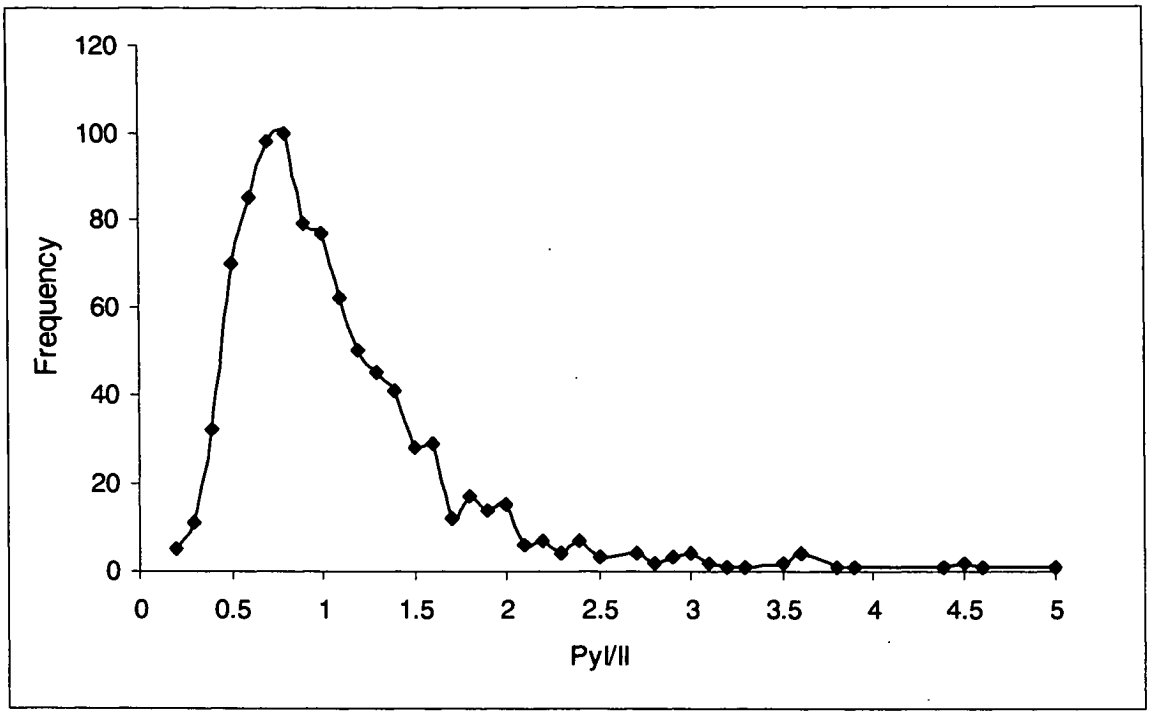

Figure 5.2.1. Distribution of PyI/II for plants assessed in the 1998 seedling trial. Figure only includes individuals with values between 0 and 5 (n=1270). Data are not included for the 18 plants with PyI/II between 5 and 41.

The distribution of the ratio of the pyrethrins to (jasmolins + cinerins) (p/jc) was also skewed (Figure 5.2.2). Values ranged from 0.7 to 12.7. However, with one exception, all plants assessed had a p/jc that was greater than one, with a population mode of 3.6. This is consistent with observations reported by Head (1967) that the concentration of pyrethrin I and II was usually greater than the corresponding jasmolin or cinerin.

PyI/II and p/jc values were transformed by taking the log (base 10) value of each ratio. This produced an approximately normal distribution of data. Data

analysis in the following sections of this chapter are of the log of PyI/II and p/jc, which will be referred to as lPyI/II and lp/jc, respectively.

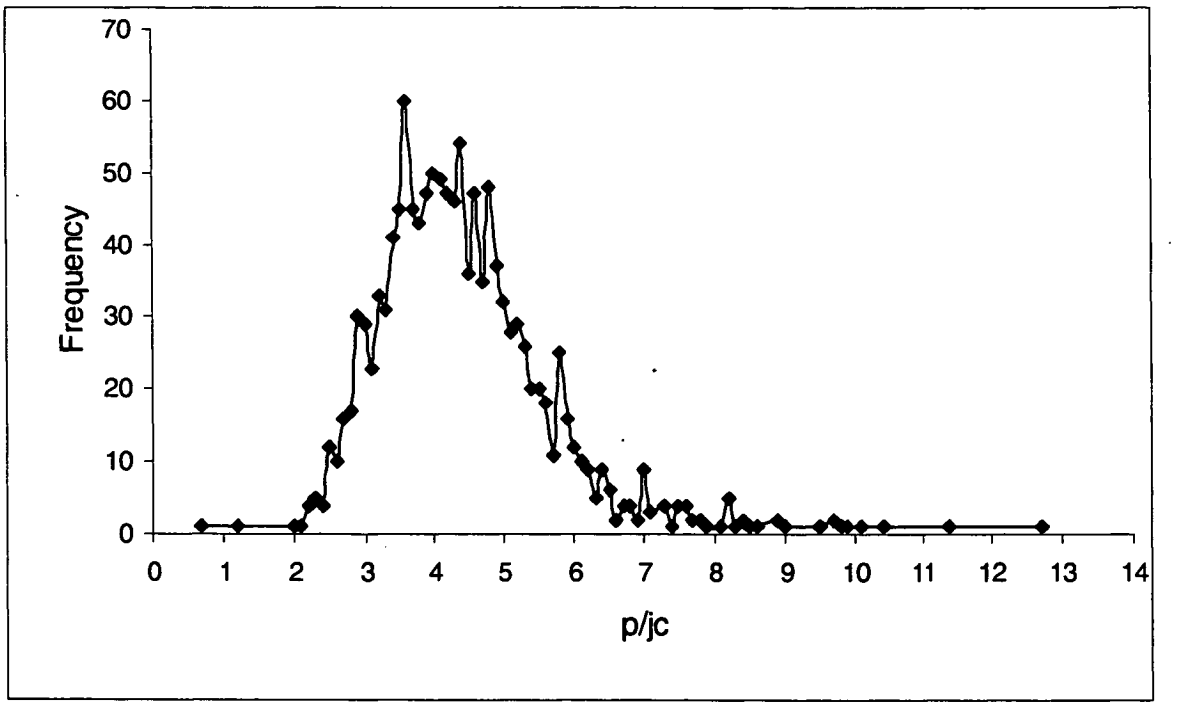

Figure 5.2.2. Distribution of p/jc for plants assessed in the 1998 seedling trial.

5.3 Genetic and environmental variation in plant survival and flowering

5.3.1 Introduction

In temperate regions, pyrethrum produces a single main flush of flowers in the summer. If plants are not fully mature at this time they do not produce sufficient quantities of flowers for an economically viable harvest (Bhat, 1995; Fulton, 1998). In Tasmania, seedlings must be at least 13 months old in the harvest season (NRE, 1998; Pylines, 1999). A reduction in the length of the initial period of vegetative growth could increase the grower's returns by decreasing the minimum time between sowing and harvest. This would be expected to reduce first year maintenance costs and allow the use of alternative, short-term crops in the summer prior to sowing of pyrethrum.

In immature (<13 months old) plants, flower yield decreases with plant age, and plants younger than 8 to 9 months remain fully vegetative (Bhat, 1995; Fulton, 1998). Fulton (1998) examined the components of the reduction in first year flower yields in young plants and reported that the incidence of flowering increased with age at harvest time, from 0% for 9 month-old seedlings to 100% for 13 month old plants. In addition, average yield of each flowering plant also increased with plant age. In contrast, sowing date has no effect on second year yields when plants are 13 months or older (Bhat, 1995; Fulton, 1998). Fulton (1998) also reported that plots of plants aged between 10 and 12 months contained both flowering and vegetative plants, and suggested that selection for the ability to flower at this age would decrease the obligate vegetative period of the crop. Phenotypic variation in the length of the obligate vegetative period has also been reported for African-grown pyrethrum (Marr, 1964a). However, as yet, it has not been determined whether the phenotypic variation in the ability of young plants to flower has a significant genetic component.

To date, there has not been any conscious selection for disease and pest

resistance in Tasmanian pyrethrum. However, genetic variation in survival can result in natural selection for this character without any conscious effort by the plant breeder (Robertson and Lerner, 1949). Therefore, it is possible that natural selection in breeding trials can result in improvement in pest or disease resistance in the breeding population.

Characters, such as survival and flowering in young plants, are phenotypically discrete. However, expression of discrete characters may be determined by an underlying variable that has a continuous distribution (Falconer, 1989). A threshold model is used for the analysis of these types of characters, with the character expressed when the underlying variable is equal or greater than a threshold value (Roff *et al.*, 2000). The underlying continuous variation can be genetic and environmental (Falconer, 1989). The genotype of an individual can be considered to determine the probability of the character being expressed in a particular environment or, alternatively, the proportion of environments in which the character will be expressed (Dempster and Lerner, 1950).

Techniques used for partitioning of total variance into environmental and genetic components do not directly apply to threshold characters (Robertson and Lerner, 1949). Instead, heritability can be estimated from the heterogeneity chi-square derived from analysis in the $2xN$ contingency table, where N is the number of genetic groups (Dempster and Lerner, 1950; Robertson and Lerner, 1949). Generalised linear mixed models can also be used to estimate heritability and predicted response for threshold characters. However, there is generally no additional benefit to using such complex methods unless the true heritability is relatively high (Lopes *et al.*, 2000).

In threshold characters, response to selection is determined by the incidence of the selection character as well as its heritability, as selection pressure is solely determined by the incidence of the desirable character (Falconer, 1989). In general, response increases as the incidence is reduced, and gains are slight

when the character incidence is greater than 0.9 (Dempster and Lerner, 1950). Therefore, improvement in the selection character depends on the ability of the breeder to choose a test environment that produces low to moderate rates of incidence of the desirable character. Previous data on flowering incidence in young pyrethrum in Tasmania (Fulton, 1998) was collected from a single trial site. Therefore, it is currently not clear whether the incidence of flowering in young plants varies substantially between environments.

The aims of the data analyses presented in this section were;

1) to determine whether there is a significant component of additive genetic variance in the variation in the survival of the plants in the 1998 seedling trial
2) to determine whether there is a significant component of additive genetic variance in the variation in the ability of young (10-month old) plants to flower,
3) to determine whether the incidence of flowering in 10-month old plants varies among growing environments.

5.3.2 Methods

Data analysed in this section consists of assessments of survival in mature (December-sown plants) and the incidence of flowering in both mature and immature (March-sown seedlings) seedlings. Survival data for immature seedlings was not analysed, as the incidence of mortality was very low (0.7%).

The incidence of survival of the mature plants was analysed to test for differences among sites, densities, populations and families. The incidence of flowering data was analysed to test for differences among sites, densities, sowing-time, populations and families.

In all cases, Chi-square analysis was used to test whether the incidence of survival/flowering varied among groups, using the methods specified by Sokal and Rolf (1987) and Zar (1996). That is, by testing the null hypothesis that the proportion surviving/flowering is the same in each group. Expected

frequencies were calculated from the overall proportion of surviving/dead (and flowering/vegetative) plants for the entire data set. Chi-square was calculated as

$$\chi^2 = \Sigma \frac{(O-E)^2}{E}$$

where O is the observed number in each class and E the expected number.

1. *Tests for site, planting density and sowing date effects.*

The effects of site, density and sowing-date were tested using data collected from all four populations of plants in the 1998 seedling trial. The effects of site-and-density and site-and-sowing-time were analysed together for the flowering data, in order to test for interactions between different effects. However, all three effects could not be analysed together because the March sowing time was only represented in one density treatment. Site and density were analysed together for survival data. The main effects for each analysis are listed on Table 5.3.1.

Table 5.3.1. Summary of the three separate analyses of density, site and planting effects on the seedling population in the 1998 trial.

Analysis	Variable	Effect A	Effect B	Seedlings included in analysis	
				Sowing-time	Density
1	Survival	density	site	Dec	high &low
2	Flowering	density	site	Dec	high &low
3		sowing-time	site	Dec & Mar	low

In each analysis, a test for independent effects was made for Effect A, B and survival/flowering by the *2x2x3* contingency table using the method specified by Zar (1996). If the effects were not independent, the data was then analysed on an individual site basis. Data from the single sites were then pooled for a combined site analysis calculating;

χ^2_{total} = the sum of the chi-squares for each site analysis

χ^2_{pooled} = the chi-square of the combined totals from all sites

$\chi^2_{heterogeneity} = \chi^2_{total} - \chi^2_{pooled}$

The heterogeneity chi-square was interpreted as the test for the interaction between effect A and site, and the pooled chi-square as the test for the main effect A (Sokal and Rohlf, 1995; Zar, 1996).

A similar analysis was conducted for testing for variation between sites. Differences between sites were initially analysed for each level of effect A. Then data for the two levels of effect A was combined. In this case, the pooled chi-square tested for site effects. All Chi-square values derived from 2*x*2 contingency tables were modified using Yates' correction (Zar, 1996).

2. *Tests for genetic variation.*

Tests for genetic variation were made by testing the hypothesis (H_0) that the proportion of survivors/dead (or flowering/vegetative) plants is the same for all families. That is;

$$H_0: p_1 = p_2 = ..= p_N$$

where p_i is the proportion of survivors/dead (or flowering/vegetative) plants in family group *i* and N is the total number of families. Rejection of the null hypothesis is interpreted as evidence for a family effect on the incidence of survival/flowering.

The half-sib and full-sib families were analysed separately, with N= 47 for the half-sibs and N=18 for the full-sibs. Expected frequencies were low for many classes so G was calculated rather than Chi-squared, where G is twice the log-likelihood ratio (Sokal and Rohlf, 1995; Zar, 1996).

Heritability (h^2) was calculated using the methods of Dempster and Lerner (1950) and Robertson and Lerner (1949), as;

$$h^2 = \frac{G - (N-1)}{0.25.n_0}$$

where

$$nr_0 = \Sigma n + \frac{\Sigma n^2}{\Sigma n} - (N-1)$$

where n is number of individuals in each family, and N the number of families.

The standard error of the estimate is sd_t/r, where;

$$sd_t = \frac{[1 + (n-1)t](1-t)\sqrt{2}}{\sqrt{n(n-1)(N-2)}}$$

and

$$t = rh^2$$

A 2x2 contingency analysis (Chi-square) was also used to test whether there were any differences in overall survival/flowering in the selected populations (current varieties and new selections) and base population (half-sibs).

Family analyses were conducted for flowering counts of March-sown seedlings, and survival counts for December-sown seedlings. Chi-square tables for data analysis in this section are presented in Appendix 3.3.

5.3.3 Results

Survival

Overall, 90.3% of the seedlings sown in December survived to harvest. The effect of planting density was statistically significant ($P<0.005$), and survival rates were consistently higher in the low density plots at each site (Table 5.3.2). Differences between sites were also significant ($P<0.01$) and there was no evidence of a site-density interaction ($P>0.25$). The average plant density at harvest time was 3.7 plants/m^2 in the low density plots and 14 plants/m^2 for the high density treatment (Table 5.3.3).

There were statistically significant differences among half-sib families ($P<0.01$) but not among the full-sib families. Heritability estimated from the half-sib families was 0.09 ± 0.05 ($n_0 = 1344.699$, $G = 77.4$). The mean survival rate for the selected population (18 full-sib families) was 90.1%, and for the base population (46 half-sib families) was 91.2%. However, these differences were not statistically significant ($P>0.05$).

Table 5.3.2. Percentage of surviving plants at each site and in each planting density.

Site	Density		
	low	high	
Omeo	91	88	ns
Kindred	91	85	*
Unifarm	94	92	ns
Total	92	88	**

ns, * and ** indicate that density effects are not significant (P>0.05), or significant at the 5% and 1% levels, respectively.

Table 5.3.3. Average plant densities at harvest.

Site	Density (plants/m^2)	
	low	high
Omeo	3.6	14.0
Kindred	3.7	13.6
Unifarm	3.8	14.8

Flowering

Approximately 8% of the December-sown plants were vegetative at harvest-time. This is very unusual and vegetative plants have not been previously reported in the University's seedling trials, even though the establishment methods and sowing times used for the 1998 trial were similar to previous breeding trials. Nonetheless, the incidence of flowering was substantially greater in the December-sown than in the March-sown seedlings. Comparisons of seedlings in the high density plots showed that, on average, 93% of the December-sown seedlings were flowering compared to only 10% of the March-sown seedlings (Table 5.3.4). The difference between the two sowing

dates was statistically significant (P<0.001) and differences between the sites were also significant (P<0.01). The incidence of flowering was greatest at the University farm and lowest at Kindred, in both December and March-sown seedlings, although there was also a significant interaction between site and sowing date (P<0.001).

Table 5.3.4. Percentage of March-sown (Mar) and December-sown (Dec) plants that were flowering at each site. Figures shown for high density plots only.

Site	Sowing date		
	Mar	Dec	
Omeo	16	98	***
Kindred	1	85	***
Unifarm	12	97	***
Total	10	93	***

** indicates that density effects were significant at the 0.1% levels.

The incidence of flowering of the December-sown seedlings in high and low density plots are shown on Table 5.3.5. Again, the differences between sites were statistically different (P<0.001). There was no evidence of a site-density interaction (P>0.05) or of a planting density effect when the data from all three sites were pooled (P>0.05). Individual site analyses found a significant density effect at the University farm (P<0.01), but not at the other two sites.

Analysis of the March-sown seedlings of the base population showed that the differences among half-sib families were statistically significant (P<0.01). Heritability was estimated at 0.14 ± 0.08 ($n_0 = 716.438$, $G = 70.2$). The differences among the full-sib families were not significant.

Table 5.3.5. Percentage of December-sown plants that were flowering at each site and in each planting density.

Site	Density		
	low	high	
Omeo	99	98	ns
Kindred	83	85	ns
Unifarm	93	97	*
Total	92	93	ns

ns indicates that density effects are not significant (P>0.05), * significant at the 5% level.

The flowering incidence of the base population seedlings that were sown in March, was 8.5%, compared to 13.3% for the selected population of full-sib families. This difference was statistically significant (P<0.05).

5.3.4 Discussion

There is clearly environmental variation in first year mortality in pyrethrum, with variation between both sites and planting densities. Mortality rates were higher in the high density plots, possibly indicating a mortality component due to root infection by *Sclerotinia minor*, as this disease affects Tasmanian-grown pyrethrum (Wong, 1994) and infection rates of sunflowers by *S. minor* are greater at high plant densities than in low (Huang and Hoes, 1980).

There is also evidence of additive genetic variation in first year mortality as there were statistically significant differences among the half-sib families, although heritability for this character appears to be low at 0.09. Therefore, there may be natural selection for increased rates of survival within breeding trials (Robertson and Lerner, 1949). However, the incidence of the selection character was high in this trial, at approximately 90%. Mass selection for a threshold character of high incidence will result in a negligible response, due to the low

selection intensity and low effective heritability, irrespective of the heritability calculated for the underlying scale (Dempster and Lerner, 1950). Therefore, if the mortality incidence observed in this trial is typical of pyrethrum breeding trials, the expected rate of genetic change in survival will be extremely low. This view is supported by the observation of a similar incidence of survival in the selected and base populations. Therefore, although there is evidence of genetic variation in survival of first year plants, the genetic improvement through natural selection in breeding trials is likely to be negligible.

The occurrence of vegetative plants in the 13-month old seedlings was unexpected, as vegetative plants have not been observed in any preceding trial of the Tasmanian breeding program. Both the rate and incidence of seedling emergence were lower for the December-sown seed, compared to the March-sown seed and to previous years. Seedling development appeared to be delayed by unusually high temperatures caused by failure of the glasshouse cooling system (Section 5.1.2). Possibly, this delay in development is the reason that some of the December-sown plants failed to flower.

There did not appear to be a consistent density effect on flowering incidence in the 1998 trial, apart from a slightly higher incidence of flowering in high density plots at the University farm. This could have been a type I statistical error that results from the 5% chance of a significant test when the null hypothesis is true (Zar, 1996). Therefore, the results suggest that the incidence of flowering may be independent of plant density but further testing is required before firm conclusions can be drawn.

There is clearly environmental variation in flowering behaviour, as site-effects were statistically significant, and the incidence of flowering was consistently greatest at Omeo and lowest at Kindred. There was also a significant interaction between site and plant age, although this 'interaction' is spurious, as the growing environment experienced by plants at each location was not completely the same for 10- and 14-month old seedlings. Within each site,

the two age groups experienced the same overall conditions from June to harvest-time. However, they experienced different environments during germination, and the young plants did not experience the field conditions from February to May as those to which the December-sown seedlings were exposed. Therefore, the apparent interaction cannot be interpreted as variation in the response of difference age groups to the same environment (site). Rather, the interaction occurs because the two age cohorts experienced different environments. This observation suggests that conditions experienced throughout the life of the plant affect the probability of flowering. That is, the significant site effect suggests that the common environment shared by 14- and 10-month old plants from May to harvest had a consistent influence on the incidence of flowering. The significant interaction suggests that the different environment for the early part of the development, for the December-sown seedlings also affected the incidence of flowering.

There is also evidence for genetic variation in the ability of young (10-month old) plants to flower, as there were significant differences in the flowering incidence of different half-sib families. This implies that there is significant level of additive genetic variation for this character in the University's base population and that selection for 'ability-to-flower-at-10-months' would increase the incidence of first year flowering in March-sown seedlings.

Brown (1992) found that pyrethrum has an extended juvenile period when it is unable to initiate flowers, and that the juvenile period lasts some 6 months in glasshouse conditions. In other species with a vernalisation requirement and rosette growth form, plant size or some other factor that is closely correlated to biomass appears to determine when a plant is sexually mature (Klinkhamer *et al.*, 1991; Prins *et al.*, 1990; Wesselingh and Klinkhamer, 1996). There is genetic variation in the minimum threshold size required for flower initiation in *Senecio jacobae* (Wesselingh and Klinkhamer, 1996) and *Cynoglossum officinale* (Wesselingh and de Jong, 1995). There also appears to be genetic variation in the

threshold temperature for vernalisation in pyrethrum (Kroll, 1964; Roest, 1976). Therefore, the variable ability of young pyrethrum to flower could be attributed to a number of causes:

- Genetic variation in the minimum size (or developmental stage) required for flower initiation.
- Genetic and environmental variation in the rate of plant growth, that results in phenotypic variation in the length of the juvenile period (or the time that threshold size is attained).
- Genetic variation in vernalisation requirements, so that plants that reach maturity in November may or may not initiate flowers depending whether conditions meet their individual vernalisation requirement. A variation in vernalisation requirements would not usually be expressed when seedlings are sown in November because all seedlings would reach the age that they can respond to vernalisation in the winter or early autumn, when conditions are sufficiently cold to meet the vernalisation requirement of all genotypes.

In the 1998 trial, the incidence of flowering was lowest at Kindred, which was the high elevation inland site, that is likely to experience the coldest conditions (BOM, 2002). This observation suggests that, in this trial, failure-to-flower was not associated with higher temperatures and, therefore, cannot be attributed to conditions that are non-vernalising to some genotypes. Instead, the relatively low incidence of flowering at Kindred is consistent with the suggestion that the individuals that failed to flower had not attained a minimum threshold size due to slow development in the cooler conditions. Similarly, the delayed development during seed germination in the December-sown seedlings was associated with an unusually high incidence of flowering failure.

The incidence of flowering in the 10-month old seedlings was higher in the selected families than in the base population. The ability-to-flower-when <13 months-old has not been used as a selection character in this population, so there appears to have been a correlated increase in flowering ability resulting from

selection for some other character. Possibly, a character, such as the rate of vegetative growth, is correlated with shorter juvenile periods and one of the main selection characters, such as UV-stage 6 pyrethrins assay.

The incidence of flowering, in this trial, ranged from 0.01 to 0.16 for 10-month old plants, falling well within the range of frequencies that allow a relatively high level of selection response (Dempster and Lerner, 1950). However, the low incidence of flowering in conditions experienced at Kindred means that a relatively large selection population (5000 plants) would be required to provide a reasonable number of selected plants. Nonetheless, response would be maximised by conditions that produce a low incidence of flowering, or by later sowing dates, which will also reduce the incidence of flowering (Bhat, 1995; Fulton, 1998). Relatively large populations are economic for selection of this character, as evaluation consists of visual assessment and costs will be low compared to yield assessment. Multiple sowing dates may also increase the probability of success, as the incident rate at each site is likely to vary between seasons and may be difficult to predict.

It seems unlikely that a crop could produce an economic yield unless flowering incidence approaches 100%. Therefore, development of a pyrethrum variety that could be harvested within 12 months of sowing would depend on increasing the incidence of flowering in young plants. Data presented in this section suggest that this could be achieved through recurrent selection. The production of economic crops in young plants will also depend on increasing the yield per plant (Fulton, 1998), and this character also needs to be assessed in order to determine whether varieties can be developed that will produce economic yields at first harvest.

5.4 Effects of plant density on pyrethrins yields and genotype rankings

5.4.1 Introduction

Although, it is usually best to carry out selection in the environment that the crop will experience in commercial conditions, it is sometimes more effective to select in some other environment (Falconer, 1952). In the case of yield improvement of crop plants, it has been argued that single-plant selection is usually more effective at lower densities than those used commercially (Fasoulas, 1984). Because, when there is competition between plants in mixed-genotype seedling trials, yield may be determined primarily by competitive ability rather than by the genes that will determine yield in a monoculture (Kawano *et al*, 1982; Spitters, 1984). Although low plant densities will eliminate competition between plants, genotype rankings may not be consistent between densities (Annicchiarico and Piano, 2000; Mallikarjun and Khanure, 1998; Sekhon *et al*, 1996) and selection at low density will not necessarily result in improved performance in commercial densities.

It is possible to regard a selection character under different environments as two different characters (Falconer, 1952), as, to some extent, different genes may control the character in each environment (Spitters, 1984; Yamada, 1962). This means that the genetic-environment interaction can be measured by the genetic correlation between the phenotypes for the same genetic group in different environments (Dickerson, 1962; Falconer, 1952; Yamada, 1962). Therefore, selection in conditions that are different to commercial areas is a type of indirect selection. Indirect selection, at a low planting density, is more effective than direct selection, at the commercial density, when the product $h_l r_g$ is greater than h_c, where h is the square root of the heritability, l refers to the density of the test environment, c to the density of commercial areas, and r_g is the genetic correlation between the character in each environment (Falconer, 1952; Searle,

1965).

The problem of choosing appropriate selection environments also occurs when the selected material will experience a variety of growing conditions. In this case, the breeder is aiming to improve the average returns in all growing environments, and the optimum number of test environments will be determined by the magnitude of the genotype-environment interaction (Dickerson, 1962). Also, heritability estimates made from plants tested in a single environment will be inflated when there is an interaction between genotype and environment, and, hence, predictions of selection response will also overestimate the potential improvement (Byrne *et al*, 1987; Cotterill and Zed, 1980; Geidel *et al*, 2000; Namkoong *et al*, 1966; Weyrich *et al*, 1988).

In Tasmania, establishment rates of direct drilled pyrethrum are quite variable, with a range of 9 to 36 plants/m^2 observed in areas sown in 1996 (Greenhill, 1997). In the target density of 16 to 32 plants/m^2 (Fulton, 1998), the flower stems form a continuous canopy at harvest, so that individual plants are difficult to separate and identify and competition effects are likely. Therefore, there may be advantages to planting breeding trials at lower densities than commercial areas, as identification of single-plants may be easier and the reduction in competition may increase differentiation between genotypes (Cannell, 1984; Fasoulas, 1984). However, selection will only be effective at low plant densities if there is a significant correlation between variety rankings in low and commercial densities. In addition, direct drilled pyrethrum crops vary both in terms of average population of plants per hectare and in the spacing between individual plants. Therefore, there may be an interaction between genotype rankings and the different plant densities of commercial areas.

Parlevliet (1968) examined the effect of plant spacing on pyrethrum in Kenya and reported that there was no evidence of any interaction between genotype and spacing. However, the range of spacings used in the Kenyan study (0.45 to 0.90m, by 0.30m) do not apply to current commercial crops in

Tasmania. To date, the effect of plant density on genotype rankings has not been investigated for Tasmanian conditions or the range of densities of current commercial areas.

Fulton (1998) examined the effect of plant densities from 4 to 48 plants/m^2 on pyrethrum grown in Tasmania. He reported that, while pyrethrins content was not affected by density, flower yields per unit area doubled over the range of 4 to 16 plants/m^2. However, Fulton's data was limited to a single trial site and only one biclonal cross. There have been a number of other studies of density on pyrethrum (Fulton, 1998; Gnadinger, 1936; Ngugi and Ikahu, 1990b; Parlevliet, 1968; Rao and Singh, 1982; Sastry *et al.*, 1989). However, these examined a range of densities that were lower than those used currently in Tasmania. None of the previous published studies have investigated the effects of planting density on the ratios of the pyrethrins esters.

The aims of the data analysis presented in this section were;

1) to determine whether Fulton's (1998) findings are generally applicable to Tasmanian environments and genotypes,
2) to determine if there is any genotype-density interaction for pyrethrins yield, its components or PyI/II .

5.4.2 Materials and methods

1. Effect of planting density on pyrethrins yield and PyI/II

The effect of planting density on the entire trial population was measured on yield data collected from mature (December-sown) plants. Plot means were calculated from unadjusted pyrethrins content (PP_H), dry flower yield (DFY) and pyrethrins yield (PyY), and for log adjusted Pyrethrins I/II (lPyI/II) and pyrethrins to (jasmolins + cinerins) (lp/jc). Yields per hectare were calculated for each plot as the product of the plot means of yield per plant and plant density at harvest time. Plot mean data are presented in Appendix 3.4.1.

Plot means were initially analysed on an individual site basis, by one-way ANOVA (Appendix 3.4.2). The differences between the smallest and largest error mean squares for each site were less than 10-fold for each character, so combining data from all three sites was considered to be acceptable (Patterson and Silvey, 1980; Williams and Matherson, 1994).

Analysis of variance of data combined from all sites was computed using the model;

$$Y_{ijkl} = \mu + S_i + B_j(S_i) + D_k + \varepsilon_{ijkl}$$

where Y_{ijk} is the plot mean, μ is the experiment mean, S_i is the effect of the ith site (i=3), B_j the effect of the jth block within the ith site (j=5), D_k the effect of planting density (k=2) and ε_{ijkl} the residual. F-tests for density effects were computed by the GLM procedure of SAS, according to the analysis of variance presented in Table 5.4.1.

Table 5.4.1. Analysis of variance for plot mean data.

Source	df	Mean square	F
Site	2		
Block(site)	12		
Density	1	MS_D	MS_D/MS_e
Error	14	MS_e	
Total	29		

2. *Estimation of genotype-environment interaction*

The genetic correlation (r_g) between character values in the high and low density plots was calculated from half-sib family means for December-sown plants. Therefore, the r_g calculated corresponds to the correlation of breeding values in the two density treatments (Falconer, 1989). As such, these estimates

can be used to predict the response of progeny measured in one plant density, to single plant selection of parents tested in the other plant density.

The genetic correlations between density treatments were calculated using the methods of Atlin and Frey (1989). Family means were calculated from individual plant values for each density-site combination, for both standardised values, raw data, and block adjusted values in the case of PP_H. Families that were not represented in each density treatment at all sites were not included in the analysis, in order to produce a balanced data set.

Analysis of variance within each density treatment was conducted according to the model;

$$Y_{ij} = \mu + S_i + G_j + S_i x G_j$$

where Y_{ij} is the mean of family j at the ith site, μ is the experiment mean, S_i is the effect of the ith site (i=3) G_j is the effect of the jth family (j=41), and $S_i x G_j$ is the interaction between them. All effects were treated as random and the genetic (family) variance within each density treatment was estimated by the MIXED procedure of SAS using the REML method.

Cross-products (*CP*) between family means in low and high density plots were calculated by the multivariate (MANOVA) option of the GLM procedure for each character. Mean cross products were calculated as *CP/df*, and genetic covariances were estimated according to the analysis of covariance presented in Table 5.4.2.

The genetic correlation (r_G) was calculated as

$$r_G = \sigma_{G(12)} / \sigma_{G(1)} \sigma_{G(2)}$$

where $\sigma_{G(12)}$ is the genetic covariance between high and low density treatments, and $\sigma_{G(1)}$ and $\sigma_{G(2)}$ are the square roots of the genetic variance for each environment.

Table 5.4.2. Analysis of covariance for half-sib family means in low and high density environments.

Source	df	Expected mean cross-products
Sites (*S*)	2	
Families (*G*)	40	$\sigma_{GS(12)} + 3\sigma_{G(12)}$
G x S	80	$\sigma_{GS(12)}$

5.4.3 Results

ANOVA tables for the effect of planting density on yield components and lPyI/II and lp/jc are in Appendix 3.4.2. Density means and the results of F-tests are summarised on Table 5.4.3 for each character.

Analysis of the combined site data failed to detect any density effect for PP_H, lPyI/II or lp/jc (Table 5.4.3). There also appears to be no evidence of any site-density interaction in PP_H or lp/jc as there was no density effect on these characters within any individual site (Table 5.4.4). This was also the case at two sites for lPyI/II (Table 5.4.4). However, the average value for this character was higher in the low density plots at Kindred ($P<0.01$).

In contrast, planting density had an effect on yields of both flowers and pyrethrins (Table 5.4.3), and yields per plant in the low density plots were more than double the yields on plants in the high density plots. The reduced yield per plant in the high density treatment was more than offset by the increased number of plants per unit area, and yields per hectare were substantially greater at the higher density (Table 5.4.3).

Table 5.4.3. Treatment means and standard errors for low and high density plots.

Character	density		
	Low	High	
PP_H (%)	1.8 ± 0.2	1.9 ± 0.2	ns
DFY (g/plant)	47 ± 9	22 ± 4	***
DFY (t/ha)	1.5 ± 0.3	2.8 ± 0.5	***
PyY (g/plant)	0.84 ± 0.14	0.41 ± 0.06	***
PyY (kg/ha)	26 ± 4	51 ± 8	***
lp/jc	0.6 ±1.2	0.6 ± 1.3	ns
lPyI/II	0.00 ± 0.05	-0.02 ± 0.03	ns

ns indicates not significant (P>0.05), *** indicates statistically significant (P<0.001).

Table 5.4.4. Site means for low and high densities for pyrethrins content (PP_H) and log values of PyI/II.

Site	PP_H (%)			Log(PyI/II)		
	Low	High		Low	High	
Omeo	1.7	1.7	ns	-0.02	-0.04	ns
Kindred	1.8	1.9	ns	0.05	-0.01	**
Uni farm	2.0	2.0	ns	-0.02	-0.01	ns

ns indicates not significant (P>0.05), * indicates statistically significant (P<0.01).

Table 5.4.5. Site means for low and high densities for flower yield (DFY) and pyrethrins yield (PyY), and the percent increase in yield in the high density plots.

Site	DFY (t/ha)			PyY (kg/ha)		
	Low	High	% increase	Low	High	% increase
Omeo	1.8	3.3	83	31	57	83
Kindred	1.4	2.5	79	25	46	84
Uni farm	1.2	2.5	108	24	50	108

Differences between high and low densities treatments within sites were all statistically significant (P<0.001)

Examination of individual site means (Table 5.4.5) shows that the response to planting density was similar at the two NW coast sites, Kindred and Omeo, at 79 and 83%, respectively. The response was a little higher at the University farm, at 108%.

Table 5.4.6 Average pyrethrins yields for full-sib families in high and low density plots, and the percentage increase in yield for plots established at 16 plants/m^2 relative to plots established at 4 plants/m^2.

Family number	Pyrethrins yield (kg/ha)		Increase in high density (%)
	Low	High	
211	34	65	90
212	46	101	119
213	27	74	170
214	29	72	149
215	47	87	87
216	40	68	69
217	27	39	45
218	32	65	105
219	36	63	73
220	27	42	57
221	20	38	94
223	28	48	75
224	32	85	161
225	31	64	105
226	19	27	43
227	27	53	93

The effect of planting density on individual full-sib families is presented on Table 5.4.6. The response of individual full-sib families was quite variable. Although all families had higher yields in the high density plots, the percentage

increase due to the higher planting density ranged from 43 to 170%.

Estimated cross products and calculations for genetic correlations are presented in Appendix 3.4.4 and the family variance estimates in Appendix 3.4.5. There was little difference between genetic correlation estimates for adjusted and unadjusted values for each character (Table 5.4.7). Estimates of genetic correlations for PP_H and logPyI/II were high (>1.0). Estimates for DFY were low, at 0.16 to 0.19, and estimates for PyY were intermediate, at 0.36 to 0.38.

Table 5.4.7. Estimates of genetic correlations between half-sib family means in high and low density plots.

Character	r_g
PP_H	1.35
PP_{Hs}	1.22
PP_{Hb}	1.29
DFY	0.19
DFY_s	0.16
PyY	0.36
PyY_s	0.38
lp/jc	1.45
lPyI/II	1.03

5.4.4 Discussion

The data presented suggest that planting density does not affect pyrethrins content, a conclusion consistent with previous studies conducted over a wide range of spacings and growing regions; specifically, Tasmania (Fulton, 1998), Kenya (Ngugi and Ikahu, 1990b; Parlevliet, 1968) and India (Rao and Singh, 1982). The estimates for the genetic correlation for this character were higher than the theoretical limit of 1.0 (Table 5.4.7). However, the variance of genetic correlation estimates is large, and the number of plants per family in this study

was substantially lower than the optimum required for reliable estimates (Robertson, 1959b). The high estimated values for r_g suggest that the true genetic correlation for PP_H is close to 1.0. Therefore, it appears that planting density has a negligible effect on pyrethrins content. It is likely that there is little, if any, interaction between breeding value and densities, so the planting density of seedling trials does not need to correspond to commercial area when the sole aim is to improve pyrethrins content.

It is also likely that PyI/II is not generally affected by plant densities in the range of 4 to 16 plants/m^2 (Table 5.4.3) and there appeared to be no genotype-density interaction, as $r_g = 1$. There was a significant density effect at one trial site (Table 5.4.4), suggesting the possibility that density effects on this character may be expressed in some, but not all, growing conditions. However, this may be a type I error (F-test significant when null hypothesis is true), as the number of degrees of freedom for the F-test are low (1/4). The ratio p/jc does not appear to be affected by planting density.

The effect of density on yield (Table 5.4.5) was consistent with previous observations that decreasing density from 16 to <1 plant/m^2 results in greater yield per plant but lower yields per hectare (Fulton, 1998; Gnadinger, 1936; Parlevliet, 1968; Sastry *et al*, 1989). Increasing the establishment plant density from 4 to 16 plants/m^2 resulted in almost a 100% increase in first year yields in the genetically diverse population in this trial (Table 5.4.3). There were some differences between the responses of individual full-sib families (Table 5.4.6). Individual families sizes are small (n = 18/treatment), so it is not clear whether the variation in response is due solely to sampling variance or indicates the presence of a true genotype-density interaction. However, all families had increased average yields/ha in the high density plots, and there is no evidence that any family does not produce higher yields in the higher density. Therefore, data confirm Fulton's (1998) observations that first year yields are substantially greater in crops established at 16 plants/m^2 compared to 4 plants/m^2, in

Tasmanian growing conditions.

Estimates of genetic correlations between low and high densities were low for flower yield. As the variance of these estimates is high (Robertson, 1959b) it is difficult to determine whether there is a small, positive genetic correlation for flower yield, or whether the true value is not significantly different from zero. The estimate for pyrethrins yield was intermediate, which is consistent with a relatively high and low r_g for the component characters, PP_H and DFY.

A previous study (Parlevliet, 1968) found that there was no clone-density interaction for flower yields of Kenyan pyrethrum. The two studies are not necessarily comparable due to the difference between flower harvesting methods used in Kenya and Tasmania and difference between r_g based on breeding values (Table 5.4.7) and clone values (Parlevliet, 1968). The Kenyan experiment may have failed to detect any interaction due to the small sample of clones (three) used for the trial. However, the densities used in the Kenyan studies were relatively low (6 to 9 plants/m^2), and it is possible that interactions are relatively small or absent in this range, and that genotype rankings only begin to change at relatively high plant densities. There is no reason why the magnitude of genotype-density interactions should be consistent between different density ranges, and these observations suggest that they are not. Therefore, the r_g values estimated for 4 and 16 plants/m^2 are not necessarily applicable to the target densities of commercial areas (16 to 32 plants/m^2).

The low values for the r_g estimates for flower yield suggest that there is a large genotype-density interaction for this character. This means that heritability will be over-estimated when data are collected from trials planted at a single density, as commercial pyrethrum experiences a range of density environments. Accurate predictions of the potential for flower yield improvement require trials planted at a number of densities that cover the range commonly experienced by commercial crops, and this should be a priority for future research.

5.5 Evaluation of selection outcomes for the University's breeding program

5.5.1 Introduction

The University's pyrethrum breeding program can be divided into three main stages, each consisting of a period of population improvement followed by selection of varieties for commercial use. The first phase (1971 to 1985) was conducted in Kashmir and Tasmania, and consisted of recurrent selection for stage 5 pyrethrins and morphological traits. The SP-clones that formed the main base population for the subsequent breeding program, were selected from the first recurrent selection generation (SP), and these included *CIG 3* and *CIG 11*, the two clonal varieties that were grown commercially in Tasmania from 1980 to 1995. The next phase (1985 to 1993) consisted of recurrent selection for stage 6 pyrethrins and visually estimated flower yield. Twelve biclonal crosses were selected from the RS2/3 population that was tested in the 1991 seedling trial. Five of these crosses were selected by industry, along with the cross between *CIG 3* and *CIG 11*. These six seedling varieties (current varieties) are currently grown by farmers under contract to Botanical Resources Australia, in Tasmania. In the third phase of the breeding program (1993 to 1997), the recurrent selection program continued using the same methods as the second phase, and an additional 12 biclonal crosses were selected from seedling trials planted after 1991.

Different methods were used for variety selection in each phase of the breeding program. In the first phase, clones were tested in a series of site-replicated trials conducted over a number of seasons (Bhat, 1982; Bhat and Menary, 1984c; Bhat and Menary, 1985; Bhat and Menary, 1986c). Clones were evaluated for pyrethrins content by UV-assay of stage 5 flowers (PP_{U5}) and flower yield by harvesting and weighing flowers at the time that the majority were at stage 5. In the second phase, the twelve seedling varieties were selected, in part,

on the basis of data collected from the 1991 seedling trial. Full-sib families (biclonal crosses) were evaluated for three seasons by UV-assay of stage 6 flowers (PP_{U6}) and visually-estimated yield. Initial selections were made on the basis of first-year data, as well as on the availability of parent material (Potts and Menary, 1993a). The five varieties were selected from the initial twelve by industry using second and third year data, and some other methods, as the final selections do not appear to have been made solely on the basis of the University's trial data. In the third phase, full-sib families were selected from each of the seedling trials planted between 1992 and 1995. These were chosen solely on the basis of their UV-stage 6 assay and visual yield scores.

The effects of the first phase of the breeding program have been evaluated by measuring the response in the recurrent selection population (Bhat and Menary, 1984b; Bhat *et al*, 1985) and the response achieved through variety selection (Bhat and Menary, 1984c). The first cycle of population improvement resulted in a significant improvement in PP_{U5} and a non-significant increase in flower yield (Bhat *et al*, 1985). A similar result was reported for the second cycle of population improvement, although the second generation was not evaluated in the same trial as the first selection generation and base populations (Bhat *et al*, 1985), so genetic and environmental effects were confounded (Gall *et al*, 1993). Nevertheless, an increase in flower yield was observed in both selection cycles. As flower yield was not used as a selection character (Bhat *et al*, 1985), Bhat (1995) suggested that there had been subconscious selection for yield when plants were visually scored for morphological characters. Alternatively, flower yield might be correlated with one of the selection characters, for example, synchronous flowering, as an improvement in this character would be expected to increase pyrethrins yield (Bhat, 1995). Evaluations of selected clones indicated that they had significantly higher flower yields and PP_{U5} than the selection population from which they were chosen (Bhat and Menary, 1984c). However, pyrethrins contents were only assessed for stage 5 flowers (Bhat and Menary,

1986a; Bhat and Pandita, 1982) and not for the entire crop which consists of a range of flower maturity stages (Faber, 1980; Tattersfield, 1931).

The six seedling varieties in current commercial production (current varieties) are reported to have higher commercial yields than the preceding clonal varieties (Greenhill, 1997; Pylines, 1999). However, seedlings in Tasmania are grown at higher population densities (>9 plants/m^2) than clones (3 plants/m^2). So, at least part of the increased yield of seedlings is due to the effect of planting density, and is not necessarily due to any genetic improvement in pyrethrins yields. The current seedling varieties have not yet been tested with the base population in order to determine whether the activities of the breeding program have produced a genetic improvement in economic yield. Similarly, the selections made after 1992 (new selections) have not been tested with the current varieties in order to determine whether there is an improvement in the economic yield for the new selections.

Examination of the pedigrees of the University's recurrent selection population (Chapter 4) suggested that the genetic base for the breeding population was quite narrow and that the Glaxo-clones had made only a minor contribution to the later generations. Small populations can suffer from inbreeding depression which would be expressed in either reduced fertility or fitness (Husband and Gurney, 1998). In addition, both response and selection limits are lower in small populations (Aggery *et al*, 1995; Campo and Turrado, 1997; Goddard, 2001; Namkoong *et al*, 1988). Therefore, potentially there can be advantages to increasing the genetic base of the breeding population by the inclusion of the progeny of previously unselected clones. However, this proposition is less attractive if the breeding values for the unselected clones are low relative to selected material.

The aims of the data analysis presented in this section were;

1) to determine whether the methods used by the University's breeding program from 1985 to 1995 have produced varieties with higher

pyrethrins yields than the population from which they originated,

2) to determine whether the varieties selected after 1992 are likely to have higher pyrethrins yields than the varieties currently in commercial use,
3) to determine whether progeny of some of the unselected base population clones should be added to the current breeding population,
4) to compare the University's base population with unselected Yugoslavian material in order to establish whether this material has higher flower and pyrethrins yields than unimproved pyrethrum.

5.5.2 Methods

Data analysed in this section consists of pyrethrins yield and its components for mature (13-month old) plants, established in both at high (16 plants/m^2) and low (4 plants/m^2) densities.

Seedlings in the 1998 trial can be grouped into four populations;

- the Yugoslavian population of unselected, unimproved pyrethrum (one polycross family),
- the University's base population, formed from a polycross of 46 clones - the 23 SP-clones (including *CIG 3* and *CIG 11*) that form the main base population for the University's breeding program and the 23 Glaxo-clones, of which only two have contributed descendants to the current recurrent selection population (46 half-sib families),
- the current varieties, which are the five biclonal crosses selected from the 1991 seedling trial and the cross between *CIG 3* and *CIG 11* (six full-sib families),
- the new varieties, which are 12 biclonal crosses selected after 1992 (12 full-sib families).

The methods used to develop each population, RS generation number and number of individuals assessed for yield are summarised on Table 5.5.1.

Table 5.5.1 Selection methods used in the development of the four populations in the 1998 seedling trial, generation number, and number of plants (n) in the trial.

Population	Generation	n	Selection criteria
Yugoslavian	-	22	Random collection of wild genotypes
Base: SP-clones	RS0	498	Pyrethrins content (stage 5, UV) Morphological characters
Glaxo clones	RS0	419	Pyrethrins content Flower yield Morphological characters
Current varieties	RS2 - RS3	151	Pyrethrins content (stage 6, UV) Visual yield Amount of parent material Unspecified methods used by industry
New selections	RS3 - RS4	232	Pyrethrins content (stage 6, UV) Visual yield

Each of the three sites was planted in a block replicated split-plot design, with density as the main-plot factor and genotype as the sub-plot factor. Main plots were divided into 56 single plant sub-plots, and individuals from the 65 families (1 polycross, 46 half-sib and 18 full-sib) were randomly assigned to sub-plots. A number of families contained insufficient numbers to be represented in all main-plots, so the trial consisted of replicated incomplete blocks.

In this analysis, variation among individual plants is attributed to the effect of population, the interaction between the main-plot and sub-plot effect (density x population) and the within plot error (Steel and Torrie, 1980). Population and density can be regarded as fixed effects so appropriate analysis of variance and F-tests are specified by Steel and Torrie's Model I split-plot model in a randomised block design (Table 16.4, 16.9, Steel and Torrie, 1980) and are shown

on Table 5.5.2. Data were analysed according to the analysis of variance in Table 5.5.2, for the following characters; pyrethrins yield (PyY), pyrethrins content (PP_H), dry matter content (DM), fresh flower yield (FFY), dry flower yield (DFY), the log of Pyrethrins I/II (lPyI/II) and pyrethrins to (jasmolins + cinerins) (lp/jc).

Table 5.5.2. Analysis of variance.

Source	df	Mean square	F
Site	2	-	-
Block(site)	12	-	-
Main-plot			
Density	1	-	-
Density*block(site)	14	-	-
Sub-plot:			
Population	3	MS_p	MS_p/MS_e
Population*density	3	MS_{pxd}	MS_{pxd}/MS_e
Error	1282	MS_e	
Total	1317		

The UV-assay of stage 6 flowers (PP_{U6}) was only assessed for plants in the low density plots. This character was analysed using the model

$$PP_{U6} = \mu + S_i + B_j(S_i) + P_k + \varepsilon_{ijkl}$$

where μ is the experiment mean, S_i is the effect of the ith site (i=3), B_j the effect of the jth block within the ith site (j=5), P_k the effect of the kth population (k=4) and ε_{ijkl} the residual. F-statistics for population effects were made by calculating F by dividing the population mean square by the error mean square.

The progeny of the Glaxo and the SP-clones were produced by pollination by a single polycross pollen mix. Therefore the two populations were not independent. In the first analysis the Glaxo and SP-progeny were assessed as a single group (base population). Data was then re-analysed treating the Glaxo and SP-clones as separate groups. Means for polycross progeny can be used to

compare breeding values of parent clones.

Data was analysed using the GLM procedure of SAS. Type III sums of squares were used as there were unequal numbers within each population-density combination (Maxwell and Delaney, 1989; Milliken and Johnson, 1984; Shaw and Mitchell-Olds, 1993; Younger, 1998). Comparisons between populations were made by paired t-tests between least-square population means.

5.5.3 Results

ANOVA tables are presented in Appendix 3.5.1 and the results of analysis are summarised on Table 5.5.3.

Differences between populations were significant for all characters ($P<0.01$) (Table 5.5.3). Generally, interactions between populations and planting densities were not significant ($P>0.05$), with the exception of dry matter content ($P<0.01$) and pyrethrins yield ($P<0.05$).

Table 5.5.3. Summary of results of statistical analysis of data.

Source	Character						
	PP_{U6}	PP_{H}	DM	FFY	DFY	PyY	lPyI/II
Population	***	***	**	***	***	***	***
Population x density	-	ns	**	ns	ns	*	ns

ns indicates differences between population means are not significant ($P>0.05$), *, ** and *** indicate significance at the 5%, 1% and 0.1% levels, respectively.

Differences between overall population means for dry matter content were generally not significant, apart from the current varieties (33%), and the base population (30%). There did not appear to be any overall trend of increasing or

decreasing dry matter with selection (Table 5.5.4). The relative values for population means were similar for fresh flower yields and dry flower yields (Table 5.5.5), so differences in dry matter content did not appear to affect population means for dry flower yields.

Table 5.5.4. Mean dry matter content (DM) and PyI/II for populations in 1998 trial. Figures represent the average of three sites and two plant densities.

Population	DM[+]	PyI/II[++]
Yugoslavian	34	1.5
Base	30	1.0
Current varieties	33	1.2
New selections	31	0.9

[+] Differences between population means are not significant (P<0.05) with the exception of the base population and current varieties.

[++] Differences between population means are all significant apart from Yugoslavian population and current varieties.

Although there were significant differences between population means for lPyI/II, there was no apparent trend in PyI/II with selection (Table 5.5.4). The average PyI/II for the Yugoslavian material was quite high at 1.5, while all the selected populations had an average PyI/II that was quite close to the optimum value of 1.0.

There was a general increase in flower yields between the Yugoslavian and base populations and the current varieties (Table 5.5.5). However, the average flower yield for the new selections is lower than either of the other two selected populations and approximately equal to the Yugoslavian material.

Table 5.5.5. Mean fresh (FFY) and dry flower yields (DFY) for populations in 1998 trial, expressed as yield relative to the current varieties. Figures represent the average of three sites and two plant densities.

Population	FFY	DFY
Yugoslavian	74^{ab}	72^{ab}
Base	86^{b}	82^{b}
Current varieties	100^{c}	100^{c}
New selections	72^{a}	71^{a}

Means followed by the same letter are not significantly different from one another (P>0.05)
Means followed by the different letters are significantly different (P<0.05).

In contrast, pyrethrins content increased for each successive selection population (Table 5.6.6), with a similar trend in both PP_{U6} and PP_{H}. Population averages were consistently 0.3 percentage units higher for PP_{U6} than for PP_{H}, with the exception of the new selections in which PP_{U6} was 0.4 units higher in value.

Table 5.5.6. Mean pyrethrins content for populations in 1998 trial, assessed for stage 6 flowers by UV-assay and whole plant samples by HPLC-assay. Figures represent the average of three sites and two plant densities.

Population	PP_{U6}	PP_{H}
Yugoslavian	1.5	1.2
Base	2.0	1.7
Current varieties	2.5	2.2
New selections	2.8	2.4

All differences between population means are significant (P<0.01).

Although there was an interaction between population and planting density for pyrethrins yield (Table 5.5.3), the population rankings (Table 5.5.7)

were the same in each density, that is;

Yugoslavian < base < new selections < current varieties.

Similarly, rankings for the average of the two densities were also the same, and the significant interaction (Table 5.5.3) is due to variation in the magnitude of the differences between populations in each density (Table 5.5.7). The main effect of the interaction is that the difference between the new selections and the base population is significant at the low density and not at the high density.

Table 5.5.7. Population means for pyrethrins yields in low density plots, high density plots and the average of both.

Population	Low density (g/plant)	High density (g/plant)	Mean (g/plant)
Yugoslavian	0.60[a]	0.20[a]	0.40[a]
Base	0.77[a]	0.37[a]	0.57[a]
Current varieties	1.20[c]	0.58[b]	0.89[c]
New selections	0.93[b]	0.44[a]	0.68[b]

Means followed by the same letter are not significantly different from one another (P>0.05)
Means followed by different letters are significantly different

The average improvement for each selection stage is shown on Table 5.5.8. On average, plants of the base population had higher pyrethrins content, flower yield and pyrethrins yields than the unselected Yugoslavian material. However, the differences in flower and pyrethrins yields were not statistically significant (P>0.05). The mean for current varieties was significantly higher than for the base population for all three characters (P<0.001). The new selections had a higher average pyrethrins content than the base population but flower yield was lower (P<0.05). Overall pyrethrins yields were higher for the new selections (P<0.005). Although the average pyrethrins content of the new selections was higher than for the current varieties, this was offset by significantly lower flower

yields and pyrethrins yields were lower for the new selections than for the current varieties ($P<0.01$).

Table 5.5.8. Percent improvement for each selection cycle, calculated from the differences between the population averages for all three sites and both planting densities.

Population	PP_H (%)	DFY (g/plant)	PyY (g/plant)
Base-Yugoslavia	39%***	14%ns	44%ns
Current varieties-Base	28%***	22%***	56%***
New selections-Current varieties	8%***	-29%**	-23%***
New selections -Base	39%**	-14%*	20%**

ns indicates differences between population means are not significant ($P>0.05$), ** and *** indicate significance at the 1% and 0.1% levels, respectively.

Mean yields for individual crosses are shown on Figures 5.5.1 and 5.5.2. and the mean PP_H are shown on Figure 5.5.3. These data are also shown in Table 3.5.2 of Appendix 3, with individual cross numbers. The new selections generally had higher pyrethrins content than the six varieties currently in commercial use (Figure 5.5.3). However, this did not result in higher pyrethrins yields. The majority of the new selections had lower mean yields than the current varieties in both the high (Figure 5.5.1) and the low density plots (Table 5.5.2), although differences were generally not significant. None of the new selections had higher pyrethrins yields than all six of the current varieties, in this trial.

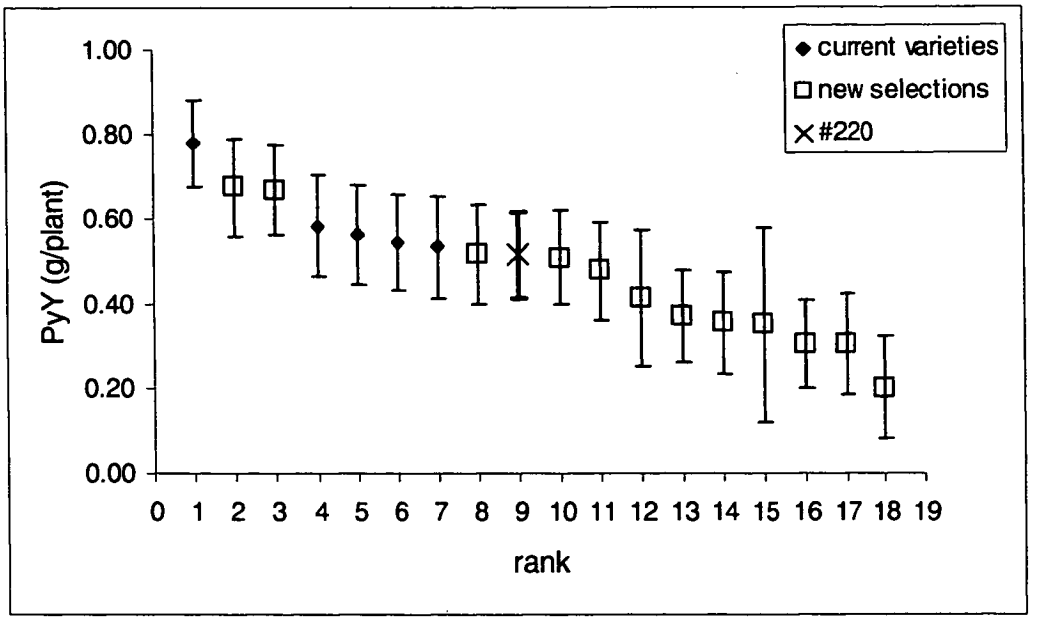

Figure 5.5.1. Family means for pyrethrins yields (g/plant) in high density plots, for each of the six current varieties and the 12 crosses selected after 1991 (new selections). *#220* is the cross between *CIG 3* and *CIG 11*. Family numbers are shown in Table 3.5.2 (Appendix 3).

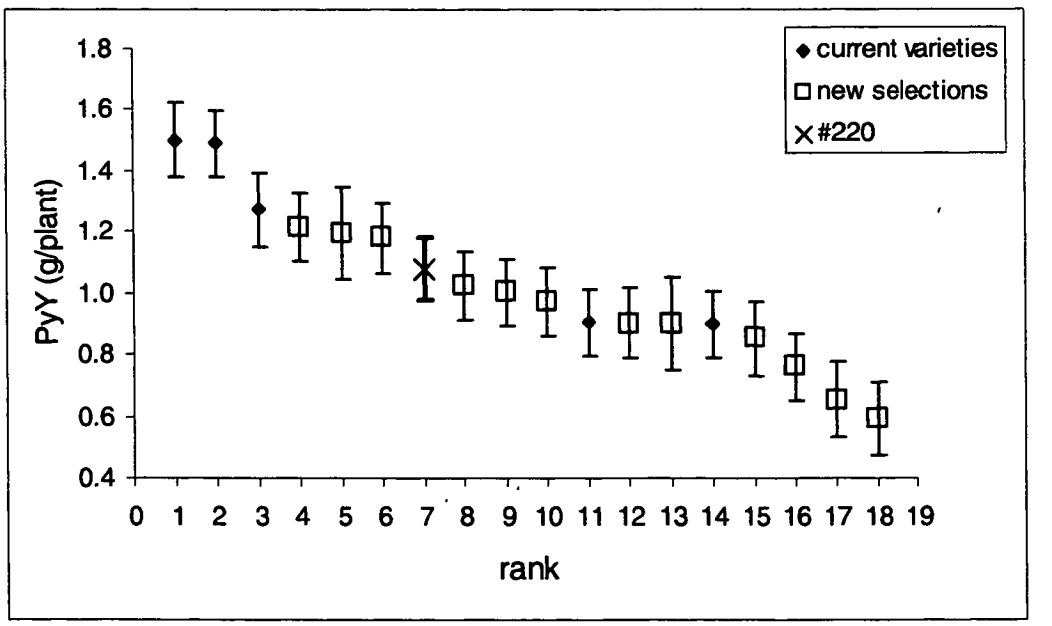

Figure 5.5.2. Family means for pyrethrins yields (g/plant) in low density plots established, for each of the six current varieties and the 12 crosses selected after 1991 (new selections). *#220* is the cross between *CIG 3* and *CIG 11*.

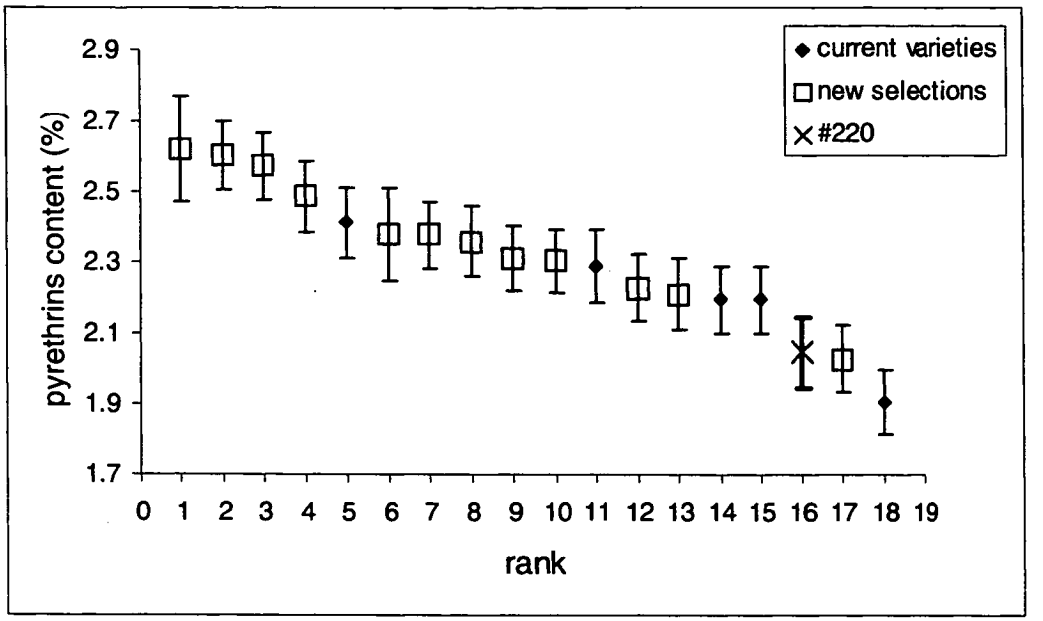

Figure 5.5.3. Family means for pyrethrins content (PP_H) for each of the six current varieties and the 12 biclonal crosses selected after 1991 (new selections). Means are the average of three sites and both planting densities. *#220* is the cross between *CIG 3* and *CIG 11*. Family numbers are shown in Table 3.5.2 (appendix 3).

The average pyrethrins yield and PP_H for the five biclonal crosses selected from the 1991 trial was approximately 10% higher than for the cross between *CIG 3* and *CIG 11* (Table 5.5.9). However, these differences were not statistically significant ($P>0.05$).

The progeny means for the SP- and Glaxo-clones were similar for PP_H (Table 5.5.10). However, the average flower yields and pyrethrins yields were higher for the the progeny of the Glaxo clones (Table 5.5.10).

Table 5.5.9. Average yields and PP_H for the cross between *CIG 3* and *CIG 11* (*#220*) and the other five current varieties that were selected from the 1991 seedling trial (*others*). Figures are least square means of three sites ± standard error.

	PP_H +	PyY		
		Low	High	Average+
#220	2.04 ± 0.09	1.1 ± 0.1	0.5 ± 0.1	0.8 ± 0.1
others	2.20 ± 0.04	1.2 ± 0.1	0.6 ± 0.1	0.9 ± 0.04
difference	8%^{ns}	10%^{ns}	10%^{ns}	12.5%^{ns}

+ average of both densities

Table 5.5.10. Progeny means for the SP- and Glaxo-clones and the average for the Yugoslavian population. Figures represent mean of three sites and two establishment densities.

Population	PP_H (%)	DFY (g/plant)	PyY (g/plant)
Yugoslavian	1.22	30	0.40
SP-clones	1.68	32	0.52
Glaxo-clones	1.74	37	0.63
SP-clones vs Yugoslavian	***	ns	ns
Glaxo vs SP	ns	**	*

Estimates of half-sib progeny means for individual clones are shown for PP_H in Table 5.5.11 and pyrethrins yields in the high density plots on Table 5.5.12. Clones are ranked from highest to lowest and clones that were used as parents in the recurrent selection program are indicated. Many of the clones that were not chosen as parents have higher values, in this trial, than individuals that were selected for breeding.

Table 5.5.11. Maternal half-sib family averages and standard errors (s.e.) for PP_H. + indicates clones that have progeny in the RS3 and RS4 generations.

Mother clone #	Population		PP_H (%)	s.e.
135	Glaxo		2.6	0.2
106	Glaxo		2.0	0.1
26	SP-clone	+	2.0	0.2
98	Glaxo	+	2.0	0.1
20	SP-clone	+	2.0	0.1
110	Glaxo		2.0	0.1
13	SP-clone	+	2.0	0.1
129	Glaxo		1.9	0.1
104	Glaxo		1.9	0.1
6	SP-clone		1.9	0.1
107	Glaxo		1.9	0.1
4	SP-clone		1.9	0.1
24	SP-clone	+	1.9	0.2
136	Glaxo		1.8	0.1
10	SP-clone	+	1.8	0.1
118	Glaxo		1.8	0.1
21	SP-clone	+	1.8	0.1
18	SP-clone		1.8	0.1
23	SP-clone	+	1.7	0.1
128	Glaxo		1.7	0.1
120	Glaxo		1.7	0.1
11	SP-clone	+	1.7	0.1
12	SP-clone		1.7	0.1
131	Glaxo		1.7	0.1
103	Glaxo		1.7	0.1
100	Glaxo		1.7	0.1
122	Glaxo		1.7	0.1
126	Glaxo		1.6	0.1
3	SP-clone	+	1.6	0.1
15	SP-clone	+	1.6	0.1
109	Glaxo	+	1.6	0.1
105	Glaxo		1.6	0.1
19	SP-clone	+	1.6	0.2
133	Glaxo		1.6	0.1
25	SP-clone		1.6	0.1
134	Glaxo		1.6	0.1
16	SP-clone	+	1.6	0.1
17	SP-clone	+	1.6	0.1
124	Glaxo		1.6	0.3
99	Glaxo		1.5	0.1
14	SP-clone		1.5	0.1
127	Glaxo		1.5	0.1
2	SP-clone	+	1.5	0.1
9	SP-clone	+	1.5	0.1
8	SP-clone		1.4	0.1
7	SP-clone		1.4	0.1

Table 5.5.12. Maternal half-sib families means and standard errors (s.e.) for Glaxo and SP-clones for PyY in the high density plots. + indicates clones that have progeny in the RS3 and RS4 generations.

Mother clone	Population		PyY (g/plant)	s.e.
105	Glaxo		0.7	0.1
13	SP-clone	+	0.6	0.1
127	Glaxo		0.6	0.1
131	Glaxo		0.6	0.1
100	Glaxo		0.6	0.1
110	Glaxo		0.5	0.1
129	Glaxo		0.5	0.2
106	Glaxo		0.5	0.2
136	Glaxo		0.5	0.1
19	SP-clone	+	0.5	0.2
134	Glaxo		0.5	0.2
20	SP-clone	+	0.5	0.1
126	Glaxo		0.5	0.1
21	SP-clone	+	0.5	0.1
16	SP-clone	+	0.5	0.1
128	Glaxo		0.4	0.1
103	Glaxo		0.4	0.2
10	SP-clone	+	0.4	0.1
118	Glaxo		0.4	0.1
133	Glaxo		0.4	0.1
122	Glaxo		0.4	0.1
12	SP-clone		0.4	0.1
120	Glaxo		0.4	0.1
23	SP-clone	+	0.3	0.1
18	SP-clone		0.3	0.1
124	Glaxo		0.3	0.4
135	Glaxo		0.3	0.2
4	SP-clone		0.3	0.1
109	Glaxo	+	0.3	0.1
107	Glaxo		0.3	0.1
26	SP-clone	+	0.3	0.2
25	SP-clone		0.3	0.1
15	SP-clone	+	0.3	0.1
14	SP-clone		0.3	0.1
17	SP-clone	+	0.3	0.1
2	SP-clone	+	0.3	0.1
98	Glaxo	+	0.3	0.2
6	SP-clone		0.3	0.1
11	SP-clone	+	0.3	0.1
9	SP-clone	+	0.2	0.1
3	SP-clone	+	0.2	0.1
8	SP-clone		0.2	0.1
24	SP-clone	+	0.2	0.2
7	SP-clone		0.2	0.1
99	Glaxo		0.2	0.1
104	Glaxo		0.2	0.1

5.5.4 Discussion

There was a consistent improvement in crop pyrethrins (PP_H) with each successive stage of selection (Table 5.5.6). Selection in the first phase of the breeding program was for stage 5 pyrethrins and in the later phases for stage 6 pyrethrins. Both selection methods resulted in an increase in population means for PP_H. Therefore, it appears that selection for UV-assay of flowers at maturity stage 5 or 6 are effective methods for increasing the pyrethrins content of mechanically harvested crops.

Population means for PP_{U6} were approximately 0.3 percentage units higher than for PP_H. This is consistent with expectations. UV-assays tend to over-estimate pyrethrins content as the UV-method is not specific for pyrethrins (Head, 1973), and this is particularly true for the University's method (Appendix 1.1). In addition, pyrethrins levels are often at a maximum in stage 5 to 6 flowers (Bhat and Menary, 1984a; Ikahu and Ngugi, 1990). Hence, the pyrethrins of the entire crop would be lower as it includes flowers at other maturity stages (Faber, 1980).

On average, the progeny means for the Glaxo-clones were higher than for the SP-clones for flower yield ($P<0.01$), while PP_H were similar for both sets of clones (Table 5.5.10). The average flower yields for the Glaxo progeny were also higher than for the Yugoslavian population. This suggests that the methods used by Glaxo for yield selection were effective. Unfortunately, the University was not provided with records of Glaxo's selection methods.

Population means for the University's base population and the unselected Yugoslavian material appear to be consistent with data reported by Bhat *et al* (1985) for the SP population and unselected material. In both cases, the mean pyrethrins content for the selected population was higher ($P<0.001$) and there appeared to be a non-significant increase in flower yield ($P>0.05$). However, in the 1998 trial, the apparent improvement in flower yield in the base population is

probably solely due to the Glaxo clones. The average flower yield for the progeny of the SP-clones, at 32 g/plant, is not significantly different to the Yugoslavian population mean of 30 g/plant ($P>0.05$). And, as the SP-clones were pollinated by pollen from Glaxo and SP-parents, the flower yields for the SP-progeny would be expected to be lower if they had been pollinated solely by other SP-clones. Therefore, the data suggest that the average flower yields for the SP-clones are similar or lower than the Yugoslavian population, and the observation of an increase in flower yield (Bhat, 1995; Bhat *et al*, 1985) was not repeated in the 1998 trial. This supports the view that the apparent increase in flower yield reported by Bhat *et al* (1985) was due to random sampling error, as indicated by the non-significant t-test (Table 1, Bhat *et al*, 1985). It is less likely that there was subconscious selection for high yielding plants in the development of the SP population, or indirect selection for flower yield through selection for another character such as synchronous flowering.

The current varieties had significantly higher pyrethrins content and yields than the base population from which they originated (Table 5.5.7 and 5.5.8). Overall, the six cultivars produced 56% more pyrethrins than the base population when the two were tested at the same planting density. The five crosses selected from the 1991 seedling trial had an improvement in pyrethrins yields of approximately 10% compared to family #220 (the cross between the two clones selected from the SP in the early 1980's). However, this difference was not statistically significant. Therefore, the data are consistent with a conclusion that the recurrent selection and variety selection methods used from 1985 to 1992, have resulted in five varieties with higher yields than the cross that was available in 1985 (#220). However, they do not provide conclusive proof of this.

In contrast, the new selections had lower flower and pyrethrins yields than the current varieties, although the average PP_H of the new selections was higher (Table 5.5.7 and 5.5.8). A reduced flower yield in the later selections could be due to a negative genetic correlation between flower yield and one or both of the

selection characters (visual yield and PP_{U6}). However, this interpretation is inconsistent with other observations. Firstly, similar selection methods, used to produce the current varieties from the base population, resulted in improved flower yields (Table 5.5.9). Secondly, there is no evidence that selection for PP_{U5} in the development of the base population resulted in any decrease in flower yield (Table 5.5.7, 5.5.9, Bhat *et al*, 1985), and a negative association between flower yield and PP_{U6}, but not PP_{U5}, seems unlikely. Similarly, the majority of previous studies failed to find evidence of a negative genetic correlation between pyrethrins content and flower yield (Bhat and Menary, 1986b; Parlevliet, 1974; Singh *et al*, 1987). Also, visual selection for yield in Kenya produced an increase in flower yields (Parlevliet, 1975). Therefore, it is unlikely that selection for visual yield and PP_{U6} have resulted in a correlated negative response in flower yield.

Instead, the low mean flower yield of the new selections relative to current varieties is probably the result of the different variety selection methods used for the two sets of seedling varieties. The new and commercial varieties were selected for high PP_{U6} and visual yield, as assessed for each full-sib family in the seedling trials. However, the current varieties were also selected on the basis of the availability of parent material (Potts and Menary, 1993a). Although factors affecting the amount of parent material available were not specified (Potts and Menary, 1993a), it is likely that more material would be available for the clones that were growing in variety-evaluation trials. Generally 10 new clones were added to the site-replicated clonal-evaluation trials each year and clones were culled or retained on the basis of performance (Potts and Menary, 1988). There were 120 replicate plants for each clone in the variety trials, compared to only five replicates for clones used solely as parents. Therefore, it seems likely that the selected crosses were between clones that were in the variety trials in 1992. In contrast, the new selections were chosen solely on the basis of full-sib family evaluations in the seedling trials. Therefore, selection of the current varieties was

based on an additional component that was absent from the selection of the new varieties. This additional component corresponds to three-stage clonal evaluation of the parents; that is, single-plant selection from seedling trials, selection from clonal-row trials, and evaluation in variety trials replicated for site, block and season.

Hence, it could be argued that the selection method used for the new selections (family selection by visual yield) is relatively imprecise, and the difference between the genetic value of the flower yield of the selections is only slightly greater than the mean for the entire seedling population. In contrast, selection of the current varieties (three-stage clonal testing of parents and family selection) is a more accurate method of identifying varieties with high genetic values for flower yield. The use of this method in 1992 resulted in selection of varieties with substantially greater flower yields than the main selection population. Therefore, it is not necessarily the selection character (visually-estimated yield) that is at fault, but rather the testing method (family selection from seedling trials).

Even when there has been an improvement in the population mean for the recurrent selection population, the best genotypes in an early generation may be superior to the population mean for succeeding generations (Gardener, 1978). Therefore, accurate genotype selection from an early generation can result in varieties with a higher genetic value than imprecise selection from the later generations. Hence, the observed decrease in flower yields of new selections relative to current varieties does not necessarily indicate a decline in the yield of the recurrent selection population.

Nevertheless, the average flower yields for the new selections were also lower than for the base population ($P<0.01$). However, there is no significant difference between the mean flower yield for the new selections and the progeny of the SP-clones ($P>0.05$), although, flower yields for the new selections are significantly lower than for the progeny of the Glaxo clones ($P<0.001$). Plants in

the RS2, 3 and 4 generations are mainly descended from SP-clones, with minimal contribution from the Glaxo material (Chapter 4). Therefore, it is possible that the University's RS1 generation had a lower average flower yield than a random selection from all the Glaxo- and SP-clones, due to the disproportionate contribution of the low yielding SP-clones compared to the high yielding Glaxo clones. If this is the case, then the mean flower yield for the new selections may be comparable to the RS1 generation.

To summarise, these results are consistent with the following interpretation:

- The population improvement methods that were used have resulted in an increase in the mean of the recurrent selection population for PP_H and a negligible change in flower yield.
- Visual selection of full-sib families evaluated at one or two sites is little more effective than random selection.
- The relatively high flower yields of the current varieties is due to selection of crosses between parent clones which had ample parental material. This corresponds to selection of parents with high visual yield scores in three stages of clonal testing.

Examination of family means (Figure 5.5.2) suggests that it is unlikely that any of the new selections will have higher yields than the current varieties. It also seems unlikely that variety selection methods used from 1993 to 1997 will lead to the development of superior new varieties in the immediate future. Therefore, the methods used to develop new varieties need to be modified, for example by;

- increasing the number of test environments,
- combining data from different trials (White and Hodge, 1989), for example, clone evaluation trials and full-sib family evaluations,
- using more accurate yield evaluation methods, for example, harvesting and weighing flowers, increasing the size of family plots.

There may be advantages to adding more progeny of the Glaxo clones to the current breeding population. This would increase the genetic diversity of the breeding population, and, although average pyrethrins would be expected to be lower for the Glaxo clones, it is possible that they have higher flower yields than the current generation of the recurrent selection population.

Overall, the data collected in the 1998 trial suggests that both PP_{U5} and PP_{U6} are effective selection criteria for the improvement of crop pyrethrins yields. The relative efficiency of PP_{U6} compared to alternate selection methods will be examined in the next two sections.

5.6 Evaluation of component or correlated character selection for yield improvement in pyrethrum. Part 1: Genetic parameter estimates for pyrethrins yield and its components

5.6.1 Introduction

Like most other pyrethrum breeding programs, the University's recurrent selection program used related characters to improve pyrethrins yield rather than direct selection for yield *per se*. The University's selection characters; UV-assay of stage 6 flowers and visually estimated yield, have a number of potential advantages. Firstly, selection by component or related characters can be more effective than direct selection for yield (Banziger and Laffite, 1997; Dolan *et al*, 1996; Richards and Thurling, 1979; Thurling, 1974). Secondly, direct selection for pyrethrins yield can result in a disproportionately high increase in flower yield compared to pyrethrins content, which can result in a low economic gain (Parlevliet, 1969). Assessment costs are low for visually estimated yield and stage 6 flower assay because it is not necessary to recover all the flowers from each plant. The University's UV-method is also less expensive than assay by more accurate methods, such as AOAC and HPLC.

However, selection by component or related characters is not always more efficient than direct selection (Caldwell and Weber, 1965; Pritchard *et al.*, 1973). The relative efficiency of indirect selection depends on the genetic correlation between the target character (yield) and selection character(s) and the relative magnitude of their heritabilities (Gallais, 1984; Hansel, 1984; Searle, 1965), and their evaluation costs (Pritchard *et al*, 1973; Utz *et al.*, 1997).

Different selection methods can be compared either by measuring the realised response following selection or by calculating the predicted response prior to selection from parameters such as heritability and phenotypic variance (Falconer, 1989). Although assessments of realised response may be the only

truly reliable method for comparing selection methods (Byth *et al.*, 1969a), this approach has a number of practical disadvantages. Firstly, a breeding program's resources must be divided among a number of selection methods, some of which may have low or negligible value. Secondly, the use of predicted response allows the optimum selection method to be chosen using data collected from an initial evaluation and selection trial. In contrast, there must be a second trial to assess realised response with the retrospective evaluation method, so there is a considerable delay before selection methods can be chosen. Thirdly, the realised response, like any biological parameter, is variable and accurate estimates of response require a number of replicate selection lines (Sen and Robertson, 1964), as well as a number of assessment environments (Byth *et al*, 1969a). Hence, accurate evaluations of realised response can be expensive and time-consuming.

Predicted response has been used to estimate the relative selection efficiency of alternate selection methods in a number of crop species (for example, Atlin and Frey, 1989; Banziger and Laffite, 1997; Geidel *et al*, 2000; Johnson *et al*, 1955; Richards and Thurling, 1979; Shankar *et al.*, 1963; Subandi *et al.*, 1975; Thurling, 1974). In general, comparisons between predicted and realised response suggest that predicted response is a reliable method for choosing between different selection techniques (Bravo *et al.*, 1980; Caldwell and Weber, 1965; Manning, 1958; Souza and Byrne, 1998), although this is not always the case. Sometimes, there is poor agreement between realised and predicted response when the predictions are made from genetic parameters that have been estimated from a single environment (Byth *et al*, 1969b; Pringle and Shaw, 1998), or when there is a non-linear relationship between characters (Moll *et al*, 1975). However, in general, the use of predicted response to selection is a well-established and generally robust method for evaluation of selection methods (Goddard, 2001; Mackay, 1996).

There are no published evaluations of the relative efficiency of direct and correlated or component character selection for yield in pyrethrum, either in

Tasmania or in any other region. Similarly, narrow-sense heritability estimates have not been calculated for pyrethrins yield for mechanically-harvested crops, and there are no estimates of the genetic correlation between yield and related selection characters. Consequently, currently there are no data available that could be used to compare indirect and direct selection for pyrethrins yield, in Tasmanian growing conditions.

Selection indices used in the University's recurrent selection program were constructed using estimates of genetic parameters that were specific to individual sites and seasons. The use of specific genetic parameter estimates can increase the efficiency of the selection index (Thurling, 1974). However, the presence of genotype-environment interactions will cause heritability to be over-estimated when data are collected from a single environment (Cotterill and Zed, 1980; Namkoong *et al*, 1966). Consequently, selection indices constructed from specific genetic parameter estimates will be less efficient than indices constructed from genetic parameter estimates derived from multisite trials, when there is a significant genotype-environment interaction. Genotype-environment interactions have been reported for clonal genotypes (Bhat and Menary, 1986a; Parlevliet, 1969), but, as yet, there has been no investigation of interactions between breeding values and environments.

The objectives of the data analysis presented in this section were;

1) to provide estimates of narrow-sense heritability and genetic correlations between characters that can be used to calculate the predicted response to alternate selection methods,
2) to determine whether there is an interaction between test environment and breeding value for pyrethrins yield and related characters.

The genetic parameters will be used to estimate predicted gains for alternate selection methods in the next section.

5.6.2 Methods

Preliminary analysis methods

Data analysed in this section consist of plant assessments made for mature (December-sown) plants from the 46 maternal half-sib families (base population). Family sizes were unbalanced and each contained a maximum of 30 individuals, with a total population size of 906. Families were assessed at three sites and in two planting densities. A population size of 1000 individuals with 20-30 in each family was aimed for as recommended by Harris (1964) and Robertson (1959a). Families were replicated at three sites, as this number can be expected to be the most efficient (Robertson, 1959b).

Characters analysed were pyrethrins yield (PyY), dry matter content (DM), fresh flower yield (FFY), dry flower yield (DFY), HPLC-assay of the entire flower crop (PP_H), UV-assay of stage 6 flowers (PP_{U6}), visually estimated flower yield (DFY_{est}), and log-adjusted values of Pyrethrins I/II (lPyI/II) and the ratio of pyrethrins to (jasmolins + cinerins) (p/jc).

Data were initially analysed on an individual site and density basis to check that residual variances among sites and densities were sufficiently homogeneous to allow data to be pooled (Williams and Matherson, 1994). Data collected for each site-density combination were analysed by;

$$Y_{ijkl} = \mu + B_i + F_j + \varepsilon_{ijkl}$$

where μ is the mean, B_i the effect of the ith block (i=5), F_j the effect of the jth family (j=46) and ε_{ijk} the residual.

Differences between the largest and smallest error variances among both site and densities were less than 10-fold (Appendix 3.6.1), so combined analyses across both sites and densities were considered acceptable (Patterson and Silvey, 1980; Williams and Matherson, 1994).

Estimation of genetic parameters

Genetic parameter estimates need to be calculated for the specific unit to which selection is applied (Dudley and Moll, 1969; Falconer, 1989). In the University's recurrent selection program, single plants were selected from seedling trials, on the basis of either first-year or second-year harvest data. Therefore, the appropriate genetic parameters to assess alternate selection characters are individual (narrow-sense) estimates generated from data collected from single plants in one harvest season.

Narrow-sense heritability can be estimated by the parent-offspring method, or by analysis of full-sibs (Falconer, 1989) or half-sibs (Nguyen and Sleper, 1983), if non-additive genetic variance is negligable. The parent-offspring method was not used because the base population parents exist as clones. Data generated from parents propagated as clones are not applicable to single plant selection in the University's breeding program, as parents were assessed as seedlings.

The use of maternal half-sib families have a number of advantages over full-sib families. In hand-crossing, pollen collection and pollination is simpler and requires less labour, so more flowers can be pollinated in total. There is only one half-sib family for each clone so a larger set of parents can be used (Nguyen and Sleper, 1983). The precision of genetic parameter estimates increases with the number of genotypes sampled (Xie and Mosjdis, 1999) and population size (Baker, 1986). The disadvantages of using maternal half-sib families are that additive genetic effects can be estimated but effects due to dominance cannot (Cotterill *et al.*, 1987), and that estimates can be biased if there are maternal effects for the character(s) assessed (Mazer and Gorchov, 1996).

Heritability was estimated using raw individual phenotype, block adjusted individual phenotype and standardised individual phenotype.

Heritability estimates specific to a single density (h^2_l, h^2_h)were calculated from variance components estimated by separate analysis of high and low density data. Variance components were estimated using the model;

$$Y_{ijkl} = \mu + S_i + B_j(S_i) + F_k + \varepsilon_{ijkl}$$

where Y_{ijk} is the individual plant value, μ is the experiment mean, S_i is the effect of the ith site (i=3), B_j the effect of the jth block within the ith site (j=5), F_k the effect of the kth family (k=46) and ε_{ijkl} the residual.

Heritability estimates applicable to variable planting density (h^2_{hl}) were estimated by analysis of the complete data-set that included plants assessed in both densities. Individual plant values were analysed by the model;

$$Y_{ijklm} = \mu + S_i + B_j(S_i) + D_k + D_k x\, B_j(S_i) + F_l + \varepsilon_{ijklm}$$

where D_k the effect of planting density (k=2), $D_k x\, B_j(S_i)$ is the density-block interaction (main-plot error), F_l is the effect of the lth family (l=46) and ε_{ijklm} the residual.

All effects were random. The total phenotypic variance (V_P) was calculated as the sum of the variances for each effect, and heritability (h^2) was calculated by;

$$h^2 = 4\, x\, V_{fam} / V_P$$

where V_{fam} is the family variance component. This assumes that the parents are not inbred, there is no epistatic variance (Nguyen and Sleper, 1983), and that families consist solely of half-sibs, that is negligible incidence of self-pollination (Squillace, 1974).

The standard error (*se*) for estimates was calculated using the square-root of the approximate variance of heritability given by Dieters *et al* (1995) as;

$$se = 4\, x\, se_{fam} / V_P$$

where se_{fam} is the standard error of the estimate of family variance.

The coefficient of family variation was estimated as the ratio of family variance (V_{fam}) to the character mean for each density and the coefficient of phenotypic variation was the ratio of phenotypic variance (V_P) to the character mean.

Variance components were estimated using the MIXED procedure of SAS (REML method), using the *covtest* option to test the significance of each variance component and to generate standard errors of estimates.

Genetic and phenotypic correlations between pairs of characters

Correlations between pairs of characters were estimated from family means, as estimates from family means are considered to be more reliable than estimates from individual data (Reeve, 1955). Family means were calculated for each site-density combination. Families that were not represented in each density treatment at all sites were not included in the analysis in order to produce a balanced data set. The data was analysed by the model;

$$Y_{ij} = \mu + S_i + G_j + \varepsilon_{ij}$$

where Y_{ij} is the mean of family j at the ith site, μ is the experiment mean, S_i is the effect if the ith site (i=3) G_j is the effect of the jth family (j=41), and ε_{ij} is the residual. All effects were treated as random. Family variance (V_F) was estimated using the MIXED procedure of SAS (REML method).

Cross-products (*CP*) between family means between each pair of characters were calculated using the MANOVA option of the GLM procedure. Mean cross products were calculated as *CP/df*, and genetic covariances were estimated according to the analysis of covariance presented in Table 5.6.1.

The genetic correlation (r_g) was calculated by;

$$r_g = \sigma_{G(12)} / \sigma_{G(1)} \cdot \sigma_{G(2)}$$

where $\sigma_{G(12)}$ is the genetic covariance between character 1 and 2, and $\sigma_{G(1)}$ and $\sigma_{G(2)}$ are the square roots of the genetic variance for character 1 and 2.

Phenotypic correlations are the Pearson's product-moment correlation between family means. Phenotypic correlations and tests of significance were also provided by the MANOVA option of the GLM procedure.

Table 5.6.1. Analysis of covariance for half-sib family means in low and high density environments.

Source	df	Expected mean cross-products
Sites (*S*)	2	
Families (*G*)	40	$\sigma_{GS(12)}+3\sigma_{G(12)}$
residual	80	$\sigma_{GS(12)}$

Analysis of genotype-site interactions

Genotype-site interactions were analysed by calculating the average genetic correlation (r'_g) between family means at different sites, for each character. As there were three sites, r'_g was estimated using Dickerson's (1962) method for estimating the average correlation between more than two environments, as;

$$r'_g = \sigma^2_G /(\sigma^2_G + \sigma^2_{GE'})$$

where σ^2_G is the variance of genotypes across environments and $\sigma^2_{GE'}$ is the interaction variance corrected for the variation of genetic scales among sites.

$$\sigma^2_{GE'} = \sigma^2_{GE} - V(\sigma_{Gi})$$

where $V(\sigma_{Gi})$ is the variance of the square root of the genetic variance within each site *i*.

Family means were calculated separately for the high and low density treatments as well as for the whole data-set, and a separate analysis conducted for the high, low and combined density data. Family means were analysed by the model;

$$Y_{ij} = \mu + S_i + G_j + \varepsilon_{ij}$$

All effects were treated as random and variance components were estimated by the MIXED procedure (REML option), to provide estimates of the family variance(σ^2_G) and the residual variance (σ^2_{GE}). Genetic correlations reported in

this section refer to the combined density data-set. Correlations specific to the low density plots are used in Section 5.7 for response predictions.

5.6.3 Results

Results of statistical analysis are presented in Appendix 3.6.2 and calculations for genetic correlations in Appendix 3.6.3.

Coefficients of variation are shown on Table 5.6.2. Flower and pyrethrins yields showed higher levels of phenotypic variation than PP_H and DM. The levels of genetic variation were highest for flower and pyrethrins yield (>15%), moderate for PP_H (8%), and very low for dry matter content (1 to 4%).

Table 5.6.2. Genetic and phenotypic coefficient of variation (CV, %) for pyrethrins yield and its components for pyrethrum established at low and high densities.

Character	Phenotypic CV		Genetic CV	
	Low	High	Low	High
PP_H	32	31	8	8
DM	27	25	4	1
FFY	57	76	15	18
DFY	53	74	13	19
PyY	63	78	20	23

There were statistically significant levels of family variance for most characters, both within each density and when the two densities were combined (Table 5.6.3). The exceptions were DM and DFY_{est} (Table 5.6.2). Family effects for DM were just significant (0.05>P>0.04) for block-adjusted or standardised scores from the combined density data-set. Family effects were almost significant (P=0.053) when DFY_{est} from both densities were combined.

Table 5.6.3. Tests for significant family variance for pyrethrins yield, its components and related characters for high and low densities environments and for data combined from both (combined).

Character	Low	High	Combined
PP_H	*	*	*
PP_{Hb}	*	*	*
PP_{Hs}	*	*	*
DM	ns	ns	ns
DM_b	ns	ns	*+
DM_s	ns	ns	*++
FFY	*	*	*
FFY_s	*	*	*
DFY	*	*	*
DFY_s	*	*	*
PyY	*	*	**
PyY_b	*	*	**
PyY_s	*	*	**
lPyI/II	**	**	***
Lp/jc	*	*	**
PP_{U6}	*	na	na
DFY_{est}	ns+++	ns	ns++++

ns, *, **, and *** are not significant, and significant at P<0.05, P<0.01 and P<0.001, respectively
na - not assessed,
+ P=0.044,++ P=0.049, +++ P =0.07, ++++ P=0.053

Standard errors for heritability estimates were all relatively high (Table 5.6.4). Heritability for DM was low with estimates ranging from 0 to 0.15. Heritability estimates for PP_H and PP_{U6} were moderate, ranging from 0.24 to 0.34. Estimates for flower yield heritability made within a single planting density, were also moderate (0.23-0.30), with similar values for fresh and dry flower yield. The estimates for DFY_{est} were within the same range, although these were not statistically significant. Heritability for PyY within a single planting density was

a little greater than for flower yield or PP_H. Estimates were quite high for PyI/II (~0.6) and moderate for p/jc (0.2-0.4).

Table 5.6.4. Narrow-sense heritability (h^2) estimates for pyrethrins yield, its components and related characters for high (h^2_l) and low (h^2_h) density environments and for data combined from both (h^2_{lh}).

Character	$h^2 \pm$ s.e.		
	h^2_l	h^2_h	h^2_{lh}
PP_H	0.24 ± 0.13	0.25 ± 0.13	0.30 ± 0.11
PP_{Hb}	0.29 ± 0.15	0.25 ± 0.13	0.32 ± 0.11
PP_{Hs}	0.25 ± 0.19	0.31 ± 0.16	0.34 ± 0.13
DM	0.08 ± 0.09	0.01 ± 0.08	0.08 ± 0.05
DM_b	0.13 ± 0.10	0.03 ± 0.08	0.10 ± 0.06
DM_s	0.15 ± 0.11	0.00 ± 0.09	0.11 ± 0.07
FFY	0.26 ± 0.13	0.23 ± 0.13	0.17 ± 0.07
FFY_s	0.29 ± 0.15	0.25 ± 0.13	0.21 ± 0.09
DFY	0.24 ± 0.12	0.26 ± 0.13	0.14 ± 0.07
DFY_s	0.30 ± 0.15	0.30 ± 0.15	0.19 ± 0.08
PyY	0.39 ± 0.17	0.36 ± 0.15	0.26 ± 0.10
PyY_b	0.39 ± 0.17	0.36 ± 0.15	0.26 ± 0.10
PyY_s	0.35 ± 0.17	0.37 ± 0.16	0.28 ± 0.16
lPyI/II	0.77 ± 0.24	0.56 ± 0.19	0.65 ± 0.17
lp/jc	0.23 ± 0.12	0.39 ± 0.16	0.36 ± 0.11
PP_{U6}	0.28 ± 0.14	na	na
DFY_{est}	0.24 ± 0.16	0.36 ± 0.30	0.18 ± 0.11

In general, the heritability estimates for each character were similar in the low and high density treatments. The exception to this was DM which had lower heritability in the high density plots. Estimates of h^2_{lh} were consistently lower than h^2_l and h^2_h for flower and pyrethrins yield. In contrast, estimates of h^2_{lh} were a little higher than the estimates specific to a single planting density for PP_H and similar for other characters (Table 5.6.4).

Phenotypic correlations between PyY and the component and correlated

characters were all significant and positive, with the exception of DM that had a negative association with PyY (Table 5.6.5). The genetic correlations showed a similar pattern. The negative association between DM and PyY appears to be due to a negative association between PP_H and DM, as the estimates of r_g and r_p between DFY and DM were approximately 0.

Table 5.6.5. Phenotypic (r_p) and genetic (r_g) correlations between characters.

Characters		r_p	r_g
PyY_b	PP_H	0.34***	0.80
	DFY	0.81***	0.80
	FFY	0.84***	0.95
	DM	-0.18*	-0.94
	PP_{U6}	0.26**	0.74
	DFY_{est}	0.64***	0.25
PP_H	PP_{U6}	0.48***	0.91
	lPyI/II	-0.16^{ns}	-0.15
	lp/jc	0.12^{ns}	-0.43
	DFY	-0.19*	0.21
	DM	-0.41***	-4.00
lPyI/II	lp/jc	0.02^{ns}	0.29
DM	FFY	-0.20*	1.30
	DFY	0.04^{ns}	0.03

The association between PP_H and DFY was relatively small (< | 0.3 |) and negative for the estimate of r_p and positive for r_g. Similarly, the association between PyI/II and PP_H was relatively small, although both r_p and r_g were negative. There was also a negative genetic correlation between p/jc and PP_H (-0.43). Genetic correlations between families means at different sites (Table 5.6.6) ranged from 0.17 to 0.30. Estimates were of a similar magnitude for pyrethrins yield and its component characters.

Table 5.6.6. Genetic (r'_g) correlations between half-sib family means at different sites for pyrethrins yield and its main components.

Character	r'_g
PP_H	0.22
PP_{Hs}	0.17
PP_{Hb}	0.24
DFY	0.23
DFY_s	0.30
PyY	0.26
PyY_s	0.26

5.6.4 Discussion

There were statistically significant levels of family variance for pyrethrins yield and its components in both densities, with the exception of DM which had marginal significance. Narrow-sense heritability estimates for PyY, PP_H and DFY were all moderate, suggesting that each of these characters can be improved through selection. Heritability estimates for flower and pyrethrins yields were similar to estimates made from data reported by Parlevliet and Contant (1970) for pyrethrum in Kenya, although these are not necessarily comparable due to the differences between growing regions. In contrast, the values for PP_H and PP_{U6} were lower in the 1998 trial than the estimate of 0.7 for pyrethrins content reported by Parlevliet and Contant (1970). Although this may be partially due to variation between populations or environments (Falconer, 1989), the higher heritability in the Kenyan trial is probably due to increased sample replication. In the Kenyan trial, pyrethrins content was the average of duplicate assays of four samples, while flower yield was based on accumulated annual yield (Parlevliet and Contant, 1970). In contrast, in the 1998 trial, pyrethrins content was estimated from an unreplicated assay for each plant. Selection accuracy and heritability increase with the number of replicates (Berdahl and Barker, 1997; Hallauer and Miranda, 1981).

Although genetic parameters may vary among populations (Falconer, 1989), estimates of genetic parameters are subject to large sampling errors (Koots and Gibson, 1996; Lin, 1978; Reeve, 1955; Xie and Mosjdis, 1999). Therefore, the use of average parameter estimates, made by combining local data with published estimates, may be preferable to the use of local estimates of genetic parameters (Cotterill and Dean, 1990; Koots and Gibson, 1996). However, the use of general estimates is probably not indicated for pyrethrum due to the significant difference between growing and harvesting systems in Tasmania and in Kenya. Potentially, the differences in climate and between continuous cropping and a single annual harvest, affect the way that genes may be expressed. More particularly, there are major differences in the way yield is assessed in the Kenyan and Tasmanian trials. Genetic parameters are specific to the selection unit (Nguyen and Sleper, 1983) and the number of replicates (Falconer, 1989; Hallauer and Miranda, 1981). Estimates that are derived from replicated assays of flowers assessed for clonal parents in Kenya, are not intrinsically comparable with unreplicated assessments of seedlings in Tasmania. Therefore, the combination of Tasmanian and Kenyan heritability estimates is not recommended.

Although there was a statistically significant, negative phenotypic correlation between PP_H and DFY, the genetic correlation was positive. Both correlations were within the range ($<|0.3|$) that can be interpreted as a negligible association between characters (Robinson *et al.*, 1951; Smith *et al.*, 1981). This is consistent with previous reports that there is little evidence of a negative genetic association between pyrethrins content and flower yield (Bhat and Menary, 1986b; Parlevliet, 1974; Singh *et al*, 1987).

Dry matter content is usually not used as a selection character in pyrethrum (Bhat, 1995; Parlevliet, 1975; Tuikong, 1984) due to low overall levels of genetic variability and the inability to detect any parent offspring correlation for this character in trials conducted in Kenya (Parlevliet and Contant, 1970). The results

of the 1998 trial generally support this practice, as the overall level of genetic variation and heritability DM are low (Table 5.6.2, 5.6.4). However, family effects were statistically significant for the combined data-set and for block and standardised data, although the level of significance was low ($0.04<P<0.05$). Therefore, there may be low levels of genetic variance in DM in the University's base population, that is only detected when the number of family replicates is relatively high and the environmental error component is minimised by data adjustment. Dry matter content appears to have a negative association with PyY and PP_H, although genetic correlation estimates for DM are not reliable due to the low genetic variance for this character (Windig, 1997).

The standard errors of the heritability estimates are too large to allow rigorous comparisons between estimates in different conditions or for adjusted and unadjusted values. However, the h^2_{hl} estimates were consistently lower than the h^2_l and h^2_h estimates for the characters that are affected by plant density (FFY, DFY and PyY). This is not the case for density-independent characters (PP_H, PyI/II and p/jc), an observation consistent with a genotype-density interaction for DFY and PyY but not for PP_H (Section 5.4).

Currently, commercial pyrethrum is established at a range of planting densities. Density varies both between and within sites, and can fall between 9 and 30 plants/m^2 (Greenhill, 1997). The existence of a genotype-density interaction for pyrethrins yield between densities of 4 and 16 plants/m^2 indicates that an interaction is also likely in the density range of Tasmanian commercial areas (9-30 plants/m^2). This finding has two main implications for selection trials. Firstly, heritability estimates generated from trials established at a single consistent planting density will overestimate the progress that can be made from selection. This is because the estimated phenotypic variance does not include the component due to the genotype-density interaction. When the selected material is intended for a growing environment of variable densities, the genetic parameter estimates need to be generated from trials established at a range of

densities that correspond to those typical of commercial areas.

Strictly, this also applies to conditions that existed prior to 1995 when plants were arranged in beds. The intra-plant spacing was 0.5m within beds and 1.0m between beds, so spacings varied for individuals in the edge and centre rows. Research in Kenya (Parlevliet, 1968) indicated that genotype-spacing interactions were negligible for a comparable range of variation (0.3m by 0.45-0.9m). However, this range of variation has not been investigated for Tasmanian conditions, and it is not clear whether the heritability estimates generated from the low density plots (consistent 0.5m spacing) can be applied to growing conditions prior to 1995.

In a number of crops, selection trials are established at lower densities than commercial areas, as genetic differentiation may be increased at low density due to a reduction in intra-plant competition (Cannell, 1984; Fasoulas, 1984). There appeared to be no evidence for any increase in genetic differentiation in the low density treatment used in the 1998 trial as differences between h^2_l and h^2_h were negligible for all characters, apart from DM.

When conditions in selection trials differ from conditions in the target environment, selection will be less effective when $h_s.r_g < h_t$, where h_s and h_t are the square-roots of the heritability in the selection and target environments and r_g the genetic correlation between them (Falconer, 1952). Heritability of PP_H does not appear to be affected by density and the r_g between low and high density is probably close to 1 (Section 5.4). Therefore, there would be no advantage (or disadvantage) to selection for PP_H in trials established at a density that is different to commercial areas. This is not the case for DFY or PyY. The r_g between densities is < 1 for these characters, so selection will be less efficient in densities that do not correspond to commercial conditions, as there is no evidence of increased genetic differentiation at the lower plant density used in this trial. Therefore, the second implication for these finding is that selection for yield will be most efficient when trials are established at densities comparable to

commercial areas.

Heritability for PyI/II was relatively high, at approximately 0.65, consistent with previous reports that the PyI/II is predominantly controlled by additive genetic affects and has high heritability (Parlevliet, 1974; Parlevliet, 1975). Although there was a negative association between PyI/II and PyY, the magnitude of the correlation was relatively low ($|<0.3|$). Again this is consistent with previous reports (Parlevliet, 1974). Breeding for PyI/II in Tasmanian crops was aimed at producing an intermediate progeny mean through disassortive mating (Potts and Menary, 1990). This appears to be a viable method for producing progeny with an intermediate PyI/II. In contrast, the negative correlation between p/jc and PP_H of -0.43 is potentially more important. It is higher in magnitude and selection would be for a high proportion of the more toxic pyrethrin I&II esters (increased p/jc). The negative genetic correlation between p/jc and PP_H indicates that selection for p/jc would have a negative effect on total pyrethrins. Therefore, selection for high p/jc would not be recommended, unless there was a clear economic advantage to high p/jc extracts.

Genetic correlations between PyY and the selection characters used by the University's breeding program were positive, at 0.7 for PP_{U6} and 0.25 for DFY_{est}. These values were a little lower than the corresponding values for PP_H and DFY of 0.8. The r_g for PP_{U6} and PP_H was also positive and high, at 0.9. Therefore, selection for any of these component or correlated characters would be expected to result in a correlated improvement in PyY.

Heritability for PP_{U6} was 0.28 which was lower than previous estimates reported for the early generations of the University's breeding program of 0.47 to 0.98 (Chapter 4). It is likely that there is a genotype-site interaction for this character, as the average genetic correlation between sites is substantially lower than 1 (Table 5.6.6). So previous estimates, which were calculated from data collected in a single environment, would be expected to over-estimate

heritability. The estimates made from the multi-site 1998 trial would be expected to produce more reliable predictions of response.

The estimate of 0.28 for heritability of PP_{U6} for the base population was greater than the estimate of 0.24 for the RS2 generation and 0.20 for the RS3 generation (Chapter 4). Although the estimates all have large standard errors, the trend of decreasing heritability of a selection character in successive generations is consistent with expectations (Falconer, 1989). Selection for PP_{U6} appears to have resulted in a correlated increase in PP_H (Section 5.6), so a comparable decline in heritability is also likely for this character. This would also be expected to result in lower heritability for PyY. Therefore, the heritability for PP_H and PyY for the current recurrent selection generation would be expected to be lower than the estimate for the base population (RS0 generation).

The significant heritability for PP_{U6} and relatively high r_g between this character and PyY and PP_H, suggests that selection for PP_{U6} should result in a correlated increase in pyrethrins yield and content. This is consistent with data presented in Section 5.5, which showed that selection for PP_{U6} had resulted in an increase in PP_H in the selected populations.

There was no evidence of any significant family variance for visually estimated flower yield. However, family effects were almost significant in the high density plots (P=0.07) and for the combined data set (P=0.053), and the regressions of visual yield on DFY were all statistically significant (Section 5.2.2). Visual yield was not evaluated at Kindred or in the high density plots at Omeo. It is possible that the lack of statistical significance is due to the substantially lower number of replicates for estimated yield compared to the other characters. Consequently, there are not sufficient data to allow a firm conclusion that visual yield selection is either an effective or ineffective method for pyrethrins yield improvement in Tasmanian growing conditions.

5.7 Evaluation of component or correlated character selection for yield improvement in pyrethrum. Part 2: Estimation of response to alternate selection methods

5.7.1 Introduction

The University of Tasmania's pyrethrum breeding program used a selection index of UV-assay of stage 6 flowers and visually estimated flower yield for pyrethrins yield improvement, during the period 1985 to 1995. Results presented in this thesis indicate that there has been an increase in the average UV-assay of stage 6 flowers in the recurrent selection population (Chapter 4) and that this increase is likely to have produced a correlated response in crop pyrethrins content (Section 5.5) and pyrethrins yields (Section 5.6). However, it has not yet been determined whether indirect selection is more efficient than direct selection for pyrethrins yield, in Tasmanian growing conditions, and whether direct selection has the potential disadvantage of a proportionally greater increase in flower yield than pyrethrins content, which has been observed for clonal selection in Kenya (Parlevliet, 1969).

Parents can be selected for breeding by individual phenotype (single-plant selection) or general combining ability, assessed by testing the progeny of potential parents (Bhat, 1995). Progeny testing increases the accuracy of selection (Falconer, 1989; Lerner, 1958), and for a fixed number of potential parents, will always be more effective than single-plant selection (Searle, 1978). However, progeny testing increases generation time (Falconer, 1989; Parlevliet, 1975) and the evaluation cost for each potential parent, as multiple offspring are assessed. Therefore, for equivalent assessment costs, fewer potential parents are evaluated and the selection differential is lower than for single-plant selection. The relative efficiency of progeny testing to single-plant selection depends on whether the increase in selection accuracy offsets the decreased selection intensity (Lerner,

1958). Generally, progeny testing is only more efficient than single-plant selection when the narrow-sense heritability of the selection character is low (Falconer, 1989; Lerner, 1958).

In Kenya, progeny testing produced substantially higher improvement in flower yields than mass selection (Parlevliet and Contant, 1970), with a greater response per unit time even though generation time was longer (Parlevliet, 1975). However, the relative efficiency, in terms of response per unit cost, was not reported (Parlevliet and Contant, 1970; Parlevliet, 1975). The relative efficiency of progeny and mass selection can be estimated from narrow-sense heritability (Lerner, 1958) and has not been examined for pyrethrum in Tasmania.

The aims of the data analysis presented in this section were to determine whether;

1) selection for the two indirect characters used by the breeding program is more efficient than direct selection for pyrethrins yield or index selection for components of yield (DFY, FFY, DM and PP_H),
2) single plant selection is more efficient than family selection (progeny testing),
3) direct selection for pyrethrins yield is likely to result in a proportionally higher increase in flower yields than pyrethrins content.

5.7.2 Methods

Alternate selection characters were compared by calculating the predicted response in pyrethrins yield from the population parameters generated from the 1998 seedling trial. Predicted response was estimated for the following selection methods;

- direct selection for pyrethrins yield (PyY),
- selection for the pyrethrins yield components (DM, FFY and PP_H),
- selection for the yield components typically used by pyrethrum breeders

(DFY and PP_H),

- selection for the characters used by the University's breeding program (DFY_{est} and PP_{U6}),
- indirect selection for each of the component and correlated characters.

For multi-character selection, characters were combined using a Smith-Hazel index as this is usually the most efficient method for selection of multiple characters (Hazel *et al*, 1994; Young, 1961).

Estimation of selection response

The response to direct selection for pyrethrins yield (R_y) was calculated as;

$$R_y = i\, h^2\, \sigma_P$$

where h^2 is heritability for PyY, i is the selection differential in standard deviation units (Appendix Table A, Falconer, 1989) and σ_P is the phenotypic standard deviation for pyrethrins yield. Following Falconer (1989), the correlated response (R_c) for DFY and PP_H was calculated as;

$$R_c = i\, h_c\, h_y\, r_g\, \sigma_c$$

Where h_c and h_y are the square-root of the heritability for the component character and pyrethrins yield, r_g is the genetic correlation between them and σ_c is the phenotypic standard deviation of the component character.

Similarly, the correlated response in PyY (R_y) to indirect selection for any other character (c) was calculated by;

$$R_y = i\, h_c\, h_y\, r_g\, \sigma_y$$

where σ_y is the phenotypic standard deviation for pyrethrins yield, h_y and h_c is the square-root of the heritabilities of PyY and c, and r_g is the genetic correlation between them.

Selection indices were constructed using the method specified by Thurling (1974) and Robinson (1951) for constructing indices of correlated characters that maximise the response in yield. Indices take the form of $b_1x_1 + b_2x_2 + ..+ b_nx_n$ where

there are n characters in the index with phenotypic values $x_1, x_2,..x_n$ and $b_1, b_2,..b_n$ are the optimum weight for each character. Optimum weights are estimated from solving simultaneous equations;

$$b_1p_{11} + b_2p_{12} + ..+ b_np_{1n} = g_{1y}$$
$$b_1p_{12} + b_2p_{22} + ..+ b_np_{1n} = g_{2y}$$
$$b_1p_{13} + b_2p_{23} + \ + b_np_{nn} = g_{ny}$$

where p_{ii} and p_{ij} are the phenotypic variance and covariance respectively, and g_{iy} is the genetic covariance between character i and pyrethrins yield. These formulae correspond to equations given by Falconer (1989; p295) for improvement of a character that is not included in the index.

The phenotypic variances and covariances were calculated by Falconer's (1989) equations as;

$$p_{ij} = r_p\sigma_i\ \sigma_j$$
$$g_{ij} = r_g h_i h_j \sigma_i\ \sigma_j$$

where σ_i and h_i are the standard deviation and square-root of the heritability of character i, and r_g and r_p are the genetic and phenotypic correlations between character i and j.

The expected response in pyrethrins yield (R) is

$$R = i\ \sqrt{(b_1g_{iy} + b_2g_{2y} + .. + b_ng_{ny})}$$

where i is the selection differential in standard units. This is the formula for response specified by Thurling (1974) and, again, can be derived from equations given by Falconer (1989; p297).

The correlated response of one of the component characters (R_1) was calculated using Falconer's (1989) method as;

$$R_1 = i\ [b_1g_{11} + b_1g_{12} + ..+ b_1g_{1n}\ ] / \sqrt{(b_1g_{iy} + b_2g_{2y} + .. + b_ng_{ny})}$$

where

$$g_{ii} = h^2{}_i\sigma^2{}_i$$

Selection responses were calculated for three indices. Index 1 was constructed from the component characters DFY and PP_H, Index 2 from FFY, DM

and PP_H and Index 3 from the correlated characters, DFY_{est} and PP_{U6}. Variances and covariances used for index construction are in Appendix 3.7.1 and the calculated values for *b* coefficients are Appendix 3.7.2.

Response was calculated firstly, for a selection intensity of 0.05, and secondly, for a selection intensity that took into account the variation in the cost of different characters (Utz *et al*, 1997). Table 5.7.1 shows relative costs per plant for each character and the number assessed (*N*) for a fixed cost. Selection intensity was calculated as *50/N*, based on selection of 50 plants.

Relative selection intensity (*RSE*) was calculated for each selection method as;

$$RSE = 100 \, . \, R_i / R_d$$

Where R_i is the predicted response to method *i* and R_d is the response to direct selection for pyrethrins yield.

Table 5.7.1. Relative costs per plant for assessment of characters in arbitrary units (CU), the number of plants that can be assessed for a fixed cost of 10 000 CU, the proportion selected (p) for N and a constant selection population size of 50, and the selection differential (i) in standard units (Appendix Table A, Falconer, 1989).

Character(s) assessed	Cost	N	p(%)	i
PyY	10.00	1000	5.00	2.063
PP_H +DFY	10.00	1000	5.00	2.063
PP_H	10.00	1000	5.00	2.063
DFY	3.66	2732	1.83	2.459
PP_{U6} (PC)	3.11	3215	1.56	2.502
PP_{U6} (CC)	4.33	2311	2.16	2.386
DFY_{est}	0.49	20 340	0.25	3.117
PP_{U6} (PC) + DFY_{est}	3.60	2778	1.80	2.459
PP_{U6} (CC) + DFY_{est}	4.82	2075	2.41	2.353

Relative selection efficiency of progeny testing

The relative efficiency of progeny testing to mass selection for pyrethrins yield ($RSE_{p/m}$) was calculated following Lerner (1958), by;

$$RSE_{p/m} = \frac{1 + (n-1)(0.25)}{\sqrt{n[1 + (n-1)t]}}$$

where n is the number of individuals in each family and t is the intraclass correlation which is equivalent to $0.25.h^2$, and h^2 is the narrow-sense heritability for pyrethrins yield. Heritability may be over-estimated for yield (Section 5.6), so $RSE_{p/m}$ has been estimated for a range of h^2 values.

Population parameters used to estimate predicted response

Two different types of growing conditions have been used in Tasmania; the system used prior to 1995 of beds with plants at a fixed spacing and the system currently used of direct drilled seedlings at relatively high and variable densities. The ranks of alternate selection methods can vary between different growing conditions (Annicchiarico and Pecetti, 1995; Gallais, 1984), so selection responses need to be individually evaluated for each production system.

Current commercial conditions consist of high and variable densities (HVD), typically 9 to 30 plants/m^2 (Greenhill, 1997). Genotype-density interactions for flower and pyrethrins yield are likely in this range (Section 5.6), so predicted response needs to be calculated from estimates of genetic parameters that take the interaction variance into account. Currently there are no estimates of genetic parameters applicable to the density range of 9 to 30 plants/m^2, so the estimates generated from the combined analysis of density treatments of 4 and 16 plants/m^2 in the 1998 trial were used, as these are the best that are currently available. Variance and standard deviations of characters in the high density plots (16 plants/m^2) were used, as these fall within the density range of current areas. Population parameters used to estimate response for

current commercial conditions (HVD) are listed on Table 5.7.2 and 5.7.3.

Table 5.7.2. Parameters used for calculation of response to selection in current commercial conditions (HVD). Parameters are heritability (h^2), genetic correlation with pyrethrins yield (r_g), phenotypic variance (σ^2_P) and phenotypic standard deviation (σ_P).

Character	h^2	r_g	Mean	σ^2_P	σ_P
PyY_b	0.28	-	0.37 g/plant	0.076	0.276
PP_{Hb}	0.34	0.80	1.74%	0.25	0.5
DM	0.11	-0.94		57	7.54
FFY	0.21	0.95		3001	54.7
DFY	0.19	0.80	22 g/plant	250	15.81
PP_{U6}	0.30	0.74		0.23	0.48
DFY_{est}	0.18	0.25		250	15.81

Table 5.7.3. Phenotypic correlations (r_p) between characters used for index construction.

Characters		r_p LD	HVD
PP_H	DFY	0.03	-0.19
PP_{U6}	Dry(e)	-0.07	-0.11
FFY	DM	-0.22	-0.20
FFY	PP_H	0.14	-0.07
DM	PP_H	-0.31	-0.42

Prior to 1995, commercial areas consisted of beds with a spacing of 0.5x0.5m for plants in the centre row of each bed and 0.5x1.0m for the edge rows. It is not clear whether genotype-density interactions occur in plants established at these two densities (Section 5.6). Therefore, selection response has been calculated for

two sets of genetic parameters for these conditions. The first (LD_{NI}) are listed in Tables 5.7.3 and 5.7.4 and assume that there is no interaction between genotype and density for any character. These population parameters were estimated from analysis of data collected in the low density plots of the 1998 trial. The second set of parameters (LD_I) assume that there is an interaction for flower and pyrethrins yields and are listed on Table 5.7.5. Estimates of variance and standard deviations were generated from analysis of low density plot data and genetic parameters from the combined density analysis.

Table 5.7.4. Parameters used for calculation of response to selection in commercial conditions prior to 1995, assuming no genotype-density interaction (LD_{NI}). Parameters are heritability (h^2), genetic correlation with pyrethrins yield (r_g), phenotypic variance (σ^2_P) and phenotypic standard deviation (σ_P).

Character	h^2	r_g	Mean	σ^2_P	σ_P
PyY_b	0.35	-	0.28 g/plant	0.229	0.478
PP_{Hb}	0.25	0.71	1.74%	0.241	0.491
DM	0.15	-0.14		61	7.81
FFY	0.29	1.08		8192	90.51
DFY	0.30	0.89	46.3 g/plant	579	24.06
PP_{U6}	0.30	0.96		0.228	0.477
DFY_{est}	0.24	0.52		579	24.06

Variance estimates for DFY_{est} were lower than for DFY in both HD and LD, an observation that appears to be counter-intuitive as the estimated values would be expected to be more variable due to the increased measurement error. This anomaly appears to result from a lower frequency of high scores (7 - 10) than would be expected from the DFY data, apparently due to an opperator bias against high scores. The variance estimate for DFY was used for DFY_{est} as this estimate more accurately reflects the variation in this character.

Table 5.7.5. Parameters used for calculation of response to selection in commercial conditions prior to 1995, assuming that there is a genotype-density interaction (LD_I). Parameters are heritability (h^2), genetic correlation with pyrethrins yield (r_g), phenotypic variance (σ^2_P) and phenotypic standard deviation (σ_P).

Character	h^2	r_g	Mean	σ^2_P	σ_P
PyY_b	0.28	-	0.28 g/plant	0.229	0.478
PP_{Hb}	0.34	0.81	1.74%	0.241	0.491
DM	0.11	-0.94		61	7.81
FFY	0.21	0.95		8192	90.51
DFY	0.19	0.80	46.3 g/plant	579	24.06
PP_{U6}	0.30	0.74		0.228	0.477
DFY(e)	0.18	0.25		579	24.06

5.7.3 Results

The predicted response and relative selection efficiency (RSE) for a fixed selection intensity are shown on Table 5.7.6, and for a fixed assessment cost on 5.7.7. The RSE of each selection character and index varied between different sets of genetic parameters. However, the indices of component characters were at least as efficient as direct selection, in all conditions, with a RSE of 100 to 125. The predicted response to the index of the selection characters used by the University (Index 3) was lower in HVD and LD_I, even when the lower cost of these characters was taken into account (Table 5.7.7). In contrast, Index 3 was the most efficient selection method in LD conditions if a negligible genotype-density is assumed for commercial spacings used prior to 1995.

Indirect selection for a single character was usually less efficient than direct or index selection, with the exception of the LD_{NI} set of genetic parameter estimates. In LD_{NI}, UV stage 6 assay was more efficient than direct selection (RSE

= 108) and dry flower yield was comparable to direct selection (RSE = 98).

Table 5.7.6. Expected response (R), in pyrethrins yield (g/plant), and relative selection efficiency (RSE), expressed as the percentage of the response to direct selection, for a fixed selection intensity (proportion selected = 0.05) and three sets of growing conditions (HVD, LD_I and LD_{NI}). Index 1 was constructed from PP_H and DFY, Index 2 from PP_H, FFY and DM and Index 3 from PP_{U6} and DFY_{est}.

Method	HVD		LD_I		LD_{NI}	
	R	RSE	R	RSE	R	RSE
Direct	0.16	100	0.26	100	0.35	100
Index 1	0.19	122	0.30	117	0.35	100
Index 2	0.20	125	0.29	106	0.39	112
PP_H	0.14	88	0.24	88	0.21	60
DFY	0.11	66	0.18	66	0.28	82
Index 3	0.13	81	0.22	81	0.35	102
PP_{U6}	0.12	77	0.21	77	0.31	89
DFY_{est}	0.03	20	0.06	20	0.15	43

Table 5.7.7. Expected response (R), in pyrethrins yield (g/plant), and relative selection efficiency (RSE), expressed as a percentage of the response to direct selection, for a selection intensity that reflects character evaluation costs.

Method	HVD		LD_I		LH_{NI}	
	R	RSE	R	RSE	R	RSE
Direct	0.16	100	0.26	100	0.35	100
Index 1	0.19	122	0.30	117	0.35	100
Index 2	0.20	125	0.29	106	0.39	112
PP_H	0.14	88	0.24	88	0.21	60
DFY	0.13	79	0.22	79	0.34	98
Index 3	0.15	92	0.27	96	0.42	121
PP_{U6}	0.14	89	0.26	93	0.37	108
DFY_{est}	0.05	30	0.08	30	0.22	65

The predicted effects of direct and index selection on the component characters (DFY and PP_H) are shown on Table 5.7.8. Predicted responses for PP_H

ranged from 13 to 20%. Responses for DFY were a little higher, ranging from 23 to 32%.

Table 5.7.8. Response in pyrethrins yield and correlated response of component characters to selection for yield by direct selection and by an index of the PP_H and DFY (Index 1). Response is expressed as percent of base population mean for each character.

Method		Response (%)		
		PyY	PP_H	DFY
Direct	HVD	40	14	24
Index	HVD	49	20	23
Direct	LD_{NI}	52	15	32
Index	LD_{NI}	47	13	26

Table 5.7.9. Relative selection efficiency ($RSE_{p/m}$) for higher PyY by progeny testing compared to mass selection for different values of narrow-sense heritability (h^2) and numbers of offspring for each parent assessed (n). Estimates assume a consistant trial size of 1000. The number of potential parents assessed (N) is 1000/n.

n	N	$RSE_{p/m}$			
		$h^2 = 0.26$	$h^2 = 0.2$	$h^2 = 0.15$	$h^2 = 0.10$
1*	1000	1.0	1.0	1.0	1.0
5	200	0.8	0.82	0.83	0.85
10	100	0.82	0.85	0.89	0.93
25	40	0.88	0.94	1.02	1.11
50	20	0.92	1.01	1.11	1.26

* n=1 corresponds to mass selection.

The relative efficiency of progeny testing and single-plant selection are shown on Table 5.7.9. For the heritability for PyY estimated from the 1998 trial (0.26 for HVD), progeny testing was less efficient than mass selection. Progeny testing would only be more efficient than mass selection if the heritability of PyY was less than 0.15.

5.7.4 Discussion

There are a number of limitations to the methods used to compare selection characters in this study. Firstly, errors in the estimates of population parameters will cause errors in the response predictions (Banziger and Laffite, 1997). Genetic parameters are difficult to estimate accurately (Falconer, 1989), and there are a number of additional factors that may decrease the reliability of the estimates generated from the 1998 trial:

- The highly variable incidence of seedling emergence caused family numbers to be unbalanced and many were smaller than the optimum size of approximately 20 (for true heritability of 0.2) (Harris, 1964; Robertson, 1959a).
- Heritability estimates generated from maternal half-sibs can be biased by maternal effects (Boettcher *et al.*, 1996; Husband and Gurney, 1998).
- The range of test densities was not the same as the density range of commercial areas in Tasmania, and the genotype-density interaction may be under-estimated.

The rankings of alternate selection methods are determined by the values of population parameters, so inaccurate parameter estimates will cause incorrect conclusions to be drawn.

Estimates in this chapter were made from the University's base population. Therefore, conclusions about the RSE of different selection methods only apply to this population, and cannot be generalised to other breeding programs. Similarly, genetic parameters change as the result of breeding (Falconer, 1989; Gallais, 1984), so rankings of alternate selection criteria will not be the same for the later generations of the University's breeding program. Selection methods need to be independently evaluated for future breeding at the University.

The magnitude of the standard error for the predicted response varies between selection methods. Estimates of genetic correlations have larger

standard errors than estimates of heritability made from the same data (Koots and Gibson, 1996; Lin, 1978; Reeve, 1955; Xie and Mosjdis, 1999), so response predictions calculated using genetic correlations have larger standard errors than predictions made solely from heritability estimates. Hence, response predictions are more reliable for direct selection than indirect or index selection. In addition, errors in the genetic parameters used to construct Smith-Hazel selection indices generally cause response to be over-estimated (Baker, 1986; Harris, 1964; Villanueva and Kennedy, 1993). Consequently, the use of Smith-Hazel selection indices are not recommended unless the predicted response is substantially greater than for alternate selection methods (Baker, 1986).

In pyrethrum, genetic correlations between some characters are inherently difficult to estimate with precision. This is because estimates of genetic correlations are least reliable when the genetic variance of the characters is low or the absolute magnitude of the true correlation is low (Robertson, 1959b; Windig, 1997). Genetic variance for DM is consistently low in pyrethrum (Section 5.6, Parlevliet and Contant, 1970), so the estimate of the genetic correlation between DM and any other character is likely to be inaccurate. Hence, the effectiveness of a selection index constructed using this parameter is likely to be reduced due to inaccurate parameter values, and there is a high probability that response will be overestimated. In addition, the absolute value of the correlation between DM and the target character (PyY) is small, so inclusion of this character will not contribute significantly to the overall effectiveness of the index, even when parameter estimates are precise (Baker, 1986). Consequently, it not desirable to include DM in a Smith-Hazel index, even when the predicted response is relatively high.

Similarly, it is difficult to make a precise estimate of the correlation between DFY and PP_H, as the magnitude of this correlation is likely to be small (Section 6.5 ,Bhat and Menary, 1986b; Parlevliet, 1974; Singh *et al*, 1987). Again, it is likely that the effectiveness of an index constructed using this parameter will be

reduced and response overestimated. A heritability-type of index is preferable to a Smith-Hazel when the magnitude of the genetic correlation is small (Geidel *et al*, 2000; Smith *et al*, 1981).

Direct selection for PyY leads to a slightly higher predicted response in flower yield (24-32%) than pyrethrins content (14%). However, the disparity was substantially less than the difference reported for clonal selection of Kenyan-grown pyrethrum (Parlevliet, 1969), and similar to the disparity predicted for component character selection (Table 5.7.8). Hence, direct selection does not appear to have any particular disadvantage compared to component character selection, in this respect, in Tasmanian-growing conditions.

Therefore, a heritability index of DFY and PP_H is probably the best selection method when;

- reliable heritability estimates are available, and
- the RSE for the selection index is greater than for direct selection.

Otherwise, there seems to be no compelling reason to prefer component character selection to direct selection for population improvement of Tasmanian-grown pyrethrum, and direct selection will be preferable as the effectiveness of selection is not reduced by inaccurate parameter values.

The results of the 1998 trial suggest that the University's selection characters (PP_{U6} and DFY_{est}) were the most efficient selection characters for low planting densities if there is no genotype-density interaction. A genotype-density interaction is unlikely between the two plant spacings used in Tasmanian growing areas prior to 1995 (0.5x1.0m and 0.5x0.5m), as interactions were absent in Kenyan-grown pyrethrum for a similar spacing range (Parlevliet, 1968). The predicted response for the University's selection characters was similar to direct selection (RSE=96) if an interaction was assumed. Therefore, the data indicate that the characters used by the University from 1985 to 1997 were probably the most efficient of the selection characters tested, for improvement of the base population for commercial conditions prior to 1995. At worst, the University's

characters were probably only slightly less efficient than direct or component character selection. However, in all three cases, the index of DFY_{est} and PP_{U6} was only slightly more efficient than selection for PP_{U6} alone, and there does not appear to be any particular advantage to the inclusion of visual yield as a selection character, irrespective of its low assessment cost.

Although the University's selection characters were the most efficient for the LD_{NI} set of genetic parameters, indices of the yield components (Index 1 and 2) were more efficient in HVD and LD_I. Hence, the University's selection characters are sometimes less efficient, and the RSE of this method needs to be assessed for each individual population and set of growing conditions. In practice, assessment of two sets of breeding characters prior to selection will increase the overall cost of the breeding program, and this increased cost is unlikely to be offset by any subsequent economic advantage of the University's selection characters. Therefore, yield evaluation by harvesting and weighing flowers and HPLC-assay is probably to be recommended, unless there is some particular reason to use of the University's selection characters. For example, small amounts of material are sampled for UV-stage 6 assays, so seed can be harvested in the same season as yield assessments. Hence, the use of UV-stage 6 assay would allow a reduction in generation time when seed is harvested from selection trials.

Progeny testing is only more efficient than single-plant selection, in terms of response per unit cost, if the heritability of the selection character is <0.15 and the number of parents evaluated is small (<100) (Table 5.7.9). This suggests that progeny testing is not appropriate for population improvement. Population improvement requires a large population size, and single-plant selection is more efficient when relatively large numbers of potential parents are assessed (Table 5.7.9). However, progeny testing might be more efficient for selecting parents for seedling varieties. A relatively small number of parents (eg 10) can be selected for variety selection, so a reasonable selection intensity can be maintained from a

small evaluation population.

Variety selection by progeny-testing is only more efficient when the heritability of the selection character is <0.15. Estimates of heritability for the economically important characters (PyY, PP_H, DFY, FFY) were generally 0.2 or greater in the 1998 trial. Therefore, in the base population, single-plant selection was probably the most efficient method for selection of parents for both population improvement and varieties. However, in the current RS4-5 generation of the breeding population, the heritability of pyrethrins content is likely to be lower than for the base population (Chapter 4, Section 5.6), and selection for pyrethrins content would also be expected to reduce the genetic variance, and heritability, of pyrethrins yield. Further, the heritability of pyrethrins and flower yield may have been overestimated in the 1998 trial, because plants were not tested over the same density range as commercial areas. Therefore, the heritability of the economically valuable characters may be 0.15 or less in the current generation. If this is the case, it would be more efficient to select parents for varieties by progeny mean. For example, in a seedling trial of 50 half-sib families, with 20 individuals per family, efficiency could be maximised by:

- Selection of parents for population improvement from seedlings in the trial by individual plant data (single-plant selection). This would allow a selection intensity of 5%, if parent population size of 50 was maintained.
- Selection of parents of varieties from the 50 parent clones by the mean of each clone's half-sib progeny. Five clones could be selected at a selection intensity of 10%.

The relative efficiency of selection of parents of varieties by progeny testing depends on the heritability of the main selection character, and this needs to be independently assessed for each generation (Falconer, 1989; Goddard, 2001; Lerner, 1958; Mackay and Gibson, 1993).

6 Assessment of seedlings from the RS4 and RS5 generations

6.1.1 Introduction

The seedling trials established by the University's pyrethrum breeding program, from 1986 to 1995, had two main purposes; they were used for single plant selection for the population improvement program, and for family selection for the development of new seedling varieties. Trials established from 1988 consisted predominantly of full-sib families produced by hand-crossing. The target variety was also a full-sib family (biclonal cross) and family selection was on the basis of family plot data. Consequently, the data collected for each trial consisted of a few hundred plot assessments and several hundred individual plant assessments.

Visual yield assessments for both plots and single plants are relatively inexpensive. In contrast, pyrethrins assays are more costly, so the collection of plot assay data increases the cost of the assessment program above the level required for single plant assessments. In addition, the plot assessment methods used by the University were relatively imprecise, consisting of a single assay sample for each family and visually assessed flower yield. Evaluations of families selected after 1992 (Section 5.5), indicated that family selection methods may be little better than random selection.

Although biclonal crosses are commonly used as varieties in pyrethrum, polycrosses between several clones are also common (Dalgety, 1975; Kroll, 1958; Parlevliet, 1975). An evaluation of single and polycross varieties led Kenyan pyrethrum breeders to recommend polycrosses in preference to biclonal crosses (Parlevliet and Contant, 1970), and varieties based on several genotypes are more typical in outcrossed species than hybrid crosses (Borojevic, 1990). Alternatively, some breeding programs that use population improvement techniques release the entire improved population as a variety (Vogel and Pedersen, 1993).

The release of the entire breeding population as a variety or a polycross of parents chosen by individual phenotype would be less expensive than the practice of full-sib family selection for a number of reasons. The expense of full-sib family assessments would be removed. Seed production costs could also be reduced, as the production of large numbers of full-sib families requires hand-pollination. Seed could be produced by a field-based polycross if full-sib families were not required. This would eliminate the cost of hand-pollination and reduce plant maintenance costs. In addition, more seed is produced by each mother plant in the field (unpublished data), as only a very small proportion of the flowers are pollinated by hand-crossing programs. Alternatively, individual phenotype data from seedling trials could be used to generate progeny means to select parents for varieties from the clones that are crossed to produce each seedling trial (Section 5.6).

A potential disadvantage to a variety produced from a large number of clones is that this type of variety may be more genetically variable than a biclonal cross. Although homogeneity is not considered to be important for most breeding characters in pyrethrum (Glynne Jones, 1968), homogeneous or synchronous flowering may be an important character for mechanically-harvested pyrethrum (Bhat, 1995). Yields may be reduced when flowering is asynchronous, due of the presence of buds and fully-mature flowers (Bhat, 1995) that have low pyrethrins compared to flowers at maturity stage 4 to 7 (Head, 1966a). There are two components to asynchronous flowering in pyrethrum crops; asynchronous flowering patterns displayed by individual plants, and variation in maturity-time among individuals. Potentially, there may be more heterogeneity in maturity-time among the progeny of a polycross than of a biclonal cross, due to the increased diversity of parent genotypes.

Selections from RS3 and RS4 seedling trials were planted in a replicated clone area in 1998, which was used for seed production. Seed collected from this area was tested in a direct-drilled seedling trial that was established in 2000. The

polycross seed from the seed production area effectively corresponds to a mixed RS4-RS5 recurrent selection generation. The main aim of the data analysis presented in this chapter is to assess the RS4-5 population as a potential variety and determine whether synthetics generated from large numbers of clones have any clear commercial disadvantage in comparison to biclonal crosses.

The pyrethrins contents of the clones in the seed production area were assessed by HPLC-assay of stage 6 flowers in the 1998 harvest season. The second aim of this trial was to evaluate the effectiveness of this character for the selection of parents for population improvement or synthetics intended for commercial use.

6.1.2 Material and methods

Seed production area

The seed orchard was established in autumn 1998 and consisted of 90 clones. The orchard was replicated at two sites (Horticultural Research Centre, Hobart and University Farm, Cambridge), with two randomised, incomplete blocks at each site. Clones in the orchard were:

- 82 clones selected from the seedling trials at Cawood, Omeo and Bellingers (Table 3.1). These clones belong to the RS3 and RS4 generations and were selected by the usual method of a selection index of UV-stage 6 assay and visually-estimated flower yield.
- The eight clones that are the parents of the six biclonal crosses in current commercial use (Appendix 3.1).

Seed was collected in February 1999 and 2000.

Pyrethrins evaluations of seed orchard clones (1998 harvest)

Seed orchard clones were assessed for pyrethrins content in December 1998.

Fifteen stage 6 flowers were harvested from each replicate and assessed by the HPLC-method (Section 3.1.4).

Seedling evaluation trial

Seed from individual mother clones was collected separately and then pooled to form two seedlots;

SL1 - sample pooled from all 90 mother clones

SL2 - sample pooled from the 20 mother clones with the highest mean pyrethrins content (HPLC-assay of stage 6 flowers, as assessed in December, 1998). None of the eight parents of commercial varieties were included in this group.

SL1 and SL2 were tested with two of the biclonal crosses in current commercial use; *#220*, which is the cross between *CIG 3* and *CIG 11*, and *#864* (*CIG 3* x clone # *922*). Seed for these crosses was provided by BRA and consisted of achenes harvested from commercial seed production areas which had been mechanically cleaned and gravity sorted. Such commercial seed consists of 70-90% fertile achenes.

The trial consisted of a total 16 plots arranged in four randomised complete blocks. Seeds were sown into raised beds in rows spaced at 0.2 m. Beds were approximately 1.1 m width, with each bed forming a single plot of 45 x 1.1 m.

Seed was initially sown at the University farm in April 2000. However, there was negligible plant establishment and the trial was re-sown in November 2000. Plant establishment after the second sowing was more successful, although highly variable, both within and between plots. Plant counts made in March 2001 ranged from 0 to 33 plants/m^2, and the majority of the trial had plant densities lower than the commercial target density of 16 to 36 plants/m^2.

Yield evaluation (2001 harvest)

Pyrethrins yields were assessed in December 2001. The initial intention was to mechanically harvest the entire trial using a mechanical flower stripper. However, there were two main problems with this harvesting method. Firstly, there was considerable lodging of flower stems into the alleys between the beds so that it was not possible for the harvester to recover all flower material. Secondly, the plant density for the majority of the trial was considerably lower than for commercial areas. Data presented in Chapter 5 indicates that flower and pyrethrins yield should be assessed at planting densities that are comparable with commercial areas. In contrast, pyrethrins content appears to independent of plant density.

Two sampling methods were chosen to deal with these problems.

1. *Yield evaluation:*

Yield evaluations were restricted to sub-plots that were at commercial densities at the time density was assessed in March. This sampling method does not violate the requirement for random sampling if the variable establishment density is assumed to be due to random variation in operation of the seed drill during sowing. If this is the case, then the areas of the trial that were at commercial density can be considered to be random samples of plant genotypes and microsite environments. None of the areas of SL1 were at commercial densities, so yield assessments were made for SL2 and one of the commercial crosses (#864). The other commercial cross was not sampled in order to minimise harvesting costs.

Yield was assessed by hand-harvesting to ensure that all flowers were recovered. A sub-plot of $6m^2$ was harvested from each plot for SL2 and cross #864. Flowers were harvested by cutting the stems at ground level and then stripping flowers from the stems by bench-combs. Fresh yield was determined by weighing flowers. Counts of flower maturity stages were determined from one 100 g subsample. Flowers were classified into maturity stages using

definitions of Potts and Menary (1987) (Table 2.3). Flower maturity index (FMI) was calculated as;

FMI = 100.(mean flower maturity stage)

and an index of variability of flower maturity (SF) as;

SF = 100.(standard deviation of flower maturity stage).

Two 200g subsamples were dried for 48 h at 50°C for determination of percentage dry matter and pyrethrins content. Pyrethrins were assayed using the HPLC method (Section 3.3). The mean of the two samples was used to estimate the pyrethrins content and percentage dry matter for each plot, and plot values for pyrethrins yield were calculated from these and total weight of fresh flowers.

The coefficient of variation was calculated for each character from the square-root of the error mean square (s) and the trial mean, and represents the variability among plots.

The number of samples (n) required to detect a 'true' difference between means was estimated using the iterative method specified by Sokal and Rolf (1995), by;

$$n \geq 2(\sigma/\delta)^2 \{t_{\alpha[v]} + t_{2(1-P)[v]}\}^2$$

where n is the number of samples required, σ the standard deviation, δ the smallest true difference that can be detected, v the degrees of freedom of the sample standard deviation with a groups and n replications per group ($v=a[n-1]$), α the significance level, P the probability the difference will be significant, $t_{\alpha[v]}$ and $t_{2(1-P)[v]}$ are the values from a two-tailed t-table with v degrees of freedom for probabilities of α and $2(1-P)$, respectively. The measured value of the difference between variety means was used for δ, and s was used as an estimate of σ (Sokal and Rohlf, 1995).

2. *Pyrethrins content evaluation.*

Pyrethrins content appears to be density independent, so samples were

taken from entire plots irrespective of plant density. Two replicate plots (45x1.1m) were harvested for each variety using a boronia header which uses a combing mechanism to stip flowers from the plants. Five 200g subsamples were taken of the flowers collected from each plot, dried for 48 h at 50°C and ground using a hammer mill. A sub-sample of each was assessed for pyrethrins content by the HPLC method (Section 3.3).

Data were analysed by the GLM procedure of SAS.

6.1.3 Results

Pyrethrins content of seed orchard clones (1998 harvest)

Mean pyrethrins contents for stage 6 flowers for the clones assessed in the seed orchard are listed on Table 6.1 and ANOVA tables are in Appendix 3.8. The population mean for the 82 RS3 and RS4 selections (2.69%) was higher than the mean for the eight parents of the current varieties (2.33%). This difference was statistically significant ($P<0.05$).

The mean pyrethrins for the 20 clones that were used to form SL2 was 3.14%, representing a selection differential of 0.47 between these clones and the entire population of clones in the orchard (2.67%).

Evaluation trial (2001 harvest)

Population means for assessments made by hand-harvesting 6m^2 plots of SL2 and #864 are shown in Table 6.2 and ANOVA tables are in Appendix 3.8. Pyrethrins content was approximately 20% higher for seedlings in the SL2 population than for #864 ($P<0.05$). The average flower and pyrethrins yields were greater for the SL2 population. However, these differences were not statistically significant ($P>0.05$), although the difference in the average pyrethrins

yield was almost statistically significant (P<0.1). There was no evidence of an increase in asynchrony of flowering in the SL2 population compared to the biclonal cross, as the mean SF for the populations were not significantly different (P>0.05). The mean PyI/II was 1.0 for the SL2 population, a value that corresponds to the ideal for this character.

Table 6.1. Average pyrethrins content (HPLC-assay of stage 6 flowers) for three populations of clones within the seed orchard. Values represent the mean of two sites.

Group	Pyrethrins content (%)	n
Trial mean	2.67	90
Parents of the current commercial seedling varieties (A)	2.33	8
RS3 and RS4 generation clones (B)	2.69	82
difference (B-A)	0.36*	
Mother clones for SL2 (C)	3.14	20
difference (C-trial mean)	0.47	

* indicates difference is statistically significant (P<0.05)

The measured difference between the mean pyrethrins yield for *SL2* and *#864* was 43 kg/ha, which corresponds to a 50% higher yield for *SL2*. If this value corresponds to the true value of the difference between the seedlots, then the minimum number of replicate plots required to detect this difference would be 7 ($\alpha = 0.05$, $P = 0.80$, $\sigma = 24$), assuming that two varieties were tested in the

trial (a=2). This is substantially greater than the four replicates used in this trial, so even quite large differences between seedlot yields would not be expected to be statistically significant. Six replicates would be required for a trial of four varieties ($\alpha = 0.05$, $P = 0.80$, $\sigma = 24$).

Table 6.2. Coefficient of variation (CV) and seedlot means and standard errors for pyrethrins yields, flower maturity index (FMI), variability of flowering index (SF) and PyI/II, based on hand-harvested plots of $6m^2$.

Character	CV(%)	*#864*	SL2	
Dry flower yield (kg/ha)	24	3460 ± 1080	4470 ± 970	ns
Pyrethrins content (%)	5	2.4 ± 0.1	2.8 ± 0.2	*
Pyrethrins yield (kg/ha)	24	82 ± 27	125 ± 32	@
FMI	3	560 ± 20	586 ± 17	ns
SF	8	98 ± 10	95 ± 9	ns
Pyrethrins I/II	10	1.1 ± 0.1	1.0 ± 0.1	ns
Density (plants/m^2)		13 ± 5	11 ± 1	ns

*, @ and ns indicate that differences between variety means are statistically significant ($P<0.05$), not quite statistically significant ($0.05<P<0.1$) or not significant ($P>0.1$), respectively.

The coefficient of variation was relatively high for pyrethrins and flower yield, at 24% (Table 6.2). The variability among plots was lower for pyrethrins content (5%), FMI, SF and PyI/II.

Average pyrethrins content and PyI/II as assessed by sub-sampling of mechanically-harvested areas of $40m^2$ are shown on Table 6.3. PyI/II ranged from 1.0 to 1.2; values that were not significantly different ($P>0.05$) and are well within the range specified for commercial extracts (Anon., 1992; Maciver, 1995).

Table 6.3. Means and standard errors for pyrethrins content and PyI/II, based on mechanically-harvested areas of 40 m^2.

Variety	Pyrethrins content (%)	PyI/II
SL2	2.7 ± 0.2 [a]	1.0 ± 0.1
SL1	2.6 ± 0.1 [b]	1.2 ± 0.2
#864	2.5 ± 0.1 [b]	1.1 ± 0.1
#220	2.2 ± 0.1 [c]	1.2 ± 0.1

Means with different letters are different according to Duncan's multiple comparison test ($P<0.05$)

Estimates of mean pyrethrins content for SL2 and #864 are not identical for the two assessment methods (Table 6.2 and 6.3). However, the estimates for the two assessment methods are within 0.1 unit of one another for each seedlot, variation that is consistent with the standard deviation of each mean (0.1-0.2).

The average pyrethrins content for SL2 was higher than for *#864* for both assessment methods ($P<0.05$). Pyrethrins content for the progeny of the selected clones (SL2) was a little higher than for the entire RS5 (SL1) population ($P<0.05$), and the cross between *CIG 3* and *CIG 11* (*#220*) had substantially lower pyrethrins content than any of the other populations ($P<0.05$).

6.1.4 Discussion

Assessment of pyrethrins of stage 6 flowers provides a non-destructive method of pyrethrins assessment which allows seed to be harvested in the same flowering season as pyrethrins evaluation. In contrast, yield assessment by stripping and weighing all the flowers at the commercial harvest stage (FMI 4.5 to 6.5) removes the achenes before seeds are mature (FMI 8), and seed cannot be

harvested until 12 months later in the following flowering season.

Selection by HPLC-stage 6 evaluations resulted in a statistically significant increase in pyrethrins content in the SL progeny of the 20 selected clones (Table 6.3). The measured selection differential of 0.47 between the HPLC-stage 6 assay of the selected clones and the population average (Table 6.1) produced a small increase of 0.1 in SL2 population over the entire SL1 population. The SL2 progeny were open-pollinated, with pollen originating from all 90 clones in the seed orchard. Consequently, the gain in pyrethrins content would be approximately twice the value for the SL2 population if the 20 selected clones were crossed in isolation (Nguyen and Sleper, 1983). Therefore, the technique of selecting clones from HPLC-stage 6 flower assays of replicated clonal material is a viable method for improving the crop pyrethrins content. A cost-benefit analysis could be used to determine whether the gain justifies the additional evaluation costs.

There was little evidence of any increased variability in the polycross population compared to biclonal crosses, as the SF values were similar for SL2 and cross #864 (Table 6.2). This indicates that the variation among flower maturity stage did not differ between the multiple-parent SL2 and two-parent cross. This is consistent with data collected for Kenyan-grown pyrethrum, which shows that the variation in characters such as flower size, pyrethrins content and PyI/II is similar for biclonal and polycrosses (Parlevliet, 1974). Therefore, there is no evidence that polycrosses have any intrinsic disadvantage over biclonal crosses in terms of homogeneity of flowering (Table 6.2) or in any other character (Parlevliet, 1974).

In this trial, the SL1 and SL2 populations had a higher mean pyrethrins content than the two commercial varieties, for pyrethrins content assessed by HPLC-assay of samples of the entire crop. This indicates that the selection for UV-stage 6 assay and visually-estimated yield has resulted in improved crop pyrethrins content, as these were the selection criteria for the 82 RS3 and RS4

clones in the seed orchard. The SL1 and SL2 were progeny of these 82 clones and the parents of six current commercial varieties, which were selections from the RS1 to RS3 generations (Table 3.2).

Flower and pyrethrins yields for the SL2 population were higher than for the commercial variety (*#864*). Although differences were not statistically significant, the number of replicates used in this trial was too low for significant differences to be detected. Therefore, the data are consistent with the proposition that selection for UV-stage 6 assay will produce an increase in crop pyrethrins yields.

The seed for all four populations was not produced at the same location, so the environment experienced during seed-set was not the same for each population. Environmental conditions experienced during seed development can influence phenotype (Biere, 1991; Mazer and Schick, 1991) and effects due to the maternal environment can persist for extended periods (Campbell, 1997; Husband and Gurney, 1998). Therefore, genetic and maternal-environment effects are confounded in this trial. It would be preferable to produce seed for control and test varieties at the same location(s) and season(s).

Variety trials clearly need to consist of more than four replicates when plots of $6m^2$ are harvested. The probability of detecting differences among varieties would be increased by using a minimum of seven plots of $6m^2$ to per variety. Alternatively, the variance among plots decreases with increasing plot size (Chaves and Filho, 1992), so that fewer replicates will be required if the size of the plots is increased (Sokal and Rohlf, 1987). In either case, the area harvested for each variety is increased and harvesting costs increase proportionally.

Typically, yield selection is most efficient when material is divided among a large number of small plots (Chaves and Filho, 1992; Loo-Dinkins *et al*, 1990; Rattunde *et al.*, 1991), as this strategy maximises the number of micro-sites sampled. However, in pyrethrum, assay costs are proportional to the number of samples. Therefore, for a fixed trial area, the advantage of increasing the number

of replicates is counterbalanced by increased assay costs. In this trial, the differences between variety means for pyrethrins content were statistically significant and the overall coefficient of variation was relatively low, at 5%, compared to 24% for pyrethrins and flower yield (Table 6.2). Therefore, for a fixed total cost, it would preferable to increase the total area harvested, as this will increase the probability that differences in flower yields will be detected. It would be less desirable to increase the number of assay samples. Alternatively, efficiency could be maximised by sowing a large number of replicate plots (for example 8 plots of $6m^2$), and assesing pyrethrins on composite samples pooled from two or more replicates.

Duplicate assay samples were taken from each plot in this trial. It may be more efficient to take a single sub-sample for assay but increase the number of replicate plots. This would increase the number of micro-sites sampled for each variety for the same total assay cost.

Theoretically, the best full-sib families within a population should be superior to the population mean. However, it is not necessarily true that selection of families is more efficient than selection of single plants as parents. The selection response for single plant selection is the product of selection intensity, individual heritability and the variance among individual plants (Falconer, 1989). The improvement from full-sib family selection is the product of selection intensity, family heritability and variance among full-sib families (White and Hodge, 1989). Family heritability is higher than individual heritability (Falconer, 1989). However, the variance among families will be lower than the variance among individual plants (Lerner, 1958). Consequently, family selection does not necessarily result in a higher selection response than single plant selection (Lerner, 1958). Hence, a polycross of single plants selected from a seedling population might be superior to the full-sib families selected from the same population. Further, family heritability is greater than individual heritability when family means are calculated from individual plant values

(Falconer, 1989; Hodge and White, 1992; Lerner, 1958). This is not necessarily the case when family selection is made by the plot assessment methods used by the University. The data from the 2001 harvest indicate that family plots need to be quite large for comparisons between families to be meaningful.

Even when the response to mass selection is lower than for family selection, mass selection might be more efficient due to the additional costs imposed by full-sib family selection. These addition costs are incurred through the need for controlled pollination to generate full-sibs and the added cost of family-plot evaluations. However, the latter can be removed by generation of family means from individual plant data.

One advantage of assessing full-sib families it that family means can be combined with individual plant values in a selection index. The use of family means results in a higher selection response than selection on individual values alone (Cotterill and Dean, 1990). However, the use of family information usually increases inbreeding as selected individuals are more likely to belong to a small number of superior families. As a result, heritability is reduced more rapidly than for mass selection (Jeyaruban *et al.*, 1995) when there are repeated selection cycles. Consequently, the use of family information produces a higher response in the short-term at the expense of a lower response in the long-term (Verrier *et al.*, 1993; Wei *et al.*, 1996). As the University's breeding population is in its sixth recurrent selection cycle, selection methods that minimise inbreeding are preferable. Further, there is probably little advantage to using the relatively imprecise estimates of full-sib family means generated from plot assessments. Nevertheless, combined family indices can utilise half-sib family means and family means can be generated from individual plant data. Therefore, combined index selection could continue to be used if full-sib family assessments were discontinued, although selection based on individual phenotype may be preferable.

Therefore, while selection of full-sib families increases the cost of the

breeding program, this strategy does not necessarily provide higher gains than those provided solely by population improvement. Consequently, selection of the entire recurrent selection generation for commercial use may be more efficient than selection of biclonal crosses from each generation. Increased genetic diversity can lead to greater stability of yield under varying environmental conditions (Allard and Bradshaw, 1964), so varieties derived from a mixture of parent genotypes may be preferable to biclonal crosses.

Although the data are restricted to a single location and season, they indicate that the SL1 population is potentially a commercially viable variety. A polycross between the 20 mother clones that produced the SL2 population is also a potentially viable variety, that would be expected to have slightly higher pyrethrins content than the population generated from all 90 clones in the 1998 seed orchard. Both synthetics can be recommended for continued testing with a view to commercial development.

7 General discussion

7.1 Summary and discussion of results

7.1.1 Methods for yield improvement of Tasmanian-grown pyrethrum

The results of this study indicate that the recurrent selection methods used by the University of Tasmania were an effective means for improving pyrethrins yields of mechanically harvested crops. In particular, the UV-stage 6 assay value appears to be an effective character for single plant selection, as there is evidence of the following;

- a genetic gain in this character in the recurrent selection population,
- a high, positive genetic correlation between UV-stage 6 assay and pyrethrins yield, of 0.75-0.96,
- a close correspondence between the improvement in crop pyrethrins content (PP_H) and the UV-stage 6 of selected varieties relative to the base population,
- the RS5 generation had a higher mean pyrethrins content than two of the current commercial varieties.

In contrast, there was no clear evidence that estimated flower yield is an effective selection character. Although the regressions of flower yield on the visually estimated values were statistically significant, heritability for estimated yield was not and there is no evidence of a genetic gain in the breeding population.

Estimates of predicted response were used to compare the efficiency of different selection characters for the improvement of pyrethrins yield. These indicated that the index of the University's selection characters was the most efficient of the methods tested for the improvement of the University's base population, for the planting densities used prior to 1995. However, this is not always the case, and the optimum selection method needs to be individually assessed for each population and type of growing conditions (Annicchiarico and

Pecetti, 1995). Consequently, any potential economic advantage of indirect selection is likely to be off-set by the initial cost of evaluating the selection method. Selection by harvesting and weighing flowers and HPLC-assay is probably preferable unless there are particular reasons to choose the University's characters.

The indirect selection characters may have practical advantages in some circumstances. Specifically, stage 6 flower assay and visually estimated yield are relatively non-destructive methods for pyrethrins evaluation which allow seed to be collected in the same harvest season. Typically, pyrethrum is sown in November and plants assessed in the second December, 13 months later. If trials are planted in fully randomised single-plant plots, seed could be collected in January-February of the first flowering season, and planted in November of the same year. This would result in a two-year breeding cycle. In contrast, yield evaluation by stripping and weighing flowers at the harvest FMI (3.5-6.5) excludes seed collection which takes place at an FMI of 8. This means that open-pollinated seed cannot be harvested until the second flowering season. Alternatively, selected plants can be lifted in autumn after the first harvest, split and replanted in a seed orchard. In both cases there would be a three year breeding cycle.

The genetic gain from collecting open-pollinated seed from selections would only be half the gain that would result from crossing the selections in an isolated seed orchard, or in the glasshouse (Nguyen and Sleper, 1983). However, establishment of a seed orchard (or glasshouse crossing) imposes an additional cost that has to be considered when calculating selection efficiency. An individual cost-benefit analysis needs to be conducted for a range of options before the optimum breeding method can be chosen.

In contrast, variety selection methods used after 1992 do not appear to be likely to produce varieties that are superior to those currently in commercial use. Varieties selected after 1992 had higher pyrethrins content but lower flower and

pyrethrins yields than the current commercial varieties. There are three possible interpretations of this observation:

1. There has been a genetic gain in the pyrethrins content of the breeding population and a decrease in flower yield. Hence, selections from the later generations have higher pyrethrins content and lower flower yields than selections from early generations.
2. There has been a genetic gain in pyrethrins content in the breeding population and a negligible change in flower yield. The lower mean flower yield of the later selections is due to random sampling variation.
3. There has been a genetic gain in pyrethrins content in the breeding population and a small or negligible increase in flower yield. Methods used to select the current varieties were more precise than the methods used to select varieties after 1992. Hence, the current varieties have significantly higher flower yields than the mean of the population from which they were selected. The new selections have flower yields similar to the mean for their generation.

If there has been a decrease in flower yield, it could be attributed to a number of causes; there may be a negative association between flower yield and one of the selection characters; random drift could result in a decline in flower yield; or reduced yield could be an expression of reduced fitness resulting from inbreeding.

The selection index method used by the University favoured UV-stage 6 assay, due to the higher economic weight and estimated heritability for this character. Therefore, there was low selection pressure for flower yield and genetic drift is possible for this character. In contrast, a negative association between UV-stage 6 assay and flower yield seems unlikely, as there does not appear to be a consistent negative genetic correlation between flower yield and pyrethrins content (Bhat and Menary, 1986a; Parlevliet, 1974; Singh *et al*, 1987), and the estimate for the genetic correlation between these characters was positive

for the University's base population. Reduced yield due to inbreeding is also possible but seems unlikely as pyrethrins content increased, and it would be expected that reduced fitness would also have a negative effect on this character.

However, while a decline in flower yields is theoretically possible, the RS5 generation had a higher mean flower yield than one of the current commercial varieties in the 2000 trial. Although this difference was not statistically significant and only applies to one site and season, it is not consistent with the proposition that flower yields declined in the recurrent selection population. Therefore, the third interpretation is the most likely. The varieties selected after 1992 were chosen by full-sib family selection using plot assessment methods (Potts and Menary, 1989). The current commercial varieties were also selected on the basis of availability of parent material (Potts and Menary, 1993a), a selection criterion that would have favoured crosses with clonal parents that were in contemporary clonal variety trials. Hence, there would have been 'automatic' selection for crosses between clones that had performed well in site-replicated clone trials. These clones would be expected to be genetically superior to clones that were culled from the trials or not selected for further evaluation. Hence, the low flower yields of the varieties selected after 1992 are more likely to be indicative of less accurate selection methods than a decline in the mean for the breeding population.

Data collected in the 2000 seedling trial indicated that differences between seedling varieties can only be detected when trial plots are large, even when yields are assessed by harvesting and weighing flowers. The plot assessment methods used from 1985 to 1997 (visual yield, UV-stage 6 assay, maximum plot size of $2m^2$) are quite imprecise and are possibly little better than random selection. Hence, selection of full-sib families from a population of some 200 families would require more accurate family evaluation methods, including increased plot size and number of family replicates (8×6 m^2 plots), more than one assay per family, by HPLC, and yield evaluation by harvesting and

weighing. Screening some 200 families using such methods would be extremely expensive, so it is likely to be more effiecient to select parents for potential varieties from single plant data collected in the seedling trials. Parents for varieties could be selected from seedling trials by either;

A. individual phenotype, or

B. progeny means for the parent clones intercrossed for the seedling trial.

Option A would be the most efficient if heritability for the main selection character is high (>0.15). Selected parents could be;

1. crossed to form a single polycross variety, or
2. crossed in a full diallel to produce full-sib families for variety testing. Two stage selection of full-sib families means that the number of families at the family selection stage is relatively small so large plot sizes, replicated trial sites and precise evaluation methods are economically feasible.

The potential advantage of option 2 is that the best full-sib families will be superior to the polycross of all selected parents when there are significant levels of specific combining effects in the control of the economically important characters. However, there is an additional cost in producing and testing full-sib families, and this needs to be offset by the additional gain for this method to be the more efficient. Specific combining effects have not yet been evaluated for the Tasmanian breeding population or growing conditions, so it is currently not possible to compare the relative efficiencies of these two strategies.

The results of the 1998 trial indicated that there is a genotype-density interaction between establishment densities of 4 and 16 plants/m^2 for pyrethrins and flower yield. There was no evidence of any increase in heritability for these characters at the lower plant density. Commercial areas are established in a density range of 9 to 30 plants/m^2, and it is reasonable to assume that a genotype-density interaction also occurs among commercial planting densities.

The finding that there is a genotype-density interaction for flower yield has a number of implications for selection for yield improvement. Firstly, the

commercial environment varies in planting density, so heritability estimates will be inflated when they are made from trials established at a single density, and predicted response will also be over-estimated. Secondly, selection is likely to be most efficient when breeding trials are established at densities that correspond to commercial areas. Thirdly, variety testing will be more efficient with more than one test density (Dickerson, 1962).

The results of the 1998 trial also confirmed previous findings that pyrethrins content is independent of plant density (Fulton, 1998; Ngugi and Ikahu, 1990b; Rao and Singh, 1982) and there is no evidence of a genotype-density interaction for this character. Therefore, if selection is solely aimed at improvement of pyrethrins content, the choice of planting density is only determined by practical considerations. For example, a low density may be preferable when selections are intended to be propagated by splitting, as this will maximise plant size. Alternatively, a high density will reduce the trial area and, hence, maintenance costs and may enhance cross-pollination which would be advantageous when open-pollinated seed is collected from selection trials.

7.1.2 Selection characters other than pyrethrins yield

The pyrethrum varieties currently grown commercially in Tasmania are unable to produce an economic yield within 12 months of sowing. Low flower yields in young (< 12 month old) plants can be attributed to a low incidence of flowering and low yields of any flowering plants (Fulton, 1998).

The low incidence of flowering in young plants appears to be due to the long juvenile period of pyrethrum seedlings (Brown, 1992). Data collected from the 1998 trial indicates that the length of the period of obligate vegetative growth is controlled, in part, by additive genes. Consequently, the incidence of flowering in 10-month old seedlings could be increased through recurrent selection. A high incidence of flowering is a prerequisite for the development of seedling varieties that can produce a commercial first-year yield. However,

economically viable first-year yields also depend on an increase in flower yield per plant, so this character also needs to be assessed. Similarly, second (and third) year yields also need to be assessed to ensure that selection for early flowering does not lead to an annual plant type.

An increase in the ratio of pyrethrins to (jasmolins + cinerins) (p/jc) might increase the value of the extract as the pyrethrins appear to be more toxic than the other esters. However, p/jc may not a desirable selection character for pyrethrum as there appears to be negative genetic association with pyrethrins content. If there is a clear economic advantage to high p/jc, then it is recommended that the character is reassessed in order to confirm whether the negative genetic correlation observed in the 1998 trial is a consistent characteristic of pyrethrum. If it is, then reliable economic weights for pyrethrins yield and p/jc should be obtained. The economic gains predicted from selection by a Smith-Hazel index could be used to determine whether an index that includes p/jc will increase the economic worth of the crop more rapidly than selection for yield (or its components). The ratio p/jc should not be used as a selection character unless it can be demonstrated that the increase in value resulting from higher p/jc will offset any reduction in yield improvement.

Data collected from the 1998 trial indicate that the ratio PyI/II has high heritability, consistent with previous observations for this character (Parlevliet, 1974; Parlevliet, 1975). The average PyI/II for the RS4-5 population was approximately 1.0. This value corresponds to the ideal for PyI/II. The genetic correlation between PyI/II and pyrethrins content was negative but low ($<|0.3|$), indicating that the average PyI/II for plants selected from the RS4-5 generation should also be approximately 1.0. This suggests that PyI/II need not be a primary selection character in the University's breeding program. Individuals with unusually high or low PyI/II need not be excluded as parents for polycrosses or population improvement unless the mean for all selections deviates substantially from 1.0.

7.1.3 Population size

Analysis of progeny data (Chapter 4) indicated that only a small number of base population clones have contributed progeny to the current breeding population. As few as 19 clones may form the actual base for the current breeding population, and at most, the effective base population consists of 29 clones.

Only two of the clones in the Glaxo population have contributed progeny to the later generations. Results from the 1998 trial show that the Glaxo population has higher flower yields than the SP-population, and it is likely that the Glaxo population has higher flower yields than the current generation of the breeding population. Therefore, the addition of progeny from the 23 extant Glaxo clones to the current breeding population could be advantageous. The Yugoslavian population is another potential source of genetic material that could be added to the current population. However, pyrethrins contents were substantially lower than for the SP- and Glaxo-clones, so addition of Yugoslavian material may be less desirable.

7.2 Limitations of current study

There are a number of limitations to the conclusions that can be drawn from this study.

1. The methods used to assess yield.

Flower and pyrethrins yields were assessed by the conventional methods used in Tasmanian pyrethrum research, which consist of stripping flowers and drying them at 50°C. This is a reasonably accurate method for assessing total pyrethrins at harvest-time, as virtually all the flower material present at harvest is collected and pyrethrins degradation is minimised by drying at 50°C (Githinji,

1973; Ngugi and Ikahu, 1990a). However, the aim of breeding is to increase the yield recovered by commercial harvesting rather than total yield *per se* (Hallauer and Miranda, 1981), and it can be argued that the assessment method used in this study does not directly measure commercially harvestable yields.

In commercial harvesting, the flower stems are cut, windrowed and dried in the field, and the achenes are recovered by mechanical threshing. There is potential for a loss of pyrethrins during field-drying and of achenes during harvesting. There is likely to be both phenotypic and genetic variation in the proportion of pyrethrins lost during drying, as the stability of pyrethrins in harvested plant material is affected by the temperature and length of the drying time (Githinji, 1973; Ngugi and Ikahu, 1990a), flower maturity stage at harvest (Boevink, 1991; Gnadinger, 1936), and microbial activity (Picone, 1999). Genetic variation in the post-harvest stability of pyrethrins has been reported (Bhat and Menary, 1984c), as well as interactions between drying temperature and genotype (Ngugi and Ikahu, 1990a).

Similarly, there may be variation in the proportion of achenes recovered from windrows. Stage 8 flowers are comparatively brittle and achenes are more easily lost than from other flower stages (Tattersfield, 1931). There may be genetic variation in flowering synchronicity (Bhat, 1995) which could affect the proportion of stage 8 flowers in a crop and, consequently, the proportions of achenes recovered.

Pyrethrins yield assessment methods that mimic commercial harvesting have practical advantages over the methods currently favoured in Tasmanian pyrethrum research. The number of plants that can be assessed in a season are limited by the time required to cut and strip flowers during the two week harvest window of crop maturity. If cut stems were dried in the field, the achenes would be removed 1-2 weeks after the date of harvest-maturity and the two activities would not take place at the same time. Further, if a small-scale mechanical thresher could be used to recover the achenes, labour costs could be reduced and

the correspondence between experimental and commercial harvesting would be greater.

Selection for total yield is a form of indirect selection for the target character of commercially recovered yield. It may be a less efficient selection character than recovered yield if there is genetic variation in the factors that determine the proportion of the total crop that is recovered by mechanical-harvesting (Subandi *et al*, 1975). Total yield may not accurately reflect the economic value of different varieties, if there is substantial genetic variation in a yield component, such as pyrethrins stability in crop windrows.

2. *The restriction of data to the first-year harvest.*

As a perennial crop, pyrethrum typically has a life-span of four seasons. Assessments of Kenyan pyrethrum found that there was a high correlation (>0.9) between first-year and three-year accumulated yields (Parlevliet, 1969). However, this result does not necessarily apply to Tasmanian growing conditions and further studies are required to assess the efficiency of selection for first-year yields for the improvement of accumulated crop yields.

3. *Test population*

Genetic parameters were estimated for the base population rather than for the latest recurrent selection generation (RS5). This allowed an evaluation of the methods that were used to improve the base population. However, the conclusions drawn cannot be applied to the current generation as the relative selection efficiency of different characters can change as the breeding program progresses. This is because the relative selection efficiency of alternate methods is determined by genetic parameters (Searle, 1965; Searle, 1978) which change as the result of selection. Genetic correlations caused by linkage will break down after a few breeding cycles (Gallais, 1984) and genetic variance, and consequently

heritability, typically decreases with repeated selection cycles (Falconer, 1989) for a number of reasons. Genetic variance decreases due to linkage disequilibrium (Bulmer, 1971; Mackay and Gibson, 1993), inbreeding and through fixation of alleles (Falconer, 1989). While it is possible to predict the rate of the reduction of genetic variance due to linkage disequilibrium (Bulmer, 1971) and inbreeding (Lerner, 1958), the overall rate of reduction is often difficult to predict (Gardener, 1978; Goddard, 2001; Hospital and Chevalet, 1993; Keightley and Hill, 1992). In general, predictions of response only apply to one or two selection cycles (Goddard, 2001; Mackay and Gibson, 1993). Therefore, genetic parameters estimated from the base population should not be used to estimate selection responses for the RS4-5 generation.

4. The use of predicted response to compare selection methods

Predicted response is a useful tool for the evaluation of selection methods. However, there are limitations to this method. Response estimates have a large standard error due to the difficulty of obtaining precise estimates of genetic parameters. Currently, there is no method for testing whether differences between response estimates are statistically significant. Another limitation stems from the fact that selection response measures the change in the population mean (Falconer, 1989). In animal breeding, a positive selection response corresponds to an increase in the average value of the herd or flock that can be expected to directly increase returns (Yonezawa *et al*, 1999). This is also true for plant breeding when the entire improved population is released as a commercial variety. However, when varieties are selected from the improved population, population improvement is part of the variety production method rather than the primary selection goal (Yonezawa *et al*, 1999). Typically, the extrema of the population increases as well as the mean, so the best genotypes from the improved population will be superior to the best of the earlier generations (Hallauer and Miranda, 1981). The outcome of variety selection will be

determined by the proportion of genotypes in the improved population that are superior to varieties in current commercial use (Yonezawa *et al*, 1999), and the accuracy of the variety selection methods (Dekkers, 1992; Falconer, 1989). Consequently, the plant breeder can only infer whether breeding goals will met from the estimated response, when the entire breeding population is released as a variety.

5. Establishment density of 1998 trial

The 1998 seedling trial was established at plant densities of 4 and 16 plants/m^2. In contrast, commercial areas fall within the range of 9 to 36 plants/m^2 (Greenhill, 1997). Genotype-density interactions may not be consistent across the range 4 to 36 plants/m^2, so the heritability estimates produced from this trial do not necessary apply to commercial conditions. Reliable estimates of heritability for pyrethrins yields require trials established at densities comparable to the range found in commercial crops.

7.3 Recommendations for future work

7.3.1 General recommendations

Initial testing indicates that the RS5 generation may be a viable commercial variety. Similarly, the polycross of 20 clones selected from the seed orchard on the basis of HPLC- stage 6 assay may also be a viable variety and potentially has higher mean pyrethrins content than the entire RS5 generation. Continued testing is recommended for both these populations. Direct comparisons of the mean yields of the 20-clone polycross and the RS5 generation should be used to assess the efficiency of selection method used to choose these clones.

Continued improvement in the breeding population depends on there being genetic variance for selection characters in the current (RS4-5) generation.

Therefore, it is recommended that genetic parameter estimates are obtained for the current population. Similarly, it still needs to be established that pyrethrins yield is heritable when plants are established at higher density than the maximum density of 16 plants/m^2 that was used in the 1998 trial.

It would be also desirable to evaluate characters associated with seed-set and seed quality. Although this study did not examine these characters directly, germination counts suggest that there is considerable phenotypic variation in the incidence of seed-set. This could affect seed yield which would affect the economic viability of a seedling variety. Sub-optimal germination temperature can cause death and secondary dormancy in pyrethrum seeds (Fulton and Clark, 1997). Variation in the susceptibility of seeds to stress during germination could influence establishment of seedlings in the field. This could also be a useful selection character if there is a significant genetic component to any phenotypic variation.

Establishment following direct drilling appears to be quite variable. An investigation of the variation in the establishment of agricultural pyrethrum and its causes would be a usefull topic for future research. Potentially, establishment could be a component of yield per unit area and improved establishment might be an additional breeding aim.

7.3.2 Trial designs for obtaining reliable estimates of genetic parameters in pyrethrum

Data collected in the 1998 trial indicates both a genotype-location and genotype-density interaction for pyrethrins yields, so reliable estimates of genetic parameters in pyrethrum require families to be replicated at more than one site and density.

Breeding trials planted from 1985 to 1995 were set out in family plots containing 3 to 15 plants. Usually, only two or three plants were assessed from

each plot. There is always some natural attrition of plants in the field, so the advantage of this type of 'redundant' design is that plots usually contain at least one surviving plant at harvest time. However, this type of design is relatively inefficient, as substantially more plants are maintained than assessed. There can also be edge effects for flower yields in some seasons (Groom and Menary, 1994), a problem which is difficult to address when trial consists of many small plots, as buffer rows for some 400 plots would substantially increase the size of the trial.

The 1998 trial consisted of fully-randomised plots containing one individual from each family, and a buffer row of edge plants that were not assessed. This type of design has the advantage of being relatively efficient, as only a small proportion of plants were not assessed. Edge effects were eliminated by the use of buffer rows and there was no family-environment covariance caused by grouping sibs in common family plots (Magnussen, 1993). However, there was the disadvantage that families were not represented in all plots, so the design was quite unbalanced. A balanced trial design is preferable (Shaw, 1987), and could be achieved by randomly locating a number of family replicates within each block (Loo-Dinkins *et al*, 1990). Replication of families within plots should increase the chance of there being at least one representative for each family in each block at harvest time.

In addition to natural attrition, unbalanced family sizes were caused by highly unbalanced seedling numbers among families. Although this appears to be caused, in part, by poor germination resulting from unusual conditions, seed-set is highly variable in pyrethrum. The only way to ensure a reasonable minimum family size is to produce more seed for each family, so that families with very low incidence of seedling emergence contain sufficient seedlings to allow full replication. In hand-crossing programs this would probably require pollination of some 10 flowers per family.

Field crossing has a number of advantages over hand-crossing; specifically reduced cost and increased seed yields. In addition, seed production and quality

parameters could also be assessed for mother clones. For example, the incidence of seed-set varies widely in hand-crossed flowers and a similar level of variation in field conditions might affect the commercial viability of some biclonal crosses. This character needs to be assessed in seed raised in the field, as seed-set in hand-crossed flowers does not necessarily reflect seed-set in the field.

Estimates of genetic parameters for pyrethrins and flower yield need to be obtained from trials established at densities that correspond to those of commercial areas. In practical terms, individual plants may be difficult to distinguish at higher densities (>16 plants/m^2), and selection may have to be on the basis of the family means assessed from family plots. Therefore, while single plant selection and randomising families within plots may be efficient, family selection and grouping sibs into family plots may be necessary for selection at densities exceeding 16 plants/m^2. Potentially, a combined selection index of individual plant phenotype at 16 plants/m^2 and family mean at a higher density could maximise progress for the full range of commercial density conditions.

7.4 Conclusions

- Data from the 1998 seedling trial indicate that the goals of the breeding program were met during the period of 1985 to 1991, as the six commercial varieties produced had superior pyrethrins yields to the initial (base) population. However, the main goal of the breeding program after 1992, of producing direct-drilled seedling varieties with superior yield to the initial selections, was not met.
- Methods used for population improvement from 1988 to 1997 appear to have been both effective and efficient. In contrast, there appear to be weaknesses in the variety selection methods.
- Although the use of the indirect selection characters (UV stage 6 assay and estimated flower yield) appears to have been more effective than direct

selection, this will not always be the case. Consequently, the indirect characters are not generally recommended unless they have some practical advantage, for example, when open-pollinated seed is collected from plants in the selection trials.

- Data collected from the University's base population indicated that there was significant genetic variation in pyrethrins yield, flower yield and pyrethrins content. However, these conclusions do not necessarily apply to the current recurrent selection generation and genetic parameters need to be re-estimated for this population.
- Plant density affects selection for flower yield but not pyrethrins content.
- Selection for a high incidence of flowering in young (<12 month old) plants could reduce the average length of the period of obligate vegetative growth in Tasmanian pyrethrum and is a potentially beneficial selection character.
- Selection for PyI/II is probably not necessary for the University's pyrethrum population. Selection for pyrethrins to (jasmolins + cinerins) is probably not desirable.

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Appendix 1. Re-analysis of previously published data.

1.1 Comparison of UV and HPLC assay methods

Bhat and Menary presented UV and HPLC-assays of the flowers of eight clones replicated at three sites (Table 1, Bhat and Menary, 1986). Data collection methods were not specified but assays were likely to be of flowers harvested when approximately 75% of the disc florets were open (Bhat *et al*, 1985).

Table 1.1.1 Pyrethrins contents for seven clonal varieties replicated at three sites. Data is presented for pyrethrins contents as assessed by HPLC (PP_H) and UV-assay methods (PP_U) and the difference between the two assays (PP_U-PP_H). Data adapted from (Bhat and Menary, 1986 Table 4, p.14).

clone	site	PP_H	PP_U	difference
CIG 3	Ouse	2.22	2.03	-0.19
O5	Ouse	1.48	1.67	0.19
O7	Ouse	1.17	1.4	0.23
B3	Ouse	1.65	1.84	0.19
CIG 11	Ouse	1.7	2	0.3
R3	Ouse	1.7	1.73	0.03
CIG 9	Ouse	1.65	1.76	0.11
S19	Ouse	1.31	1.54	0.23
CIG 3	Bushy Park	2.27	2.49	0.22
O5	Bushy Park	1.42	1.98	0.56
O7	Bushy Park	1.54	1.88	0.34
B3	Bushy Park	1.64	2.22	0.58
CIG 11	Bushy Park	1.7	2.45	0.75
R3	Bushy Park	2.14	2.45	0.31
CIG 9	Bushy Park	2.29	2.32	0.03
S19	Bushy Park	1.94	2.15	0.21
CIG 3	HRC	2.34	2.57	0.23
O5	HRC	1.25	1.68	0.43
O7	HRC	1.12	1.93	0.81
B3	HRC	1.31	2.52	1.21
CIG 11	HRC	1.82	2.36	0.54
R3	HRC	1.63	2.08	0.45
CIG 9	HRC	1.82	2.71	0.89
S19	HRC	1.25	2.07	0.82
MEAN		1.68	2.08	0.39

In general, the UV-assay (PP_U) was higher than the HPLC-assay (PP_H) for each sample, with an average pyrethrins content for the data set of 2.1% using the UV-method and 1.7% for the HPLC-method (Table 1.1.1). The correlation between the two assay-methods was 0.6 (Table 1.1.2), a value that was statistically different from zero ($P<0.01$). There was a negative correlation between pyrethrins content as assessed by HPLC and the difference between the two assays (Table 1.1.2).

Table 1.1.2. Product-moment correlation coefficient (r) and $P(H_o: |r| = 0)$ for pyrethrins content determined by the UV and the HPLC methods and the difference between the UV-assay and the HPLC-assay (*difference*).

Traits	r	P
HPLC pyrethrins content - UV & pyrethrins content	0.60	0.002
HPLC pyrethrins content & *difference*	-0.48	0.02

Data was structured as a two-way ANOVA without replication. Hence it was possible to test for site and clone effects but not the interaction between them. Differences between clones and sites were significant for both assay methods (Table 1.1.3). Analysis of the difference between the HPLC and UV-assays for each sample showed that site effects were statistically significant ($P<0.01$). Differences between clones were not significant ($F = 1.97$, $P = 0.13$). However, the possibility of a genetic component of variation in this character should not be dismissed, as the F-value was reasonably high.

Table 1.1.3 ANOVA tables for analysis of data in Table 1.
HPLC

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
clones	2.23	7.00	0.318	9.50	0.0002
sites	0.42	2.00	0.211	6.30	0.011
Error	0.47	14.00	0.033		
Total	3.12	23.00			

UV

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
clones	1.19	7	0.171	6.34	0.0017
sites	1.31	2	0.653	24.25	0.0001
Error	0.38	14	0.027		
Total	2.88	23			

diff

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
clone	0.63	7	0.089	1.97	0.13
site	1.15	2	0.577	12.74	0.0007
Error	0.63	14	0.045		
Total	2.41	23			

1.2 Calculation of realised heritability from Kashmir breeding population

Realised heritability (h^2) can be calculated as the ratio of the selection response (R) to the selection differential (S):

$h^2 = R/S$ (Falconer, 1989).

Pandita and Bhat (1984) assessed the base population (BP) in Srinagar, Kashmir, and reported an average pyrethrins content of 1.01%. The average of the selected plants was not specified but the selections had a minimum pyrethrins content of 1.5%, so heritability has been calculated for two possible values for the selections, 1.5% and 1.75%, giving S values of 0.5 and 0.75 respectively. The progeny of the selections (SP) and the BP were assessed at three sites in Tasmania. The average pyrethrins content of the BP was 1.3% in these trials and the response to selection (difference between the SP and BP) was 0.13 per cent pyrethrins, an increase of 10%. This gives us a response of 0.13. If we assume that would also have been a selection response of 10% if the SP and BP had been assessed together in Srinagar, and that the BP and the same mean pyrethrins content (1.01) as in the original trial, then the response would have been 0.10 per cent pyrethrins (10% or 1.01). Heritabilities have been calculated using both response values. These values of R and S give heritability estimates of 0.13 to 0.26 (Table 1.2.1).

Table 1.2.1 Heritability estimates for two values of S and R.

	heritability	
	S	
R	0.5	0.75
0.13	0.26	0.17
0.10	0.20	0.13

Appendix 2. List of parents used for seedling trials planted from 1986 to 1994.

SP refers to the selected population imported from Kashmir, Glaxo to clones that originated from the Glaxo collection, RS1(HRC) are RS1 clones that were evaluted at the HRC, RS2(HRC) are RS2 clones that were evaluated at the HRC. In all other cases population refers to the seedling trial that the clone was originally selected from, trial codes are listed on Table 4.2.1.

		number of	number of families in each trial									
clone #	population	trials	CM	CP-K	E	N-M	FF	G	C-D	J	B	O
1	SP	1		4								
2	SP	1		1								
3	SP	1		1								
9	SP	1		5								
10	SP	1		1								
13 (CIG 3)	SP	4		12		17	13	7				
15	SP	1		4								
16	SP	1		1								
17	SP	1		1								
19	SP	1		1								
20	SP	1		1								
21	SP	1		1								
22	SP	1		1								
23	SP	1		1								
24 (CIG 11)	SP	3		4			4	1				
26	SP	1		1								
29	RS2(HRC)	1		1								
30	RS2(HRC)	1		1								
31	RS2(HRC)	1		1								
33	RS2(HRC)	1		1								
36	SP	1		1								
48	RS2(HRC)	1		1								
53	RS1(HRC)	1				1						
60	RS1(HRC)	2		5		7						
62	RS1(HRC)	4		2		1	11	1				
67	RS1(HRC)	1		1								
69	RS1(HRC)	2	1	2								
71	RS1(HRC)	5	1	2	25		4	1				
72	RS1(HRC)	2	1	1								
73	RS1(HRC)	4		1	22		3	2				
74	RS1(HRC)	4	1	1	19	8						
75	RS1(HRC)	4	1	1	19	5						
76	RS1(HRC)	2	1	1								
78	RS1(HRC)	3	1	2		10						
79	RS1(HRC)	1		1								
80	RS1(HRC)	2	1	1								
81	RS1(HRC)	4	1	1	23	13						
82	RS1(HRC)	2	1	2								
83	RS1(HRC)	3		2			24	3				
84	RS1(HRC)	5	1	2	18	1	2					
85	RS1(HRC)	2	1	1								
86	RS1(HRC)	3		2			22	4				
89	RS1(HRC)	4	1	2			15	10				
95	RS1(HRC)	1				1						
98	Glaxo	1		4								
99	Glaxo	1		1								
100	Glaxo	1		1								

		number of			number of families in each trial							
clone #	population	trials	CM	CP-K	E	N-M	FF	G	C-D	J	B	O
103	Glaxo	1		3								
104	Glaxo	1		1								
105	Glaxo	1		1								
107	Glaxo	1		1								
109	Glaxo	3		1		11	8					
110	Glaxo	1		2								
118	Glaxo	1		1								
122	Glaxo	1		1								
126	Glaxo	1		1								
128	Glaxo	1		1								
129	Glaxo	1		1								
135	Glaxo	1		1								
200	RS2(HRC)	1		1								
201	RS2(HRC)	1		1								
202	RS2(HRC)	1		1								
203	RS2(HRC)	1		1								
204	RS2(HRC)	1		1								
205	RS2(HRC)	1		1								
206	RS2(HRC)	1		1								
207	RS2(HRC)	1		1								
208	RS2(HRC)	1		1								
209	RS2(HRC)	1		1								
210	RS2(HRC)	1		1								
211	RS2(HRC)	1		1								
212	RS2(HRC)	1		1								
213	RS2(HRC)	1		1								
214	RS2(HRC)	1		1								
215	RS2(HRC)	1		1								
216	RS2(HRC)	1		1								
218	RS2(HRC)	1		1								
219	RS2(HRC)	1		1								
602	RS2(HRC)	1						9				
722	RS2(HRC)	1			1							
901	RS1(HRC)	1		1								
902	RS1(HRC)	1		1								
903	RS1(HRC)	1		1								
904	RS1(HRC)	2		1		19						
905	RS1(HRC)	2	1	1								
906	RS1(HRC)	4	1	1		8	4					
907	RS1(HRC)	2	1	1								
908	RS1(HRC)	2	1	1								
909	RS1(HRC)	4	1	1	18		3					
910	RS1(HRC)	1		1								
911	RS1(HRC)	1		1								
912	RS1(HRC)	1		1								
913	RS1(HRC)	1		1								
914	RS1(HRC)	2	1	1								
915	RS1(HRC)	1		1								
916	RS1(HRC)	1		1								
917	RS1(HRC)	1		1								
918	RS1(HRC)	2	1	1								
919	RS1(HRC)	1		1								
920	RS1(HRC)	1		1								
921	RS1(HRC)	2	2	1								

		number of			number of families in each trial							
clone #	population	trials	CM	CP-K	E	N-M	FF	G	C-D	J	B	O
922	RS1(HRC)	5	2	1	19		6	3				
923	RS1(HRC)	1		1								
924	RS1(HRC)	1		1								
925	RS1(HRC)	2	2	1								
926	RS1(HRC)	1		1								
928	RS1(HRC)	1		1								
930	RS1(HRC)	1		2								
931	RS1(HRC)	1		1								
932	RS1(HRC)	1		1								
933	RS1(HRC)	1		1								
934	RS1(HRC)	2	1	1								
935	RS1(HRC)	2	1	1								
936	RS1(HRC)	2	1	1								
937	RS1(HRC)	1		2								
938	RS1(HRC)	1		1								
939	RS1(HRC)	1		1								
940	RS1(HRC)	1		1								
941	RS1(HRC)	1		1								
942	RS1(HRC)	1		1								
943	RS1(HRC)	2	1	1								
945	RS1(HRC)	1		1								
946	RS1(HRC)	1		1								
947	RS1(HRC)	1		1								
948	RS1(HRC)	1		1								
949	RS1(HRC)	1		1								
950	RS1(HRC)	1		1								
951	RS1(HRC)	5	1	1	17	7	4					
952	RS1(HRC)	1		2								
953	RS1(HRC)	3	1	1		19						
954	RS1(HRC)	2		1	16							
955	RS1(HRC)	1		1								
956	RS1(HRC)	2	1	1								
957	RS1(HRC)	4		2		8	6	6				
958	RS1(HRC)	1		1								
959	RS1(HRC)	1		1								
960	RS1(HRC)	1	1									
961	RS1(HRC)	1		1								
962	RS1(HRC)	1		1								
963	RS1(HRC)	1		1								
964	RS1(HRC)	1		1								
965	RS1(HRC)	2		1	20							
966	RS1(HRC)	2		1	19							
967	RS1(HRC)	1		1								
968	RS1(HRC)	1		1								
969	RS1(HRC)	2		1		23						
970	RS1(HRC)	2		1	25							
971	RS1(HRC)	1		1								
972	RS1(HRC)	1		1								
973	RS1(HRC)	1		1								
974	RS1(HRC)	1		1								
975	RS1(HRC)	1		1								
976	RS1(HRC)	2		1		16						
977	RS1(HRC)	1		1								
978	RS1(HRC)	3		1			22	3				

		number of			number of families in each trial							
clone #	population	trials	CM	CP-K	E	N-M	FF	G	C-D	J	B	O
983	RS1(HRC)	1				2						
1008	CM	1						15				
2703	CM	1						11				
3011	RS2(HRC)	2					31	2				
3101	RS2(HRC)	1					30					
3302	RS2(HRC)	2					21	5				
5333	K	2						2	19			
6411	K	1						14				
8412	K	1									3	
9233	RS2(HRC)	1										4
9251	K	1							10			
11151	K	1							1			
11242	K	1						3				
11342	K	1							3			
12232	K	2						4	11			
12351	K	1						13				
15111	K	2								1	1	
16142	K	2								3	1	
16213	K	1							5			
16313	K	1							9			
16322	K	1							6			
20002	K	1						12				
20004	K	2					28	1				
20006	K	2					30	1				
20011	K	2					17	2				
20704	K	1						11				
134211	K	2						4	3			
134221	K	1							9			
134312	K	1						3				
134432	K	1							2			
139121	K	1						3				
139142	K	1							13			
139323	K	2						12	6			
174121	K	2						20	9			
181432	K	1							4			
182332	K	1						10				
220251	K	2						1	3			
223322	K	1						5				
224241	K	2						14	17			
230333	K	1							4			
304212	E	1						4				
307412	E	2								15	1	
309351	E	1							11			
309421	E	1							9			
317131	E	2								9	3	
321343	E	1							17			
323131	E	3						3		4	2	
333452	E	2								3	3	
351253	E	1								3		
365111	E	1						8				
374252	E	1							8			
374442	E	1									3	
387152	E	1						6				
387331	E	1						3				

		number of			number of families in each trial							
clone #	population	trials	CM	CP-K	E	N-M	FF	G	C-D	J	B	O
388431	E	2						12	9			
392112	E	1						10				
393123	E	1								3		
401243	E	1							9			
401422	E	1							7			
401423	E	2						3	3			
401432	E	2								5	1	
402411	E	2								5	1	
404452	E	2						2	9			
406242	E	2								6	6	
407113	E	1							5			
407143	E	1							2			
407423	E	3							3	6	1	
415142	E	2						8	8			
415351	E	1							7			
419142	E	2								5	7	
420153	E	2						15	11			
420333	E	2								4	6	
422243	E	2								10	5	
422252	E	1						2				
426232	E	1						1				
505021	CP	1							4			
505811	CP	1							9			
506112	CP	1							14			
506221	CP	1							9			
506231	CP	1							11			
511813	CP	2								6	2	
514732	CP	2								13	3	
515132	CP	2								6	4	
515711	CP	1							5			
516233	CP	1							17			
516423	CP	1							10			
520411	CP	2								5	1	
520611	CP	2								5	3	
603421	N/M	1										7
605222	N/M	1							4			
609223	N/M	1									6	
609343	N/M	1									9	
615141	N/M	1									5	
615342	N/M	1							4			
615441	N/M	1							9			
617241	N/M	2								5	1	
617643	N/M	1										3
617721	N/M	1									7	
619312	N/M	1								2		
619331	N/M	1									9	
619621	N/M	2								3	1	
625251	N/M	1									12	
629341	N/M	2								2	2	
630811	N/M	1								4		
634721	N/M	2								11	2	
634823	N/M	1									5	
642211	N/M	1									4	
645731	N/M	1									12	

		number of			number of families in each trial							
clone #	population	trials	CM	CP-K	E	N-M	FF	G	C-D	J	B	O
646221	N/M	2								10	4	
648143	N/M	2								8	2	
648522	N/M	2								15	6	
648613	N/M	2								8	6	
649111	N/M	2								4	2	
651411	N/M	1							10			
652243	N/M	2								6	4	
653351	N/M	2								6	1	
654233	N/M	1							12			
655313	N/M	1									13	
655451	N/M	2								5	1	
657131	N/M	1							2			
658233	N/M	2								7	3	
659212	N/M	2								4	4	
660233	N/M	2								4	4	
662121	N/M	1							1			
662212	N/M	2								7	7	
662241	N/M	2								5	1	
663211	N/M	2								2	3	
664123	N/M	1									6	
678441	N/M	2								6	1	
682112	N/M	2								5	1	
682421	N/M	1							18			
682433	N/M	1									7	
688313	N/M	1									4	
688331	N/M	2								5	4	
688442	N/M	1									5	
690132	N/M	1							21			
710122	FF	1									5	
714113	FF	1									8	
718223	FF	1										1
734121	FF	1								4		
745112	FF	2								7	1	
745213	FF	1									4	
745332	FF	1									6	
753121	FF	1										2
753131	FF	1										7
764123	FF	1									3	
765221	FF	1								3		
780112	FF	2								7	4	
793122	FF	1										6
805223	FF	1								2		
810131	FF	1										3
810321	FF	1									6	
819112	FF	1								2		
823412	FF	2								16	6	
827032	FF	1									6	
835312	G	1									7	
840313	G	1									16	
851112	G	1										6
851253	G	1									6	
851321	G	1										5
853331	G	1									4	
857431	G	1									5	

		number of	number of families in each trial									
clone #	population	trials	CM	CP-K	E	N-M	FF	G	C-D	J	B	O
859151	G	1										2
863111	G	1									3	
901343	G	1										2
903211	G	1										3
906453	G	1										6
922131	G	1									10	
922333	G	1										6
924132	G	1										3
928113	G	1									5	
930412	G	1									10	
940223	G	1									7	
944212	G	1									10	
945213	G	1										4
948322	G	1										9
954221	G	1									15	
958121	G	1										3
959242	G	1									6	
959351	G	1									6	
963452	G	1									20	
964123	G	1									8	
964213	G	1									5	
967411	G	1									9	
973442	G	1									6	
975313	G	1									6	
976132	G	1										4
978332	G	1									6	
980312	G	1									12	
986243	G	1									7	
987211	G	1										7
988212	G	1										10
989122	G	1									9	
989413	G	1									4	
993342	G	1									9	
993442	G	1										6
996323	G	1										4
998321	G	1									13	
1017433	CD	1										7
1018251	CD	1										5
1027642	CD	1										7
1072141	CD	1										2
1126412	CD	1										6
1127232	CD	1										7
1147921	CD	1										9
1152441	CD	1										5
1170433	CD	1										3
1174223	CD	1										3
1176353	CD	1										1
B3P1-23	SP	1	1									
Ellesmere polycross		1						1				
Ellesmere polycross		1						1				
Glaxo polycross	Glaxo	1	1									

		number of			number of families in each trial							
clone #	population	trials	CM	CP-K	E	N-M	FF	G	C-D	J	B	O
H14P2-2	SP	1	1									
O5P-8	SP	1	1									
O7P2-8	SP	1	1									
R3P2-15	SP	1	1									
R3P2-19	SP	1	1									
R3P2-47	SP	1	1									
R3P2-6	SP	1	1									
RS0	SP	0										
S17P2-32	SP	1	2									
S17P2-45	SP	1	1									

3. Appendix 3

3.1 Parent clones for 1998 trial

Table 3.1.1 SP-clones. Table shows University numbers, clone names (where applicable), clones that were used as parents for the first and second RS1 generations and clones that currently extant at the HRC.

University number	Clone name	Descendants in the First RS1	Descendants in the Second RS1	Extant
1	*CIG 7*		√	
2	*CIG 4*		√	√
3	*CIG 8*		√	√
4				√
5				
6				√
7				√
8				√
9	*CIG 2*		√	√
10			√	√
11			√	√
12				√
13	*CIG 3*	√	√	√
14				√
15			√	√
16			√	√
17			√	√
18				√
19			√	√
20			√	√
21		√	√	√
22	*CIG 9*	√	√	
23		√	√	√
24	*CIG 11*	√	√	√
25				√
26			√	√
27				

Table 3.1.2. Surviving Glaxo clones. Table shows University numbers, Glaxo numbers, clones that were used as parents for the second RS1 generation, and clones that have descendants in the RS3 generation.

University number	Glaxo number	Population	Descendants in RS1	RS3
98	MSB23	P11	√	√
99	MSB24	P11	√	
100	MSB25	P11	√	
103	MSB75	P11	√	
104	MSB101	P11	√	
105	MSB143	P11	√	
106	MSB155	P11		
107	MSB196	P11	√	
109	MSB233	P11	√	√
110	SLG5	P9	√	
118	SLG146		√	
120	SLG261	P11		
122	SLG269	P11	√	
124	SLG A11	P8		
126	SLG A16	P8	√	
127	SLG A17	P8		
128	SLG A19	P8	√	
129	SLG A21	P8	√	
131	SLG D5	P9		
133	SLS1/1			
134	SLS13/1			
135	SLS48/3		√	
136	SLS57/1			

* The P8 population is recorded as originating from India , P9 from New Guinea, and P11 consists of second generation open-pollinated seedlings from high pyrethrins clones held by the University.

Table 3.1.3. Biclonal crosses in 1998 trial. Table shows cross number, parent clones and RS generation number.

Cross number	Parent clones			
	Clone	Generation	Clone	Generation
Current varieties				
220	*CIG 3*	RS0	*CIG 11*	RS0
958	6411	RS2	224241	RS2
989	365111	RS2	420153	RS2
840	2703	RS2	365111	RS2
864	*CIG 3*	RS0	922	RS1
963	224241	RS2	365111	RS2
New selections				
1129	*506221*	RS2	*401423*	RS2
1147	*516423*	RS2	*9251*	RS2
3234	*688442**	RS2	*664123*	RS2
3239	*714113*	RS2	*664123*	RS2
3256	*922131*	RS3	*664123*	RS2
3312	*645731*	RS2	*964123*	RS3
3323	*710122*	RS2	*964123*	RS3
3351	*993342*	RS3	*745332*	RS2
3352	*993342*	RS3	*964123*	RS3
4012	*922333*	RS3	*1018251*	RS3
4019	*948322*	RS3	*1018251*	RS3
4040	*988212*	RS3	*1027642*	RS3

* male sterile

3.2 Tables for Section 5.2.

3.2.1 Regression analysis of DFY and Y-scores.

Omeo - low density plots

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	61736.84097	61736.84097	302.340	0.0001
Error	237	48394.59702	204.19661		
C Total	238	110131.43799			

Root MSE	14.28974	R-square	0.5606
Dep Mean	57.07741	Adj R-sq	0.5587
C.V.	25.03572		

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	10.425576	2.83775802	3.674	0.0003
Y	1	11.506488	0.66175140	17.388	0.0001

Omeo - high density plots

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	43320.21523	43320.21523	655.447	0.0001
Error	224	14804.74212	66.09260		
C Total	225	58124.95735			

Root MSE	8.12974	R-square	0.7453
Dep Mean	26.36106	Adj R-sq	0.7442
C.V.	30.83994		

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	-2.276221	1.24243412	-1.832	0.0683
Y	1	10.081037	0.39376429	25.602	0.0001

University farm - low density plots

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	55999.68321	55999.68321	565.068	0.0001
Error	216	21406.14014	99.10250		
C Total	217	77405.82335			

Root MSE	9.95502	R-square	0.7235
Dep Mean	38.74725	Adj R-sq	0.7222
C.V.	25.69221		

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	4.071237	1.60702473	2.533	0.0120
Y	1	7.907291	0.33264213	23.771	0.0001

3.2.2 Regression analysis for HPLC standards.

Pyrethrins I.

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	3.74116E11	3.74116E11	4396.03	<.0001
Error	187	15914270260	85103050		
Uncorrected Total	188	3.900302E11			

Root MSE	9225.13141	R-Square	0.9592
Dependent Mean	38062	Adj R-Sq	0.9590
Coeff Var	24.23689		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
c1	1	605168	9127.35801	66.30	<.0001

Pyrethrins II.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	2.15524E11	2.15524E11	2032.99	<.0001
Error	187	19824445073	106013075		
Uncorrected Total	188	2.353485E11			

Root MSE	10296	R-Square	0.9158
Dependent Mean	28709	Adj R-Sq	0.9153
Coeff Var	35.86411		

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
c2	1	598925	13283	45.09	<.0001

3.2.3 Block means and block adjustments

Table 1. Block means for PP_H for low density plots.

Site	block					Site mean
	1	2	3	4	5	
1	2.07	1.57	1.80	1.58	1.61	1.72
2	1.51	1.89	1.83	1.79	2.06	1.81
3	2.11	1.96	2.04	1.94	1.76	1.95

Table 2. Block adjustments for PP_H for low density plots.

Site	block				
	1	2	3	4	5
1	-0.353	0.147	-0.085	0.134	0.107
2	0.305	-0.084	-0.015	0.022	-0.254
3	-0.157	-0.010	-0.091	0.009	0.093

Table 3. Block averages for percent dry matter content (DM) for site-treatment combinations that showed statistically significant block effects. (A is low density and B is high density).

Site-treatment	block					
	1	2	3	4	5	mean
2B	29.2	30.7	33.1	32.9	31.4	31.3
3A	25.8	27.7	28.3	27.9	29.8	28.0
3B	29.2	27.2	30.6	27.8	28.3	28.6

Table 4. Block adjustment values for DM for site-treatment combinations that showed statistically significant block effects.

block	1	2	3	4	5
2B	2.1	0.6	-1.8	-1.5	0.0
3A	1.1	-1.4	-4.8	-5.5	-5.0
3B	-1.0	-2.0	-3.0	-4.0	-5.0

3.2.4 ANOVA tables for analysis of block effects

site	density	trait	Source	df	SS	MS	F	Pr > F
1	low	PyY	Block	4	4.12	1.03	4.24	0.003
			Error	238	57.75	0.24		
			Total	242	61.87			
1	low	DM	Block	4	1,139	284.73	1.64	0.164
			Error	238	41,249	173.32		
			Total	242	42,388			

site	density	trait	Source	df	SS	MS	F	Pr > F
1	low	DFY	Block	4	649	162.35	0.34	0.849
			Error	238	112,845	474.14		
			Total	242	113,494			
1	low	FFY	Block	4	21,300	5,324.92	0.62	0.646
			Error	239	2,042,189	8,544.73		
			Total	243	2,063,489			
1	low	PP_H	Block	4	8.80	2.20	7.45	0.000
			Error	239	70.58	0.30		
			Total	243	79.38			
1	low	lPyI/II	Block	4	1.21	0.30	0.80	0.529
			Error	239	90.53	0.38		
			Total	243	91.74			
2	low	PyY	Block	4	0.38	0.10	0.24	0.916
			Error	193	76.72	0.40		
			Total	197	77.10			
2	low	FFY	Block	4	53,173	13,293	1.06	0.375
			Error	193	2,409,783	12,486		
			Total	197	2,462,956			
2	low	DM	Block	4	226	57	1.91	0.111
			Error	193	5,716	30		
			Total	197	5,942			
2	low	DFY	Block	4	5,410	1,352	1.38	0.244
			Error	193	189,717	983		
			Total	197	195,126			
2	low	PP_H	Block	4	6.62	1.65	4.89	0.001
			Error	193	65.32	0.34		
			Total	197	71.93			
2	low	lPyI/II	Block	4	0.42	0.10	0.29	0.881
			Error	193	68.40	0.35		
			Total	197	68.82			
3	low	PyY	Block	4	1.24	0.31	1.56	0.185
			Error	213	42.15	0.20		
			Total	217	43.39			

site	density	trait	Source	df	SS	MS	F	Pr > F
3	low	DM	Block	4	388	97	3.66	0.007
			Error	213	5,650	27		
			Total	217	6,038			
3	low	DFY	Block	4	2,159	540	1.53	0.195
			Error	213	75,247	353		
			Total	217	77,406			
3	low	FFY	Block	4	16,316	4,079	0.73	0.572
			Error	215	1,199,199	5,578		
			Total	219	1,215,515			
3	low	PP_H	Block	4	3.38	0.85	2.51	0.043
			Error	215	72.33	0.34		
			Total	219	75.71			
3	low	lPyI/II	Block	4	2.27	0.57	1.41	0.232
			Error	215	86.62	0.40		
			Total	219	88.89			
1	high	PyY	Block	4	0.40	0.10	0.96	0.428
			Error	228	23.75	0.10		
			Total	232	24.15			
1	high	DM	Block	4	338	84	0.61	0.658
			Error	228	31,728	139		
			Total	232	32,065			
1	high	DFY	Block	4	1,621	405	1.57	0.183
			Error	228	58,743	258		
			Total	232	60,364			
1	high	FFY	Block	4	22,903	5,726	1.63	0.167
			Error	229	803,878	3,510		
			Total	233	826,781			
1	high	PP_H	Block	4	2.54	0.63	1.43	0.225
			Error	229	101.62	0.44		
			Total	233	104.16			
1	high	lPyI/II	Block	4	0.59	0.15	0.47	0.761
			Error	229	72.50	0.32		
			Total	233	73.09			
2	high	PyY	Block	4	0.53	0.13	1.24	0.294
			Error	194	20.63	0.11		
			Total	198	21.16			

site	density	trait	Source	df	SS	MS	F	Pr > F
2	high	FFY	Block	4	23,310	5,827	1.71	0.148
			Error	194	659,611	3,400		
			Total	198	682,920			
2	high	DM	Block	4	416	104	3.39	0.010
			Error	194	5,945	31		
			Total	198	6,360			
2	high	DFY	Block	4	2,053	513	1.63	0.169
			Error	194	61,187	315		
			Total	198	63,240			
2	high	PP_H	Block	4	0.88	0.22	0.70	0.592
			Error	194	60.72	0.31		
			Total	198	61.60			
2	high	lPyI/II	Block	4	0.64	0.16	0.60	0.666
			Error	194	52.43	0.27		
			Total	198	53.08			
3	high	PyY	Block	4	0.30	0.07	0.96	0.432
			Error	224	17.49	0.08		
			Total	228	17.79			
3	high	FY	Block	4	14,713	3,678	1.49	0.206
			Error	224	552,330	2,466		
			Total	228	567,043			
3	high	DM	Block	4	320	80	2.84	0.025
			Error	224	6,315	28		
			Total	228	6,635			
3	high	DFY	Block	4	730	183	1.02	0.398
			Error	224	40,091	179		
			Total	228	40,821			
3	high	PP_H	Block	4	1.45	0.36	1.58	0.181
			Error	224	51.48	0.23		
			Total	228	52.93			
3	high	lPyI/II	Block	4	0.52	0.13	0.42	0.794
			Error	224	69.96	0.31		
			Total	228	70.48			

3.3 Tables for Section 5.3

3.3.1 Survival data.

Test whether variation in the incidence of plant survival in low (A) and high (B) density plots is greater than expected by random sampling variation.

For each sample and for the pooled sample: H_0: Plants are sampled from populations with the same incidence of survival.

For heterogeneity testing: H_0: All samples are from the same population.

Expected frequencies shown in brackets.

Site	Missing		Alive		df	χ^2	P
	A	B	A	B			
Omeo (1)	30 *(35.5)*	41 *(35.5)*	306 *(300.5)*	295 *(300.5)*	1	1.906	0.17
Kindred (2)	29 *(39.5)*	50 *(39.5)*	307 *(296.5)*	286 *(296.5)*	1	6.266	0.012
Uni farm (3)	19 *(22.5)*	26 *(22.5)*	317 *(313.5)*	310 *(313.5)*	1	1.131	0.29
χ^2_T					3	9.031	0.01
χ^2_P	78 *(97.5)*	117 *(97.5)*	930 *(910.5)*	891 *(910.5)*	1	8.306	0.005
χ^2_H					2	1.0	>0.25

χ^2_P - pooled chi-square

χ^2_T - total chi-square (sum of site chi-squares), test whether main effect significant

χ^2_H - heterogeneity chi-square (λ^2_T -λ^2_P), test whether interaction is significant.

Test whether variation in the incidence of plant survival among sites is greater than expected by random sampling variation.

density	missing			alive			df	χ^2	P
	1	2	3	1	2	3			
A	30 *(26.0)*	29 *(26.0)*	19 *(26.0)*	306 *(310)*	307 *(310)*	317 *(310)*	2	3.085	0.21
B	41 *(39)*	50 *(39)*	26 *(39)*	295 *(297)*	286 *(297)*	310 *(297)*	2	8.528	0.01
χ^2_T							4	11.62	0.005
χ^2_P	71 *(65)*	79 *(65)*	45 *(65)*	601 *(607)*	593 *(607)*	627 *(607)*	2	10.76	0.004
χ^2_H							2	0.085	0.96

Analysis of variation among half-sib families:
Test whether variation in the incidence of plant survival among half-sib families is greater than expected by random sampling variation.

H_0: Plants are sampled from populations with the same incidence of survival.

Number of families = 47

	n	p	G	P
Missing	141	0.0991	62.8	0.0025
Alive	1282	0.9001		
total	1420			

Analysis of variation among full-sib families:
Test whether variation in the incidence of plant survival among full-sib families is greater than expected by random sampling variation.

H_0: Plants are sampled from populations with the same incidence of survival.

Number of families = 18

	n	p	G	P
Missing	51	0.0878	18.8	0.34
Alive	530	0.9122		
total	581			

Test whether variation in the incidence of plant survival between the base and selected populations is greater than expected by random sampling variation.

H_0: Plants are sampled from populations with the same incidence of survival.

Population	Missing	Alive	χ^2	P
Selections	141 *(136.5)*	1282 *(1286.7)*	0.6008	0.44
Base	51 *(55.7)*	530 *(525.3)*		

3.3.2 Analysis of flowering incidence

Analyses

Analysis	Effect 1	Effect 2
1	Sowing time	Site
2	Density	Site

Analysis 1. Effects of sowing time and site on flowering incidence of plants in high density plots.

Test whether variation in the incidence of flowering in March (C) and December-sown (B) plants is greater than expected by random sampling variation (for plants assessed in high density plots).

Site	Vegetative C	Vegetative B	flowering C	flowering B	df	χ^2	P
Omeo (1)	279 *(151.3)*	6 *(133.7)*	55 *(182.7)*	289 *(161.3)*	1	419	<0.0001
Kindred (2)	328 *(199.3)*	43 *(171.7)*	4 *(132.7)*	243 *(164.1)*	1	448	<0.0001
Uni farm (3)	294 *(156.6)*	8 *(145.4)*	40 *(177.4)*	302 *(164.6)*	1	471	<0.0001
χ^2_T					3	1338	
χ^2_P	901 *(506.6)*	57 *(451.4)*	99 *(493.4)*	834 *(439.6)*	1	1320	<0.0001
χ^2_H					2	18	<0.0001

Test whether variation in the incidence of flowering among sites (1, 2 and 3) is greater than expected by random sampling variation for March (C) and December-sown plants(B), tested in high density plots..

density	vegetative 1	vegetative 2	vegetative 3	flowering 1	flowering 2	flowering 3	df	χ^2	P
B	6 *(18.6)*	43 *(18.3)*	8 *(19.8)*	289 *(276.1)*	243 *(267.7)*	302 *(290.2)*	2	52.55	<0.001
C	279 *(301)*	328 *(300)*	294 *(301)*	55 *(33.1)*	4 *(32.9)*	40 *(33.1)*	2	45.9	<0.001
χ^2_T							4	98.45	
χ^2_P	285 *(65)*	371 *(65)*	302 *(65)*	344 *(607)*	247 *(607)*	342 *(607)*	2	32.57	0.004
χ^2_H							2	65.88	<0.001

Analysis 2. Effects of establishment density and site on plants sown in December.

Test whether variation in the incidence of flowering in low (A) and high density plots (B) plants is greater than expected by random sampling variation (for plants sown in December).

Site	Vegetative		flowering		df	χ^2	P
	A	B	A	B			
Omeo (1)	4 *(5.1)*	6 *(4.9)*	302 *(300.9)*	289 *(290.1)*	1	0.441	0.51
Kindred (2)	51 *(48.7)*	43 *(45.3)*	256 *(258.3)*	243 *(240.7)*	1	0.2645	0.61
Uni farm (3)	21 *(14.7)*	8 *(14.3)*	296 *(302.3)*	302 *(295.7)*	1	5.719	0.02
χ^2_T					3	6.425	
χ^2_P	76 *(67.9)*	57 *(65.1)*	854 *(302.3)*	834 *(295.7)*	1	2.117	0.15
χ^2_H					2	4.106	0.043

Test whether variation in the incidence of flowering among sites (1, 2 and 3) is greater than expected by random sampling variation for low density (A) and high density(B) plots, tested in high density plots.

density	vegetative			flowering			df	χ^2	P
	1	2	3	1	2	3			
A	4 *(25)*	51 *(25.1)*	21 *(25.9)*	302 *(281)*	256 *(281.9)*	296 *(291.1)*	2	49.3	<0.001
B	6 *(18.9)*	43 *(18.3)*	8 *(19.8)*	289 *(276.1)*	243 *(267.7)*	302 *(290.2)*	2	52.56	<0.001
χ^2_T							4	101.9	
χ^2_P	10 *(43.9)*	94 *(43.3)*	29 *(45.8)*	591 *(557.1)*	499 *(549.7)*	598 *(581.2)*	2	98.88	<0.001
χ^2_H							2	3.04	>0.05

Analysis of variation among half-sib families in flowering incidence of March-sown plants:

Test whether variation in the incidence of flowering among half-sib families is greater than expected by random sampling variation.

H_0: Plants are sampled from populations with the same incidence of survival.

Number of families = 47

Site	df	G	P
1	42	56.2	0.07
2	42	62.5	0.02
3	42	13.5	1.00
χ^2_T	124	162.6	
χ^2_P	42	77.8	0.002
χ^2_H	82	54.4	0.98

Analysis of variation among full-sib families in flowering incidence of March-sown plants:

Test whether variation in the incidence of flowering among full-sib families is greater than expected by random sampling variation.

Number of families = 18

Site	df	G	P
1	20	8.0	0.99
2	20	11.2	0.94
3	20	4.0	1.00
χ^2_T	60	23.2	
χ^2_P	22	19.8	0.60
χ^2_H	38	3.4	1.0

Test whether variation in the incidence of flowering (March-sown) between the base and selected populations is greater than expected by random sampling variation.

Population	flowering	vegetative	χ^2	P
Selections	33 *(24)*	216 *(225)*	4.98	0.026
Base	66 *(75)*	715 *(705)*		

3.4 Tables for Section 5.4

3.4.1 Plot means

Plot means for the entire mature (December-sown) seedling population in the 1998 trial.

site	DFY (g/plant)		PP_H (%)		IPyI/II		PyY (g/plant)	
	low	high	low	high	low	high	low	high
1	59.69	29.10	2.07	1.77	-0.030	-0.06	1.22	0.50
1	55.59	24.39	1.57	1.65	-0.007	-0.05	0.87	0.41
1	55.34	22.77	1.80	1.89	-0.057	-0.01	1.00	0.47
1	56.85	23.96	1.57	1.65	-0.021	-0.05	0.91	0.39
1	56.82	29.61	1.61	1.67	0.029	-0.01	0.88	0.50
2	52.77	17.84	1.51	2.01	0.063	-0.01	0.80	0.36
2	38.31	21.69	1.89	2.00	0.030	0.00	0.72	0.42
2	46.44	16.37	1.83	1.95	0.063	-0.01	0.84	0.32
2	43.87	18.37	1.79	1.88	0.085	-0.05	0.78	0.31
2	40.07	25.18	2.00	1.85	0.036	0.02	0.81	0.44
3	33.61	16.21	2.11	2.07	0.006	0.00	0.71	0.34
3	37.49	20.69	1.96	2.08	-0.010	-0.05	0.73	0.42
3	43.01	19.86	2.04	2.08	-0.043	0.00	0.88	0.42
3	41.28	19.83	1.89	1.89	-0.097	0.02	0.81	0.38
3	37.02	21.25	1.75	2.02	0.031	-0.01	0.65	0.43

site	log (p/jc)		Density (plants/m^2)		Flower yield (t/ha)		Pyrethrins yield (kg/ha)	
	low	high	low	high	low	high	low	high
1	0.61	0.60	3.8	15.4	2.26	4.49	46.37	77.09
1	0.59	0.60	3.9	15.4	2.14	3.76	33.37	63.28
1	0.62	0.63	3.1	12.3	1.70	2.80	30.63	57.70
1	0.59	0.64	3.9	14.3	2.19	3.42	35.05	55.43
1	0.56	0.60	3.8	11.7	2.15	3.47	33.50	58.69
2	0.63	0.59	3.8	14.0	2.00	2.50	30.23	50.28
2	0.62	0.60	3.8	15.1	1.45	3.28	27.09	63.20
2	0.63	0.64	3.9	12.3	1.82	2.01	33.17	39.05
2	0.65	0.63	3.4	13.1	1.50	2.41	26.58	41.13
2	0.66	0.63	3.4	12.9	1.37	3.24	27.72	56.27
3	0.65	0.66	3.9	14.6	1.30	2.36	27.49	49.10
3	0.69	0.68	3.9	15.1	1.45	3.13	28.26	63.02
3	0.72	0.69	3.6	14.3	1.54	2.84	31.60	60.42
3	0.66	0.68	3.7	15.4	1.53	3.06	30.08	58.51
3	0.66	0.65	3.9	14.6	1.43	3.10	25.20	62.21

3.4.2 ANOVA Table for analysis of plot mean data on an individual site basis.

PP_H - site 1

Source	SS	df	MS	F	P-value
density	0.00005	1	0.0000493	0.0017	0.968
error	0.23085	8	0.03		
Total	0.23090	9			

PP_H - site 2

Source	SS	df	MS	F	P-value
density	0.044	1	0.044	2.22	0.175
error	0.159	8	0.020		
Total	0.203	9			

PP_H - site 3

Source	SS	df	MS	F	P-value
density	0.015	1	0.015	1.13	0.318
error	0.107	8	0.013		
Total	0.122	9			

DFY - site 1

Source	SS	df	MS	F	P-value
density	2,386	1	2,386	369	<0.001
error	52	8	6		
Total	2,438	9			

DFY - site 2

Source	SS	df	MS	F	P-value
density	1,489	1	1,489	66	<0.001
error	180	8	23		
Total	1,669	9			

DFY - site 3

Source	SS	df	MS	F	P-value
density	894	1	894	101	<0.001
error	71	8	9		
Total	965	9			

PyY -site 1

Source	SS	df	MS	F	P-value
density	0.68	1	0.682	55.52	<0.001
error	0.10	8	0.012		
Total	0.78	9			

PyY -site 2

Source	SS	df	MS	F	P-value
density	0.44	1	0.440	159.94	<0.001
error	0.02	8	0.003		
Total	0.46	9			

PyY -site 3

Source	SS	df	MS	F	P-value
density	0.33	1	0.328	68.67	<0.001
error	0.04	8	0.005		
Total	0.37	9			

LogPyI/II -site 1

Source	SS	df	MS	F	P-value
density	0.00097	1	0.0010	1.23	0.300
error	0.00634	8	0.0008		
Total	0.00731	9			

LogPyI/II -site 2

Source	SS	df	MS	F	P-value
density	0.00988	1	0.0099	16.71	0.003
error	0.00473	8	0.0006		
Total	0.01461	9			

LogPyI/II -site 3

Source	SS	df	MS	F	P-value
density	0.00046	1	0.0005	0.30	0.596
error	0.01197	8	0.0015		
Total	0.01243	9			

DFY (t/ha) -site 1

Source	SS	df	MS	F	P-value
density	5.62	1	5.62	26.30	0.0009
error	1.71	8	0.214		
Total	7.32	9			

DFY (t/ha) -site 2

Source	SS	df	MS	F	P-value
density	2.80	1	2.80	14.81	0.005
error	1.51	8	0.19		
Total	4.32	9			

DFY (t/ha) -site 3

Source	SS	df	MS	F	P-value
density	5.25	1	5.25	93.24	<.0001
error	0.45	8	0.06		
Total	5.70	9			

PyY (kg/ha) -site 1

Source	SS	df	MS	F	P-value
density	1,777	1	1,776.5	31.49	0.0005
error	451	8	56.4		
Total	2,228	9			

PyY (kg/ha) -site 2

Source	SS	df	MS	F	P-value
density	1,106	1	1,105.6	20.01	0.002
error	442	8	55.3		
Total	1,548	9			

PyY (kg/ha) -site 3

Source	SS	df	MS	F	P-value
density	2,269	1	2,268.7	120.83	<0.001
error	150	8	18.8		
Total	2,419	9			

Log(p/jc) -site 1

Source	SS	df	MS	F	P
density	0.000845	1	0.0008	1.78	0.22
error	0.003806	8	0.0005		
Total	0.004652	9			

Log(p/jc) -site 2

Source	SS	df	MS	F	P
density	0.0012	1	0.0012	3.14	0.11
error	0.003057	8	0.0004		
Total	0.004257	9			

Log(p/jc) -site 3

Source	SS	df	MS	F	P
density	1.99E-05	1	2E-05	0.04	0.84
error	0.003853	8	0.0005		
Total	0.003873	9			

3.4.3 ANOVA tables for analysis for plot data combined from all three sites

PP_H

Source	df	SS	MS	F	P-value
site	2	0.350	0.175		
block(site)	12	0.291	0.024		
density	1	0.038	0.038	2.45	0.1397
Error	14	0.218	0.016		

DFY

Source	df	SS	MS	F	P-value
site	2	832	416		
block(site)	12	124	10		
density	1	4,589	4,589	179	<.0001
Error	14	358	26		

PyY

Source	df	SS	MS	F	P-value
site	2	0.124	0.062		
block(site)	12	0.090	0.007		
density	1	1.408	1.408	198.42	<.0001
Error	14	0.099	0.007		

LogPyI/II

Source	df	SS	MS	F	P-value
site	2	0.0130	0.0065		
block(site)	12	0.0078	0.0007		
density	1	0.0039	0.0039	2.29	0.1524
Error	14	0.0235	0.0017		

DFY (t/ha)

Source	df	SS	MS	F	P-value
site	2	3.01	1.51		
block(site)	12	1.95	0.16		
density	1	13.39	13.39	93.36	<.0001
Error	14	2.01	0.14		

PyY (kg/ha)

Source	df	SS	MS	F	P-value
site	2	468	234	6.67	
block(site)	12	658	55	1.56	
density	1	5,045	5,045	144	<0.0001
Error	14	491	35		

Log(p/jc)

Source	df	SS	MS	F	P-value
site	2	0.02530	0.0127		
block(site)	12	0.00834	0.00070		
density	1	0.00001	0.00001	0.04	0.85
Error	14	0.00469	0.00033		

3.4.4 Calculations for r_g (genetic correlations of half-sib family means in high and low density plots).

Character	Cross products		df		Mean cross products		Genetic covariance
	error	family	error	family	error	family	
DFY	666.73	772.27	80	40	8.334	19.307	3.657
DFY_s	1.90	2.14	80	40	0.024	0.054	0.010
PyY	0.47	0.68	80	40	0.006	0.017	0.004
PyY_s	2.54	4.69	80	40	0.032	0.117	0.029
PP_H	0.55	2.47	80	40	0.007	0.062	0.018
PP_{Hs}	2.61	9.57	80	40	0.033	0.239	0.069
PP_{Hb}	0.51	3.55	80	40	0.006	0.089	0.027
l PyI/II	-0.16	1.26	80	40	-0.002	0.032	0.011
lp/jc	0.174	0.106	80	40	0.004	0.001	0.001

trait	Genetic covariance	Genetic variance		r_g
		low	high	
DFY	3.657	22.16	16.03	0.19
DFY_s	0.010	0.05	0.07	0.16
PyY	0.004	0.02	0.01	0.36
PyY_s	0.029	0.07	0.08	0.38
PP_H	0.018	0.01	0.02	1.35
PP_{Hs}	0.069	0.04	0.07	1.22
PP_{Hb}	0.027	0.01	0.04	1.29
lp/jc	0.0013	0.001	0.0005	1.45
lPyI/II	0.011	0.01	0.01	1.03

3.4.5 Estimates of family variance for low (1) and high (2) density plots.

Character	Parameter	Estimate	Std-Error
PP_H 1	family	0.0113	0.0100
	site	0.0055	0.0076
	residual	0.0856	0.0135
	total	0.1024	
PP_{Hc} 1	family	0.0445	0.0383
	site	0.0000	.
	residual	0.3271	0.0511
	total	0.3716	
Character	Parameter	Estimate	Std-Error
PP_{Hb} 1	family	0.0119	0.0099
	site	0.0000	.
	residual	0.0839	0.0131
	total	0.0958	
PP_H 2	family	0.0162	0.0123
	site	0.0212	0.0236
	residual	0.1006	0.0159
	total	0.1379	
PP_{Hs} 2	family	0.0721	0.0481
	site	0.0000	.
	residual	0.3736	0.0584
	total	0.4457	
PP_{Hb} 2	family	0.0383	0.0260
	site	0.0000	.
	residual	0.2035	0.0318
	total	0.2418	
DFY1	family	22.1622	26.8496
	site	81.4440	87.5010
	residual	248.1240	39.2318
	total	351.7302	
DFY_s 1	family	0.0508	0.0428
	site	0.0000	.
	residual	0.3634	0.0568
	total	0.4142	
DFY2	family	16.0279	10.9848
	site	4.1219	6.2313
	residual	86.1222	13.6171
	total	106.2720	
DFY_s2	family	0.0722	0.0435
	site	0.0000	.
	residual	0.3221	0.0503
	total	0.3942	

Character	Parameter	Estimate	Std-Error
PyY1	family	0.0162	0.0113
	site	0.0122	0.0144
	residual	0.0889	0.0141
	total	0.1173	
PyY_s1	family	0.0718	0.0438
	site	0.0000	.
	residual	0.3268	0.0510
	total	0.3986	
PyY2	family	0.0064	0.0037
	site	0.0001	0.0007
	residual	0.0264	0.0042
	total	0.0329	
PyY_s2	FAM	0.0779	0.0426
	SITE	0.0000	.
	Residual	0.2986	0.0466
	total	0.3765	
lPyI/II1	FAM	0.0142	0.0047
	SITE	0.0017	0.0021
	Residual	0.0184	0.0029
	total	0.0342	
lPyI/II 2	FAM	0.0083	0.0030
	SITE	0.0000	.
	Residual	0.0145	0.0023
	total	0.0228	

3.5 Tables for Section 5.5

3.5.1 ANOVA Tables

DM

Source	df	SS	MS	F Value	Pr > F
site	2	4,947	2,473		
block(site)	12	1,208	101		
density	1	0	0		
density*block(site)	14	1,595	114		
population	3	989	330	4.54	0.0036
density*population	3	812	271	3.73	0.011
Error	1282	93,078	73		
Corrected Total	1317	102,846			

DFY

Source	df	SS	MS	F Value	Pr > F
site	2	38,192	19,096		
block(site)	12	5,597	466		
density	1	48,157	48,157		
density*block(site)	14	14,674	1,048		
population	3	14,115	4,705	11.61	<.0001
density*population	3	1,861	620	1.53	0.2047
Error	1282	519,573	405		
Corrected Total	1317	802,237			

PyY

Source	df	SS	MS	F Value	Pr > F
site	2	5.36	2.68		
block(site)	12	4.30	0.36		
density	1	15.57	15.57		
density*block(site)	14	4.04	0.29		
population	3	14.98	4.99	28.99	<.0001
density*population	3	1.90	0.63	3.67	0.0119
Error	1282	220.80	0.17		
Corrected Total	1317	315.75			

PP_H

Source	df	SS	MS	F Value	Pr > F
site	2	18.77	9.39		
block(site)	12	12.45	1.04		
density	1	0.06	0.06		
density*block(site)	14	9.91	0.71		
population	3	104.94	34.98	142.28	<.0001
density*population	3	0.31	0.10	0.42	0.7385
Error	1286	316.16	0.25		
Corrected Total	1321	464.06			

FFY

Source	df	SS	MS	F Value	Pr > F
site	2	376,354	188,177		
block(site)	12	60,602	5,050		
density	1	594,982	594,982		
density*block(site)	14	160,975	11,498		
population	3	137,043	45,681	7.85	<.0001
density*population	3	15,080	5,027	0.86	0.4592
Error	1286	7,481,662	5,818		
Corrected Total	1321	10,847,521			

lPyI/II

Source	df	SS	MS	F Value	Pr > F
site	2	0.59	0.29		
block(site)	12	0.42	0.03		
density	1	0.14	0.14		
density*block(site)	14	0.90	0.06		
population	3	2.65	0.88	14.22	<.0001
density*population	3	0.21	0.07	1.11	0.3459
Error	1286	79.76	0.06		
Corrected Total	1321	84.77			

Glaxo and SP-clones

DFY

Source	df	SS	MS	F Value	Pr > F
site	2	22,457	11,228	27.84	<.0001
block(site)	12	9,057	755	1.87	0.0343
density	1	139,046	139,046	344.8	<.0001
density*block(site)	14	13,445	960	2.38	0.0029
population	1	4,865	4,865	12.06	0.0005
density*population	1	239	239	0.59	0.4411
Error	882	355,674	403		
Corrected Total	913	549,637			

PyY

Source	df	SS	MS	F Value	Pr > F
site	2	1.66	0.83	5.52	0.0041
block(site)	12	4.45	0.37	2.47	0.0035
density	1	36.34	36.34	242.18	<.0001
density*block(site)	14	3.53	0.25	1.68	0.0547
population	1	2.45	2.45	16.3	<.0001
density*population	1	0.06	0.06	0.4	0.5283
Error	882	132.33	0.15		
Corrected Total	913	180.59			

PP_H

Source	df	SS	MS	F Value	Pr > F
site	2	13.12	6.56		
block(site)	12	9.29	0.77		
density	1	1.40	1.40		
density*block(site)	14	8.31	0.59		
population	1	0.78	0.78	3.09	0.0789
density*population	1	0.26	0.26	1.04	0.3074
Error	885	222.07	0.25		
Corrected Total	916	254.75			

PP_{U6}

Source	df	SS	MS	F Value	Pr > F
site	2	7.04	3.52		
block(site)	12	6.64	0.55	2.51	0.0032
population	3	63.05	21.02	95.13	<.0001
Error	573	126.59	0.22		
Corrected Total	590	206.50			

3.5.2 Full-sib family means for current varieties (CV) and new selections (new) for PyY in high and low density plots and PP_H (average of both densities).

number		PyY(high)			PyY(low)			PP_H		
		mean	se	rank	mean	se	rank	mean	se	rank
220	CV	0.5	0.1	9	1.1	0.1	7	2.0	0.1	16
840	CV	0.78	0.1	1	1.5	0.1	2	1.9	0.1	18
864	CV	0.6	0.1	4	0.9	0.1	14	2.2	0.1	14
958	CV	0.5	0.1	6	0.9	0.1	11	2.2	0.1	15
963	CV	0.6	0.1	5	1.5	0.1	1	2.4	0.1	5
989	CV	0.5	0.1	7	1.3	0.1	3	2.3	0.1	11
1129	New	0.3	0.1	16	0.8	0.1	16	2.3	0.1	10
1147	New	0.5	0.1	8	1.0	0.1	9	2.0	0.1	17
3234	New	0.5	0.1	11	1.2	0.1	4	2.4	0.1	7
3239	New	0.4	0.1	14	0.9	0.1	15	2.5	0.1	4
3256	New	0.3	0.2	17	0.7	0.2	17	2.2	0.1	13
3312	New	0.7	0.2	3	1.0	0.2	10	2.3	0.2	9
3323	New	0.4	0.1	13	0.9	0.1	12	2.6	0.1	3
3351	New	0.7	0.1	2	1.0	0.1	8	2.2	0.1	12
3352	New	0.5	0.1	10	1.2	0.1	6	2.6	0.1	2
4012	New	0.2	0.1	18	0.6	0.1	18	2.4	0.1	8
4019	New	0.4	0.1	12	0.9	0.1	13	2.4	0.1	6
4040	New	0.3	0.2	15	1.2	0.3	5	2.6	0.2	1

3.6 Tables for Section 5.6

3.6.1 Estimates of residual variance for analysis of individual site and density combinations.

Table 1. Residual variance for analyses of data for each individual site and planting density. Site 1 is Omeo, 2 Kindred, 3 is the University farm, and L is low density and H is high.

character	Residual variance					
	1L	2L	3L	1H	2H	3H
PP_H	0.23	0.22	0.22	0.33	0.21	0.13
DM	116	23	19	95	33	25
FW	6581	9864	6141	3132	2972	2261
DFY	417	858	368	236	310	157
PyY	0.16	0.28	0.19	0.07	0.08	0.07
Y	1.74	na	3.99	1.44	na	na
PP_{U6}	0.22	0.16	0.21	na	na	na

na not assessed.

3.6.2 Variance components used to calculate heritability.

1. Estimates of variance, the standard error of the estimate (se) and the probability that the estimate is zero for low (A) and high (B) density plot data, all effects random. Family is the effect due to half-sib family and fam*site is the interaction between half-sib family and site.

density	trait	parameter	variance	se	Z	Pr > |Z|
A	DFY_a	family	0.078	0.038	2.04	0.0412
		site	0.000	.	.	.
		fam*site	0.000	.	.	.
		Residual	0.948	0.068	13.91	0.0001
		TOTAL	1.026			

density	trait	parameter	variance	se	Z	Pr > |Z|
B	DFY_a	family	0.064	0.042	1.53	0.125
		site	0.000	.	.	.
		fam*site	0.052	0.051	1.02	0.308
		Residual	0.930	0.072	12.84	0.0001
		TOTAL	1.046			
A	FFY_a	family	0.072	0.037	1.98	0.0474
		site	0.000	.	.	.
		fam*site	0.000	.	.	.
		Residual	0.922	0.066	13.94	0.0001
		TOTAL	0.994			
B	FFY_a	family	0.055	0.037	1.49	0.135
		site	0.000	.	.	.
		fam*site	0.028	0.047	0.6	0.5469
		Residual	0.913	0.071	12.88	0.0001
		TOTAL	0.997			
A	PyY_a	family	0.094	0.046	2.06	0.0398
		site	0.000	.	.	.
		fam*site	0.000	.	.	.
		Residual	1.001	0.072	13.82	0.0001
		TOTAL	1.096			
B	PyY_a	family	0.089	0.042	2.12	0.0337
		site	0.000	.	.	.
		fam*site	0.010	0.044	0.23	0.8201
		Residual	0.900	0.070	12.85	0.0001
		TOTAL	0.999			
A	PP_{HB}	family	0.017	0.010	1.62	0.1051
		site	0.011	0.013	0.87	0.3843
		fam*site	0.008	0.013	0.58	0.5593
		Residual	0.217	0.018	12.02	0.0001
		TOTAL	0.252			
B	PP_{HB}	family	0.012	0.011	1.04	0.2978
		site	0.023	0.025	0.91	0.3625
		fam*site	0.030	0.016	1.93	0.0537
		Residual	0.225	0.018	12.65	0.0001
		TOTAL	0.290			
A	PP_{U6}	family	0.016	0.009	1.79	0.0734
		site	0.024	0.026	0.93	0.3526
		fam*site	0.000	.	.	.
		Residual	0.211	0.016	12.97	0.0001
		TOTAL	0.250			

density	trait	parameter	variance	se	Z	Pr > |Z|
A	lPyI/II	family	0.013	0.004	3.05	0.0023
		site	0.002	0.003	0.83	0.4054
		fam*site	0.001	0.003	0.41	0.6806
		Residual	0.054	0.004	12.63	0.0001
		TOTAL	0.070			
B	lPyI/II	family	0.007	0.003	2.6	0.0093
		site	0.000	.	.	.
		fam*site	0.002	0.002	0.81	0.4187
		Residual	0.045	0.003	12.93	0.0001
		TOTAL	0.054			
A	PyY_B	family	0.024	0.010	2.29	0.0221
		site	0.010	0.011	0.88	0.3807
		fam*site	0.000	.	.	.
		Residual	0.203	0.015	13.79	0.0001
		TOTAL	0.237			
B	PyY_B	family	0.007	0.004	2.05	0.0404
		site	0.001	0.001	0.53	0.5961
		fam*site	0.002	0.004	0.46	0.6452
		Residual	0.075	0.006	12.83	0.0001
		TOTAL	0.085			
B	PP_{HB}	family	0.013	0.011	1.19	0.236
		site	0.023	0.025	0.91	0.3608
		fam*site	0.030	0.015	1.98	0.0475
		Residual	0.215	0.017	12.64	0.0001
		TOTAL	0.281			
A	DM_a	family	0.028	0.032	0.88	0.3794
		site	0.000	.	.	.
		fam*site	0.033	0.051	0.65	0.5185
		Residual	0.928	0.075	12.42	0.0001
		TOTAL	0.988			
B	DM_a	family	0.000	.	.	.
		site	0.000	.	.	.
		fam*site	0.015	0.041	0.37	0.7143
		Residual	0.936	0.074	12.69	0.0001
		TOTAL	0.951			

density	trait	parameter	variance	se	Z	Pr > |Z|
A	DFY	family	41.1	22.571	1.82	0.0689
		site	90.3	93.868	0.96	0.3361
		fam*site	2.0	27.968	0.07	0.9425
		Residual	537.1	43.347	12.39	0.0001
		TOTAL	670.5			
B	DFY	family	13.7	10.165	1.34	0.1789
		site	7.1	9.120	0.78	0.4348
		fam*site	12.5	13.256	0.94	0.3447
		Residual	241.1	18.823	12.81	0.0001
		TOTAL	274.4			
A	PyY	family	0.023	0.010	2.26	0.0241
		site	0.010	0.011	0.88	0.3798
		fam*site	0.000	.	.	.
		Residual	0.208	0.015	13.79	0.0001
		TOTAL	0.242			
B	PyY	family	0.007	0.004	2.05	0.0404
		site	0.001	0.001	0.53	0.5961
		fam*site	0.002	0.004	0.46	0.6452
		Residual	0.075	0.006	12.83	0.0001
		TOTAL	0.085			
A	FFY	family	594	325	1.83	0.0672
		site	1,151	1,202	0.96	0.3384
		fam*site	73	385	0.19	0.8489
		Residual	7,452	595	12.52	0.0001
		TOTAL	9,271			
B	FFY	family	151	115	1.31	0.1905
		site	112	135	0.83	0.4084
		fam*site	109	155	0.7	0.4823
		Residual	2,914	226	12.87	0.0001
		TOTAL	3,285			
A	DFY(e)	family	17.374	11	1.54	0.124
		site	201.500	287	0.7	0.4832
		fam*site	0.000	.	.	.
		Residual	249.288	22	11.42	0.0001
		TOTAL	468.162			

density	trait	parameter	variance	se	Z	Pr > |Z|
B	DFY(e)	family	19.23	15.679	1.23	0.22
		site	0.00	.	.	.
		fam*site	0.64	0.087	7.31	0.0001
		Residual	157.57	21.561	7.31	0.0001
		TOTAL	177.44			
A	$DFY(e)_a$	family	0.068	0.044	1.55	0.1214
		site	0.000	.	.	.
		fam*site	0.000	.	.	.
		Residual	0.967	0.085	11.44	0.0001
		TOTAL	1.035			
B	$DFY(e)_a$	family	0.114	0.093	1.23	0.2196
		site	0.000	.	.	.
		fam*site	0.004	0.001	7.31	0.0001
		Residual	0.931	0.127	7.31	0.0001
		TOTAL	1.049			

2. Data combined from both densities, all effects random.

Trait	Parameter	Variance	se	Z	Pr > |Z|
PP_{Hb}	family	0.020	0.009	2.21	0.0274
	site	0.016	0.017	0.93	0.3502
	fam*density	0.016	0.008	1.95	0.0509
	density	0.002	0.004	0.57	0.5701
	Residual	0.222	0.012	19.2	0.0001
	TOTAL	0.275			
FFY	family	204	145	1.41	0.16
	site	481	499	0.96	0.3349
	fam*density	189	157	1.2	0.2295
	density	4,055	5,758	0.7	0.4813
	Residual	5,319	267	19.89	0.0001
	TOTAL	10,247			
FFY_a	family	0.040	0.025	1.61	0.1065
	site	0.000	.	.	.
	fam*density	0.026	0.025	1.01	0.3109
	density	0.000	.	.	.
	Residual	0.928	0.047	19.92	0.0001
	TOTAL	0.994			

Trait	Parameter	Variance	se	Z	Pr > |Z|
DFY	family	8.8	10.7	0.82	0.4097
	site	36.5	37.9	0.96	0.3346
	fam*density	20.2	13.1	1.55	0.1221
	density	301.5	428.4	0.7	0.4816
	Residual	400.0	20.1	19.85	0.0001
	TOTAL	767.1			
DFY_a	family	0.028	0.027	1.05	0.296
	site	0.000	.	.	.
	fam*density	0.049	0.031	1.56	0.1176
	density	0.000	.	.	.
	Residual	0.958	0.048	19.89	0.0001
	TOTAL	1.034			
DM_a	family	0.026	0.016	1.61	0.1067
	site	0.000	.	.	.
	fam*density	0.000	.	.	.
	density	0.000	.	.	.
	Residual	0.943	0.046	20.39	0.0001
	TOTAL	0.969			
PyY	family	0.007	0.005	1.51	0.1322
	site	0.003	0.004	0.87	0.3858
	fam*density	0.008	0.005	1.57	0.1164
	density	0.078	0.111	0.7	0.4825
	Residual	0.142	0.007	19.78	0.0001
	TOTAL	0.239			
PyY_a	family	0.055	0.030	1.81	0.0706
	site	0.000	.	.	.
	fam*density	0.035	0.029	1.21	0.2266
	density	0.000	.	.	.
	Residual	0.954	0.048	19.83	0.0001
	TOTAL	1.044			
PyY_b	family	0.007	0.005	1.44	0.1511
	site	0.003	0.003	0.86	0.3903
	fam*density	0.009	0.005	1.65	0.0988
	density	0.079	0.112	0.7	0.4825
	Residual	0.140	0.007	19.78	0.0001
	TOTAL	0.238			

Trait	Parameter	Variance	se	Z	Pr > |Z|
lPyI/II	family	0.010	0.003	3.51	0.0005
	site	0.001	0.001	0.8	0.4257
	fam*density	0.000	0.001	0.44	0.662
	density	0.000	.	.	.
	Residual	0.050	0.003	19.95	0.0001
	TOTAL	0.062			

3.6.3 Calculations for genetic correlations between pairs of characters.

1. Analysis of family means for data combined from high and low density plots

Trait 1	Trait2	Family CP	Family df	Error CP	Error df	Family MCP	Error MCP	Family covariance
PyY_b	PP_{Hb}	2.30	41	1.20	82	0.056	0.015	0.014
PyY_b	DFY	98.33	41	116.62	82	2.398	1.422	0.325
PyY_b	FFY	397.73	41	439.58	82	9.701	5.361	1.447
PyY_b	DM	-8.70	41	-12.01	82	-0.212	-0.146	-0.022
PyY_b	UPP_{U6}	1.44	41	0.04	80	0.035	0.001	0.012
PyY_b	DFY(e)	58.51	41	37.11	41	1.427	0.905	0.174
PP_{Hb}	DFY	5.67	41	-24.52	82	0.138	-0.299	0.146
PP_{Hb}	DM	-27.91	41	-16.43	82	-0.681	-0.200	-0.160
DM	FFY	-1,221	41	-3,547	82	-29.79	-43.26	4.489
DM	DFY	44.19	41	-319.08	82	1.078	-3.891	1.656

Trait 1	Trait2	Variance (trait 1)	Variance (trait 2)	r_g
PyY_b	PP_{Hb}	0.010	0.030	0.80
PyY_b	DFY	0.010	16.502	0.80
PyY_b	FFY	0.010	231.7	0.95
PyY_b	DM	0.010	0.054	-0.94
PyY_b	UPP_{U6}	0.010	0.024	0.74
PyY_b	DFY(e)	0.010	0.677	2.11
PP_{Hb}	DFY	0.030	16.50	0.21
PP_{Hb}	DM	0.030	0.054	-3.97
DM	FFY	231.7	0.054	1.26
DM	DDFY	231.7	16.502	0.03

2. Analysis of family means for low density plots only.

Trait 1	Trait2	Family CP	Family df	Error CP	Error df	Family MCP	Error MCP	Family covariance
PyY_b	PP_{Hb}	4.951	40	6.5609	80	0.124	0.082	0.0139
PyY_b	DM	-20.0318	40	-19.45	80	-0.501	-0.243	-0.0859
PyY_b	FFY	953.42	40	1394.1	80	23.836	17.427	2.1362
PyY_b	DFY	250.07	40	372.7	80	6.252	4.659	0.5310
PyY_b	UPP_{U6}	2.0465	38	0.797	76	0.054	0.010	0.0145
PyY_b	DFY(e)	68.93	40	91.83	40	1.723	2.296	-0.1908
UPP_{U6}	DFY(e)	-15.675	38	-15.67	40	-0.413	-0.392	-0.0103
PP_{Hb}	DFY	51.707	40	54.863	80	1.293	0.686	0.2023

Trait 1	Trait2	Variance (trait 1)	Variance (trait 2)	r_g
PyY_b	PP_{Hb}	0.01773	0.02139	0.71
PyY_b	DM	0.01773	0.7903	-0.73
PyY_b	FFY	0.01773	250.17	1.01
PyY_b	DFY	0.01773	19.2127	0.91
PyY_b	UPP_{U6}	0.01773	0.02456	0.69
PyY_b	DFY(e)	0.01773	9.9034	-0.46
UPP_{U6}	DFY(e)	0.02456	9.9034	-0.02
PP_{Hb}	DFY	0.018	19.2127	0.34

3.6.4 Calculations for genetic correlations among family means at different sites.

Data sets are low density (l), high density (h) and combined from both densities (c).

Character	Data set	$V(\sigma_{Gi})$	σ^2_G	σ^2_{GE}	$\sigma^2_{GE'}$	$r\text{-}g'$
DFY	c	22.286	16.502	80.570	58.283	0.22
DFY	l	40.417	22.162	248.12	207.70	0.10
DFY	h	4.049	16.028	86.122	82.073	0.16
DFY_a	c	0.0000	0.0364	0.1724	0.1724	0.17
DFY_a	l	0.0001	0.0508	0.3634	0.3633	0.12
DFY_a	h	0.0002	0.0722	0.3221	0.3219	0.18
PP_H	c	0.0044	0.0160	0.0563	0.0519	0.24
PP_{Ha}	c	0.0000	0.0620	0.2126	0.2126	0.23
PP_{Hb}	c	0.0201	0.0262	0.0822	0.0621	0.30
lPyI/II	c	0.0002	0.0110	0.0078	0.0076	0.59
PyY	c	0.0045	0.0096	0.0314	0.0268	0.26
PyY	l	0.0124	0.0162	0.0889	0.0765	0.17
PyY	h	0.0004	0.0064	0.0264	0.0260	0.20
PyY_a	c	0.0001	0.0580	0.1671	0.1670	0.26
PyY_a	l	0.0000	0.0718	0.3268	0.3268	0.18
PyY_a	h	0.0003	0.0779	0.2986	0.2983	0.21

3.7 Tables for Section 5.7

3.7.1 Variance and covariances used for calculation of selection indices

Phenotypic (p_{ij}) and genetic (g_{ij}) variances and phenotypic (p_{ij}) and genetic covariances (g_{ij}) used to calculate Index 1 for HVD (high and variable density).

i\j	P_{ij} DFY	PP_H	g_{iy}
DFY	250	-1.502	0.805
PP_H		0.25	0.034

Phenotypic (p_{ij}) and genetic (g_{ij}) variances and phenotypic (p_{ij}) and genetic covariances (g_{ij}) used to calculate Index 1 for LD_{NI} (low density, transplanted beds; no interaction between genotype and density in inner an outer rows).

i\j	P_{ij} DFY	PP_H	g_{iy}
DFY	579	0.342	3.32
PP_H		0.241	0.049

Phenotypic (p_{ij}) and genetic (g_{ij}) variances and phenotypic (p_{ij}) and genetic covariances (g_{ij}) used to calculate Index 1 for LD_I (low density, transplanted beds; interaction between genotype and density in inner an outer rows).

i\j	P_{ij} DFY	PP_H	g_{iy}
DFY	579	0.343	2.122
PP_H		0.241	0.058

Phenotypic (p_{ij}) and genetic (g_{ij}) variances and phenotypic (p_{ij}) and genetic covariances (g_{ij}) used to calculate Index 2 for HVD.

i\j	P_{ij} FW	DM	PP_H	g_{iy}
FW	3001	-82.6	-1.91	3.48
DM		57	-1.58	-0.34
PP_H			0.250	0.034

Phenotypic (p_{ij}) and genetic (g_{ij}) variances and phenotypic (p_{ij}) and genetic covariances (g_{ij}) used to calculate Index 2 for LD_{NI}.

i\j	P_{ij} FW	DM	PP_H	g_{iy}
FW	8192	-156	6.22	16.11
DM		61	-1.19	-0.12
PP_H			0.241	0.0.049

Phenotypic (p_{ij}) and genetic (g_{ij}) variances and phenotypic (p_{ij}) and genetic covariances (g_{ij}) used to calculate Index 2 for LD_I.

i\j	P_{ij} FW	DM	PP_H	g_{iy}
FW	8192	-156	6.22	9.97
DM		61	-1.19	-0.616
PP_H			0.241	0.053

Phenotypic (p_{ij}) and genetic (g_{ij}) variances and phenotypic (p_{ij}) and genetic covariances (g_{ij}) used to calculate Index 3 for HVD.

i\j	P_{ij} DFY(e)	PP_{U6}	G_{iy}
DFY(e)	250	-0.835	0.245
PP_{U6}		0.23	0.028

Phenotypic (p_{ij}) and genetic (g_{ij}) variances and phenotypic (p_{ij}) and genetic covariances (g_{ij}) used to calculate Index 3 for LD_{NI}.

i\j	P_{ij} DFY(e)	PP_{U6}	G_{iy}
DFY(e)	579	-0.803	1.733
PP_{U6}		0.228	0.071

Phenotypic (p_{ij}) and genetic (g_{ij}) variances and phenotypic (p_{ij}) and genetic covariances (g_{ij}) used to calculate Index 3 for LD_I.

i\j	P_{ij} DFY(e)	PP_{U6}	G_{iy}
DFY(e)	579	-0.803	0.645
PP_{U6}		0.228	0.049

3.7.2 Coefficients (b_i) for selection indices.

	Index	Index character			coefficient		
		1	2	3	b_1	b_2	b_3
HVD	1	DFY	PP_H		0.0042	0.161	
HVD	2	FW	DM	PP_H	0.0012	0.004	0.143
HVD	3	DFY(e)	PP_{U6}		0.00140	0.127	
LD_{NI}	1	DFY	PP_H		0.0056	0.195	
LD_{NI}	2	FW	DM	PP_H	0.0018	0.0062	0.189
LD_{NI}	3	DFY(e)	PP_{U6}		0.00344	0.324	
LD_I	1	DFY	PP_H		0.00353	0.2135	
LD_I	2	FW	DM	PP_H	0.00082	0.0029	0.185
LD_I	3	DFY(e)	PP_{U6}		0.0014	0.219	

4. Appendix 4. Tables for Chapter 6.

Raw Data and Data Analysis

Method a evaluations

RAW DATA: plot averages (average of two 3m² subplots)

BLOCK	VAR	DFY	HPP	PYG	PYR	FMI	SF	DENS
1	Hypy	421	2.53	10.66	0.91	603	7.15	12.0
1	pyper	192	2.28	4.40	1.15	539	8.33	16.1
2	Hypy	476	3.04	14.46	1.07	573	8.31	11.6
2	pyper	370	2.36	8.72	1.14	569	7.21	17.1
3	Hypy	329	2.66	8.76	1.02	591	8.70	9.2
3	pyper	411	2.35	9.67	1.09	582	8.70	8.5
4	Hypy	562	2.87	16.06	1.12	578	8.47	10.8
4	pyper	412	2.46	10.15	1.01	549	8.46	8.3

Difference between PC-hypy and Pyper in each block
Values = (plot value for PC-hypy) - (plot value for Pyper)

BLOCK	DFY	HPP	PYG	FMI	SF	DENS
1	230	0.25	6.3	64	-1.18	-4.1
2	106	0.68	5.7	4	1.09	-5.5
3	-82	0.31	-0.9	8	0.00	0.7
4	150	0.40	5.9	29	0.01	2.5
MEAN	101	0.41	4.3	26	-0.02	-1.6
stdev	132	0.19	3.4	27	0.93	3.8

VAR - variety
DFY - dry flower yield (g/m²)
HPP - pyrethrins content, evaluated by HPLC (%)
PYG - pyrethrins yield (g/m²)
PYR - pyrethrins I/II
SF - index of variability of flower maturity
DENS - plants / m²

ANOVA TABLES

DRY FLOWER YIELD

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	55690.50000000	13922.62500000	1.60	0.3650
Error	3	26149.37500000	8716.45833333		
Corrected Total	7	81839.87500000			
	R-Square	C.V.	Root MSE		DFY Mean
	0.680481	23.53910	93.36197477		396.62500000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
VAR	1	20301.12500000	20301.12500000	2.33	0.2244
BLK	3	35389.37500000	11796.45833333	1.35	0.4048

PYRETHRINS CONTENT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.45505000	0.11376250	6.29	0.0813
Error	3	0.05423750	0.01807917		
Corrected Total	7	0.50928750			
	R-Square	C.V.	Root MSE		HPP Mean
	0.893503	5.234406	0.13445879		2.56875000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
VAR	1	0.34031250	0.34031250	18.82	0.0226
BLK	3	0.11473750	0.03824583	2.12	0.2770

PYRETHRINS YIELD

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	72.86070000	18.21517500	3.07	0.1921
Error	3	17.82070000	5.94023333		
Corrected Total	7	90.68140000			
	R-Square	C.V.	Root MSE		PYG Mean
	0.803480	23.52567	2.43725939		10.36000000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
VAR	1	36.12500000	36.12500000	6.08	0.0904
BLK	3	36.73570000	12.24523333	2.06	0.2838

PYRETHRINS I/II

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.01495000	0.00373750	0.37	0.8220
Error	3	0.03063750	0.01021250		
Corrected Total	7	0.04558750			

R-Square	C.V.	Root MSE	PYR Mean
0.327941	9.500062	0.10105691	1.06375000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
VAR	1	0.00911250	0.00911250	0.89	0.4145
BLK	3	0.00583750	0.00194583	0.19	0.8967

FMI

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1965.50000000	491.37500000	1.33	0.4254
Error	3	1112.50000000	370.83333333		
Corrected Total	7	3078.00000000			

R-Square	C.V.	Root MSE	FMI Mean
0.638564	3.360739	19.25703335	573.00000000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
VAR	1	1404.50000000	1404.50000000	3.79	0.1468
BLK	3	561.00000000	187.00000000	0.50	0.7059

SF (FLOWER STAGE VARIABILITY INDEX)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1.44235000	0.36058750	0.83	0.5840
Error	3	1.30063750	0.43354583		
Corrected Total	7	2.74298750			

R-Square	C.V.	Root MSE	SF Mean
0.525832	8.062966	0.65844197	8.16625000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
VAR	1	0.00061250	0.00061250	0.00	0.9724
BLK	3	1.44173750	0.48057917	1.11	0.4673

DENSITY

Source	DF	Type I SS	Mean Square	F Value	Pr > F
VAR	1	5.19	5.19	0.72	0.4598
BLK	3	50.85	16.93	2.34	0.2518
Error	3	21.75	7.25		

Pyrethrins content (method b)

RAW DATA

var	blk	pyr	Hpp
hypy	4	0.9	2.92
hypy	4	1.0	2.79
hypy	4	0.9	2.81
hypy	4	1.0	2.78
hypy	4	0.9	2.75
PC	4	1.0	2.75
PC	4	1.0	2.73
PC	4	1.0	2.48
PC	4	0.9	2.54
PC	4	0.9	2.70
pyper	4	1.1	2.42
pyper	4	1.0	2.52
pyper	4	1.0	2.44
pyper	4	1.1	2.59
pyper	4	1.2	2.67
pyoneer	4	1.2	2.28
pyoneer	4	1.2	2.26
pyoneer	4	1.2	2.14
pyoneer	4	1.2	2.23
pyoneer	4	1.1	2.35
hypy	2	1.1	2.84
hypy	2	1.1	2.56
hypy	2	1.1	2.71
hypy	2	1.2	2.37
hypy	2	1.1	2.60
pyoneer	2	1.2	2.23
pyoneer	2	1.2	2.20
pyoneer	2	1.2	2.22
pyoneer	2	1.2	2.29
pyoneer	2	1.2	1.81
pyper	2	1.1	2.35
pyper	2	1.2	2.56
pyper	2	1.1	2.45
pyper	2	1.1	2.41
pyper	2	1.1	2.30
PC	2	1.6	2.46
PC	2	1.2	2.58
PC	2	1.2	2.55
PC	2	1.5	2.59
PC	2	1.3	2.40

ANOVA TABLES

PYRETHRINS CONTENT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	1.60455750	0.22922250	15.21	0.0001
Error	32	0.48232000	0.01507250		
Corrected Total	39	2.08687750			
	R-Square	C.V.	Root MSE		HPP Mean
	0.768880	4.929042	0.12277011		2.49075000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
VAR	3	1.41352750	0.47117583	31.26	0.0001
BLK	1	0.17822250	0.17822250	11.82	0.0016
VAR*BLK	3	0.01280750	0.00426917	0.28	0.8371

Tests of Hypotheses using the Type I MS for VAR*BLK as an error term

Source	DF	Type I SS	Mean Square	F Value	Pr > F
VAR	3	1.41352750	0.47117583	110.37	0.0014

PYRETHRINS I/II

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	0.63600000	0.09085714	13.98	0.0001
Error	32	0.20800000	0.00650000		
Corrected Total	39	0.84400000			
	R-Square	C.V.	Root MSE		PYR Mean
	0.753555	7.198444	0.08062258		1.12000000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
VAR	3	0.15000000	0.05000000	7.69	0.0005
BLK	1	0.25600000	0.25600000	39.38	0.0001
VAR*BLK	3	0.23000000	0.07666667	11.79	0.0001

Tests of Hypotheses using the Type I MS for VAR*BLK as an error term

Source	DF	Type I SS	Mean Square	F Value	Pr > F
VAR	3	0.15000000	0.05000000	0.65	0.6330