

**Agronomic and seed quality studies in
pyrethrum
Tanacetum cinerariaefolium Sch. Bip.**

School of Agriculture

by

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Submitted in fulfilment of the requirements for the Degree of Doctor of Philosophy

University of Tasmania, December 1998

I declare that this thesis contains no material which has been accepted for a degree or diploma by the University or any other institution, except by the way of background information and duly acknowledged in the thesis, and to the best of my knowledge and belief no material previously published or written by another person except where due acknowledgement is made in the text of this thesis.

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Abstract

At the time this research project was initiated, the Tasmanian pyrethrum industry was attempting to establish crops by sowing rather than by planting of 'splits' or seedling plugs. This thesis investigated plant density and sowing times required for maximum yield. Studies were also conducted to improve chances for successful crop establishment from seed. That work investigated environmental requirements for germination and aspects of seed quality and seed production.

Previous studies have examined the influence of density on yield of pyrethrins, but none had been conducted in sown trials, in cool temperate environments, over a wide range of plant densities. Furthermore, the influence of plant density on components of yield had not been intensively investigated. This work identified that maximum yield was achieved in the first season at between 16 and 39 plants/m² and at or above 16 plants/m² in the second season following establishment. Yield was a function of dry flower yield rather than due to change in percentage of pyrethrins in the flowers. Higher flower yield was associated with greater above- ground dry matter production. The yield component which increased with plant density was number of flowering tillers/m². Yield components decreasing with density included number of flowering tillers/plant, flowers/tiller and dry weight/flower. Other aspects of changing plant morphology and development with density investigated included, crop height, mean flower maturity and plant survival. The recommendation to industry was to aim for a plant population of above 16 and below 39 plants/m². Yields achieved in this work were unprecedented in the pyrethrum agronomy literature.

No reported studies have examined the influence of time of sowing on pyrethrins yield. Field studies showed that sowings later than mid-November led to significant reductions in yield in the first flower harvest. Yield reductions were associated with decreased dry matter production/plant and flowering tillers/plant. Later sowings resulted in plants failing to flower or in significant reductions in the extent of flowering. Sowing earlier than mid-November resulted in no significant increases in yield.

As pyrethrum crops have not been traditionally established by sowing, only scant information was available on requirements for germination, or seed quality. A field study and three laboratory trials investigated the influence of temperature and seed quality on germination and emergence. Results demonstrated that rate and final emergence varied significantly at different times of year. In general, the proportion of viable seed sown that emerged and survived was very low. Both rate of emergence and final emergence

percentage were associated with temperature. Laboratory investigation of germination percentage, rate and uniformity of germination of a seed lot under a range of constant temperatures confirmed the previously reported findings relating to germination characteristics of this temperate species. Unexpected though was the high proportion of dead seed found at temperatures only several degrees higher than the temperature for optimal germination rate. Six seed lots were subsequently germinated at low, medium and high temperatures which provided some insight into the seed death phenomenon as well as documenting the range of behaviours from different seed lots. An explanation for differences in germination behaviour involving seed maturity was proposed for different seed lots and this was tested in a subsequent study. Finally, the effects of an 18 month storage period on the germination characteristics seed lots was investigated in a laboratory trial. There was little change in most germination parameters except for time to complete germination which increased in all seed lots after storage and uniformity of germination which decreased. Changes were assumed to be due to satisfaction of an afterripening requirement.

Laboratory studies investigated the influence of seed mass on various germination and seedling development characteristics. Variation in seed mass within seed lots was identified but this had little impact on rate of germination or other germination parameters. However, a following study revealed that heavier seedlings emerged from seeds that germinated earliest within seed lots. Furthermore, heavier seedlings demonstrated faster development than did lighter seedlings. Although seedling mass was found to be associated with rate of germination within seed lots, this factor failed to explain significant differences in mean time to germinate between seed lots. It was subsequently recommended to industry that cleaning on a size or mass basis could improve seed quality.

The influence of harvest date on seed quality and quantity were investigated. Results indicated that losses of larger achenes from the harvested flowers were occurring with lateness of harvest. Characteristics of the seed including mean seed mass, proportion of viable seed in the sample, germination percentage and rate of germination were found to vary considerably with harvest date. Data generated in this work were found to be consistent with the model which proposed that variability in germination parameters was due largely due to maturity of seed at harvest. A following study revealed that relative position on the capitulum also had a profound influence on germination parameters of the seed. The recommendation for industry emanating from the harvest date work was that flowers should be cut at a field capitulum moisture of 25%.

Finally, variation in seed quality and quantity was evaluated both within and between capitula. This study revealed that small flower heads produced fewer and smaller seed than larger heads. Outer achenes, regardless of whether they came from large or small capitula were found to germinate more rapidly than inner achenes. Peripheral achenes weighed more than central achenes and gave rise to heavier seedlings.

The methods and results in this study will serve as a valuable source of information for agronomists and plant breeders working on improvement of pyrethrum production. The investigations on seed quality and seed production provide a sound base for future efforts to improve seed quality and crop establishment. The findings presented provide the Tasmanian pyrethrum industry with critical information with respect to target plant densities, sowing times, seed quality and seed production. It is expected that implementation of findings from the study will prove to be pivotal in continued industry profitability and expansion.

Acknowledgements

I would like to thank the following people and organisations for their assistance and support:

Professor Robert Clark, my academic supervisor, for his enthusiasm, guidance and support through this project.

Brian Chung (Research Manager, Botanical Resources Australia, Pty. Ltd.) my industry supervisor, for his helpful discussions in trial design and his facilitation of financial support.

Richard Holloway, who helped with trial implementation and maintenance and provided sound practical advice.

Staff at Forthside Vegetable Research Station, Lyndon Butler and Vaughan Trebilco, who maintained the area around the trial, irrigated trials and were always willing to facilitate activities on the farm.

Staff of Botanical Resources Australia, particularly Trish Bourke for her collection of samples and help with trial harvest and maintenance, and Mark, Neville, Evelyn, and others for their willing help in harvesting of trials.

Alistair Gracie who assisted with pyrethrins content analysis.

Dr. N. Mendham for his helpful comments on the density study.

The Horticultural Research and Development Corporation and the Australian Post Graduate Award (Industry) for their financial support.

My mother, Mrs. Lies Fulton, who was a continuous support to me and my family.

My wife, Amabel Fulton who assisted with the conduct of trials and editing, and shared our challenges through the time this work was conducted.

I dedicate this work to

My wife Amabel,

to Rebecca, our little girl (deceased)

and our son Thomas Michael Townshend who brings joy into our lives.

A little seed

A little seed

For me to sow ...

A little earth

To make it grow ...

A little hole,

A little pat ...

A little wish,

And that is that.

A little sun,

A little shower,

A little while,

And then - a flower!

Mable Watts

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Chapter 1: Introduction

1.1 General description of the pyrethrum plant

Pyrethrum, *Tanacetum cinerariaefolium* Sch. Bip. (previously known as *Chrysanthemum cinerariaefolium* L.) is a tufted perennial herb, belonging to the family Asteraceae. Pyrethrum is found naturally along the east coast of the Adriatic sea extending from Italy to Albania and into the mountainous regions of Croatia, Bosnia and Herzegovina (Bhat, 1995). The plant has a rosette growth habit with leaves divided into leaflets. The flowering tillers terminate in white daisy-like flowers and attain a height of about 750 mm (Plate 1.1). Pyrethrum is grown as a perennial crop for the production of pyrethrins which are active both as insecticides and insect deterrents. Pyrethrins' rapid knockdown and kill of insects is due to the ability of these compounds to interfere with the normal insect nerve impulse transmission (Soderlund, 1995). Pyrethrum is grown commercially in highland tropical climates where it flowers in many flushes through the year, or in cool temperate climates at low altitude where it flowers in one annual flush.

About 90% of the pyrethrins produced by the plant are found in the ovary and on the achenes of open composite flowers (Purseglove, 1974). The composite flower, or capitulum, is 30-40 mm in diameter and consists of two types of florets: peripheral ray florets possessing white corollas (petals) and yellow disc florets (Plate 1.2). The disc florets are hermaphrodite whereas the ray florets are female only (Purseglove, 1974). Both ray and disc florets possess single achenes. The achene is therefore an indehiscent dry fruit developing from a monocarpellary ovary. In fact, the achene is better described as a cypsela since it develops from an inferior ovary and therefore includes non-carpellary tissues. The achenes are homomorphic, since there is only one distinct achene shape found under disc and ray florets (Heywood and Humphries, 1977). Pyrethrum achenes are approximately 4 mm long, have five ribs and are often tapering. Achenes appear to range in size and can be slightly curved (Plate 1.3).

The seed of *Tanacetum spp.* generally possess a papery or thin seed coat. The seed coat does not completely deteriorate in the achene and retains an exotestal palisade called a cynareae (Corner, 1976). There are no reports of the development of the ovule, embryo, or seed coat in pyrethrum. There is a description of flower morphology and a discussion of the effect of rain on seed development (Brewer, 1968).

1.2 Active constituents of pyrethrum flowers

The six insecticidal esters (Pyrethrin I, Cinerin I, Jasmolin I, Pyrethrin II, Cinerin II, Jasmolin II) are extracted from pyrethrum flowers and are collectively known as the

pyrethrins (Crombie, 1995). Pyrethrins are extracted from dried, ground, then pelletised flowers (or in Tasmania, achenes) (Plate 1.4) using organic solvents such as hexane (Plate 1.5) to produce an oleoresin concentrate containing 20-35% pyrethrins. Until recently two companies processed most of the world's oleoresin, removing pigments, waxes and resins. The processes used, which are based upon proprietary solvent extraction, produce the low staining light coloured product sought by industry. In Tasmania, a pyrethrum refining process using carbon dioxide in a super-critical-fluid extraction (Plate 1.6) has been developed, thus avoiding exposure of the product to heat and risk of flashing of the solvents (Carlson, 1995).

1.3 World production of natural pyrethrins

Pyrethrum has been used as an insecticide for over 200 years. Unlike many synthetic insecticides it has not been rendered ineffective due to resistance, nor has it been found to be dangerous for either humans or the general environment. It has survived as a major commercial insecticide despite strong competition from DDT, chlorinated hydrocarbons and most recently from synthetic pyrethroids. There have been significant advances in broadening the usage pattern of pyrethrins in recent years, contributing to its increasing share of the \$7 500 million world-wide insecticide industry (Elliot, 1995). Recent technical developments have contributed to an increasing market share of natural pyrethrins-based products in; stored product insect control, medical and veterinary pest control, protection of food, control of insect infestations of feed and farm animals, and finally in domestic insect control (Casida and Quisad, 1995).

The main countries producing pyrethrum in 1992 were Kenya, Tanzania, Rwanda, Australia (Tasmania) and Papua New Guinea. Total world dry flower production was estimated to be 18 000 tonnes in 1992, with Kenya producing about 69% of that total (Wainaina, 1995). Production in that country is carried out by 50 000 to 60 000 small scale farmers who depend on the crop for cash income. African production declined from about 90 000 tonnes per annum in the early 1970's to a low of 13 000 tonnes in 1991. The reduction in production over that period was largely due to a decrease in world demand as a result of intense competition from synthetic pyrethroids and a lowering of price due to a build up of stock. Current resurgence in production, projected to increase to previous highs, is attributed to the recent general shift towards natural products (Wainaina, 1995).

1.4 The pyrethrum industry in Tasmania

Pyrethrum production in Australia and in particular, Tasmania, has been recently reviewed (Mac Donald, 1995). Interest in pyrethrum was generated in 1978 by Professor

Robert Menary, of the University of Tasmania, who was searching for new high value, low volume horticultural crops for Tasmania. He secured the services of Dr. K. Bhat, a pyrethrum plant breeder who provided the University with seed for a base pyrethrum population. Several critical traits were identified and selected for by Bhat and Menary that were considered essential for mechanical harvesting, including synchronous flowering, lodging resistance and high yield per plant. By 1980 the research team had produced a high yielding pyrethrum clone which was subsequently patented as 'Hypy' by the University of Tasmania. By 1992 improvements in tissue culture, planting, agronomy and harvesting contributed to commercial production of 2 000 tonnes of flowers from 1 200 ha. MacDonald, 1995 reported that "for the first time a fully mechanized and intensively managed pyrethrum production system had emerged". In the following years, confidence in the industry declined due to failure of the company to return profits to the multinational owner, British Oxygen Company (BOC). The business was subsequently sold to local interests and the privately owned company, Botanical Resources Australia (BRA) is now Tasmania's pyrethrum producer. BRA is now Australia's sole natural pyrethrins producer. The industry is dependent upon technologically advanced growers who produce a range of other food and industrial crops including potatoes, peas, poppies and onions.

In the past the crop has been planted at various times of the year. Flowers are mature by early January and crops begin to lose their brilliant white appearance (Plate 1.7). At this stage the crop is cut at ground level and rowed (Plate 1.8). After a period of a week or more, depending on the dryness of the flowers, achenes are harvested using combine harvesters. The resumption of vegetative growth (Plate 1.9) after harvest is dependent upon summer breaking rains.

There has been a diverse effort to increase efficiency of pyrethrum production in order to increase the industry's competitive ability, both for land on a local level, and price on the international market. Over the last six years the industry has changed its method of crop establishment from dependence upon clonal vegetative material, to planting of plug seedlings (Plates 1.10, 1.11 and 1.12) to the current practice of direct sowing. Changing the crop establishment system from vegetative splits to seedling plugs eliminated the need for vegetative nursery propagation, but required the development of new seed-based technologies. The new establishment technique was a significant breakthrough for the industry and reduced the cost of crop establishment from around \$3 500/ha to \$2 500/ha.

Initial efforts to establish commercial pyrethrum crops through direct sowing had mixed success, but improved sowing practices and seed quality have improved the level of success. Commercial trial sowings were conducted in late 1995 and were harvested for the first time in January 1997. In sites where sowing was successful, yields were between 40 and 50 kg pyrethrins/ha. Such yields are double the highest research yields reported outside of Tasmania. In January 1998, a crop produced the unprecedented yield of 72 kg pyrethrins/ha, with several others producing yields of over 50 kg/ha. The Tasmanian industry is currently in an expansion phase and aims to increase its area of commercial pyrethrum crops from 1 200 ha to 2 000 ha by the year 2 000. Prime cropping land along the north coast of the island from Table Cape in the west to Winnaleah in the east will be required for this expansion, with over 110 growers participating. The industry aims to supply up to 40% of the world demand for natural pyrethrins.

Pyrethrum seed crops are currently grown in 1.0-1.5 ha plots located on Tasmania's north coast, and in the south east regions of the Coal River and Derwent River Valleys. Parental material is clonally propagated and planted into 1.6 m wide beds at a plant density of 4-6 plants/m². Weed control, nutrition and irrigation husbandry practices are the same as those used for commercial crops. Special effort is taken in timely and frequent application of fungicides to reduce the risk of disease in the seed. In an attempt to increase the chance of outcrossing, a selected mate line is planted in the adjacent bed. The crop is not isolated from other pollen sources. Often several seed lines are produced in the same seed production area and pollen transfer is not limited to within the desired crosses. The seed crop is windrowed at some time after flowers lose their petals and allowed to field dry prior to the achenes being threshed by a combine harvester. Achenes are cleaned on a gravity table (Plate 1.13) which effectively separates seed from hollow achenes. Seed is stored at 4°C for sowing several months later.

1.5 Tasmania - location, climate and soil

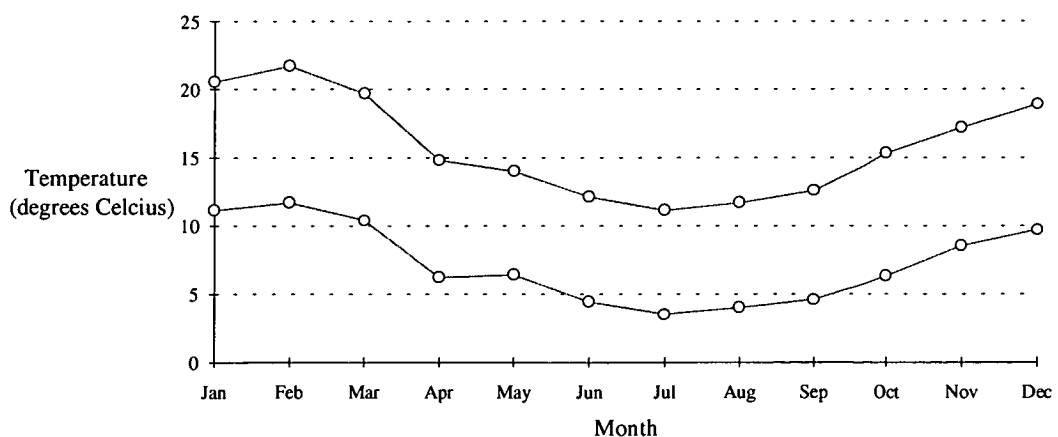
Tasmania is the most southerly, permanently inhabited island of Australia. The island state, lying some 40° to 43° latitude, covers an area of approximately 64 000 square kilometres and has a population of approximately 450 000 (Plate 1.14). The main economic activities are mining, vegetable and industrial cropping, dairy, wool and livestock production, forestry, aquaculture, fishing and tourism.

The climate of Tasmania varies significantly between regions, but may be broadly described as cool temperate. Maximum summer temperatures generally average between 18°C and 20°C while average maximum winter temperatures are 10°C to 12°C. The climate is moderated by the surrounding seas and prevailing north westerly winds. In

February, surface sea temperatures average 17°C and 15°C in the north and south of the island respectively. By August, surface sea temperatures are 13°C and 10.5°C in the north and south respectively (Gentili, 1972). Average annual rainfall varies from 1 400 mm in the west and north eastern highlands to 500 mm in eastern parts of the state (Gentili, 1972). Rainfall is generally higher in winter, but summer rains are still significant.

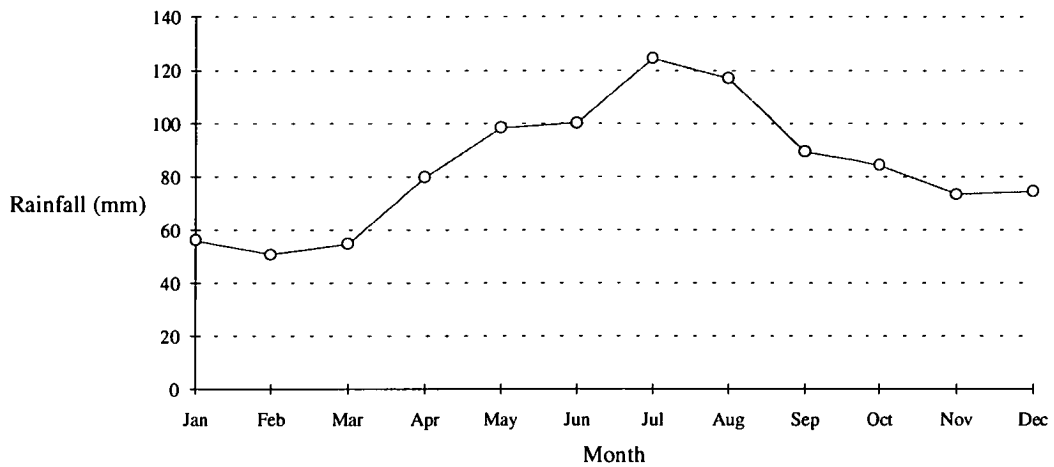
Pyrethrum production in Tasmania is restricted to the north coast of the island, although successful crops have been established in the Coal River and Derwent River valleys in the island's south east. The main environmental factor thought to limit suitability of certain regions for pyrethrum production is frost, particularly spring frosts which may freeze developing flower buds. Much of the central region of the island is therefore considered unsuitable or risky for pyrethrum production, although this has not been critically evaluated (Plate 1.15). The north coast of the island is classified as 'humid warm' using Thornwaite's method (Gentili, 1972). Although some pyrethrum crops are located on the easterly part of the north coast, most of the production is on the north coast which has a slightly higher rainfall and a higher population of land owners who are accustomed to intensive cropping practices. Pyrethrum is grown predominantly on the low-lying hills behind coastal towns located along this most agriculturally-productive region of the island. Trials in this thesis were located in the heart of the pyrethrum production region on the north coast at Forthside Vegetable Research Station (FVRS), mid-way between two population centres; Devonport and Ulverstone (Plate 1.14). Mean daily maximum and minimum temperatures recorded at the weather station at FVRS are presented in Figure 1.1. Figure 1.2 presents mean monthly rainfall data for FVRS.

Figure 1.1 Mean maximum and minimum monthly temperatures for Forthside Vegetable Research Station (FVRS)



Note (1) Monthly figures are thirty year averages.

Figure 1.2 Mean monthly rainfall at FVRS



Note (1) Monthly figures are thirty year averages.

A major factor which contributes to the value of the north coast as a highly productive region is its soil. Most of the cropping is conducted on ferrosols, which are referred to locally as krasnozems. Krasnozems are the predominant soil type of the region and are well regarded for their favourable agronomic properties (Plate 1.14). These soils are derived from extruded volcanic deposits of basic volcanic rock such as basalt. Isbell (1994) in reviewing the characteristics of this soil described them as red to brown, acid, strongly structured clay soils ranging in depth from 1 to 7 m. Moody (1994) reviewed the chemical fertility of krasnozems and referred to previous studies which reported deficiencies of N, P, K, Ca, Mg and various micro-nutrient deficiencies and toxicities. Moody (1994) concluded that sustainable use of krasnozems depended upon enhancement of organic matter levels, regular remediation of low pH, minimisation of erosion and replacement of nutrients. Bridge and Bell (1994) investigated the effects of continuous cropping on krasnozems and reported significant reductions in water infiltration rates, bulk density, organic carbon, aggregate stability, water holding capacity and crop water extraction. In the krasnozems examined, water content of the soil at -0.01 MPa to -1.5 MPa decreased from approximately 300 to 200 l/m³. This available moisture appeared to change little with respect to cropping history and soil depth. In summary, krasnozems are known for their capacity to maintain structure under intensive cropping, thus their initially high infiltration rates and available moisture. They are also known for their relatively poor chemical fertility, and more recently their degradation has been recognised through non-sustainable patterns of utilisation.

1.6 Study objectives and thesis structure

This thesis aims to provide information that will allow industry to increase yields and decrease the incidence of low and erratic crop establishment. At the time of initiation of this study in June 1994, successful commercial pyrethrum sowings had not been accomplished. Sowing techniques, nutrition, weed and disease control techniques had not been developed. Amid this host of unknowns, work initially focused on the influence of plant density and sowing time on pyrethrins yield in sown pyrethrum crops. Trials were established with the following objectives:

Density trials

- i. To evaluate the yielding potential of sown pyrethrum crops
- ii. To identify plant densities under which pyrethrins yield is maximal
- iii. To investigate how various yield components change with increased density.

Time of sowing trial

- i. To investigate the influence of month of sowing on pyrethrins yield
- ii. To investigate how yield is influenced by sowing time

To improve chances of successful crop establishment from seed, studies needed to be conducted on environmental requirements for germination and factors affecting seed quality. Trials were established with the following objectives:

Germination and seed quality trials

- i. To assess the performance and germination characteristics of pyrethrum seed
- ii. To investigate the effect of seed mass on germination characteristics of the seed
- iii. To investigate the effect of seed maturity at harvest on germination characteristics of the seed
- iv. To investigate the extent to which individual seed quality in a seed lot of known maturity, varies between and within flower heads.

The thesis is divided into chapters based on experimental work in each of the areas of plant density, time of sowing, germination, seed mass, seed maturity, and seed location on the capitulum. Each chapter begins with a literature review which overviews general theory and examines other factors of importance to the area of investigation. The literature review attempts to provide a theoretical framework within which the current work can be viewed. Research questions are posed for each area, and the experimental work conducted to investigate these questions is described. The results are reported and discussed in relation to the literature, as appropriate. The concluding general discussion

chapter outlines the findings in relations to the study objectives and their implications for industry. General methods are presented in appendices.



Plate 1.1 A grower and his pyrethrum crop in flower and ready to harvest at Table Cape on Tasmania's north coast

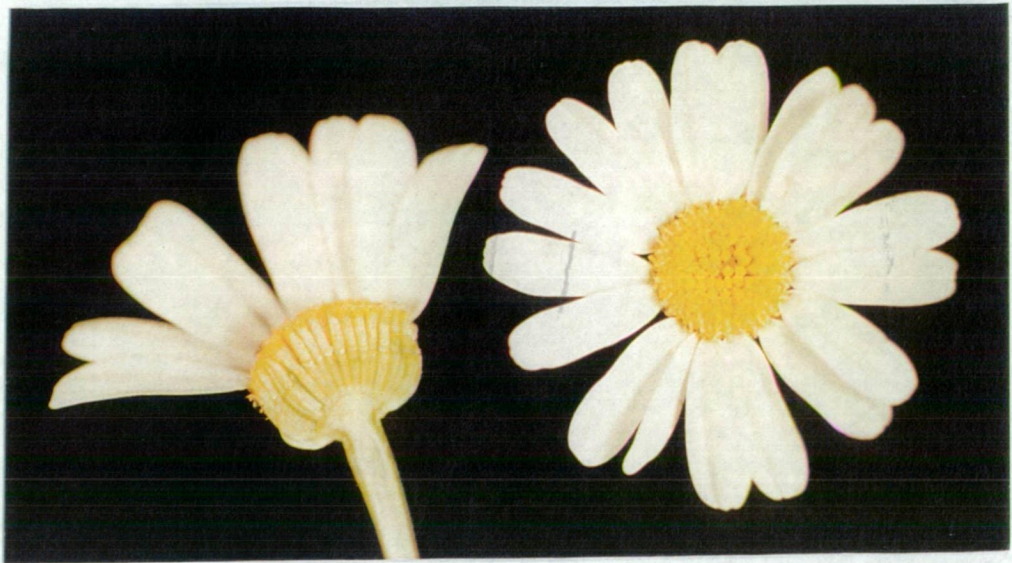


Plate 1.2 The pyrethrum flower (1.5x magnification) showing the white ray florets, and the yellow disc florets subtending individual achenes.



Plate 1.3 A range in achene sizes from within a commercial seed lot (10x magnification)



Plate 1.4 Pelletised pyrethrum achenes (magnification 0.5x)

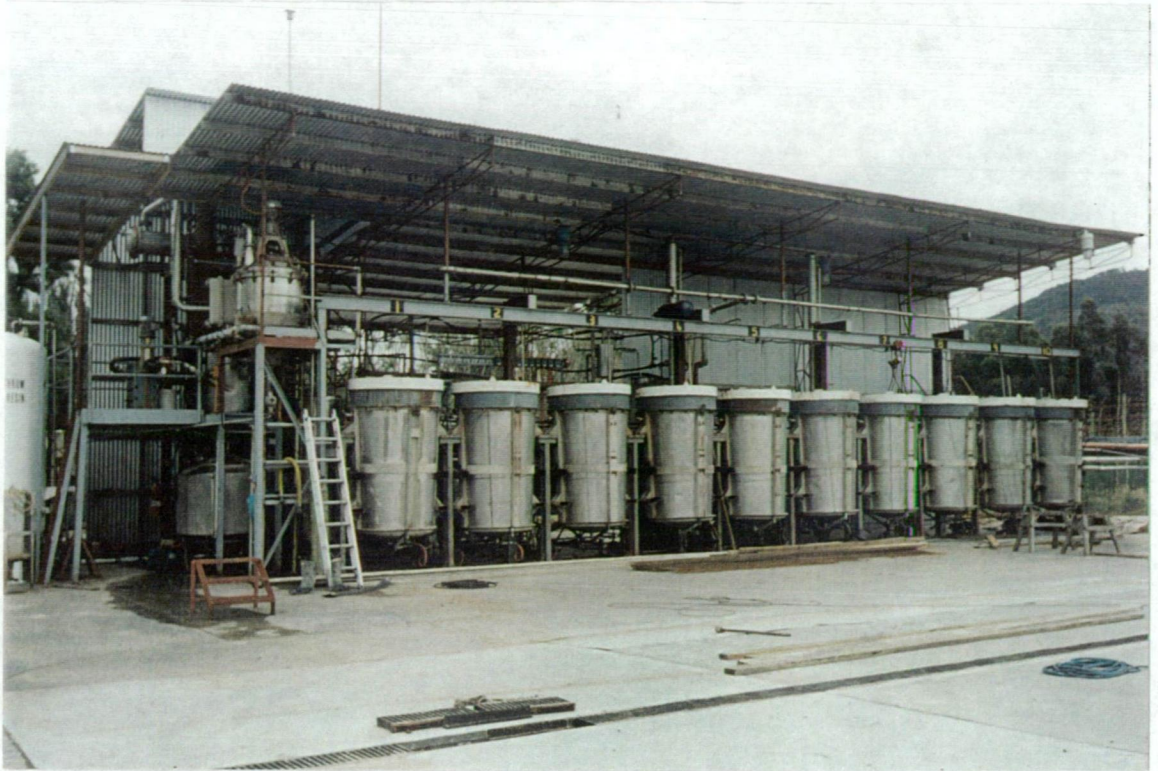


Plate 1.5 Extraction of oleoresin. Pellets are sequentially rinsed through nine vats of hexane. Once all the pyrethrins have dissolved in the hexane it is boiled and condensed off, leaving oleoresin.



Plate 1.6 A carbon dioxide super critical fluid extraction column (on left) used for processing oleoresin to achieve a low staining light coloured product by removing pigments, waxes and resins



Plate 1.7 Pyrethrum crops in full flower on the north coast of Tasmania, just prior to optimum harvest time



Plate 1.8 Cutter-rower in operation in a pyrethrum crop



Plate 1.9 A grower (left) and field officer in a crop several months after harvest. The crop is showing vegetative regrowth.



Plate 1.10 Production and collection of seedling plug trays in preparation for planting



Plate 1.11 Planting of seedling plugs



Plate 1.12 Planting of individual seedling plugs



Plate 1.13 Gravity table in operation, separating hollow non-seed from seed

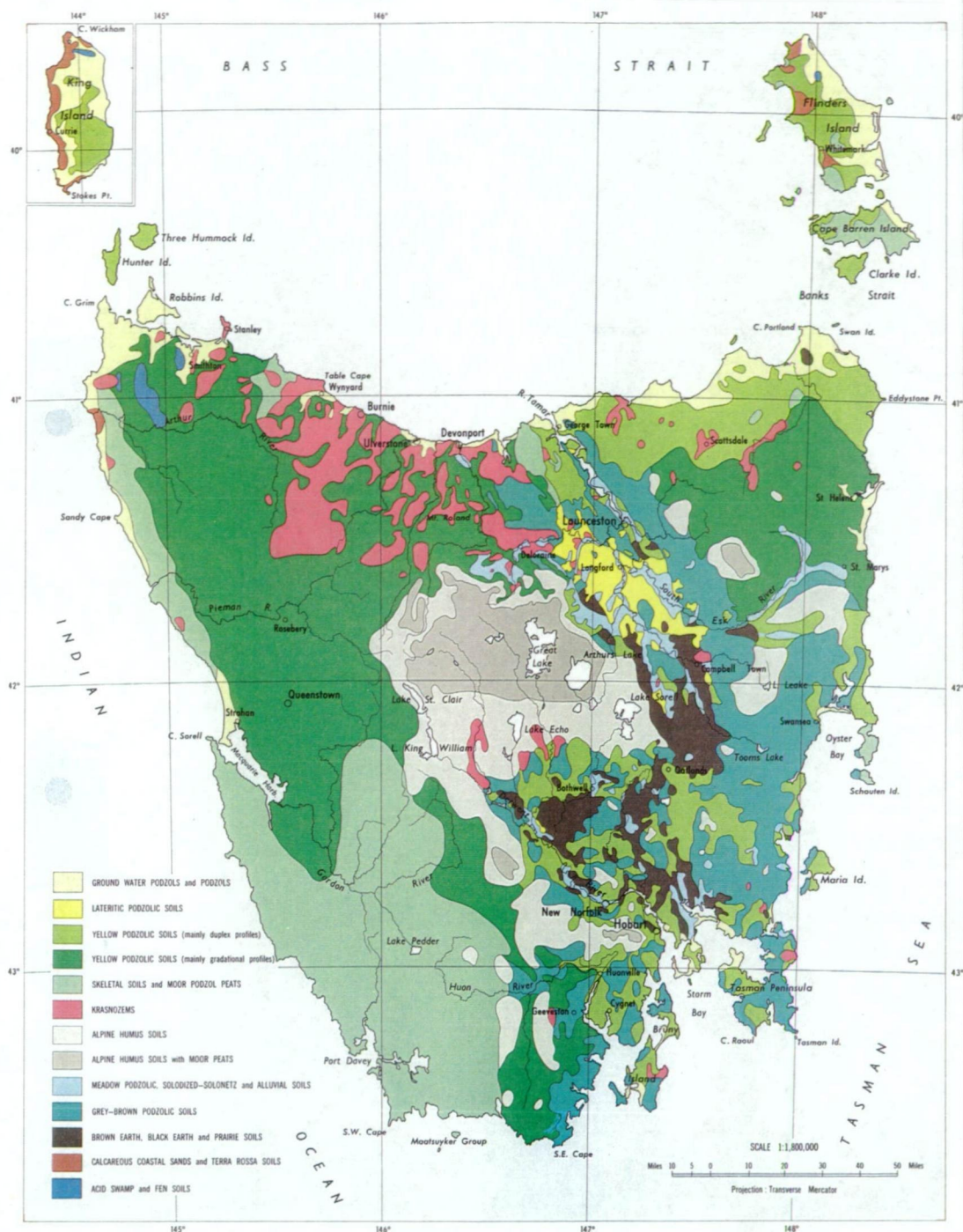


Plate 1.14 A map of Tasmania displaying soil types (Source: Nichols and Dimmock, 1965 p26)

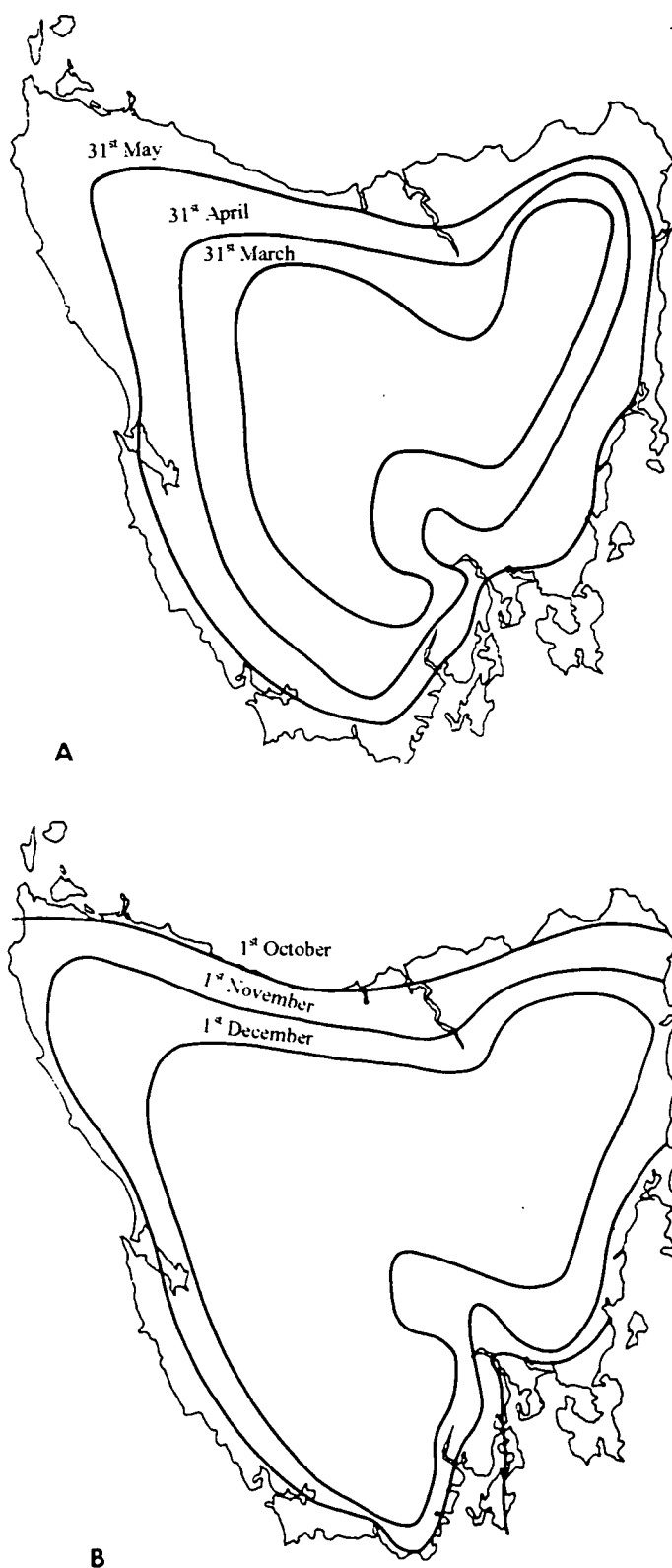


Plate 1.15 Average dates for (A) first occurrence and (B) last occurrence of air frosts (Source: Langford, 1965 p10).

Chapter 2: The effect of plant density on pyrethrins yield

2.1 Introduction

Since 1997, Tasmanian pyrethrum crops have been established by sowing seed directly into prepared soils. Choice of plant density is no longer limited by the costs associated with planting splits or seedling plugs. The current work investigates opportunities for increasing yield of pyrethrins through optimising plant density. The work reviews the effects of competition in crops in general before discussing past density studies in pyrethrum.

Two density trials conducted on the north coast of Tasmania are described. The first, a randomised block design trial established from sown seed; the second a fan or Nelder systematic design established from seedling plugs. The fan trial was established largely as an insurance policy in case the sown trial failed. Therefore only preliminary analyses for this trial are presented. Plant densities investigated in previous studies ranged from 2 to 9 plants/m². The current studies investigated densities ranging from 2 to over 100 plants/m². Two seasons of yield data are presented and compared with yields generated in other pyrethrum-producing regions and another local trial. The changes in individual yield components with density are then described.

Various other factors of potential importance to production including aspects of crop maturity, crop height and lodging, inequity between productivity of individual plants and differences in productivity of flowers from different parts of the plant are discussed. The results from the study have significant commercial cropping implications.

2.2 Literature review: The influence of plant density on yield

Discussion in this review firstly outlines the competition effect in relation to other possible between-plant interactions. Competition in cropped plants is then investigated. Changes in the size distribution of plants, self thinning and the over-riding importance of light as a limiting factor in competition are discussed. Partitioning of plant components is discussed before finally describing previous density studies conducted in pyrethrum.

2.2.1 *Interactions between plants*

In plant communities, most interactions occur through intermediaries such as physicochemical resources, herbivores, mutualists, toxins, and microbial symbionts. Goldberg (1990) suggests that "such indirect interactions consist of two distinct

processes: one or both plants has an *effect* on the intermediary and a *response* to the changes in the abundance of that intermediary". Various types of indirect interactions are presented in Table 2.1.

Table 2.1 Types of indirect interactions among plants^a

Type of interaction	Intermediary	Effect	Response	Net
Exploitation competition	Resources	-	+	-
Apparent competition	Natural enemies	+	-	-
Allelopathy	Toxins	+	-	-
Positive facilitation	Resources	+	+	+
Negative facilitation	Resources	-	-	+
Apparent facilitation	Natural enemies	-	-	+

^a In this classification, resources of plants include mutualists such as pollinators or disperses as well as abiotic resources such as light, water, mineral nutrients, CO₂. + and - in the Effect, Response, and Net columns indicate the effect of plants on the intermediary, the response of some 'target' plant to the abundance of the intermediary, and the net effect of plants on the 'target' plant, respectively.

(Source: Goldberg, 1990 p29)

Furthermore, Goldberg (1990) comments that both the effect and the response components of competition must be significant and of an appropriate sign for competition to occur. Agricultural cropping systems may be generally regarded as managed, even-aged monocultures. In such systems, environmental and plant dependent factors are homogeneous and synchronous in comparison to natural plant communities. The plants in such systems generally possess very similar life histories and genetic characteristics (Radosevich and Roush, 1990). In agriculture we are primarily interested in how interactions between plants influence crop productivity. Most of the plant interaction studies investigating competition fall into the 'exploitation competition' type proposed in Table 2.1.

Radosevich and Roush (1990) suggest that agricultural scientists investigating competition are generally not concerned with understanding ecological processes especially if the results of their studies are definitive. Although this may be true, such empirical and phenomenological studies have long been used in the interpretation of findings from more complex natural systems.

2.2.2 Competition between plants in even-aged monocultures

Many studies have shown that changing plant density can drastically effect growth, survivorship and reproductive output (Harper, 1977). With increasing intensity of competition several phases of competition are recognised:

- i. *Low - density*, where individuals do not compete for resources;

- ii. *Medium - density*, where plants respond to moderate density through a reduction in size and reproductive output;
- iii. *High - density*, where self thinning begins to decrease plant population; and
- iv. *Extremely high - density* where flowering and seed production may be suppressed while no loss in over-all dry weight production occurs.

Antonovics and Levin (1980) suggested that those phases also reflect a time course of individuals as they grow and develop within the monoculture.

Li *et al.* (1996) reported that in even-aged monocultures, it is recognised that there are at least three main effects of increasing plant density, the competition density effect, alteration in the size structure of the population, and self-thinning. These effects along with competition for light and the influence of competition on partitioning of plant parts are discussed below.

The competition - density effect

In the previous section it was proposed that for competition to be present, there has to be a negative or depleting *effect* on resources. This is usually described as a per individual change in availability of that resource. Under some circumstances, increasing plant density may in fact increase resources available per plant (Hunter and Aarren, 1988) but this will not be discussed here. The *response* of plants to limiting resources in a competitive environment is generally observed as a decrease in plant dry weight. Other plant responses include increased resource uptake, decreased resource loss, and increased conversion of internal stores to new growth (Chapin *et al.*, 1987).

A series of papers, the first of which was authored by Kira *et al.* (1953) are regarded as seminal studies for density research in even-aged monocultures. Kira *et al.* (1953) proposed that plant mass can be used as a measure of competition, where the smaller the mean plant mass the more intense the competition within the population. In a later work in that series, Shinozaki and Kira (1956) proposed the following reciprocal equation to describe the consequences of competition over a range of plant densities (Equation 1) where y is the yield per unit area, D is density and a and b are fitted parameters.

$$y = D/a + bD \text{ (Equation 1)}$$

This equation accounts for yield at very low densities differentiating it from equations proposed earlier in that series. This widely used equation, which defines an asymptotic yield-density relationship is often referred to as the law of 'constant final yield' (Li *et al.*, 1996). Many studies demonstrate that the yield density function may be asymptotic as

described, or parabolic (Willey and Heath, 1969). To account for the possibility of the parabolic relationship, Watkinson (1980) reworked an earlier attempt (Bleasdale and Nelder, 1960) at incorporating that potentiality into an equation (Equation 2) where w_m , a , and b are fitted parameters and w_m is the predicted mean weight of a plant when $D < 1/a$.

$$y = w_m D / (1 + aD)^b \text{ (Equation 2)}$$

The value of b determines whether at high density the yield decreases. When $b = 1$, Equations 1 and 2 are equivalent (Watkinson, 1980).

A range of other equations describing the yield density function have been proposed since Kira *et al.* (1953) but none, including those presented here, are universally accepted (Li *et al.*, 1996). Some noteworthy examples of other equations describing the yield-density relationship include; the reciprocal yield function of Farazdaghi and Harris (1968) which accounts for both asymptotic and parabolic situations, the inverse quadratic equation of Nelder (1966) and the exponential equation of Gillis (1979) which both account for a depression of yield per plant observed at low density.

The biological significance of parameters in Equation 1 has been reviewed (Willey and Heath, 1969). They demonstrated that as density approached zero, parameter a of Equation 1 approached a^{-1} . Willey and Heath (1969) suggested that assuming 'a' gives some indication of yield per plant in a non-competitive environment, then 'a' may be considered as a measure of the genetic potential of a crop in a particular environment. Using Equation 1 again, as density increases to its asymptotic limit, yield approaches b^{-1} , which is considered to be a measure of potential of that environment (Willey and Heath, 1969; Khah *et al.*, 1989).

Size structure of the population

The equations proposed in the previous section described models of variation in yield with density but the influence of density on variation between individual plants was not considered. Variation in plant mass within a population can arise from a number of sources and stages in the life of the plant (Benjamin and Hardwick, 1986). Variation in plant size is present initially with populations of seed and seedlings displaying normal mass distributions (Koyama and Kira, 1956). Normal distribution becomes progressively more skewed towards the higher mass end (positively skewed) upon further growth and development (Rogers, 1977; Firbank and Watkinson, 1990). Skewness of the population is also found to be greater in higher density populations (Firbank and Watkinson, 1990). As plants increase in age and as plant density increases, Coefficient of Variation (CV) also tends to increase (Benjamin and Bell, 1985 ; Stoffella and Fleming, 1990).

Firbank and Watkinson (1990) cited works which described changes in population weight distribution with increasing density in various ways. Examples include; an increase in bimodality prior to self thinning of the smaller plants (Ford 1975), increasing skewness (Weiner and Solbrig, 1984; Hara, 1988), and increase in Gini coefficient (Weiner, 1985; Weiner and Thomas, 1986). Other measures used for describing variation in the population include; coefficient of variation (CV) (Li *et al.*, 1996) and kurtosis (Rabinowitz, 1979). Bendel *et al.*, 1989 compared the skewness coefficient, CV, and Gini coefficients as measures of inequity within populations and made recommendations under which these measures are most appropriately used. Bendel *et al.* (1989) argued that in the expected situation where the underlying distribution of the variable follows the two-parameter log normal model, all three measures are equally valuable. Although this was suggested to be so, they recommended that in situations where relative precision is required to assess inequality, CV was the measure of choice.

The extent of variability among plants within populations was addressed in the review of Benjamin and Hardwick (1986). They cited various works in which CV's of less than 10% were achievable where plants were grown under controlled conditions and in non-competitive environments. Conversely, in high density populations, CV's of 100-200% were reported (Benjamin and Hardwick, 1986).

Firbank and Watkinson (1990) suggest that there are numerous causes of variation in plant size of even-aged monocultures including; variation in seed size (see Grey *et al.*, 1991), variation in sowing depth, variation in growth rate, duration of growth and herbivore and pathogen attack. Competition, as a major cause of variation in growth rate, will be the only factor discussed here. Variation in relative growth rate (RGR) between plants generated by uneven sharing of limited resources has been reviewed (Weiner, 1985; Benjamin and Hardwick, 1986). Benjamin and Hardwick (1986) report that variation in RGR between plants must be due to variability in resources available to the plants and/or variability between plants in their ability to use those resources. In that review, models explaining variability in RGR include; RGR as a function of mass of different plants (Westoby, 1984), RGR influenced by space per plant (Mithen *et al.*, 1984), RGR as a function of space per plant and the influence of neighbouring plants (Mead, 1971).

Self thinning

With plants increasing in size and differences in growth rate occurring between individual plants, some become increasingly dominant while others become suppressed. Aikman and Watkinson (1980) proposed that once a plant's growth rate became negative, and competition was 'one-sided' (Hara, 1988) self thinning was generated. The basic theory relating to self-thinning is attributed to Yoda *et al.* (1963) who observed that mortality increased at high densities in spite of there being no visible causes. They (Yoda *et al.*, 1963) proposed that once a population reached a maximum density, mortality occurred as described in Equation 3 where ω is mean weight per plant, c is a species dependent constant, N_s is the final density. The constant k is reported to take the value of 3/2 for a wide range of species (Westoby, 1984).

$$\omega = cN_s^{-k} \text{ (Equation 3)}$$

This equation describes how density declines as mean yield per plant increases in a monoculture. The equation effectively states that in a self-thinning population, the mean weight per plant will be proportional to plant density. There is significant dispute over that value of k (Weller, 1987) and dispute over other aspects of the self-thinning rule (Weller, 1990; Lonsdale, 1990; and Weller, 1991).

Self thinning occurs among the smallest plants in the population (Weiner and Thomas, 1986) which can have the outcome of decreasing variability or skewness in the population. Mohler *et al.* (1978) reported that during self thinning of a pure stand of trees, skewness was greatest at the onset of self thinning and decreased as mortality proceeded. Antonovics and Leven (1980) suggested that greatest mortality often coincides with periods of most rapid growth and that this may be due to increased intensity of competition. Weiner and Thomas (1986) proposed that: "The observation that size inequality decreases during self thinning is consistent with the hypothesis that competition asymmetry and self thinning are due to shading". Weiner (1988) states that there is significant evidence that asymmetric competition which accentuates size inequality and density dependent mortality is driven by shading. Evidence that light is often the resource limiting growth with increased density is presented below.

Light as a major limiting factor

Although research has been undertaken investigating competition for soil based resources (e.g., Baldwin, 1976) most research has investigated light as the major factor limiting growth in the competitive environment (Donald, 1961; Aikman and Benjamin, 1994). Numerous studies have demonstrated that nutrient availability had little or no influence on the establishment of plant size hierarchy within populations, which is seen as

an indicator of competition between plants (Weiner and Thomas, 1986; Li *et al.*, 1996). Weiner and Thomas (1986) suggest that prior to closure of the canopy, plants are able to compete for limited resources, including light in relation to 'some aspect of their size'. Conversely, upon canopy closure, smaller plants receive less than their light requirement for a sustained RGR which results in greater size inequity, even less light availability, and may ultimately result in self thinning.

As light in the 390-760 nm wave band is used most efficiently by green plants for the fixation of carbon, this light is known as photosynthetically active radiation (PAR) and measurement of light is in photosynthetic photon flux density units (PPFD). Although efficiency of light interception depends on many factors, an optimum leaf area of uniform distribution, which is achievable through an appropriate plant density, is the main factor that influences PPFD interception (Papadopoulos and Pararajasingham, 1997). An equation (Equation 4) exists relating PPFD interception in the closed crop canopy to leaf area index (LAI) which is the ratio of leaf area to ground area. I and I_0 of Equation 4 are the total PPFD at points above and within the canopy respectively and k is the direct beam extinction coefficient.

$$\ln(I) = \ln(I_0) - kLAI \text{ (Equation 4)}$$

If $k = 1$, and $LAI = 1$, then all leaves (presented horizontally and distributed randomly between the points of I and I_0) will intercept 63% of the incident PPFD (Papiadopoulos and Pararajasingham, 1997). The value of k is a function of the orientation of the leaves forming the canopy, therefore species with more vertically oriented leaves, such as grasses, are found to have smaller values of this constant than species with broad horizontal leaves (Acock, 1991).

The relationship between PPFD interception and rate of biomass production is well recognised. Papiadopoulos and Pararajasingham (1997) cite several examples of crops grown at a range of densities in which higher yields were generated at higher densities. Such higher yields were reportedly due to the higher density treatments achieving maximum PPFD interception earlier in the life of the crop than lower density treatments. Scaife and Jones (1976) proposed that in the absence of other limiting factors, biomass production may be determined by level of radiation (Equation 5). Where w is the shoot dry weight at time t , w_0 is the shoot dry weight at time 0, and w_{max} is the asymptotic value of w , which is the value which would be reached by a plant grown in isolation and k is the early relative growth rate of isolated plants. This equation is effectively an extension of Equation 1.

$$w^I = w_0^{-I} e^{-kt} + w_{max}^{-I} + bD \text{ (Equation 5)}$$

Recent works on crop competition for light include crop growth models based on leaf expansion and light interception (Goudriaan and Monteith, 1990), and crop growth modelling under varying conditions in a uniform canopy (Aikman and Scaife, 1993; Tei *et al.*, 1996; Li *et al.*, 1996).

Rather than investigating the influence of competition for light and plant density on the yield of a crop, which assumes equal, or two sided competition between individuals, models have been developed which describe the influence of self shading on individual plants, for example by Aikman and Benjamin (1994).

Components of plant yield

Since in many circumstances the aim of crop production is not to maximise dry matter yield, but rather to maximise production of some anatomical part or some secondary product from the plant, a knowledge of how that component varies with density is necessary. Harper (1961) cited a range of classic papers in which the influence of plant density on yield components was investigated. Harper (1961) suggested that plants with determinate and indeterminate growth systems respond very differently to density. Harper (1961) proposed that where flowering apices do not arise directly from the major vegetative apices, plants respond to density by decreasing the number of floral parts. Conversely, where conversion of a vegetative apex into a flowering one occurs at an early stage, the plant responds to density by reducing the size of the flowering parts. Not surprisingly, seed weight is found to be one of the least plastic of the yield variables, while seed number per plant changes considerably. Harper (1961) noted that there are exceptions to this such as the sunflower, a determinate plant which displays significant plasticity in seed weight. Harper (1961) suggests that plant stage of development at which competition becomes intense may influence yield components.

A simple technique for evaluating the percentage of product in the total yield of crop, or economic yield is commonly expressed as the harvest index (Equation 6).

$$\text{Harvest Index} = 100 \times (\text{Economic Yield}) / \text{Biological Yield} \quad (\text{Equation 6})$$

Biological yield is usually considered as the total above-ground dry matter of the crop although Beadle (1987) stated that a better understanding of crop performance would be achieved if total dry matter were used as the denominator in Equation 6. Bleasdale (1967a) suggested that although the asymptotic yield function (Equation 1) was suitable for describing vegetative yield and parabolic for reproductive yield, it was recognised that significant variation from these generalisations occurred.

Evaluation of a 'partitioning coefficient' is another commonly used approach (Duncan *et al.*, 1978) for describing proportions of vegetative and reproductive growth in some crops. The ratio of reproductive to vegetative growth yield is presented in Equation 7 where p is the partitioning coefficient, W_f is the fruit (or seed) yield, W_t is the total shoot dry weight, and W_0 is the total shoot dry weight at the start of fruit growth.

$$W_f = p(W_t - W_0) \text{ (Equation 7)}$$

Craufurd (1996) suggested that plant density studies create variation in W_t and W_0 and provide a useful way of investigating p . Furthermore (Craufurd, 1996) proposed that regression of W_f on W_t generated from different density sowings can provide an intercept value, W_v which gives the minimum vegetative biomass required before DM can be allocated to reproductive sinks.

The systematic growth of a plant organ in relation to the whole plant can be described as allometric growth as in Equation 8 where Y is the mass of the organ, x is the mass of the whole plant, α is the growth coefficient of the organ and b is a constant.

$$Y = bx^\alpha \text{ (Equation 8)}$$

$$\text{alternatively } \ln Y = \ln b + \alpha \ln x$$

It is on the basis of Equation 6 that Barnes (1979) described carrot yield in relation to whole carrot plant yield. The allometric description was used again (Li *et al.*, 1996) with carrots but on that occasion a time function was included which allowed valid description of relative root growth at different stages of crop development.

Plant density may not only affect components of yield but also (a) position on the plant on which that yield is generated (Grey *et al.*, 1983), (b) types of seed generated by the plant (Baker and O' Dowd, 1982), and (c) maleness of the resultant flowers (Ackerly and Jasienski, 1992). Density therefore has the capacity to influence many aspects of partitioning in plants.

Factors other than plant density can also have a significant influence on partitioning of yield in the plant. Perennial species generally allocate a significantly lower proportion of reserves to reproductive effort (Larcher, 1995) than do annuals. Plants of the same species growing at different altitudes may have vastly different partitioning strategies (Larcher, 1995). Date of sowing can have a significant influence on harvest index (Adisarwanto and Knight, 1997).

2.2.3 Density studies in pyrethrum

Density studies conducted on pyrethrum have investigated plant populations in the range two to nine plants/m². Yield increases were reported for plantings at the highest density, but the advantages were not considered economic due to the high cost of manual crop establishment (Parlevliet *et al.*, 1968a and b). A more recent study (Rajeswara Rao and Singh, 1982) investigated the influence of a similar range of plant densities on pyrethrins yield and reported optimum yield at 5.5 plants/m². A range of plant parameters were evaluated in this trial including plant height, 100-flower weight and flower diameter, none of which were influenced by plant density. The only yield parameter that varied greatly with plant density was flower weight per hectare due to greater numbers of flowers being produced. No significant differences in pyrethrins concentration were observed for the different density treatments. Further work (Sastry *et al.*, 1992) reported an optimum plant density of 4.4 plants/m² and attributed the high yield to an increase in the number of flowers per plant and dry matter production efficiency.

All of the past research investigating optimum plant density in pyrethrum has been conducted on a narrow range of plant densities and on highland crops where flowering occurs in a number of flushes through the year. There are no reports investigating the influence of very high plant density on yield. Nor were there any studies where trials were conducted in temperate environments which promote the occurrence of a single annual flowering. Previous studies provided little information into pyrethrum performance at high density in a cool temperate climate.

2.3 Materials and methods

2.3.1 Randomised complete block density trial (block trial)

Range of densities selected for investigation

A wide range of population densities was investigated since no research had been conducted investigating pyrethrum plant density in temperate, low altitude environments and where density was not limited by labour or cost of vegetative establishment.

Two plants/m² was chosen as the lowest density as this was a density at which little or no competition was expected (at least in the first year). Most densities evaluated were chosen to investigate densities below 32 plants/m² since previous studies demonstrated optimum yields below that density. High density treatments of 64 and 100 plants/m² were included with the expectation that significant self thinning would occur in those plots.

Trial site and ground preparation

The trial was located at Forthside Vegetable Research Station (FVRS) (41°10'S, 146°40'E) in northern Tasmania. Preparation of the field included mouldboard ploughing six weeks before sowing. Two weeks prior to sowing, the ground was worked with a power rotary hoe. Basal fertiliser (N:P:K) was applied with a drill (14:16:11 750 kg/ha) and followed by a final pass of the rotary hoe in order to thoroughly incorporate fertiliser and prepare a fine, firm seed bed.

Sowing

Sowing was conducted on 5/10/95 using a tractor mounted Ojoid trial drill (Plate 2.1). The cone drill was found to be suitable for precise sowing of small plots. The seed provided by British Oxygen Company (BOC) was Pyper 3A95F and was reported to have a germination capacity of above 80%. Details of densities and row spacings are presented in Table 2.2 (see also Plates 2.2 to 2.8).

Table 2.2. Block density trial plant arrangement details

Code	Density (plants/m ²)	Between row spacing (B) (mm)	Plants per linear metre	Within row spacing (A) (mm)	Rectang- ularity (B) : (A)	Plot length (m)
A	2	300	0.6	1666	0.18:1	8
B	4	300	1.2	833	0.36:1	16
C	8	300	2.4	416	0.72:1	8
D	16	300	4.8	208	1.44:1	16
E	24	150	3.6	277	0.54:1	8
F	32	150	4.8	208	0.72:1	8
G	64	150	9.6	104	1.44:1	8
H	100	150	15	67	2.24:1	8

Four rows were sown in the 300 mm between-row spacing treatments, while nine rows were sown in the higher density treatments. Between-row spacing was reduced at higher densities in order to maintain approximate rectangularity. High sowing rates (8.0 kg/ha) were used to ensure nominal treatment densities were achieved (the thinning procedure is described in a later section). Seed was sown at a depth of 10 mm to 15 mm.

Irrigation

The trial was irrigated with sprinklers on risers attached to aluminium pipes (as in Plate 2.10). Immediately after sowing 10 mm of irrigation was applied. This quantity of water was applied every second day on seven occasions in order to maintain a moist environment for the germinating seed. After 14 days many of the seeds had germinated but very few had emerged. In order to maximise emergence, water was applied on two

more occasions over the next two weeks. Water was then replenished for a 1:1 deficit with scheduling based on a cumulative deficit of 35 mm assessed with a pan evaporimeter.

Weed and disease control

The non-selective, non-residual herbicide Roundup® (a.i. Glyphosate 33%) was applied 14 days after sowing which was prior to any significant emergence of the pyrethrum. As there was no recognised program for selective weed control in seedling pyrethrum, hand weeding of the site for broad-leaved weeds was conducted six weeks after sowing and again four weeks later. Directly after the second hand weeding, by which time most plants had at least two true leaves, a combined application of a dinitroaniline herbicide, Stomp 330E® (a.i. Pendimethalin, 33%) at 3.0 l/ha. and a nitrile herbicide Totril EC® (a.i. Ioxynil, 25%) at 300 ml/ha was applied. This had both pre-emergent and foliar contact activity and was active on a wide range of weed species. That herbicide combination, along with regular hand weeding, was the main broad leaf weed control strategy used in the trial. The herbicides were subsequently applied each March and October to coincide with expected autumn and spring weed emergence. Perennial grass weed control was achieved using boom or spot spray applications of either of the aryloxyphenoxy herbicides, Verdict EC® (a.i. Haloxyfop, 10.4%), or Fusilade EC® (a.i. Fluazifop-p, butyl 21.2%), at recommended rates.

Diseases which infect the flower and require fungicidal treatment to prevent flower dry weight losses include *Sclerotinia sclerotiorum*, *Sclerotinia minor*, and *Mycosphaerella ligulicola*. A spray program to control these diseases was initiated at first flower opening (approximately 1% of flowers achieving stage 3, as described in Appendix 1.1). Four weekly applications of chemical were conducted. Either the benzimidazole fungicide Benlate WP® (a.i. Benomyl 50%) at 1.5 kg/ha or the conazole fungicide Folicur EC® (a.i. Tebuconazole 25%) at 1.0 l/ha were applied in alternate weeks over a one month period.

Thinning out procedure

Thinning of plants to the nominal densities was conducted using long (3.00 m) measuring sticks with evenly spaced markers representing the required plant spacing. Thinning was undertaken firstly to achieve the correct density and secondly, to obtain regular spacing between the remaining plants within the rows. The procedure was conducted 12 weeks after sowing when plants remaining were expected to have a high chance of survival but well before any expected competition. Thinning out was not conducted on the highest

density treatment since the those plots were assessed as being within the appropriate range.

Experimental design, replication

A randomised complete block design trial with six blocks (replicates) was used. Data were analysed using Microsoft Excel 4.0™ and checked with Systat 5.2.1™ software.

2.3.2 Vegetative assessment

A destructive harvest was conducted prior to the onset of winter (25/5/96) nearly eight months after sowing. At that time the plants had been in the ground for the warmest months of the year, but had not yet been through a winter (Plate 2.9). The four highest densities had achieved closed canopies. The evaluation had two aims: firstly, to confirm the plant densities prior to winter (during which time, losses of weaker plants were considered possible) and secondly to investigate differences in mean plant dry weight between densities.

In the four lowest densities, a number of plants were randomly selected for harvest from within the middle beds of the plot, whereas in the highest densities, a quadrat area (447 mm × 447 mm) was randomly selected (again excluding outside rows) towards the end of each plot. Whole plants were harvested, weighed and dried for 24 hours at 70°C to obtain dry weights. The number of plants harvested in the lowest densities were 2, 4, 8, and 8 in the 2, 4, 8, and 16 plants/m² treatments respectively. As it was clear from observing the trial that there was no plant loss in the lowest density treatments, nominal densities were regarded as actual plant densities. Only four of the six replicates were assessed in this vegetative assessment since differences between treatments were observed to be large. Therefore, harvesting of the remaining two replicates was regarded as unnecessary. Harvested replicates were in no way preferentially selected:

2.3.3 First season flower harvest

Three sequential harvests were conducted over a one month period during the first flowering of the trial (1/1/96, 16/1/96, and 1/2/96). Sequential harvests were conducted to accommodate for the possibility that flower maturity varied between density treatments.

Either a number of plants were harvested (four lowest densities) or a set area was harvested (highest densities) for each harvest date. Plant numbers or areas harvested are presented in Appendix 1.4. In the low density treatments, where individual plants were found to be missing or dead, a replacement plant was harvested and the area of harvest

and calculation of plant density adjusted accordingly. Samples were taken from all six replicates on each harvest occasion. Mean dry weight of the above ground parts of the harvested plants was assessed after 24 hours at 70°C.

Components of yield (Table 2.3) were investigated at harvest, although pyrethrins concentration of samples (Appendix 1.2) was not evaluated until the second harvest season 18 months later. Methods used for evaluation of components B, C, D, and E of Table 2.3 are described below.

Table 2.3. Components of yield of a pyrethrum crop.

Code	Yield component
A	plants/hectare
B	flowering tillers/plant
C	flowers/tiller
D	dry weight/flower
E	pyrethrins concentration/flower
$A \times B \times C \times D \times E = \text{pyrethrins yield/hectare}$	

Estimation of pyrethrins yield per hectare

Pyrethrins yield was calculated by the multiplication of individual plot yield component data. That product was then subject to ANOVA analysis.

Assessment of flowering tiller number per plant

Plants were harvested with hand shears as close to the crown of the plant as practically possible. The crown from which the leaf and all of the flowering tiller initials originate, remains relatively prostrate. The arms of the crown extend horizontally and at their highest point are only 10 to 15 mm above the soil surface. The number of flowering tillers arising from the arms of the crown were counted for all plants sampled. All tillers cut were primary since investigation of the crown after harvest revealed that no branching of the tillers occurred below the level of harvest.

Assessment of flower number per flowering tiller

The technique used for assessing flower number per tiller in harvests one and two was to randomly select six tillers from harvested plants. The number of plants assessed in this way is presented in Appendix 1.5. In an effort to decrease the labour involved in the third harvest, total number of tillers per plot were counted, the flowers stripped from those tillers, and a calculation of mean flowers per tiller conducted.

Assessment of mean dry weight per flower

In assessing dry weight per flower in harvests one and two, all the flowers from the tillers sampled for flower number per flowering tiller were plucked from the branches and oven

dried at 70°C for 24 hours. Mean dry weight per flower was calculated in the third harvest from a sub-sample of approximately 150 flowers obtained by 'cone and quartering' flowers combined from all the plants in the plot.

Pyrethrins concentration

Only some treatments and replicates were selected for assay. Pyrethrins were assayed in 4, 8, 16, 24 and 64 plants/m² treatments and in four of the six replicates. As differences between treatments were found to be minimal and variability between assays was low, it was not deemed necessary to conduct assays on the remaining treatments and replicates. Flower samples were dried at 50°C for 24 hours and stored in sealed, labelled plastic bags at -18°C. Analysis of total pyrethrins (Appendix 1.2) was conducted on the stored samples after the second harvest. Storage of dried pyrethrum flowers even at room temperature does not result in decreased pyrethrins (Ikahu and Ngugi, 1990).

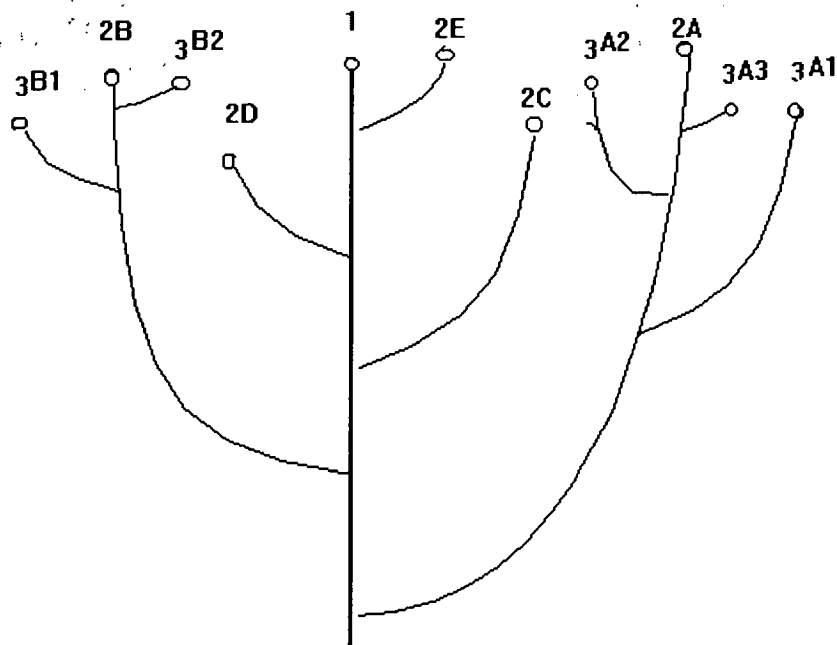
Flower maturity

Prior to drying for mean dry weight analysis, flowers were sorted into different developmental stages and mean flower weight was investigated. Mean flower maturity and the standard deviation around those means was evaluated (Appendix 1.1).

Flower position on tiller

The percentage of main flowering tillers possessing flowers in various positions (according to the classification in Figure 2.1) was evaluated in the first of the sequential harvests. Tillers collected for assessment of flowers/tiller were used for this evaluation.

Figure 2.1 Tiller branching hierarchy classification.



Variability in tiller number between plants

Variability in tiller number between plants within treatments was investigated in both first and second seasons. This was examined by evaluating skewness on an individual plot basis. Skewness as a measure of variability in plant populations is well recognised in the literature (Benjamin and Hardwick, 1986; Bendel *et al.*, 1989) and is an embedded function in Microsoft Excel 4.0™ spread sheets.

2.3.4 Second season flower harvest of the block trial

A harvest was conducted with the aim of assessing actual flower yields rather than calculated yields (evaluation one). In the first season pyrethrins yield was calculated by the multiplication of yield components. That product was then subject to ANOVA analysis. This same technique was used again in the following season (evaluation two) Since plant density was found to have little or no influence flower maturity in the first season, harvesting was only conducted on a single occasion in the second season.

Evaluation one

As in the previous season either a number of plants were harvested (four lowest densities) or a set area was harvested (highest densities). Plant numbers or areas harvested are presented in Appendix 2.3. In the low density treatments, where individual plants were found to be missing or dead, a replacement plant was harvested. A new calculation of density was conducted which accounted for the the lower population in that plot. Samples were taken from all six replicates.

Flowers were stripped from plants and combined with other flowers from the plot thus providing yield data for the plot. Two sub-samples were taken from the bulked flowers by cone and quartering, sample 'A' a sample of approximately 180 g of flowers, and sample 'B' which contained exactly 200 flowers.

Sample A was weighed and dried at 50°C for 36 hours to obtain dry weights for each treatment. Drying the flowers at 50°C ensured that no loss of pyrethrins occurred during the drying process. The dry flower sample allowed for the calculation of mean dry weight of flowers per plant. In order to confirm the findings in the previous season, for four replicates of treatments B, D, and F, mean flower maturity was evaluated prior to drying (Chapter 8.1.1). The number of replicates and treatments evaluated for flower maturity were limited since no significant differences between densities were identified in the previous season. Obtaining % dry matter data from sample A allowed the mean dry weight/flower to be calculated from sample B. Sample B was used to estimate flowers/plant through calculating mean fresh weight per flower.

Evaluation two

It was considered unnecessary to harvest all the eight density treatments to investigate changes in yield components with density. Therefore, plants were harvested from all six replicates but only from treatments 4, 8, 16, 32, and 64 plants/m². The number of plants or area harvested was 4, 6, 8, 0.5 m² and 0.5 m² respectively. The above treatments were selected for this analysis since optimum plant density was suspected to be somewhere within the range examined.

Tillers from each of the harvested plants were counted. A maximum of six plants was randomly selected from those harvested for further dissection. From each selected plant, six flowering tillers were randomly selected, giving 36 primary tillers which were individually assessed for flower number per tiller.

To investigate the influence of plant density on yield of flowers located differently on the tiller a further investigation was undertaken. Primary and 2B flowers (Figure 2.1) were collected from the flower number per tiller investigation, and assessed for stage of development, mean flower dry weight and pyrethrins assay.

2.3.5 Fan trial

This trial was located next to the randomised block density trial (Plate 2.10). Irrigation, basal fertiliser, and fungicide program were the same as those used in the block trial. Apart from a single pre-planting, pre-emergent herbicide application of the diphenyl ether herbicide Goal EC® (a.i. Oxyfluorfen 24%) at 4.0 l/ha and Stomp at 6.0 l/ha applied and incorporated in the top 100 mm of soil just prior to planting, the weed control program was the same as for the block trial. Flower disease control measures were the same as those used in the block trial.

Seedling plugs were sown on 11/10/94 (less than a week after the sowing of the block trial) by a local company specialising in seedling plug production. The seed was from the same seed lot as used in the block trial. Seedling plugs were delivered to the trial site and planted on 12/1/95.

The 'fan' or 'Nelder' systematic design employed for this trial maintained a 1:1 rectangularity. The design described firstly by Nelder (1962) as a Type 1a arrangement was used in the current work. Calculations used in determining the dimensions for the design follow those outlined by Bleasdale (1967). Plant densities evaluated in the trial are presented in Table 2.4. Five fans (replicates) were planted at various compass orientations but in close proximity to one another within the trial site (Plate 2.10).

Table 2.4 Plant densities assessed in the fan trial.

Treatment	
Code	Plants/m ²
A	3.5
B	4
C	5
D	6
E	8
F	9
G	11
H	14
I	17
J	21
K	25
L	31
M	38
N	46
O	57
P	70

In planting the seedling plugs, care was taken to minimize any trafficking within the fan to avoid soil compaction. Planting was conducted from a long plank therefore avoiding any foot marks within the fan itself (Plate 2.11). Survival in the initial planting was low with approximately 10% of the plants found to be dead after one week. Plant death was suspected to be due to high soil temperatures encountered between planting and the initial irrigation, which was approximately four hours after commencement of planting. Dead plants were replaced with spare plants from the initial planting, planted into the field just outside the fan area (Plate 2.10). Although a small number of seedling plug deaths occurred (<0.5% of total plants) in the following weeks, these were not replaced since they may have been significantly smaller than their neighbours.

First season flower harvest

Each fan contained a minimum of 15 plant positions (excluding buffers) at each of the chosen densities. As in the block trial, three adjacent harvests (31/1/96; 15/1/96; and 1/2/96) were conducted in the first season. For each harvest, plants from five sequential positions along each common density arc were collected. Missing or dead plants were recorded for the individual plant positions. If a plant was missing or dead, an extra plant was not harvested to replace it. Only four of the five fans were harvested in the first season since preliminary analyses revealed consistent trends in those harvested.

Each plant was harvested to the level of the crown as described for the block trial. Individual plants were assessed for number of primary tillers/plant. Three of the five plants in treatments 4, 8, 17, 25 and 31 were randomly selected and six tillers were again randomly selected from those plants for investigation of flowers/tiller. Only some

treatments were selected since the data collected were only intended to demonstrate changes with density.

Samples of approximately 100 flowers were retained from treatments 4, 8, 17, 25, 31, 38 and 46 plants/m² for both flower maturity and mean flower weight analysis as described for the block trial.

Second season flower harvest

In the second season, plant losses were recorded, but the position of the dead or missing plant was not. A single harvest was conducted on the 23/1/97. Plants from all five fans were harvested from 4, 8, 11, 17 and 25 plants/m². As the plants were large in low densities (4 and 8 plants/m²), six and eight plants respectively were harvested from those treatments. All the plants in the arc were harvested in the higher density treatments.

Flowers/tiller was evaluated by randomly selecting six tillers from each of six (randomly selected) plants in each treatment. Samples A and B (as described in Evaluation 1 of second season block trial) were collected from each treatment in order to determine total flower number harvested and mean dry weight per flower.

2.4 Results

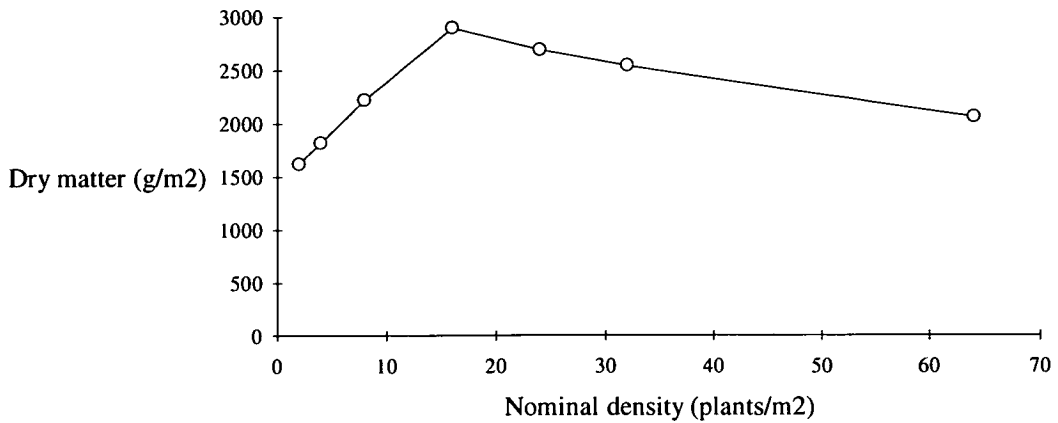
2.4.1 Estimation of yield (Block Trial)

This section provides results from assessment of the first season above-ground dry matter yield, harvest index of dry flowers and calculated yield from individual yield components. Second season yield data are then presented making special reference to the four highest density treatments which self-thinned to varying final plant densities. Finally, second season calculated yields (Evaluation 2) are compared with harvested yields from the second season (Evaluation 1).

First season

Above-ground dry matter/m² at harvest time indicates that production reached a maximum at 16 plants/m² and declines at higher densities (Figure 2.2). An increase in dry matter production of 59% was observed from the industry standard density of 4 plants/m² to the higher density of 16 plants/m². Actual densities (presented in Figure 2.8) are consistent with nominal densities in every treatment except for the highest density depicted (64 plants/m²). At that density self thinning reduced the mean plant population to 40 plants/m².

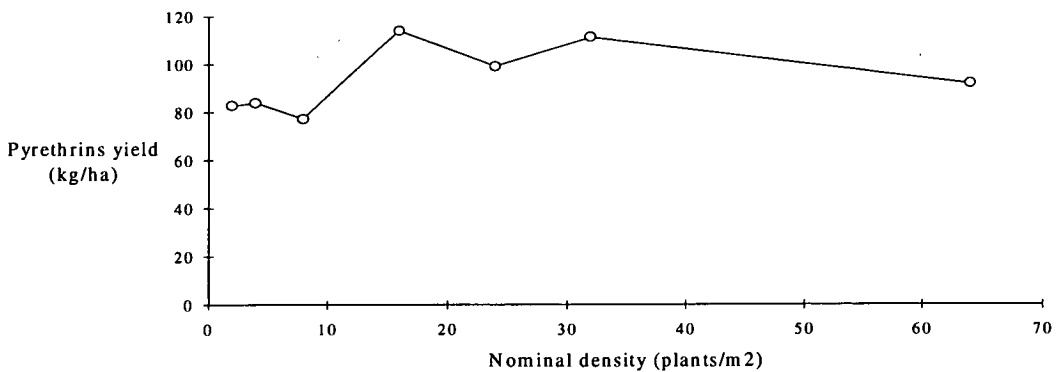
Figure 2.2 Mean above-ground dry matter per square metre vs plant density in the block trial, combined first season harvests



Note (1) Differences between some treatments are significant, $P < 0.001$, $LSD = 500 \text{ g/m}^2$. (2) Data used in the analysis were the mean dry weights of plants taken over three harvests.

An evaluation of Harvest Index (flower yield $\times 100$ /above ground total yield) identified no significant differences between density treatments (data not presented). Mean harvest index of 20.5% \pm SE of 0.98, occurred in all the treatments. Pyrethrins yields calculated from individual yield components for various densities are presented in Figure 2.3. Data indicate an increase in yield from 8 plants/m² to higher densities. Variability within density treatments responsible for the high LSD was thought to be due to multiplication of errors from individual yield components.

Figure 2.3 Calculated pyrethrins yield vs plant density in the block trial, first season

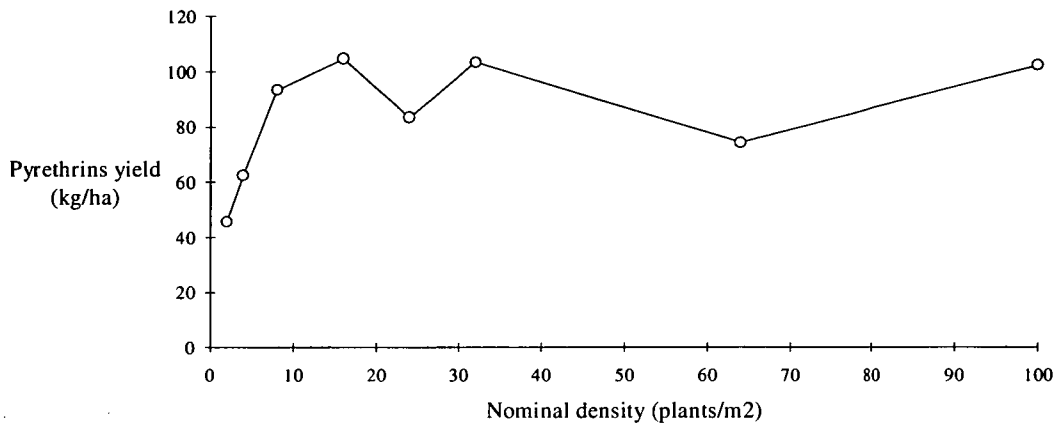


Note (1) Differences between some treatments are significant, $P = 0.04$, $LSD = 25.2 \text{ kg/ha}$. (2) Where harvest time had no influence on yield component data individual harvests were combined (e.g. plant density and flowering tillers/plant). (3) Where components altered with harvest date, data from harvest two and three were used since these times were considered to be just prior to and post time of maximum yield. Components analysed in this way were mean flower number/tiller and mean flower dry weight (see Figures 2.22 and 2.26).

Second season

Second season actual harvested pyrethrins yield data are presented in Figure 2.4. This demonstrates an increase in yield up to 16 plants/m². Self thinning occurred in all the remaining higher density treatments and appeared to reduce plant populations in a variable way regardless of initial density.

Figure 2.4 Pyrethrins yield vs plant density in the block trial, second season, Evaluation 1



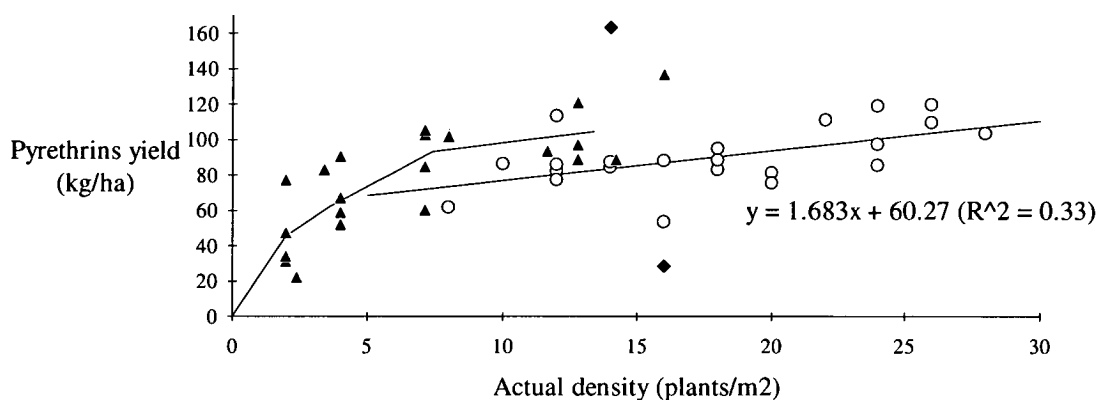
Note (1) Differences between some treatments are significant, $P < 0.001$, $LSD = 25 \text{ kg/ha}$. (2) Variability in yields among the four highest density treatments may be due to variable populations resulting from self thinning.

Figure 2.5 presents yield data against actual plot density. The lowest four density treatments (with 300 mm row spacing) demonstrated little plant loss and therefore maintained their intended densities. Conversely, all the higher density treatments (150 mm row spacing) displayed significant and variable plant loss such that different density treatments were no longer distinguishable. The highest four density treatments were therefore analysed separately from the four lowest density treatments. ANOVA analysis of yields from the lowest four, non-self-thinned density treatments was conducted while regression analysis of individual plot yields of self-thinned plots was conducted in the high density treatments (Figure 2.5).

While analysis of the lowest density treatments in this manner merely increased the level of significance of differences between treatments in the initial analysis (Figure 2.4), regression of individual plot data from high density treatments provided further information. Where plant population remained high, greater pyrethrins yields were also achieved.

Data also indicate that non-self-thinned treatments at a given density may generate higher yields than self-thinned treatments, although this would have to be verified in further studies.

Figure 2.5 Pyrethrins yield vs actual density for low density treatments (solid symbols) and for self thinned individual plots (open symbols) in the block trial, second season, Evaluation 1



Note (1) ANOVA analysis of four lowest density treatments (closed triangles): Treatment means are significantly different, $P = 0.0002$, $LSD = 21.2$ kg. (2) Regression analysis of self-thinned plots (open circles) statistics indicate that coefficient x is $1.683 \pm \text{s.e. } 0.539$ ($P = 0.0052$) suggesting that yield increases with actual density. Two outlier points (closed squares) were removed from the data set prior to regression analysis. (3) The curve and the line are not intended to intersect.

Yield was calculated from individual yield components in Evaluation 2 of the second harvest season. Table 2.5 shows that calculated yield data followed a similar trend to actual yield data reported above in Evaluation 1.

Table 2.5 Yield components and calculated yield of block trial, Evaluation 2

Variable (treatment means)	Treatment			
	B	C	D	E
nominal density	4	8	16	32
actual density	3.421	7.175	14.455	18
tillers/plant	62.75	46.306	32.617	25.498
flowers/tiller	5.493	4.093	4.481	4.226
dw/flower	.239	.246	.233	.257
assay	.0228	.0228	.0228	.0228
Calculated yield (Evaluation 2)				
Pyrethrins (kg/ha)	57.98	74.26	111.87	98.94
Yield (Evaluation 1)				
Pyrethrins (kg/ha)	62.59	93.35	104.64	103.43

Note (1) Yield component data presented are treatment means and are presented here only for descriptive purposes. (2) Differences between some treatments of Evaluation 2 were statistically significant $P = 0.005$, $LSD = 34$ kg/ha. Data subject to ANOVA were the product of individual plot yield components.

2.4.2 Vegetative harvest (block trial)

Plant densities were evaluated prior to their first winter in order to ascertain whether any self thinning had occurred and to investigate variability in mean plant dry weight. Table 2.6 presents data relating to plant densities in the higher density treatments which reveal that no plant loss had occurred in the eight months after sowing.

Table 2.6 Plant population assessment prior to winter

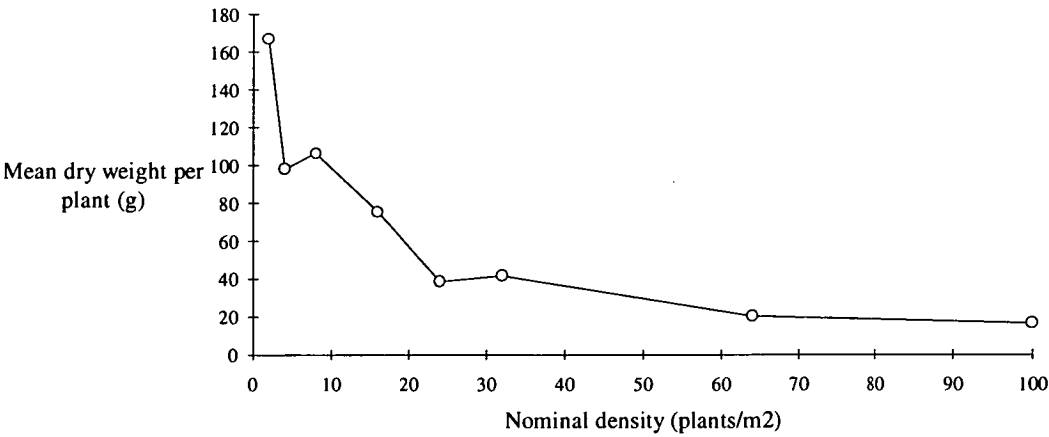
Treatment		Assessed Densities (pre-winter harvest)				
Code	Nominal density	Rep1	Rep.2	Rep.3	Rep.4	mean
A	2	-	-	-	-	2
B	4	-	-	-	-	4
C	8	-	-	-	-	8
D	16	-	-	-	-	16
E	24	28	32	32	32	31
F	32	40	36	36	32	36
G	64	72	76	76	68	73
H	100	116	120	84	120	110

Note; a single (447×447 mm) quadrat was used to assess actual plot densities.

Values for assessed densities in treatments A-D are not presented since in these treatments a number of plants were harvested rather than a given area. Since much care was taken in thinning the first treatments and no plant loss was evident, nominal density values can be considered as actual plant densities.

Figure 2.6 reports mean plant dry weight in the different density treatments. There was a typical reduction in plant weight with increasing density.

Figure 2.6 Mean plant dry weight vs plant density in the block trial, vegetative harvest



Note (1) Differences between some treatments are significant, $P < 0.001$, $LSD = 10.65$ g/plant (2) Treatments A and B were removed from the analysis due to the low plant number harvested and the high variance encountered in these treatments.

2.4.3 Changes in plant population

Block trial

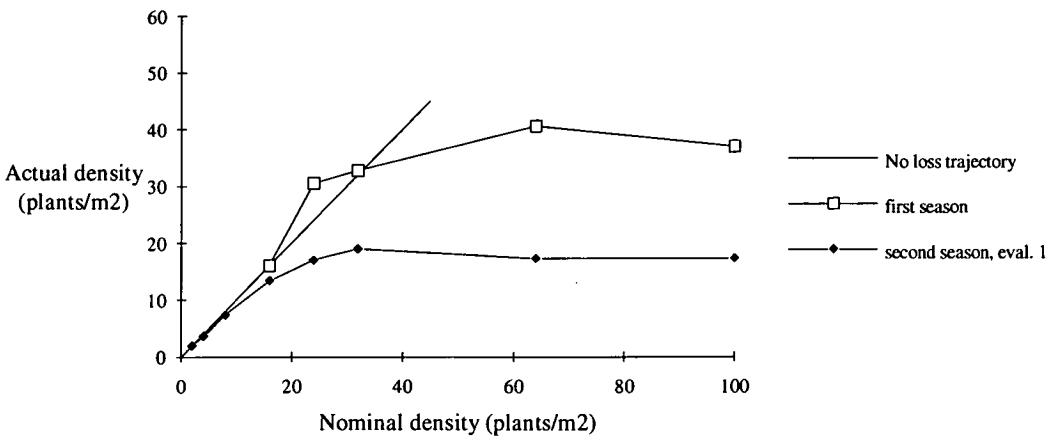
This section summarises both seasons' plant density evaluations. Table 2.7 presents all plant density evaluations conducted on the block trial.

Table 2.7 Summary of plant population assessments, block trial

Treatment		Veg. harvest	First season harvest (sequential harvests)				Second season harvest		
Code	Nominal density	Pre-winter density	H1	H2	H3	Mean	Eval. 1	Eval. 2	Mean
A	2	2				2	2.00	-	2.00
B	4	4				4	3.638	3.421	3.53
C	8	8				8	7.407	7.175	7.29
D	16	16				16	13.376	14.455	13.92
E	24	31	30	26.7	35	30.56	17.00	-	17.00
F	32	36	37	31.7	30	32.78	19.00	18.00	18.50
G	64	73	38	45	38.33	40.56	17.33	-	17.33
H	100	110	40	32.5	-	36.25	17.33	-	17.33

Figure 2.7 presents a summary of data from Table 2.7. Data indicate that plant population thinned to less than approximately 40 plants/m² by harvest time in the first season. By the second season, plant population had declined to a mean of less than 20 plants/m². Although mean plant density appeared consistent between original plant densities in which self thinning occurred, CV's reveal high levels of variability in actual plot densities between plots in those treatments.

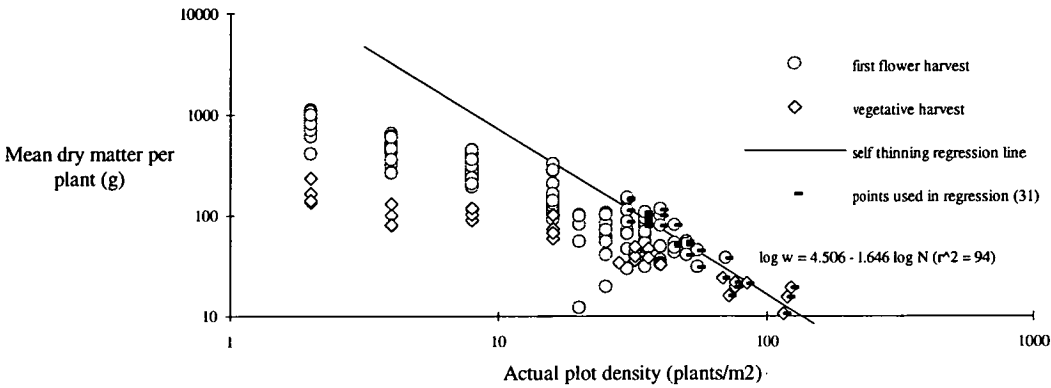
Figure 2.7 Actual density vs nominal density in the block trial, first and second season



Note (1) First season treatment densities were calculated from the mean of all samples (18) taken in that harvest. CV's for the treatments D to H were 20.1%, 18.3%, 29.3%, 30.7%. (2) Second season CV's for replicates in treatments A to H respectively were 0%, 17.8%, 6.2%, 11.4%, 40.6%, 30.2%, 24.9%, and 37.7%.

Plant density of individual plots was evaluated against mean yield per plant for both the vegetative harvest and the first season flower harvest (Figure 2.8). The highest dry matter producing plots at densities displaying self thinning were marked and a self-thinning line was fitted through those points.

Figure 2.8 Mean total dry matter per plant vs actual plant density assessed during vegetative growth and at first flowering in the block trial



Fan trial

The percentages of plants missing or dead at first harvest of the fan trial are presented in Figure 2.9. Data indicate no losses in plant numbers that could be attributed to increasing plant density. Second year data presented in Figure 2.10 demonstrate a tendency towards plant loss in the highest density treatments (Plate 2.12).

Figure 2.9 The mean percentage of plants missing or dead at the time of first harvest vs planted density in the fan trial, first season

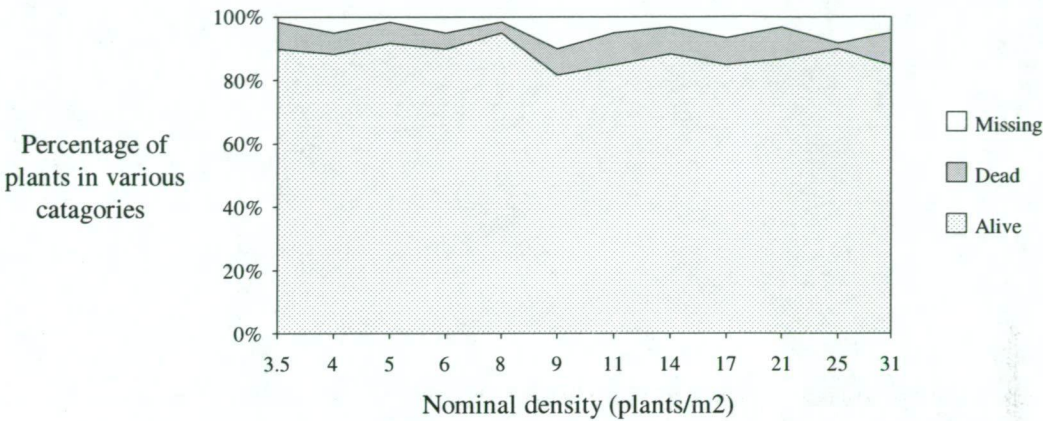
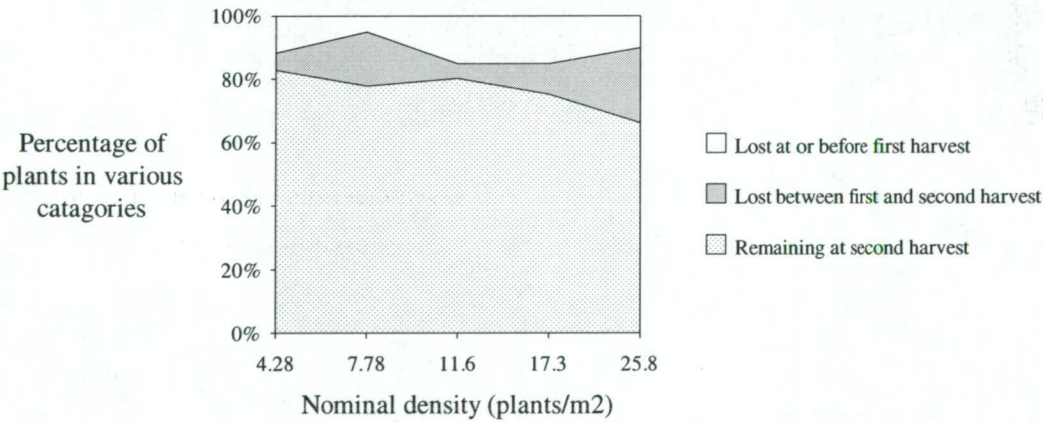
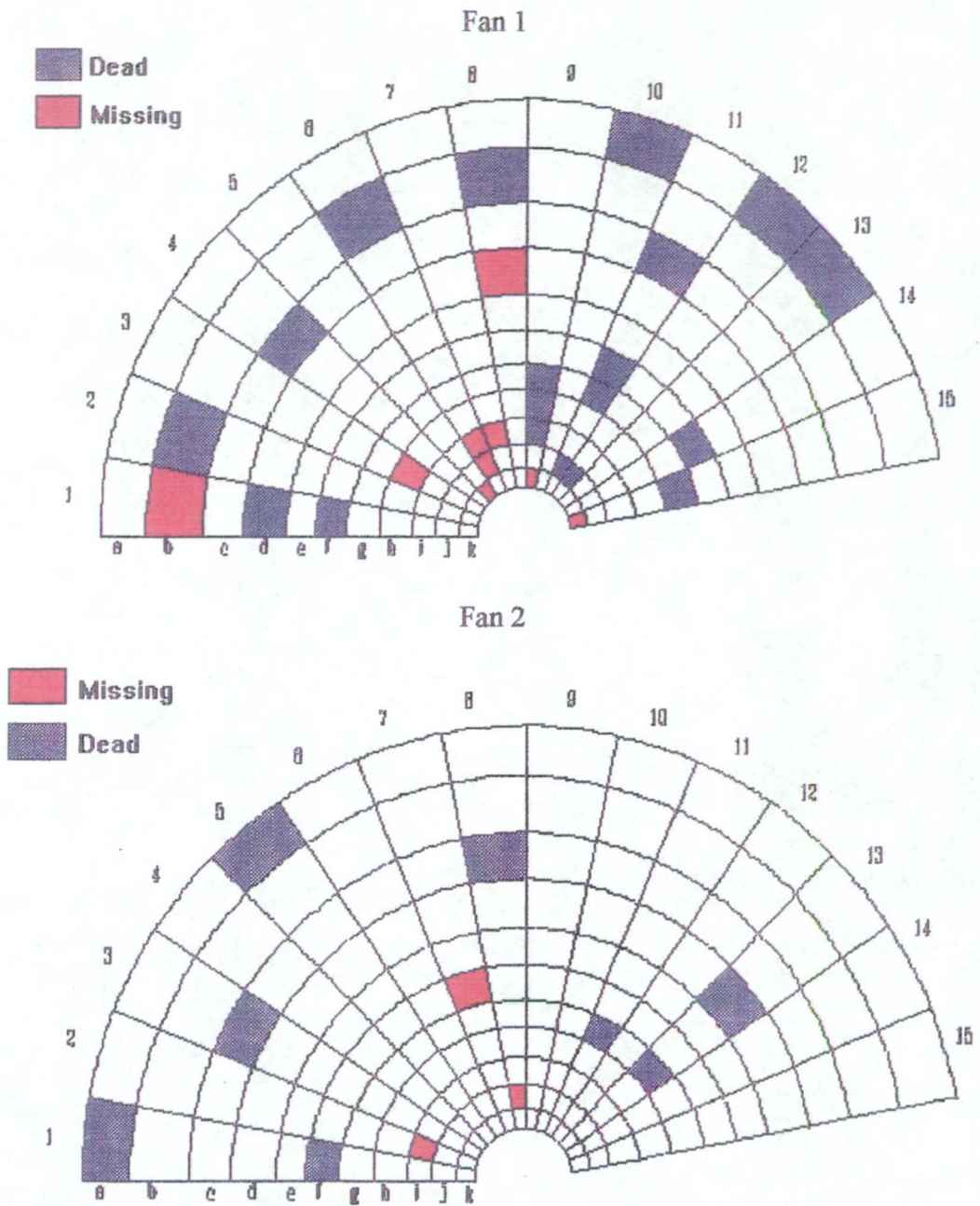


Figure 2.10 The mean percentage of plants in various categories vs nominal density in the fan trial, second harvest



The positions of dead and missing plants in two example fans are presented in Figure 2.11. There does not appear to be any change in the likelihood of a plant being dead or missing depending on the status of its nearest neighbours. Therefore, transfer of disease to neighbouring plants within a single season was not apparent.

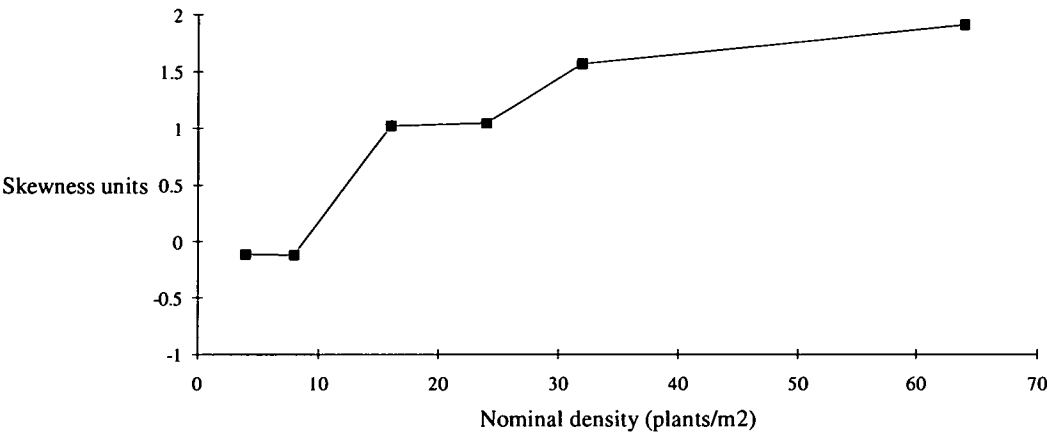
Figure 2.11 Positions of dead and missing plants in two fans, one with minimal loss, the other with significant loss, first harvest



2.4.4 Variability between plants

Variability in tiller number per plant within plots was assessed using the skewness statistical function. Skewness characterises the degree of asymmetry of a distribution around its mean. Positive skewness indicates a distribution with an asymmetric tail extending towards higher values while negative skewness indicates a distribution with an asymmetric tail extending towards lower values. Data presented in Figure 2.12 demonstrate significant changes in skewness of the population with increasing density.

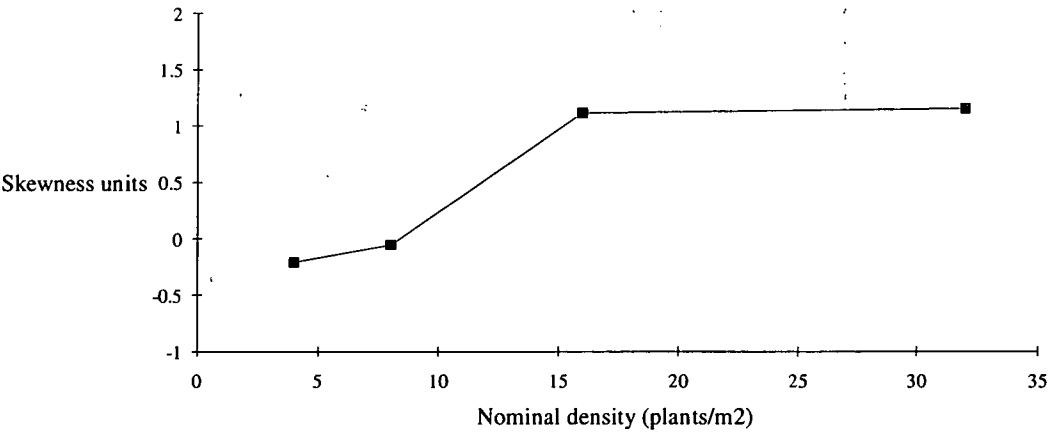
Figure 2.12 Skewness of stem number per plant vs density in the block trial, first season



Note (1) Differences between some treatments are significant, $P < 0.0001$, $LSD = 0.82$ units. (2) Treatments A and H were excluded from the analysis and are not presented in the figure.

Second season data (Figure 2.13) indicate some increase in skewness of plant stem population with increasing density.

Figure 2.13 Skewness of plant stem number vs nominal density in the block trial, second season, Evaluation 2



Note (1) Differences between some treatments are significant, $P < 0.01$, $LSD = 0.93$ units.

Although statistically significant, the appropriateness of assessing skewness of a sample of six plants is questionable. Replicate data were therefore combined and various statistics associated with the bulked replicate population are presented in Table 2.8.

Table 2.8 Statistics describing the population of plants with respect to tiller number per plant, block trial, second season, bulked replicate samples

Statistic	Density treatments			
	4/m ²	8/m ²	16/m ²	32/m ²
Mean	62.750	46.306	32.617	25.019
Skewness	0.248	0.456	0.915	1.294
CV	65.270	64.312	76.080	69.663
Kurtosis	-0.834	-0.561	0.363	2.497
plants in sample	36	36	60	54

Data presented in Table 2.8 indicate that skewness does in fact tend to increase in the higher density treatments. Coefficient of Variation (CV) measures variability relative to the individual treatment means. No appreciable changes in this statistic were apparent between density treatments. Kurtosis describes the relative peakedness or flatness of a distribution compared to the normal distribution. Positive Kurtosis indicates a peaked distribution, while negative Kurtosis indicates a relatively flat distribution. Data indicate an increase in Kurtosis with higher density treatments.

2.4.5 Yield components

Flowering tillers per plant

Mean number of flowering tillers per plant was assessed in the block and fan trials in both first and second seasons (Figures 2.14 to 2.17). Tiller number per plant responded in an expected way to increases in plant density, increasing dramatically at low densities. Pyrethrum plants displayed great plasticity with respect to this trait over a wide range of densities. Data from both trials and years changed similarly with density, although tiller numbers at given densities vary. This is particularly evident between the first and second seasons of the block trial (Figure 2.14 and 2.16).

Flowering tillers per square metre

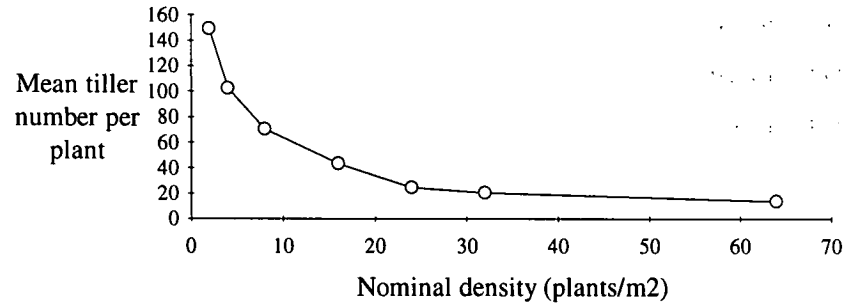
Flowering tillers/m² was calculated by multiplication of actual plot density with mean tiller number per plant (Figures 2.18 to 2.21). Results indicate tillers/m² nearly double from 4 to 16 plants/m². Above 16 plants/m², tiller number increased, decreased or remained constant depending on the trial and season.

Flower number per tiller

Flower number per tiller declined significantly in the plant density range from 2 to 8 plants/m² (Figure 2.22 to 2.25). In this range of densities, flowers/tiller declined from about 7 to 4.5. At densities higher than 8 plants/m² no significant or consistent variation

Figures 2.14 to 2.17 Flowering tiller number/plant

Figure 2.14 Mean flowering tiller number per plant vs density in the block trial, first season



Note (1) Differences between some treatments are significant, $P < 0.001$, $LSD = 13.4$ tillers per plant. (2) Figures are means of the three sequential harvests

Figure 2.15 Mean flowering tiller number per plant vs density in the fan trial, first season

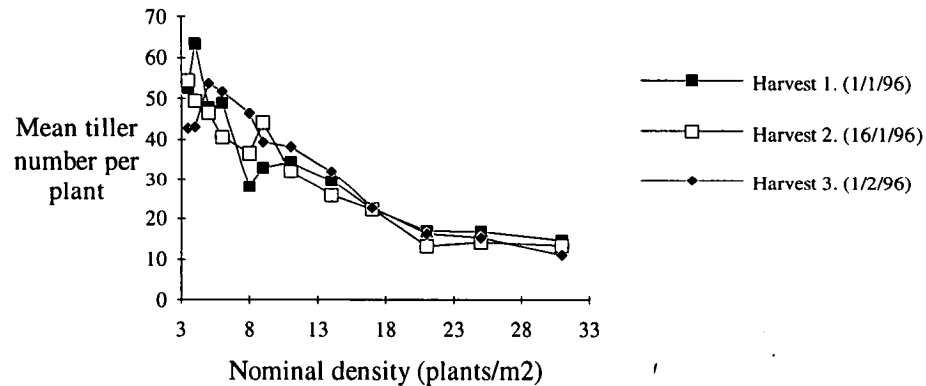
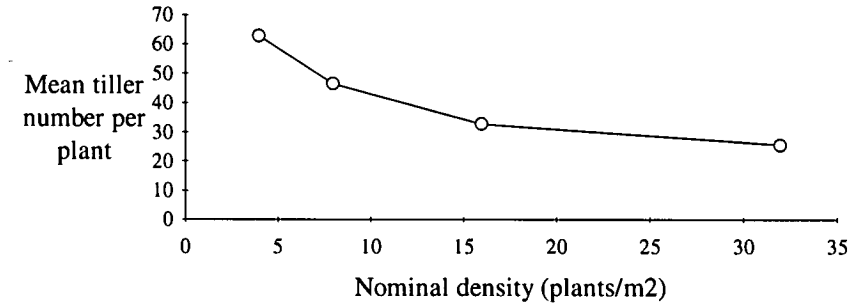
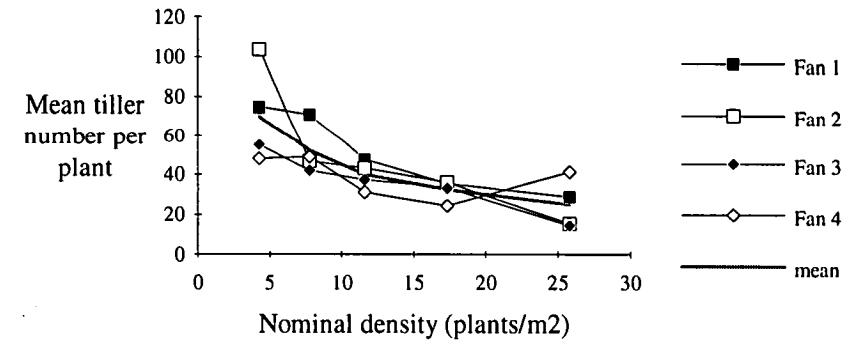


Figure 2.16 Mean flowering tiller number per plant vs density in block trial, second season, Evaluation 2



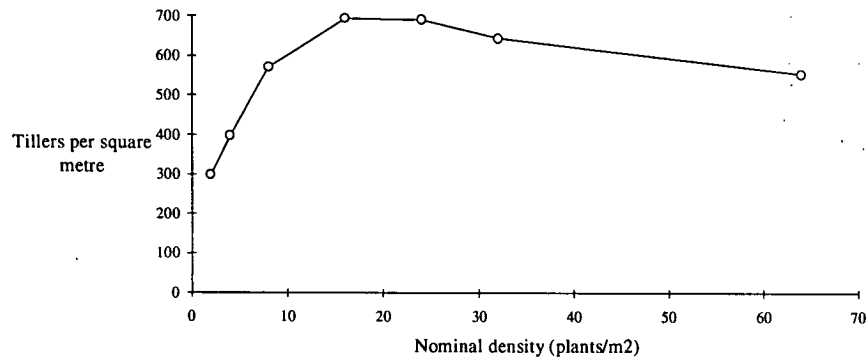
Note (1) Differences between some treatments are significant, $P < 0.001$, $LSD = 15.1$ stems per plant. (2) Treatments assessed were; B, C, D, and F.

Figure 2.17 Mean flowering tiller number per plant vs density in the fan trial, second harvest



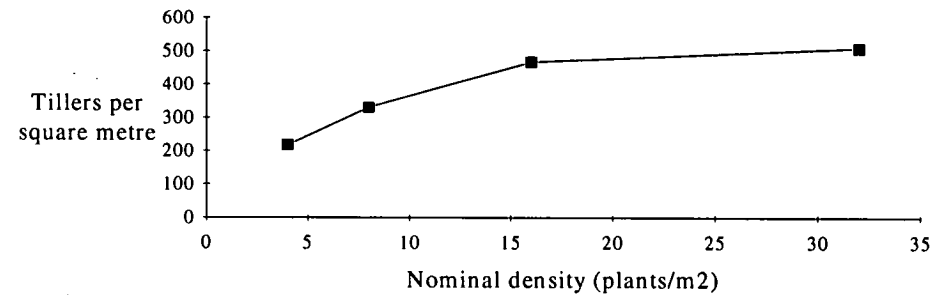
Figures 2.18 to 2.21 Flowering tillers/m²

Figure 2.18 Mean flowering tiller number per square metre vs density in the block trial, first season



Note. (1) Differences between some treatment means are significant, $P > 0.01$ level, $LSD = 93.3$ tillers/m².

Figure 2.20 Mean flowering tiller number per square metre vs density in the block trial, second season, Evaluation 2



Note (1) Differences between some treatment means are significant, $P = 0.0025$, $LSD = 141$ tillers/m².

Figure 2.19 Flowering tiller number per square metre vs density in the fan trial, first season

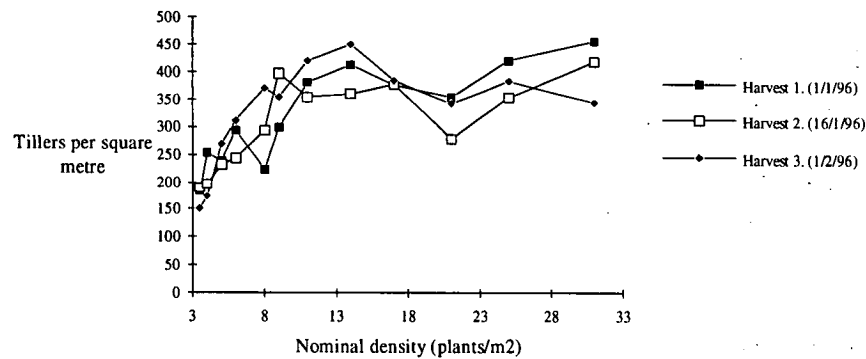
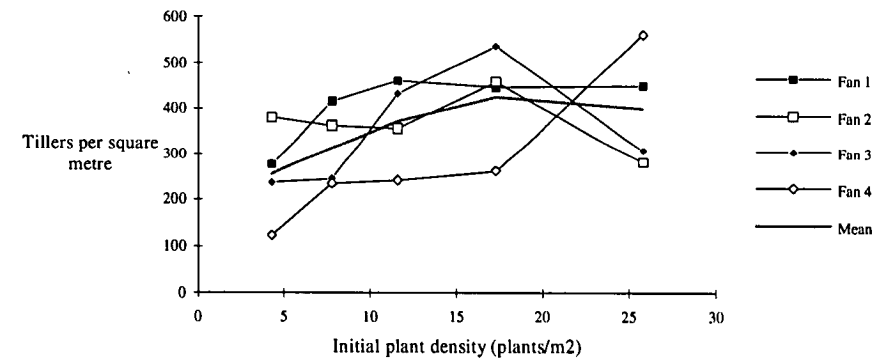
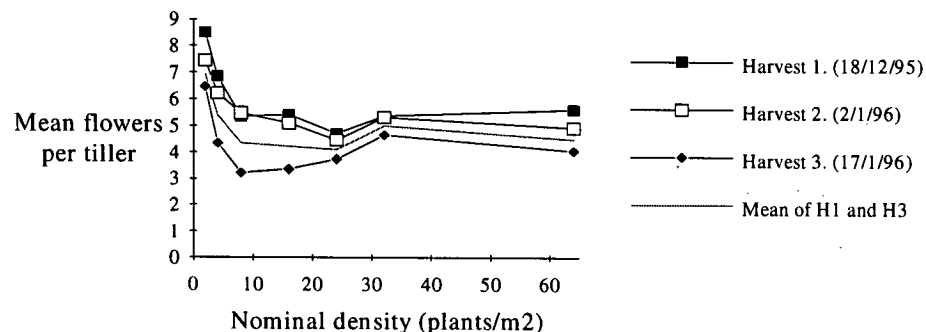


Figure 2.21 Flowering tillers per square metre vs density in the fan trial, second season



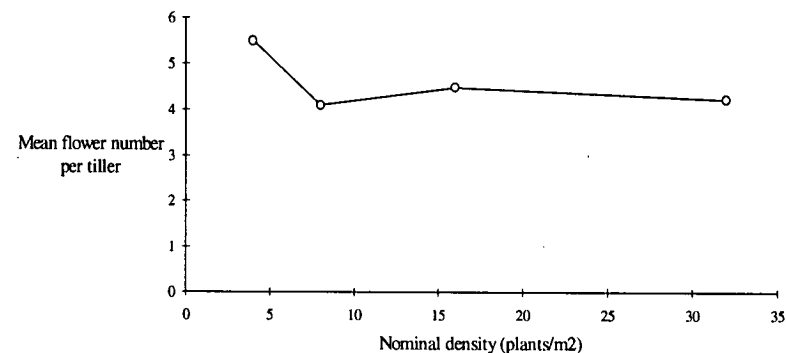
Figures 2.22 to 2.25 Flowers per tiller

Figure 2.22 Mean flower number per tiller vs density in the block trial, first season



Note (1). Analysis refers to mean of H2 and H3 data as these were prior to and post the expected optimum harvest time. Some treatment means are statistically different at $P < 0.001$, $LSD = 0.93$ flowers/tiller. (2) The highest density treatment (100 plants/m²) mean was not included in ANOVA as it was not assessed in harvest 3.

Figure 2.24 Mean flower number per tiller vs density in the block trial, second season, Evaluation 2



Note (1) Differences between treatments were not significant, although a $P = 0.091$ is evidence of a trend.

Figure 2.23 Mean flower number per tiller vs density in the fan trial, first season

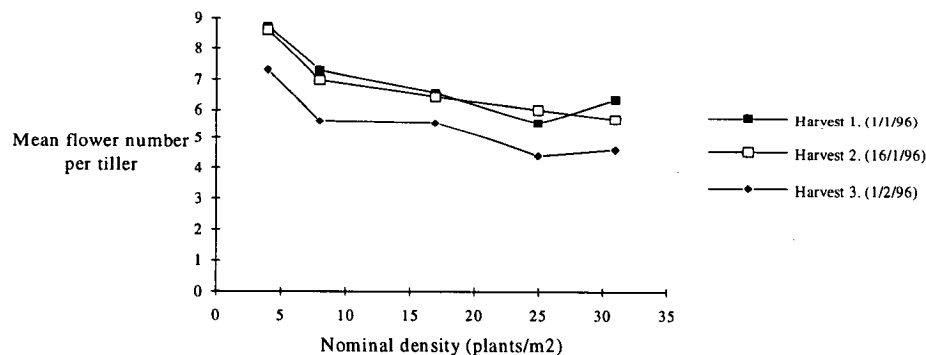
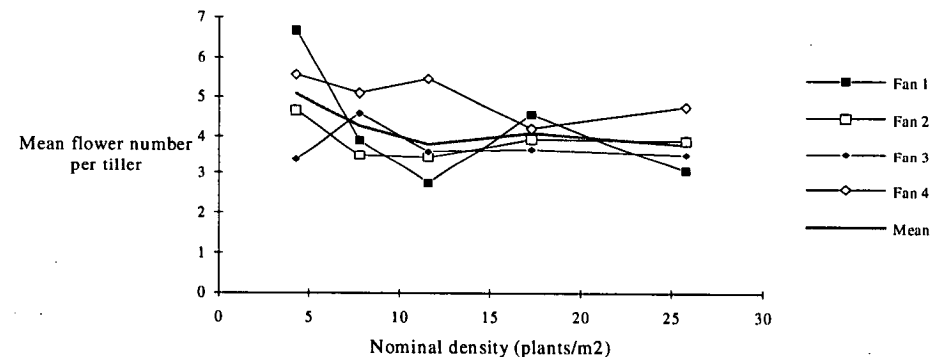
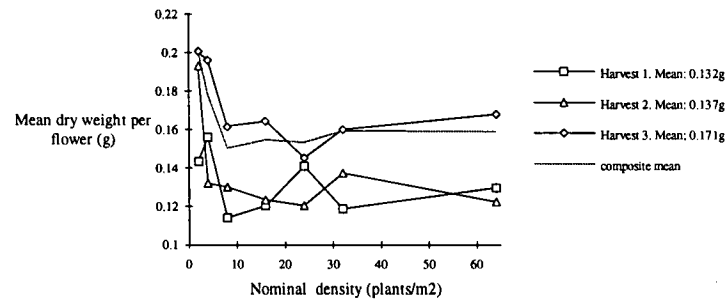


Figure 2.25 Flower number per tiller vs density in the fan trial, second season



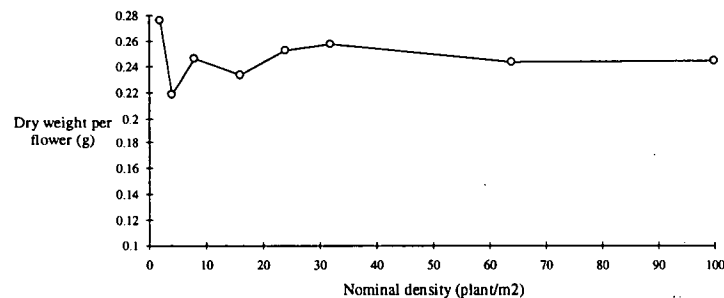
Figures 2.26 to 2.27 Mean flower weight

Figure 2.26 Mean dry weight per flower vs density in the block trial, first season



Note (1) Density treatment means within harvest dates were not significantly different in either harvest 1 or harvest 3. (2) In harvest 2, the lowest density treatment was significantly different from all other treatments ($P = 0.013$), $LSD = 0.039g$. (3) Composite values were calculated making each plot a percentage of overall mean for that harvest, averaging the resultant plot data for harvest 2 and 3 before multiplying the data to make the values up to those of mature flowers. Treatment means were statistically different, ($P < 0.0001$, $LSD = 0.024 g$).

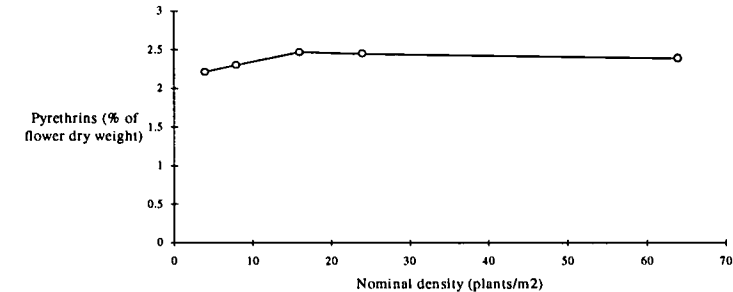
Figure 2.27 Mean dry weight per flower vs density in the block trial, second season, Evaluation 1



Note (1) Differences between some treatments are significant at $P = 0.002$, $LSD = 0.024 g/flower$

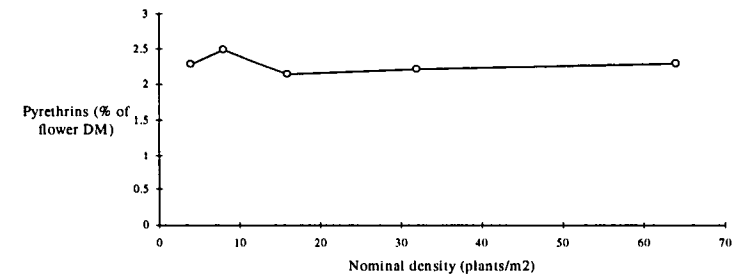
Figures 2.28 to 2.29 Pyrethrins assay

Figure 2.28 Pyrethrins assay vs density in the block trial, first season



Note:(1) Differences between treatments were not significant at $P = 0.05$ (2) Treatments assessed were B, C, D, E and G, standard errors for which were 0.087, 0.090, 0.156, 0.160 and 0.043 respectively.

Figure 2.29 Pyrethrins assay vs density in the block trial, second season, Evaluation 1



Note:(1) Differences between treatments were not significant at $P = 0.05$ (2) Treatments assessed were B, C, D, E and G; standard errors for which were 0.078, 0.142, 0.110, 0.069 and 0.160 respectively.

in flower number/tiller was observed. Data indicate that level of branching, the determinant of flower number per tiller, is a means by which pyrethrum plants modify their flower productivity only at densities at which interplant competition is minimal.

Mean flower dry weight

Flower weight was evaluated in both block and fan trials but only block trial data are presented since all the data demonstrated similar findings. Mean flower weights for different density treatments in the first and second season harvests are presented in Figures 2.26 and 2.27. Flower weight was found to decline in the density range of 2 to 4 plants/m². At densities above this, flower weight remained constant. The decrease in flower weight was substantial, decreasing by 20-30%. Differences in flower maturity were suspected to be responsible for the significant differences but upon investigation, weight differences could not be attributed to that factor.

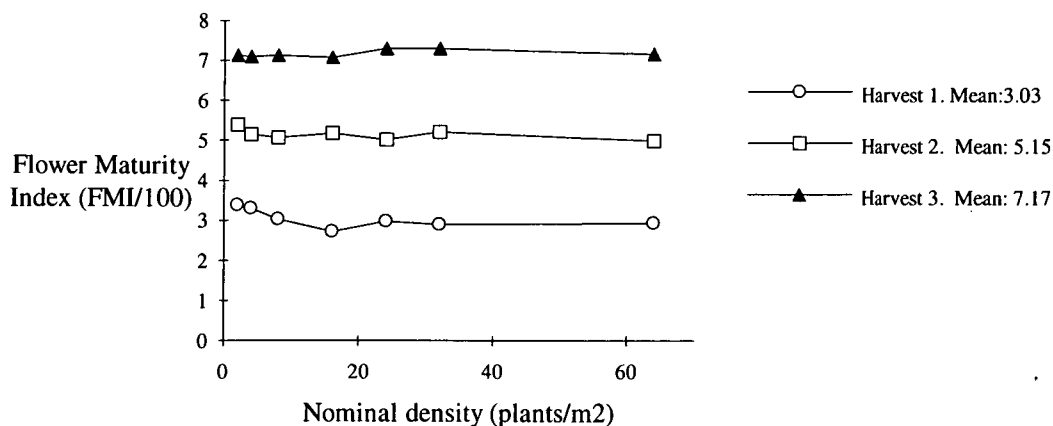
Pyrethrins concentration

Pyrethrins concentration was assessed in both seasons of the block trial (Figures 2.28 and 2.29). In both seasons pyrethrins concentration was not found to vary with respect to plant density. Pyrethrins concentrations were found to be above 2.0% of DM in both seasons, a result consistent with plant breeding data.

2.4.6 Flower maturity

Mean flower maturity was evaluated in both the block and fan trials but since results were consistent between trials, only block trial results are reported. In the first season flower maturity was assessed on each of the three sequential harvests (Figure 2.30).

Figure 2.30 Mean flower maturity vs density for sequential harvests of the block trial in the first season

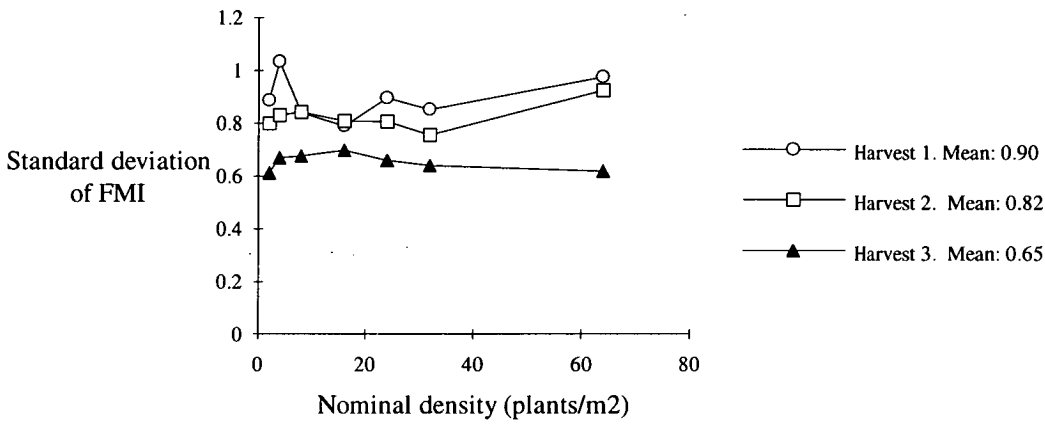


Note (1) Density treatment means within harvest times are not significantly different at $P = 0.05$

Results demonstrate that plant density had no significant influence on mean flower maturity.

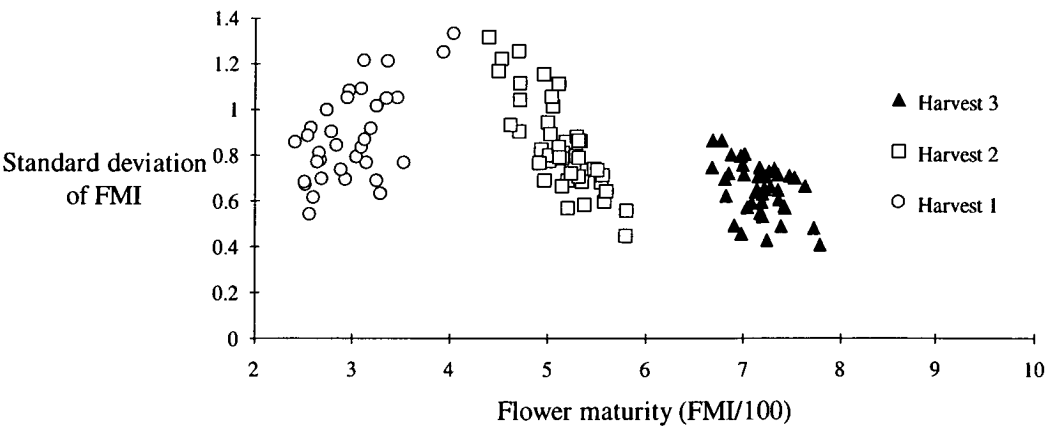
Spread in flower maturity (Figure 2.31) was also investigated and again, density had no influence on this potentially important characteristic. Although density had no influence on uniformity of flower maturity (as measured by SD) this characteristic was found to decline in the last of the three sequential harvests.

Figure 2.31 Standard deviation of FMI vs density in the block trial, first season



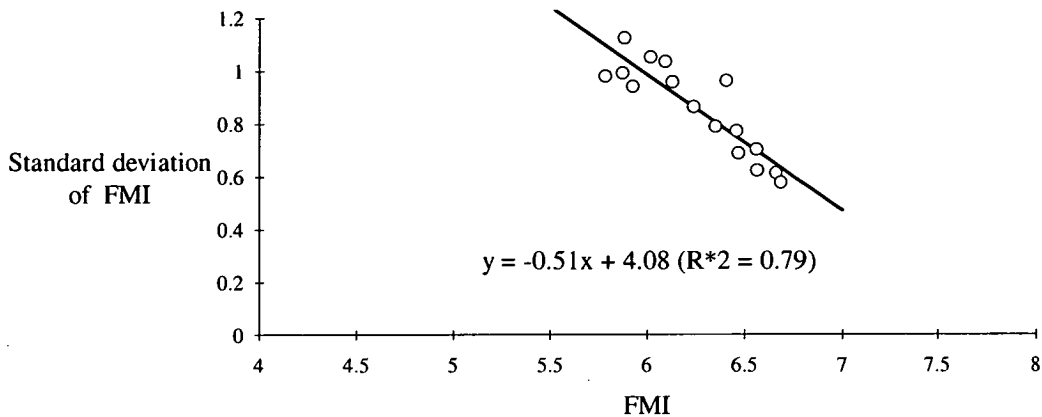
Standard deviation of FMI in individual plots in the three sequential harvests was consequently examined and presented in Figure 2.32. Data indicate that uniformity of flower maturity may increase in a consistent manner in the maturity range from 400 to 800.

Figure 2.32 Standard deviation of FMI vs FMI for plots of three sequential harvests in the block trial, first season



Mean flower maturity and standard deviation of flower maturity data from the second season again provided no evidence that plant density had any significant influence on these characteristics (data not presented). Variability in flower maturity between individual plots was investigated as for the previous years data (Figure 2.33). Again variability in flower maturity was found to decline over a range of mean flower maturities.

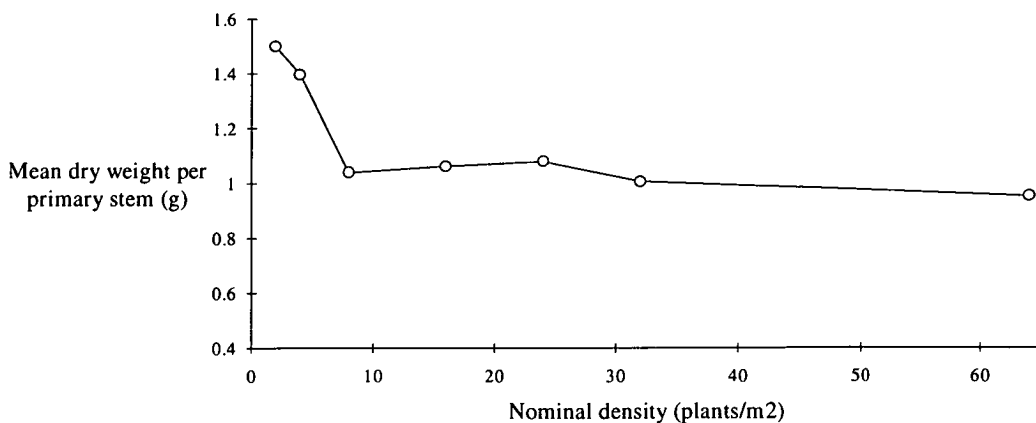
Figure 2.33 Standard deviation of FMI vs FMI in the block trial, second season, Evaluation 1



2.4.6 Changes in length and weight of the flowering tiller (primary stem)

Mean primary tiller dry weight data are presented in Figure 2.34. Results demonstrate that significant decreases in main tiller mass occur with increases in density in the range of 2 to 8 plants/m². Primary tiller length data (Figure 2.35) demonstrate that crop height increases from 2 to 8 plants/m² and then begins to decline again at densities above 16 plants/m².

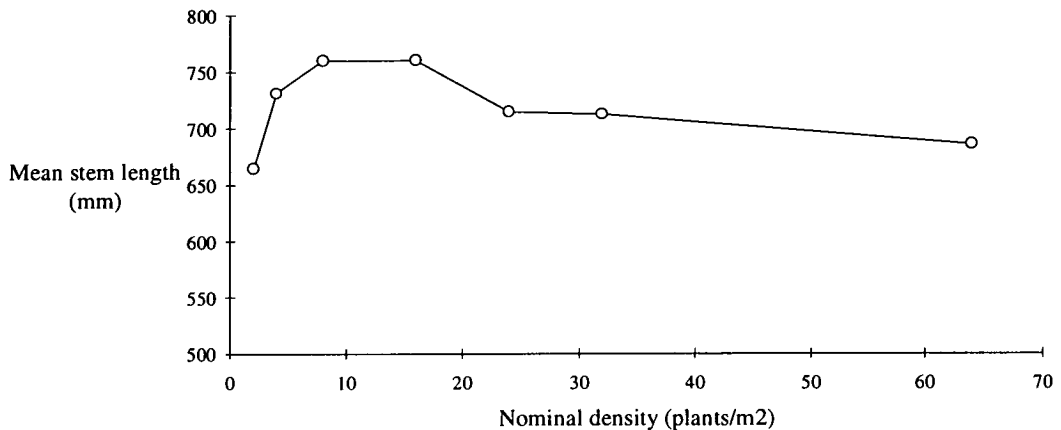
Figure 2.34 Mean dry weight per tiller vs density in the block trial, harvest 1, first season



Note (1) Differences between some treatments are significant, $P = 0.034$, $LSD = 0.37\text{g/ tiller}$.

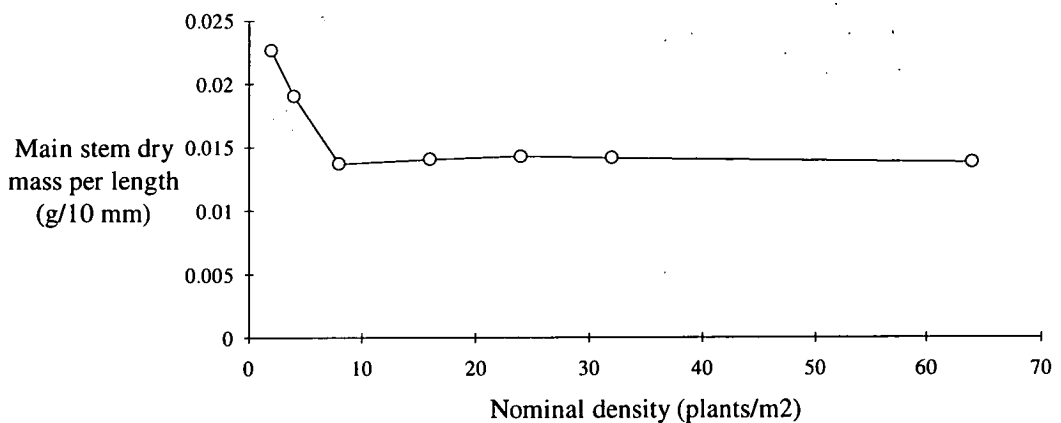
Tiller dry mass per unit length, which was calculated from previously described tiller mass and length data, is shown in Figure 2.36. Results from this investigation follow a similar path to those for tiller mass, where mean tiller mass declined significantly from 2 to 8 plants/m².

Figure 2.35 Mean primary tiller length vs density in the block trial, harvest 1, first season



Note (1) Differences between some treatments are significant, $P = 0.039$, $LSD = 63$ mm.

Figure 2.36 Main tiller dry mass per length vs density in the block trial, harvest 1, first season



Note (1) Differences between some treatments are significant, $P = 0.002$, $LSD = 0.0046$ g/10 mm.

2.4.7 Branching of the flowering tiller

Changes in branching pattern of the flowering tiller occurred with increasing density. Those changes were quantified in the earlier section which examined flower number/tiller. Branching behaviour was investigated using the branching hierarchy proposed in Figure 2.1. Occurrence of higher order branches are presented in Figure 2.37 while lower order occurrence is shown in Figure 2.38. Data presented indicated that the incidence of occurrence of the most basal secondary branches (2A, 2B) varied little with

density (Figure 2.37) but incidence of lower order secondaries (2C, 2D) decreased to some extent (Figure 2.38). The main change with density was the substantial decrease in incidence of tertiary branches (Figure 2.38). This decline occurred regardless of whether the tertiary branches were positioned on high or low order secondaries.

Figure 2.37 Mean percentage of primary tillers having branches of various hierarchy vs density in the block trial, first season, harvest 1

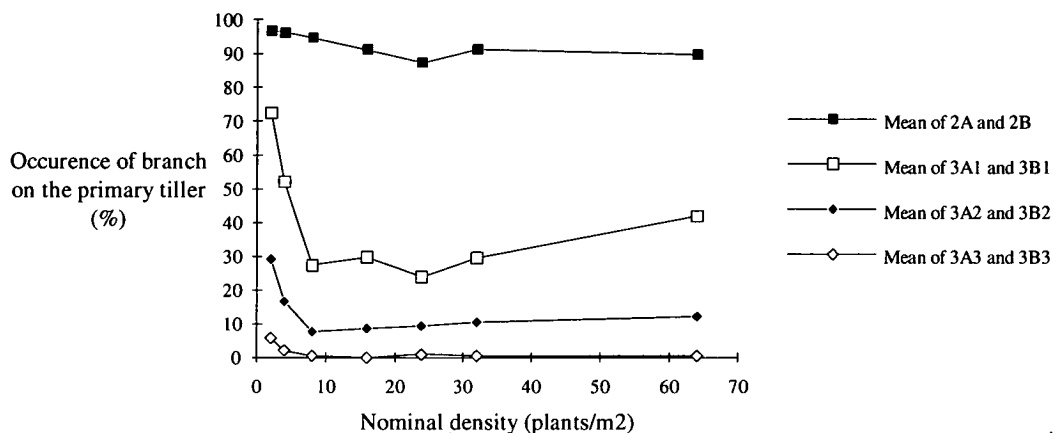
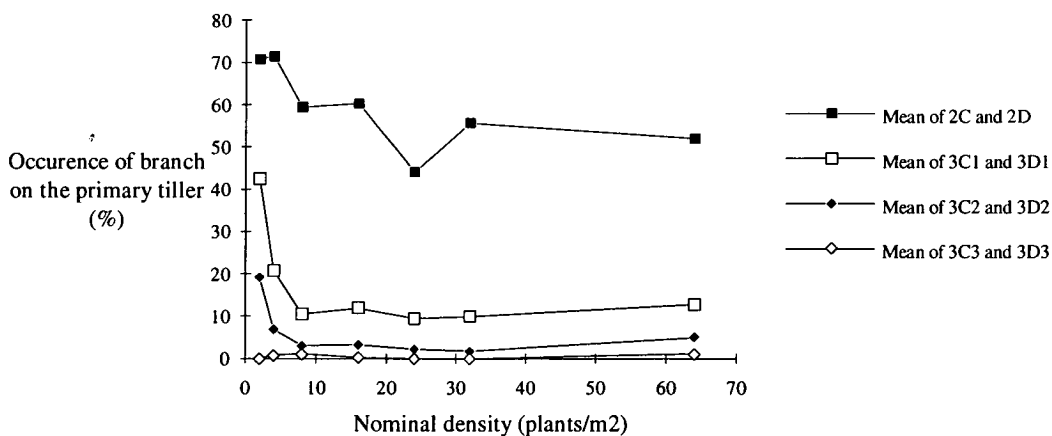


Figure 2.38 Mean percentage of primary tillers having branches of various hierarchy vs density in the block trial, first season, harvest 1



2.4.8 Yield of flowers from differing branching position

Pyrethrins yield from a flower is determined by its dry weight and its pyrethrins concentration. Flower maturity may also influence both dry weight and concentration. Table 2.9 reports the influence of density on differently positioned flowers, with respect to flower maturity; flower weight and pyrethrins concentration.

Table 2.9 Flower maturity, flower weight and pyrethrins concentration of primary and secondary flowers in the block trial, second season, Evaluation 2

Variable	Hierarchy	Actual density (plants/m ²)				Significance
		3.4	7.17	14.5	18.0	
FMI/100	Primary	6.91	6.79	6.91	9.8	N.S
	2B	6.85	6.67	6.68	6.73	
Flower weight (g)	Primary	0.32	0.30	0.24	0.29	N.S
	2B	0.30	0.29	0.26	0.27	
Pyrethrins assay (% DM)	Primary	2.21	2.27	2.28	-	N.S
	2B	2.38	2.33	2.37	-	

Note (1) Significances refer to flower positional treatment differences, not significant at $p=0.05$.

2.5 Discussion

This discussion compares in a general way yield from the current study with yields generated in other locations and in studies by other workers. Changes in plant population are investigated followed by discussion relating to changes in yield components and aspects of crop maturity with increasing density. Following this, crop height and other measures of tiller strength are examined. Finally, the effect of density on the yielding characteristics of flowers of different hierarchy is discussed.

2.5.1 Estimation of yield

Flower yields reported in the current work are unprecedented in pyrethrum agronomy literature. In highland tropical regions where the crop flowers in a number of flushes through the year, dry flower yields of 1 000 kg/ha are considered to be high (Van Rijn, 1974). More recently, field trials in Kenya reported dry flower yields of 1 766 kg/ha (Wanjala, 1991). In Australia, efforts at Black Mountain, Australian Capital Territory, in 1931 resulted in flower dry matter yields from 1 200 to 1 400 kg/ha (Gullickson, 1995).

In 1992, the average flower yield of Tasmanian crops was nearly 1 700 kg/ha (Mac Donald, 1995). In Tasmania during the early 1990s, plants were established at approximately 5/m² and the highest commercial yields of achenes rarely exceeded 2 000 kg/ha. As achenes make up approximately 80% of the weight of the flower, this is equivalent to whole flower yields of 2 500 kg/ha. In a trial on the northern coast of Tasmania, achene yields of 2 400 kg/ha (flower yield of 3 000 kg/ha) have been reported (Salardini *et al.*, 1994a). That high yield was of a second season crop established from splits. The first year achene yield from that same trial was 1 300 kg/ha (flower yield of

1 600 kg/ha). First season crops established from splits yielded significantly less than in following years. Data indicate that even though the plant only flowers in one single flush in the cool Tasmanian climate, yields are as high, if not higher than those obtained when the crop is grown in highland tropical regions and flowers many times through the year.

In the block trial, first and second season flower yields were 3 491 kg/ha and 2 745 kg/ha respectively at 4 plants/m². These results are significantly higher for first flower yield and comparable to yields (second season) reported for a trial established from splits (Table 2.10).

Table 2.10 Flower yields from trials established from splits or seed

Trials (source)	Planting or sowing date	Estab- lishment material	Density (plants/ m ²)	Dry flower yield (kg)			
				Season 1	Season 2	Season 3	Total
Salardini <i>et al.</i> (1994a)	May. '90	splits	4.46	1 600 (Jan. '91)	3 000	2 700 (est.)	7 300
Current trial (block)	Early Oct. '94	sowing	4.0	0	3 491 (Jan. '96)	2 745	6 236
Current trial (block)	Early Oct. '94	sowing	16.0	0	4 807 (Jan. '96)	4 589	9 396

The table indicates that at equivalent density and over a period of three harvest seasons, expected total yield from splits may be approximately 1 000 kg/ha more than seed-established crops over the same period. The split crop was in the ground for five months longer, required harvesting on three occasions (rather than twice) and cost significantly more to establish. Any financial advantage in achieving a marginally higher yield over the three year period through the use of splits may therefore be lost through additional land rental, crop establishment and harvest costs. This comparison provides some insight into the relative benefits of different establishment techniques. However, no scientifically valid comparisons can be made based on these data.

Of further importance to industry, and more in line with the objectives of the current study, was the identification of considerable increases in flower yield with higher plant densities (Table 2.10). In fact, flower dry weights increased to an optimum well beyond the maximum plant densities evaluated in any of the previously reported studies. In the first season, dry flower yields increased from 3 491 to 4 807 kg/ha in the 4 to 16 plants/m² treatments, a 38% increase in yield. The greatest increase occurred between 8 and 16 plants/m². Similar results were obtained in the second harvest with yields increasing from 2 745 to 4 589 kg/ha from 4 to 16 plants/m², a 67% increase in yield. In

the second season a greater increase was observed from 4 to 8 plants/m² than from 8 to 16 plants/m² (Figures 2.3 and 2.4).

The increase in flower yield with density followed an increase in above-ground dry matter production (investigated in the first season) (Figure 2.1). A dry flower harvest index of 20.5% did not vary with plant density suggesting that in this trial, dry flower yield was limited by biological yield. A constant harvest index also indicates that any effort to increase plant DM may positively influence flower yield.

Varieties with average pyrethrins concentrations of greater than 2.0% were developed during the 1980s by the University of Tasmania's pyrethrum plant breeding program (Bhat, 1995). The current work demonstrates that with increasing density and higher flower yields, pyrethrins concentration remains constant. Pyrethrins yield was therefore directly related to flower yield. In the first season, mean concentration of 2.36% was determined for flowers from the second sequential harvest taken just prior to the time considered optimum for harvest. In the second season pyrethrins concentration was 2.28%.

Pyrethrins concentration appears to be a plant characteristic that varies little under different plant densities (Rajeswara Rao and Singh, 1982), under plants generating significantly different dry flower yields due to plant immaturity (Bhat, 1995), and from first to subsequent flowering seasons (Bhat, 1995). A notable exception to these examples is found in potassium deficiency studies which indicated that low potassium availability may be responsible for substantial reductions (23%) in concentration (Salardini *et al.*, 1994b). Pyrethrins concentration appears to be a varietal characteristic that varies little between different densities.

Pyrethrins yield data generated in the first season were calculated by multiplication of yield components of individual plots as described in a range of other crops (Harper, 1961). Any error incurred in estimation of any given parameter would therefore be carried on to a similar error in the final yield. This error (if presumed to be random) may have been eliminated by using treatment means, means common to treatments that were not significantly different, or by the multiplication of fitted curves generated from yield component treatment means. Such techniques were not used since they may have hidden real trade-offs (e.g., less flowers per tiller resulting in larger flowers) that may have occurred within plots (Bhat, 1995) thereby dismissing real density effects for the sake of the production of a smooth yield curve. Therefore a common pyrethrins concentration was used in the yield calculation as there is significant evidence that this yield component

is not linked to flower yield (Bhat, 1995). Providing confidence in the pyrethrins yield data from the first season harvest was the observation that dry matter yield paralleled pyrethrins yield. Harvest Index for flower dry weight remained constant throughout the density range. Two harvests in the second season, one to evaluate yield components, the other to evaluate actual yield, generated similar yield data and further increased confidence in the techniques used to evaluate yield in the first season.

The pyrethrins yields reported in the current work of over 100 kg/ha are unprecedented in the scientific literature. The highest recorded pyrethrins yield is 48 kg/ha for a Tasmanian trial (Salardini *et al.*, 1994a) and the highest trial yields in Africa do not exceed 25 kg/ha (Bhat, 1995). Yields as low as 7 kg/ha in an Indian study (Rajeswara Rao and Singh, 1982) have been reported. Extremely high yields are not restricted to trial data. The first harvest of a sown commercial site returned 72 kg/ha in January 1998 (pers comm. Mr M. Greenhill, Field Manager BRA Pty Ltd.). As this was only the second season of attempting to establish pyrethrum crops by sowing, further increases in yield with improved seed production and sowing techniques may be expected in future seasons. The following discussion investigates how various components of yield varied with density.

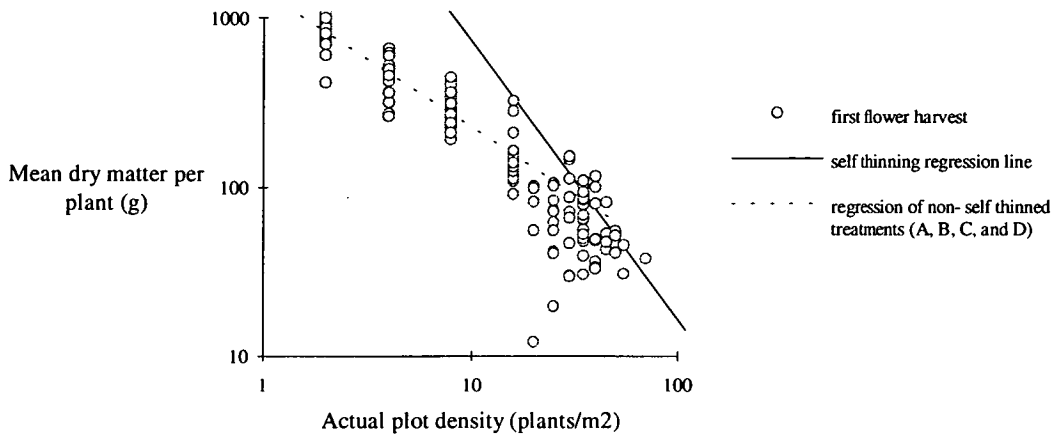
2.5.2 Changes in plant population

Data presented from the pre-flowering harvest of the block trial indicate that no plant losses occurred in the first eight months after sowing. Although this was so, large differences between density treatments in mean whole plant dry weight were evident indicating significant competition at that time (Figure 2.5).

Data from the first season of the block trial indicate that significant reductions in plant density occurred only in the two highest density treatments. The value of the constant k of the self-thinning line was 1.646 (Figure 2.8), which is reasonably close to the value $3/2$ proposed to be constant for a wide range of species (Westoby, 1984). Plant populations remaining in the self-thinned treatments indicate that maximum plant densities sustainable up to the first harvest may be 35 to 40 plants/m². The intersection point of the self thinning regression line and a regression line generated from the four lowest, non-self-thinned density treatments is presented in Figure 2.39. This was found to occur at 39.5 plants/m² indicating that this density would be the maximum population that would persist without self thinning occurring. At this density, plants would be expected to produce 75.47 g DM/plant, or 2 981g DM/m². Since a mean above ground yield of 2 894 g/m² was achieved at the much lower density of 16 plants/m², establishing plant densities greatly above this population would be expected to provide little or no increase

in productivity. In summary, evidence indicates that maximum dry matter production at plant densities above 16 and below 39 plants/m² first year was achieved without any significant self thinning.

Figure 2.39 Mean dry matter per plant vs density in the block trial, first season



Note (1) Regression line equation for self thinning line: $\log y = -1.646 \log N + 4.506$ ($R^2 = 0.94$). (2) Regression line for mean DM/plant for treatments A, B, C, and D: $\log y = -0.80184 \log N + 3.158$ ($R^2 = 0.83$) number of observations: 72. (3) Point of intersection was calculated by elimination of 'y' and solving for N.

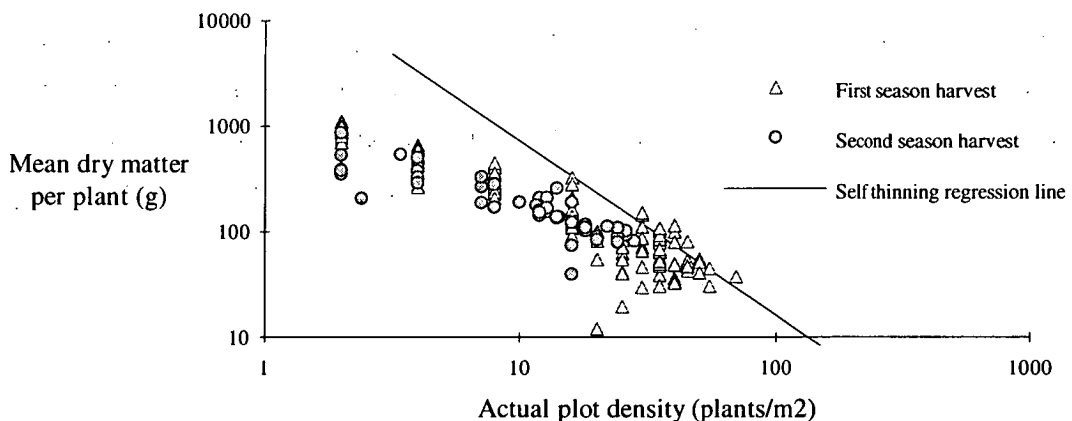
Although in the fan trial plant losses had occurred by the time of the first harvest, descriptive data presented indicate that losses were not density dependent. Unfortunately plant survival data at densities above 31 plants were not consistently collected, therefore no evidence on self thinning was available from this trial. Although densities up to 70 plants/m² were established, treatments higher than 31 plants/m² became increasingly entangled and difficult to identify. Furthermore, the harvest areas involved became increasingly small owing to the decreased area of arc at higher densities.

Plant survival data presented nevertheless indicate that plant losses occurred for reasons other than competition. Plants died at various stages of development, some at the vegetative stage, others during tiller extension and some when flowers had opened. The main agent of plant death was *Sclerotinia minor*. Infected plants displayed pin head sized black resting bodies (sclerotia) located mostly around the base of the tillers. Investigation of the distribution of dead and missing plants as presented in the results revealed no consistent 'clumping' of dead or missing plants that would indicate disease transfer from the infected plant to nearest neighbours. Similar observations were made in the highest density treatments of the block trial where single infected plants touched neighbouring foliage but failed to transfer the disease (see Plate 2.13). In summary, disease appeared to be restricted to a limited number of individuals with little evidence of disease transfer

within the season. Disease remained localised around the crown of infected plants and apparently interfered with water transport to upper parts, resulting in plant death. No major disease problems are foreseen that would preclude the use of significantly higher plant densities than those currently used.

Plant density was evaluated again in the second flower harvest of the block trial. Although population had decreased in all treatments (except treatment A) competition could not be held responsible for plant losses in any of the four lowest density treatments. Figure 2.40 indicates that in that second season, dry matter production was not as high as in the first. Therefore DM/plant data points were not as close to the self thinning line as in the previous season. The pressure for further self-thinning by the end of the second harvest therefore was lower than that observed in the first. Two factors may account for this decrease. Firstly, a decrease in plant numbers to levels that do not allow maximum dry matter production to be achieved and secondly a shorter period of time available for growth between first and second harvest than between sowing and first harvest.

Figure 2.40 Mean dry matter per plant vs density in the block trial, first and second season



Plant populations in self-thinned plots at the time of the second harvest were variable, with values ranging from 8 to 28 plants/m². The decreased yields observed where self thinning resulted in less than optimal population (Figure 2.40) is evidence that self thinning contributes to decreased yields in second season harvests. As the population and distribution of plants remaining after self thinning was variable, self thinning population levels should be avoided. Second season fan trial data indicate that at densities above 17.3 plants/m² a lowering of plant survival was observed which is in agreement with block trial data.

2.5.3 Variability between plants

Variability between individual plants was investigated by assessing flowering tiller number per plant. Skewness was measured to evaluate how the shape of the population distribution changed with plant density. Data presented for the first season harvest of the block trial reveal a significant increase in positive skewness with increasing density treatments. High skewness coefficient indicates an increased tendency for the presence of a few large and many small individuals in the population, a distribution in which further self thinning would be expected if growth were to continue.

Skewness of flowering tiller number was evaluated again in the second season harvest and significant differences between treatments were identified. Combined replicate data investigating skewness, CV and Kurtosis demonstrated significant variability between plants with increasing density. These results from both seasons agree with the changes described by Naylor (1976).

2.5.4 Yield components

So far discussion has focused on overall yield, changes in plant population and various measures of inequity of production between individuals within populations. The following discussion investigates how components of yield vary with density.

Flowering tillers

With increasing density, the number of tillers produced per plant decreased significantly, as demonstrated in both block and fan trials. Variation in plant tiller numbers appears to be a major mechanism by which plants respond to competition.

Mean number of tillers produced by the plant at a given density appeared to decrease from the first to the second harvest in the block trial while the opposite appeared to be so in the fan trial. Variations in the first season tiller number per plant are thought to be due to the different period of time available for growth and development in the two trials. The influence of sowing time on first season yields is investigated in the Chapter 3.

When tillers/m² data are calculated using actual plot plant densities, it is evident that significant increases are observed up to densities of 16-20 plants/m². At densities higher than this, tillers/m² decreased, remained constant or increased slightly depending on the trial and harvest season. Although actual increase varied depending on trial and season, tiller number/m² approximately doubled from 4 to 16 plants/m². Further examination reveals that tiller number/m² is the only yield component to increase with plant density

(other components either decreased or remain constant). Increasing yields with density can therefore be attributed to increasing tiller numbers/m².

Flower number per tiller

Flower number/tiller was assessed in both seasons of the block and fan trials. Data presented show that flowers/tiller decreased significantly in the density range of 2 to 8 plants/m². Generally over this range flowers/tiller decreased from approximately 8 to 5, or 40%. Flowers/tiller did not vary significantly from 8 plants/m² to the highest densities evaluated. There is evidence, therefore, that increasing flowers/tiller is a means by which the plant regulates its productivity at densities below 8 plants/m².

In the first season flowers were harvested from both the block and fan trials in three sequential harvests. In each, the data demonstrate that significant losses of flowers occur in the last of the three harvests, independently of density. As flowers mature and dry they become increasingly brittle resulting in significant losses in achenes. Timing harvest such that losses through flower shatter are kept to a minimum should be an important consideration.

Flower weight

As with flower number/tiller, mean dry weight/flower was only found to vary at low density. In fact, only flowers from 2 plants/m² were significantly heavier than those from higher densities. Heavier flowers at the lowest density were found in both seasons of the block trial. The possibility that flowers were more mature in the lowest density treatment, thus influencing flower weight, was investigated and found not to be responsible for this effect. Flower dry weight was assessed in the fan trial and found not to vary with plant density, but the lowest density evaluated in that trial was 3.5 plants/m², a density not low enough to display the increase observed in the block trial.

Higher weight/flower only occurred at densities well below those required for high commercial yields and is therefore of no significance in commercial crops. However higher flower dry weights at the low density may be due to production of heavier achenes and seed. Therefore this phenomenon should be investigated further in the pursuit of larger, higher quality seed.

Pyrethrins concentration

Concentrations provided no indication of any change in pyrethrins concentration with plant density. As alluded to in an earlier section, pyrethrins concentration appears to remain remarkably constant across a wide range of environmental- and plant-based

variables. Some examples of where this generalisation has been demonstrated include, 'in-field time' having little influence on concentration at first flowering (Bhat, 1995), insignificant change in concentration when clones were evaluated in different seasons (Parlevliet, 1969), and little variation under differing nitrogen (Rajeswara Rao and Singh, 1982) and phosphorus (Salardini *et al.*, 1994a) regimes. Conversely, factors such as temperature (Kroll, 1964) and rainfall (Parlevliet, 1970) have been reported to influence pyrethrins concentration, as have stage of flower development (Ikahu and Ngugi, 1989), flower development and clone (Bhat and Menary, 1984, and Ikahu and Ngugi, 1990), and severe potassium deficiency (Salardini *et al.*, 1994b). Since in the present study no differences in pyrethrins concentration were identified with increasing density, the outcome is clear: increased flower yield with density accounts completely for higher pyrethrins yields.

2.5.5 Flower maturity

One of the concerns at the initiation of the current work was that plant density would impact on flower maturity either by changing mean flower maturity or by decreasing uniformity of flower maturity. The block trial FMI was evaluated in the three sequential harvests of the first season and again in Evaluation 1 of the second season. FMI was also evaluated in the first season of the fan trial. All data demonstrate that density had no significant impact on FMI. Standard deviation (SD) of FMI was also investigated in order to evaluate the influence of density on uniformity of maturity. Again no significant differences in this statistic were identified. Therefore plant density had no significant influence on either mean or spread of flower maturity.

The potentially useful observation that SD of FMI appeared to decline substantially and in a linear way with FMI, may have significant industry implications. If this characteristic is found to vary in a consistent way in other pyrethrum crops, it may be developed as an improved means of determining optimum harvest time. Currently, FMI is used for determination of 'cutting time' but the measure is found to be unreliable, particularly at the crop maturity stages thought to be optimal for yield.

2.5.6 Changes in length and weight of flowering tiller

In the first season of the block trial some leaning of the flowering tillers was noted in the two highest density treatments. This was considered to be of only minor concern since plants in the currently accepted density of 4.0 plants/m² presented more prostrate flowering tillers than the high density treatments. Nevertheless, an attempt to investigate any factors influencing change in lodging tendency was undertaken. Data were collected

on the mean dry weight of the main tiller, mean tiller length, and tiller weight per length in the different density treatments.

Dry weights of the main tiller in the two lowest density treatments (2 and 4 plants/m²) were between 40 and 50% heavier than in higher density treatments. Previously discussed data revealed that more branching (more flowers) were also present only in these two lowest density treatments.

Mean length of the tillers increased significantly from 660 mm in the lowest density to 760 mm in 8 and 16 plants/m² after which it declined. The increase in crop height did not appear to be related to lodging since greater lodging (leaning of tillers) was apparent at densities higher or lower than the tallest treatments.

Combining the tiller mass and length "i.e. mass per unit length- Figure 2.36" provides a measure which may be related to tiller strength. Tiller strength was higher in the two lowest density treatments than in all other densities. The measure therefore fails to provide any explanation for increased leaning in some of the highest density plots. The explanation for incidence of leaning in high density plots may therefore be associated with increased individual plant skewness. A few large plants within high density treatments may have behaved similarly to plants in the lowest density treatments.

2.5.7 Yielding capacity of flowers of different hierarchy

In an earlier section it was revealed that flower number per tiller decreased with increasing density. Those data provide no information on presence of flowers from different positions with increasing density. Position of flowers present on the tiller was evaluated in the first season of the block trial. A decrease in the incidence of tertiary flowers was predominantly responsible for changes in flower number per tiller. The incidence of tertiary flowers decreased significantly within the density range 2 to 8 plants/m².

Differences in pyrethrins concentration, flower maturity and mean flower dry mass were investigated in flowers of different position. Results of this investigation revealed that no significant differences in these characteristics were apparent. These results complement the overall yield component data presented in earlier discussion.

2.5.8 Summary

In other producing regions, and until recently in Tasmania, density of planting has been largely determined by the high cost of plant material or the significant labour involved in

establishing higher populations. The recent advances in crop establishment from seed in Tasmania mean that high crop establishment costs are avoided and plant density could now be selected that would maximise yield. The current work demonstrates that appreciable increases in yield are achievable if plant densities are increased from the current standard of 4 to 16 plants/m². Densities higher than this, but below 40 plants/m², will avoid self thinning and are recommended for maximum first season yields.

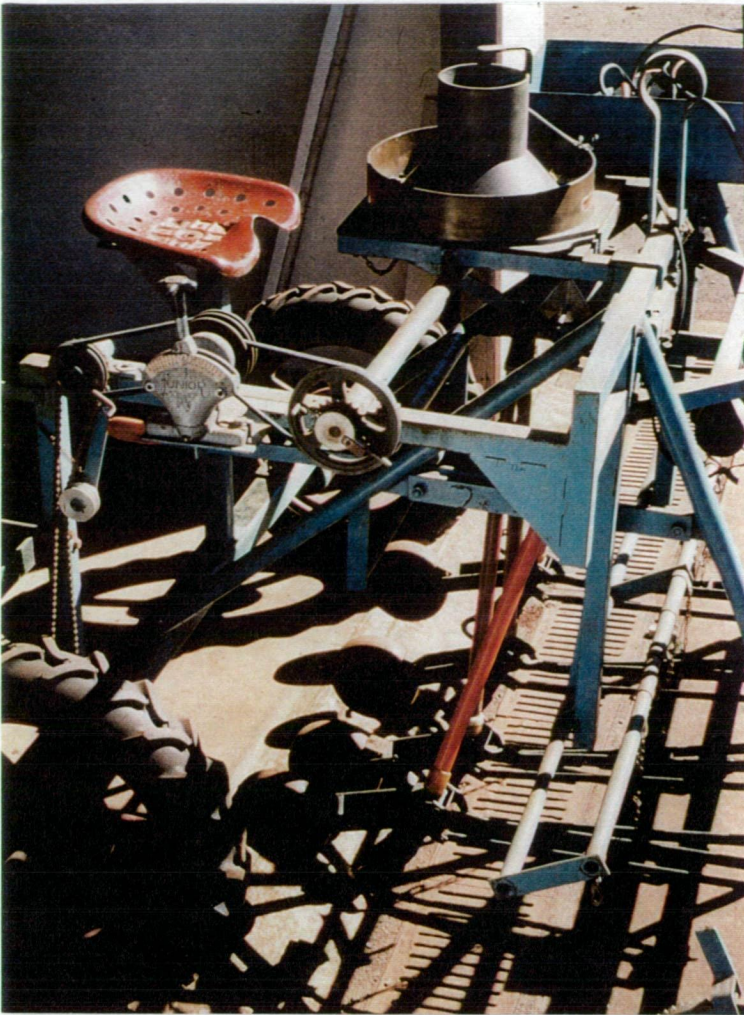


Plate 2.1 (top) The Ojoid cone trial seeder used to sow the block trial

Plate 2.2 (bottom) The block trial site at Fortside Vegetable Research Station (FVRS)



Plate 2.3 (top left) Treatment A in the block trial, 2 plants/m².

Plate 2.4 (top right) Treatment C in the block trial, 8 plants/m².

Plate 2.5 (bottom left) Treatment D in the block trial, 16 plants/m².

Plate 2.6 (bottom right) Treatment E in the block trial, 24 plants/m².



Plate 2.7 (top left) Treatment G in the block trial, 64 plants/m²

Plate 2.8 (top right) Treatment H in the block trial, 100 plants/m²

Plate 2.9 (bottom) A range of plant sizes in the 100 plants/m² and 2 plants/m² treatments of the block trial, five months after sowing (magnification of 0.2 ×)



Plate 2.10 (top) The fan trial site at FVRS showing spare plants in foreground and the irrigation system

Plate 2.11 (bottom) A plank was used for preventing compaction during planting of the fans



Plate 2.12 (top) The harvested fan trial, second season

Plate 2.13 (bottom) A single pyrethrum plant in a high density plot displaying symptoms of *Sclerotinia* wilt

Chapter 3: The effect of sowing time on yield

3.1 Introduction

This chapter investigates the influence of sowing time on pyrethrins yield. There are no reported studies on the influence of sowing time on pyrethrins yield. To investigate this, plots were sown at monthly intervals in both spring and autumn. Yield component data provide insight into how sowing time limits production. The effect of time of sowing on plant development and time available for plant growth are generally reviewed prior to discussing previous work on planting time and flowering in pyrethrum.

3.2 Literature review: The effect of plant growth and development on yield

Regardless of whether the plant is an annual, biennial, or a perennial, certain environmental and endogenous requisites have to be satisfied before reproductive yield is achieved. Requirements may vary with stage of growth or development and become more or less critical for normal flowering with time. Growth requisites may be considerably different from those for development. They may be obligate, where development or growth will not occur unless conditions are met, or they may be quantitative, where development is delayed or reduced if conditions are not completely satisfied. This work firstly outlines well-recognised factors determining growth and development in plants. Following this, literature on the effect of time of planting splits on pyrethrins yields is reviewed.

3.2.1 Development requisites

Yield can be significantly reduced in plants harvested for their reproductive parts due to inadequate development. A plant's reproductive behaviour can be influenced by a variety of factors including photoperiod, temperature and juvenility.

Responses to photoperiod vary greatly both within and between species. Generally plants are categorised as day neutral, short or long day plants and photoperiod requirements may be obligate or quantitative. Photoperiods such as the base (P_b); ceiling (P_{ce}); and critical (P_c) describe periods of light above or below which flowering is prevented or slowed (Roberts and Summerfield, 1987).

Flowering behaviour may be modified if the plant has a vernalisation requirement. This may be either obligate or quantitative and satisfied in the imbibed seed or during the

growth of the plant. Vernalisation is typically satisfied at temperatures below 16°C (Thomas and Vince-Prue, 1984).

Temperature is found to influence progress towards flowering in a range of species. Cardinal temperatures describing this progress include: the temperature below which progress towards flowering is zero (T_b); the temperature at which progress is maximal (T_o); and temperature at, and above which progress is zero (T_{ce}) (Roberts and Summerfield, 1987). Interactions between photoperiod and temperature may occur but will not be discussed here.

In addition to factors such as photoperiod and temperature, the size of the plant during the vegetative phase may also influence its capacity to flower. The juvenile phase, i.e., the period when a plant is too small to respond to flowering stimuli, varies greatly between and within species (Waring, 1987).

3.2.2 Growth requisites

Where flowering is not limited through incompetence to flower, yield may still be restricted through lack of time for growth. It may also restrict the period for reproductive growth in progressively later sowings in a temperate environment. Matching the genotypic adaptation of a crop to its environment is a basic aim where crop yield is of critical importance (Shorter *et al.*, 1991).

Plants of different sowings may vary in their capacity to grow and take full advantage of available environmental resources. Barnes (1977) following on from original studies (Shinozaki and Kira, 1956) incorporated a time factor along with plant density in plant growth equations. This recognised the contribution of 'prior time for growth' to the yielding potential of a crop.

For annual crops in particular, later sowings may result in shorter periods of time in various phases of development including reproductive stages. For example, increasingly later sowings resulted in a decrease in flowering and pod fill time prior to maturity in faba bean, *Vicia faba* (Adisarwanto and Knight, 1997). Not surprisingly, this agrees with the day degree hypothesis (Johnson and Thornly, 1985) which assumes that growth rate in a particular stage of plant development is determined by a function in which temperature has significant influence.

Polycarpic perennial plants, including pyrethrum, only ever convert a certain proportion of their buds to flowers. In some perennials, flowers are produced from the

primary or central shoot tip while older nodes remain vegetative. In other perennial species, the opposite of this is true (Salisbury, 1963). Each year, herbaceous perennial plants are therefore able to continue to produce leaves during flowering, or resume a vegetative phase after flowering. In this way, perennial plants may undergo what may be regarded as a juvenile-like phase each season (Waring, 1987). Studies on flowering behaviour in perennial plants are largely restricted to tree and orchard crops of economic significance. Salisbury (1963) proposed that this was largely due to the difficulty and time constraints of perennials in comparison to annuals that are much easier to study. Even so, there appears to be little evidence that mechanisms preventing flowering in perennial plants vary greatly from those that operate in annuals or monocarpic perennials.

3.2.3 Flowering in pyrethrum

Vernalisation, photoperiod and juvenility in pyrethrum have been investigated. Glover (1955) identified that intensity of flowering was directly related to number of hours at or below 16°C in the previous three months. To initiate flowering, temperature must fall below 16°C for six weeks. During this time if the plant was exposed to temperatures above 24°C for a week, bud initiation was inhibited (Glover, 1955). Where plants were cultivated in low-land tropical environments, they were often found to remain vegetative or 'blind' as they failed to receive the cool period essential for flower initiation. Vernalisation is therefore an essential requirement for flower initiation in pyrethrum. In light tunnel studies, Brown (1992) identified that with day temperature in the 20°C to 30°C range, the minimum vernalisation requirement was two weeks at 6°C or 12°C for three weeks. Night temperatures of 18°C were found to not satisfy the plant's vernalisation requirement. Furthermore, Brown (1992) identified that the vernalisation response in pyrethrum was quantitative since increasing periods in vernalising conditions resulted in more rapid initiation and development, larger numbers of flowers, and longer stems.

The influence of photoperiod on flowering in pyrethrum has been investigated (Brown, 1992). Light tunnel experiments demonstrated that increasing photoperiod had a quantitative positive effect on both initiation and subsequent flower development. Increased flowering was considered to be a result of the higher daily light integral rather than due to any photoperiodic effect. As the plants examined were considered to be insensitive to photoperiod, the worker proposed that pyrethrum could be regarded as a day-neutral species.

Tasmania's cool temperate climate, summer maximum temperatures average 18-20°C and winter maximum temperatures average 10-12°C. As such, the vernalisation required for flower initiation is satisfied (Mac Donald, 1995). In fact temperatures required for vernalisation reported by Brown (1992) indicate that in Tasmania vernalisation requirements are met in every month of the year. Therefore satisfaction of vernalisation requirement does not explain the single annual flowering in Tasmania rather than the periodic flushing observed in highland tropical regions. An explanation for the single annual flowering observed in cool temperate regions may be associated more with growth rate variability in the highly seasonal climate. Further research would be required to fully understand variability in flowering behaviour.

A period in which both seedlings and splits are not able to respond to flowering-inductive conditions (a juvenile phase) has been described by Brown (1992):

"During the period of juvenile like growth, the plants were not competent to respond to normally inductive treatments. The juvenile-like phase lasted until the plants had reached a minimum size, or stage of development, but did not depend on chronological age."

Brown (1992) observed that attainment of meristem competence to flower was linked to the release of lateral buds from apical dominance. Concurrent to this, older axillary meristems (further away from the meristem) lose their competence. In his light tunnel study, splits were observed to become competent to flower after 71 days and after producing 30 leaves. Seedlings were observed to take significantly longer, only being competent to initiate flowers after 261 days and after producing 20 leaves.

Even if a sown pyrethrum plant is competent to flower, yield may be restricted due to the size of the young plants. Commercial planting of splits or seedling plugs is conducted in Tasmania during the autumn months, April-May. The first flowering from those sites occurs some nine months later (mid January) and the flower yields are generally significantly lower than those obtained in subsequent seasons (Salardini *et al.*, 1994a and b). Bhat (1995) investigated the effect of planting date of splits on flower yield (Table 3.1) and found reduced dry flower production with later plantings.

Table 3.1 also demonstrates that pyrethrins concentration was not affected by planting date. This result is in general agreement with earlier work (Parlevliet, 1969) in which 110 clones were evaluated over a three year period. In that study pyrethrins concentration was found not to vary between first and subsequent harvest years, while dry flower yield was found to be significantly lower in the first than in subsequent years.

Table 3.1 Effect of planting date on pyrethrins content, dry flower yield and pyrethrins yield

Planting date	Pyrethrins content (%)	Dry flower yield (kg/ha)	Pyrethrins yield (kg/ha)
28/09/90	1.82	1,210	22
16/10/90	1.98	739	15
28/11/90	1.80	550	10
07/01/91	1.84	440	8
25/01/91	1.67	379	6

Source: Bhat, 1995 p.83

Data presented indicate flower production rather than pyrethrins concentrations determine yield since pyrethrins concentrations appear to only vary little between treatments. Furthermore, production capacity in such crops appears not to be determined by juvenile-like incompetence or an unsatisfied vernalisation requirement since sequentially-later sowings all flowered.

Since commercial pyrethrum crops are not established by sowing in other regions, no data on yielding capacity of sown crops were found. Although no production information is available, data from plant breeding methods (Bhat, 1995) indicated that if seed is sown directly after flowering, seed may again be collected from the resultant plant by the same time in the following year. Production of a pyrethrins yield within an annual cycle is therefore possible.

Light tunnel studies which compared competence to flower of splits and seedlings revealed that seedlings were very slow to respond to inductive conditions, taking some 190 days longer to initiate flowers than did the splits (Brown, 1992). Some evidence therefore exists indicating that lower first season yields may result if crops are sown rather than being planted from splits. As first season yields from split-established crops themselves are marginal, the likelihood of an economic yield being generated from crops established from seed within the one season may be low. The current work investigates the impact of sowing time on aspects of plant growth, development and yield of pyrethrum crops.

3.3 Materials and methods

3.3.1 Time of sowing field trials

Two trials were established at Forthside Vegetable Research Station (FVRS) (41°10'S, 146°40'E) on the western part of Tasmania's north coast (Plate 1.14). The first was a spring trial, when seed was sown in mid September, October, November and December

of 1995. In the second trial, seed was sown in autumn, in January, February, March and April of 1996. Each trial was laid out in four randomised complete blocks (Plate 3.1). Plots were 18.0 m long and beds were 1.6 m wide. Four rows per bed were sown with a between-row spacing of 300 mm. Preparation of the field included mouldboard ploughing in September (four months prior to the first sowing) and a drill application of 750 kg/ha of 14:16:11 (N:P:K) and subsequent incorporation. Sowing was conducted as described in Chapter 4.3. Two to three months following sowing, when plants were rosettes of approximately 40 mm diameter, treatments were thinned to a common density of 10 plants/m² as described in Chapter 2.3.

The trials were irrigated using solid set micro-irrigation sprinklers in a square arrangement 5.0 m apart (Plate 3.1) which applied water at 10 mm/hr. The weed control programme was the same as that described for the density trial (Chapter 2.3) except that the non-selective herbicide glyphosate was not used in order to eliminate the possibility of drift onto neighbouring plots. Disease control was the same as that described for the density trials. Data were analysed using the programme embedded in Microsoft Excel™ 4.0 entitled ANOVA: Two factor without replication.

3.3.2 First season flower harvest

First season assessments were limited to the autumn trial, since only this trial was flowering by January '96. All plants from a six linear metre length of each bed (30-40 plants) were harvested except the April sowing, where twice the area was harvested due to the low density encountered in those plots. Plants were harvested at ground level, weighed and assessed as being 'flowering' if tillers elongated beyond the leafy rosette zone. The tiller number and fresh weight per plant were assessed. Samples of leaf, flower and tiller material were taken to estimate plant dry weights. Flowers were collected from individual plants, bulked with others from the plot, and evaluated for mean flower maturity (Appendix 1.1).

3.3.3 Second season flower harvest

A single harvest from both trials was conducted in the second season. Plants were harvested from two middle row lengths (each 3.0 m) in each plot. Twice that length was harvested in the April sowing due to the low plant density. Tiller number per plant was counted. Flowers from plants, which were harvested at ground level, were stripped, weighed and bulked with flowers from other plants. Before stripping flowers, one third of the plants were randomly selected and six tillers were again randomly selected from each to obtain data on mean flower number per tiller. Flowers from the selected tillers were subsequently stripped and returned to the bulked sample of flowers. Mean flower

dry weight was determined by obtaining a 200 flower sample and drying at 70°C for 24 hours. Approximately 200 g of fresh flowers were sub-sampled from the bulked flowers and dried at 50°C before being placed in sealed plastic bags and stored at -18.0°C ready for grinding and pyrethrins assay (Appendix 1.2). A further sample of 200 g of fresh flowers was taken from each plot for analysis of mean flower maturity as measured by FMI (Appendix 1.1). Harvesting plots as described above allowed for both assessment of actual plot yield and calculation of yield from yield components.

3.4 Results

Table 3.2 presents data on the period from sowing to harvest time in the first and second seasons after sowing. Only some of the treatments flowered in the first season while all treatments flowered by the time of second harvest (Plate 3.2).

Table 3.2 In field time of treatments in time of sowing trials, FVRS

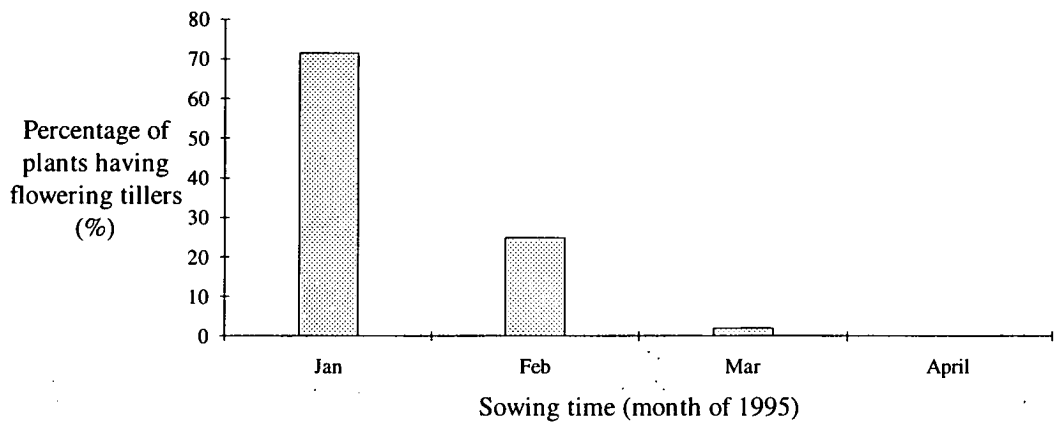
Sowing date (15-20th of each month)	Period from sowing to first harvest on 31/1/96 (months)	Extent of flowering by first harvest	Period from sowing to second harvest on 15/1/97 (months)
Autumn trial			
Jan. 1995	13	some flowers	24
Feb. 1995	12	very few	23
Mar 1995	11	trace	22
Apr. 1995	10	none	21
Spring trial			
Sept. 1995	6	none	17
Oct. 1995	5	none	16
Nov. 1995	4	none	15
Dec. 1995	3	none	14

3.4.1 First harvest results

The percentages of the plant population flowering in the autumn trial are presented in Figure 3.1. Results indicate significant decreases in flowering plants with lateness of sowing. The mean number of flowering tillers on the flowering plants was also investigated (Figure 3.2) and found to decline with later sowings. Autumn trial mean plant dry weight (Figure 3.3) declined with later sowings.

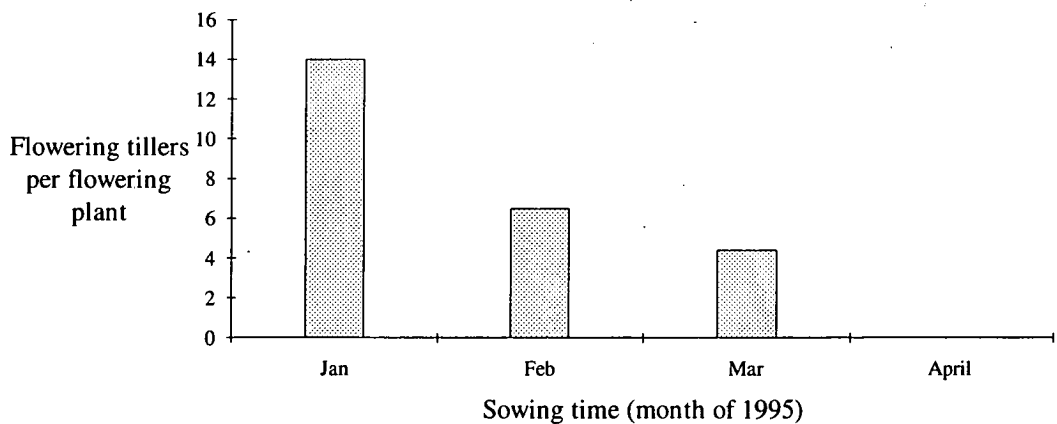
Mean dry weight of non-flowering plants (Figure 3.4) revealed plants were significantly lighter in the January than the February sowing. This indicates only the smaller plants in the January sowing remained vegetative.

Figure 3.1 Percentage of plants flowering vs sowing time, autumn trial, first harvest, January 1996



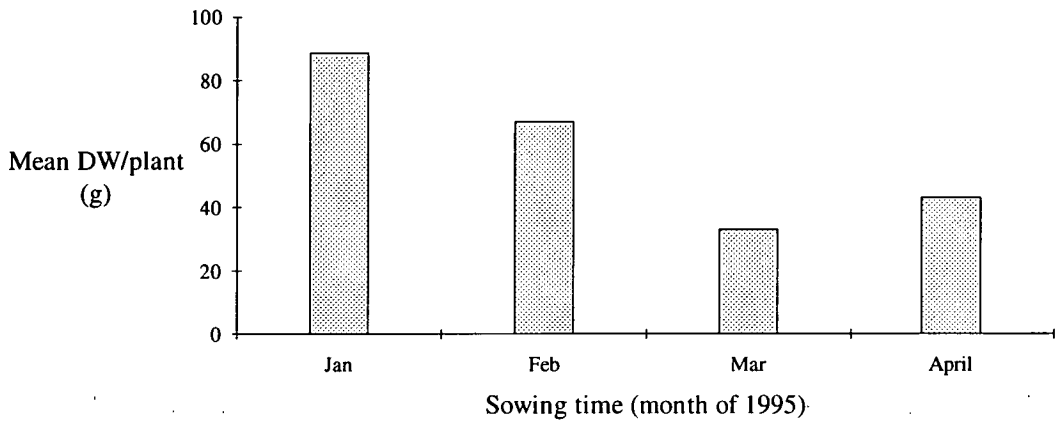
Note (1) Differences between some treatments are significant, $P = 0.001$, $LSD = 18.6\%$. (2) April was not included in the analysis.

Figure 3.2 Mean flowering tiller number per plant vs time of sowing, autumn trial, first harvest, January 1996



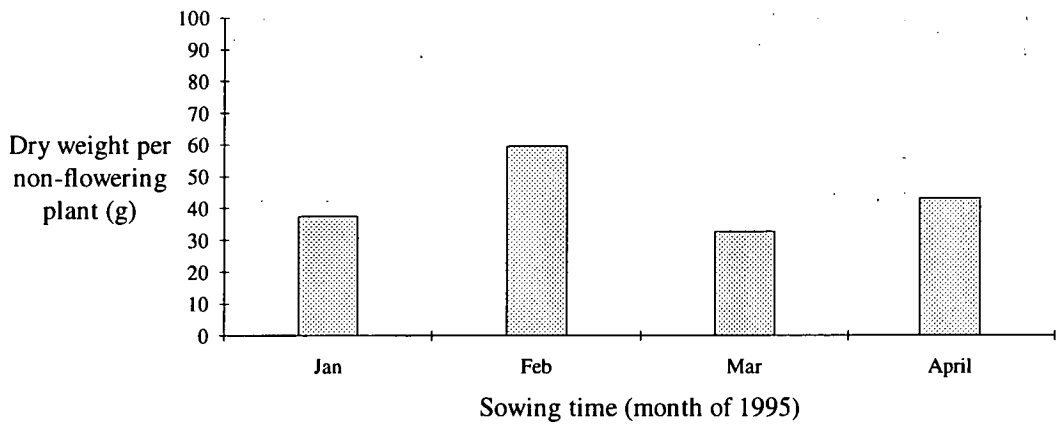
Note (1) Differences between some treatments are significant, $P = 0.019$, $LSD = 7.9$ tillers. (2) April was not included in the analysis.

Figure 3.3 Mean dry weight per plant vs time of sowing, autumn trial, first harvest, January 1996



Note (1) Differences between some treatments are significantly different, $P < 0.0001$, $LSD = 11.85$ g/plant.

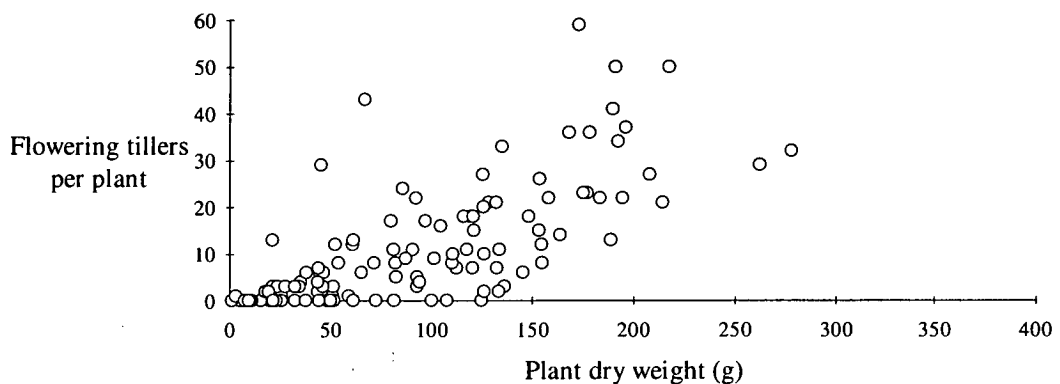
Figure 3.4 Mean dry weight per non-flowering plant vs sowing time, autumn trial, first harvest, January 1996



Note (1) Differences between some treatments are significant, $P = 0.027$, $LSD = 16.8$ g/plant.

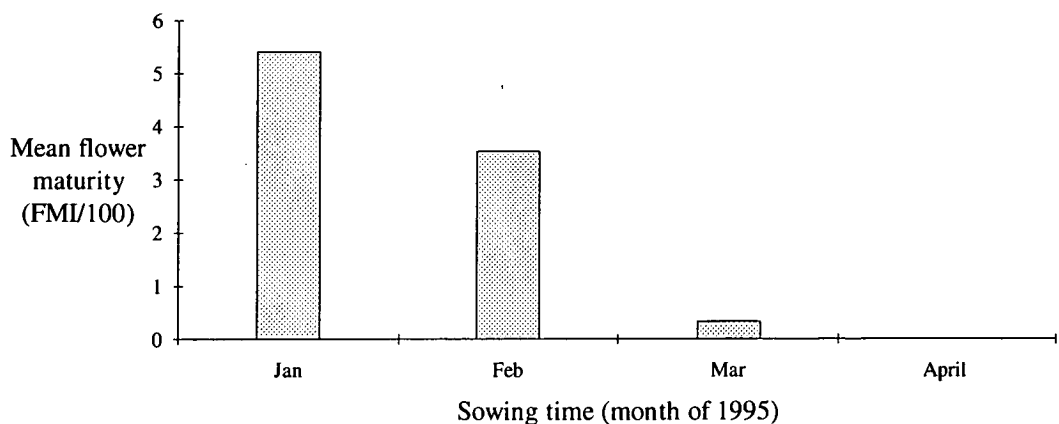
Investigation of the flowering behaviour of individual plants in the January sowing (Figure 3.5) demonstrated a poor relationship between whole plant weight and flowering tiller number per plant (Figure 3.5). Although it is acknowledged that the two variables are not independent, all plants above 125 g flowered providing some evidence for a threshold plant weight per flowering plant. Furthermore there was significant variability in extent of flowering tillers in plants of a given dry weight.

Figure 3.5 The relationship between flowering tiller number and plant dry weight at harvest time, January 1996 for the January 1995 sowing



Mean flower maturity was assessed and found to decline with lateness of sowing (Figure 3.6) indicating developmental delay occurred with lateness of sowing.

Figure 3.6 Mean flower maturity vs sowing time, autumn trial, first harvest, January 1996



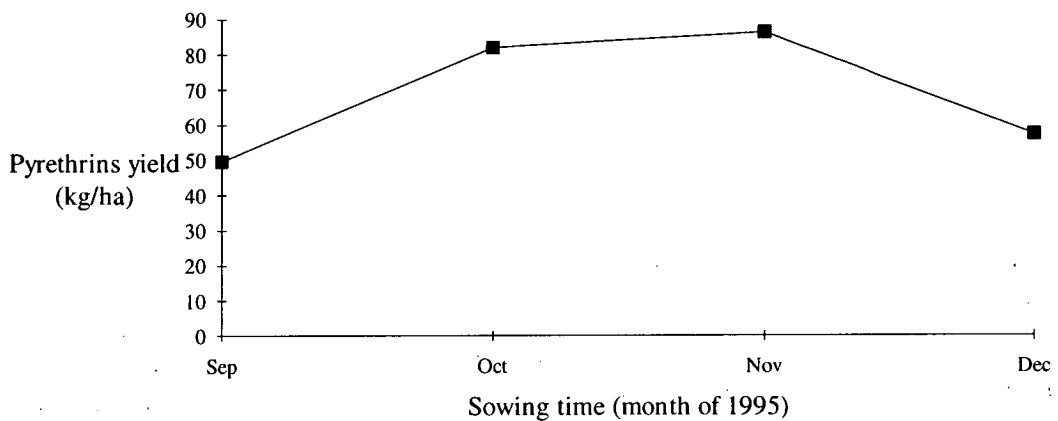
Note (1) Differences between some treatments are significant, $P = 0.012$, $LSD = 0.97$ units. (2) April was not included in the analysis. (3) FMI = flower maturity index

3.4.2 Second harvest results

By the time of the second season harvest, the autumn trial had been in the ground for between 21 and 24 months and had been harvested in the previous year. The spring trial had been in the ground for between 14 and 17 months and had not been harvested in the previous year. (Table 3.2).

Spring trial yields (Figure 3.7) increased from September to October and decreased again from November to December.

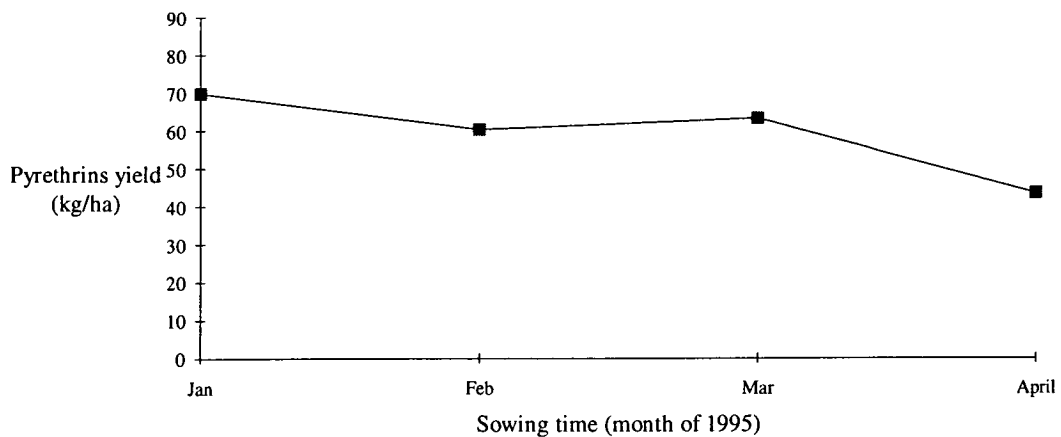
Figure 3.7 Mean pyrethrins yield vs sowing time, spring trial, second harvest, January 1997



Note (1) Differences between some treatments are significantly different, $P < 0.001$, $LSD = 12.75$ kg/ha.
 (2) Mean pyrethrins assay; 1.93%

Autumn yields only decreased in the last (April) of the sequential sowings (Figure 3.8).

Figure 3.8 Mean pyrethrins yields vs sowing time, autumn trial, second harvest, January 1997



Note (1) Differences between some treatments are significantly different, $P < 0.033$, $LSD = 16.87$ kg/ha.
 (2) Mean pyrethrins assay; 1.86%

Plant density in the various treatments, presented in (Table 3.3) indicate that low yields in September and April may be attributed to low plant density. Conversely, differences in population cannot account for the low yield in the December sowing. Differences in yield between November and December treatments were not visibly discernible (Plate 3.3).

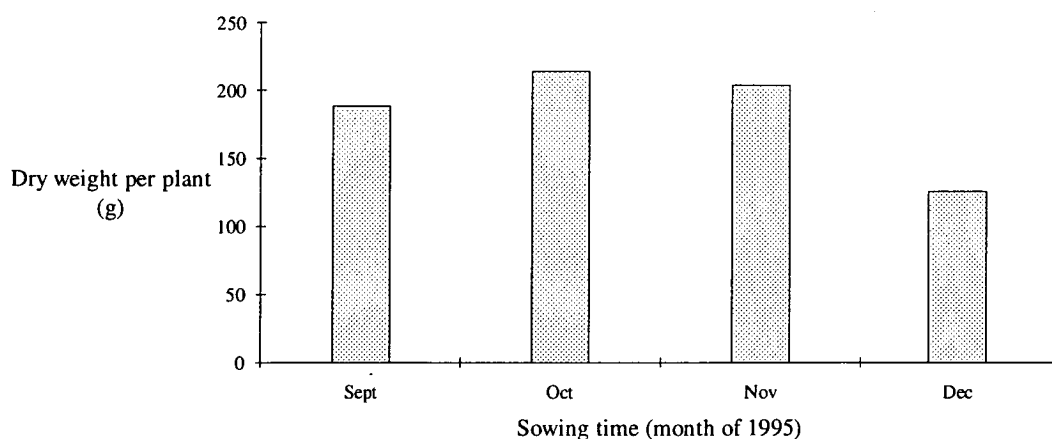
Table 3.3 Plant densities in time of sowing trials at second harvest, January 1997

Block	Autumn trial plant density (plants/m ²)			
	Jan	Feb	Mar	April
1	12.77	11.67	15.55	3.05
2	11.67	10.55	23.33	3.61
3	12.22	11.67	9.44	1.94
4	10.0	10.0	8.89	1.67
Mean	11.67	10.97	14.3	2.57

Block	Spring trial plant density (plants/m ²)			
	Sept	Oct	Nov	Dec
1	5.55	9.44	10.55	13.89
2	5.55	10.0	11.11	11.10
3	12.22	12.22	11.11	9.44
4	7.22	8.88	11.11	13.89
Mean	7.63	10.13	10.97	12.0

Figure 3.9 investigates plant dry weight in spring sowings and demonstrates a reduction in plant dry weight in the December treatment.

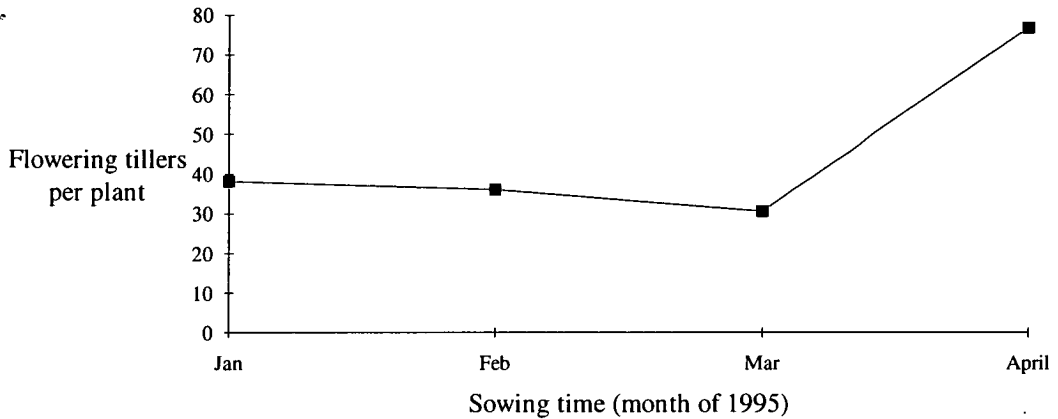
Figure 3.9 Mean dry weight per plant vs sowing time, spring trial, second harvest, January 1997



Note (1) The September treatment was excluded from the analysis due to variability in plant density in that treatment. (2) Differences between some treatments are significantly different, $P < 0.05$, $LSD = 68.5$ g/plant.

Mean number of flowering tillers per plant in the autumn trial (Figure 3.10) was found to only vary significantly in the low density April sowing.

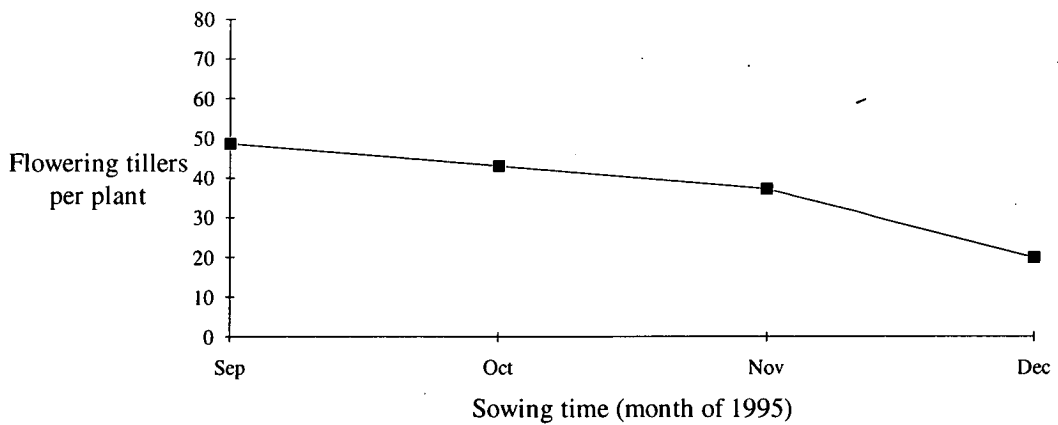
Figure 3.10 Mean number of flowering tillers per plant vs sowing time, autumn trial, second harvest, January 1997



Note (1) Differences between some treatments are significantly different, $P < 0.0001$, $LSD = 9.28$ tillers/ plant.

Flowering tiller number was also investigated in spring sowings and found to decline significantly from November to December (Figure 3.11, Plate 3.4).

Figure 3.11 Mean number of flowering tillers per plant vs sowing time, spring trial, second harvest, January 1997



Note (1) Differences between some treatments are significantly different, $P < 0.0005$, $LSD = 9.71$ tillers per plant.

The yield component of flower number per tiller was investigated in both autumn and spring sowings (Table 3.4) and found not to vary between sowings in either trial. Mean flower maturity was assessed for sowings from each trial (Table 3.4) and no large differences between treatments were identified. Likewise, no large differences in

pyrethrins concentration between sowings in either autumn or spring trials were identified (Table 3.4).

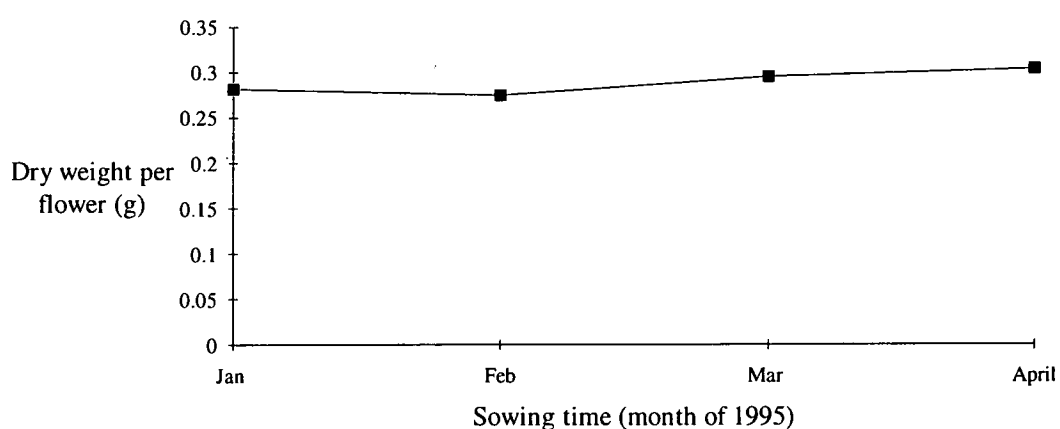
Table 3.4 Flowers per tiller, flower maturity index and pyrethrins assay for the autumn and spring trials

Yield component	Autumn trial				Mean and SE or LSD 0.05
	Jan	Feb	Mar	April	
Flowers per tiller	3.66	3.16	3.60	4.69	(NS) 3.78+/-0.72
Flower maturity index	688	678	685	706	P=0.04, LSD=18
Pyrethrins assay (% DM)	1.82	1.90	1.83	1.90	(NS) 1.86+/-0.11
	Spring trial				
	Sept	Oct	Nov	Dec	
Flowers per tiller	4.28	4.51	4.16	4.48	(NS) 4.36+/-0.32
Flower maturity index	678	660	657	659	(NS) 664+/-15
Pyrethrins assay (% DM)	1.90	1.94	2.00	1.91	(NS) 1.94+/-0.08

Note (1) DM=dry matter (2) SE = standard error

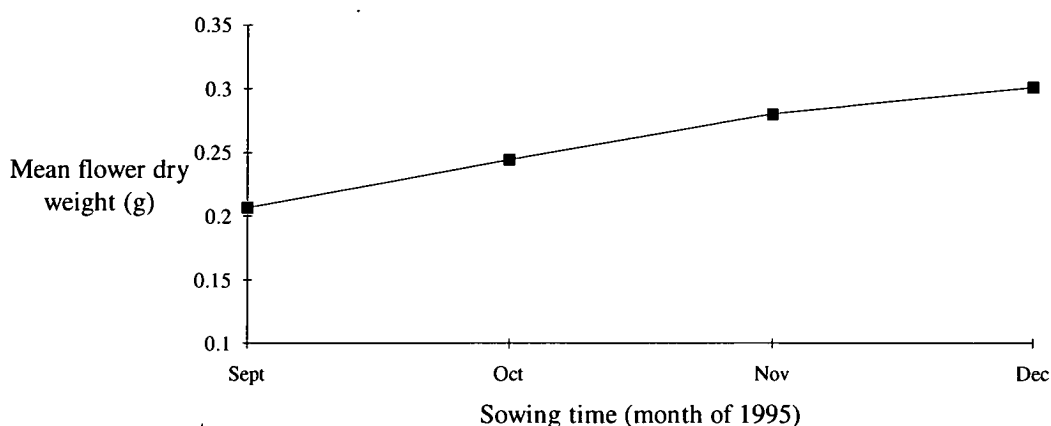
No differences in mean treatment flower dry weight were found between autumn sowings (Figure 3.12), but flower weight increased significantly with lateness of sowing in the spring trial (Figure 3.13).

Figure 3.12 Mean dry weight per flower vs sowing time, autumn trial, second harvest, January 1997



Note (1) Differences between treatments are not significantly different at, $P = 0.05$. Standard errors for sequentially later treatments were consistently less than 0.0003 g/flower.

Figure 3.13 Mean flower dry weight vs sowing time, spring trial, second harvest, January, 1997



Note (1) Differences between some treatments are significantly different, $P < 0.0005$, $LSD = 0.033$ g/flower.

Table 3.4 compares actual pyrethrins yields to yields calculated from multiplication of yield components. Results indicated that calculated yields overestimate actual yields but follow the same trend. Least significant differences were consistently higher for the calculated yields.

Table 3.4 Actual and calculated pyrethrins yields from time of sowing trials, second season, January 1997

Assessment type	Autumn trial pyrethrins yields				Statistical significance
	Jan	Feb	Mar	Apr	
Actual harvest (Fig. 3.8)	69.9	60.4	63.2	43.4	$p < 0.03$, $LSD = 16.9$
Calculated from components	93.1	69.8	85.8	51.0	$p < 0.05$, $LSD = 30.1$
Spring trial pyrethrins yields					
	Sept	Oct	Nov	Dec	
Actual harvest (Fig. 3.7)	49.5	82.0	86.3	57.4	$p < 0.0001$, $LSD = 12.8$
Calculated from components	61.2	94.7	95.9	64.0	$p < 0.027$, $LSD = 27.2$

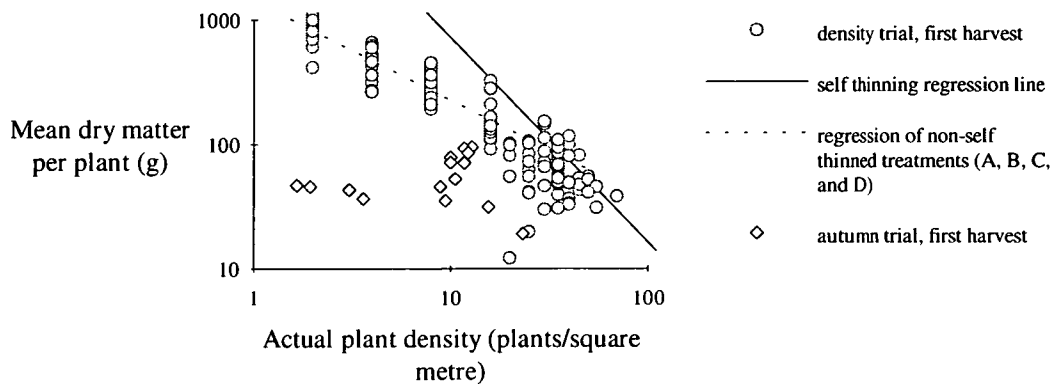
3.5 Discussion

3.5.1 First season results

The first season results demonstrate that the January sowing, with an in-field time of nearly 13 months at time of first harvest, failed to generate a significant flower yield. Later autumn sowings displayed significantly less flowering than the January sowing. Spring trial sowing, which had been in the ground for six or less months, remained completely vegetative and were, therefore, not harvested.

The mean dry matter per plant in treatments of the autumn trial were substantially less than those observed in the adjacent density trial which was sown in early October (Figure 3.20).

Figure 3.20 Mean dry matter per plant vs actual plant density for the first harvests of the density trial and the autumn time of sowing trial



If all the developmental requirements for flower production were satisfied in the January sowing, yield would still be significantly reduced due to the plants being small. In the situation where all the plants were flowering adequately, density may be significantly increased to generate higher yields in January sowings. As only 70% of the plants in the January treatment flowered, inadequate development also limited crop productivity. Providing some evidence of juvenility was the observation that plants remaining in the vegetative phase had a lower mean weight than did flowering plants. However other requirements for flowering may also have not been fully satisfied.

Plotting individual plant dry weights against tiller number/plant for the January sowing revealed only a weak relationship between these two variables. Even so, it did demonstrate that all the plants above a certain dry weight (125 g) always produced flowers. Below this weight, both flowering and vegetative plants were present.

Sequentially later sowings had fewer flowering plants, those plants possessed fewer flowering tillers, and they were developmentally delayed. All these factors indicate later and less complete satisfaction of flowering requisites with later sowings. The observed flowering behaviour in the January sowing may be explained by the combined influence of juvenility and a quantitative vernalisation requirement as described for pyrethrum (Brown, 1992). These two factors may be of importance in future plant selection, sowing time, and plant density choices, but such matters are beyond the scope of the current study. Although the importance of satisfaction of a quantitative vernalisation requirement

is well recognised in pyrethrum plant breeding (Bhat, 1995) there is no reference to juvenility in the pyrethrum breeding literature.

In summary, both limited growth and delayed plant development contributed to low yield in the January sowing and later sowings. First year flower yields of January-sown crops will remain low until it is possible to achieve rapid germination of plants which can grow quickly and also possess low thresholds for vernalization and, therefore, uninhibited flower initiation and subsequent development.

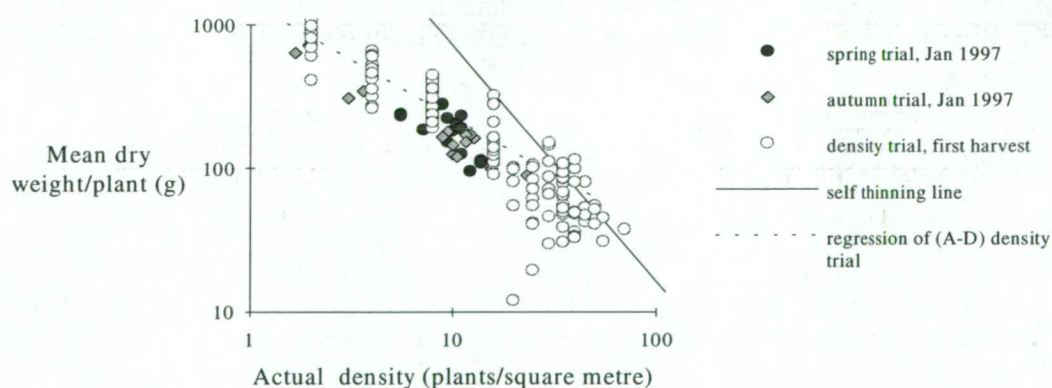
3.5.2 Second season harvest

Spring trial

Pyrethrins yields of treatments in the spring trial were assessed for the first time in January 1997. By that time treatments had been in the ground for between 14 months (December treatment) and 17 months (September treatment). As discussed in an earlier section, these plants were too immature and therefore failed to flower in the previous season.

Comparison of mean dry matter per plant of the sowing time treatments with previous season dry matter production in the adjacent plant density trial, revealed similar productivity (Figure 3.21).

Figure 3.21 Mean dry matter per plant vs actual density in time of sowing and density trials



Differences in pyrethrins yield between treatments within the spring sown trial were significant. The highest yields were achieved in the October and November sowings and lower yields in the September and December sowings. Lower yields in the September treatment were in part due to lower plant densities in three of the four replicates. Plants in the September treatment apparently responded to the lower density by increasing tiller number per plant, but this increase was not sufficient to compensate for the lower density. The number of

flowers/tiller did not vary from the September to the high yielding October and November treatments whereas mean flower weight did, with weights increasing with sequentially later sowings. Pyrethrins concentration and mean flower maturity did not vary between sowing times. Therefore low plant density and lighter flowers were responsible for low yield in the September sowing.

The low yield in December (the treatment in the ground for the shortest period of time) could not be explained by low plant density or low dry weight per flower. In fact plant density and weight per flower were higher in December than in all other treatments. The dry weight per plant (125 g in December) was significantly less than in the preceding two sowings (200 g in October and November). Tillers/plant was the yield component responsible for lower yield in the December treatment. Counteracting the influence of lower tillers/plant was a significant increase in mean flower dry weight. In summary low yield in December was due to smaller plants producing a lower number of flowering tillers. Determination of whether plants can actually compensate for low tiller numbers by increasing mean flower weight, or whether the observed flower weight increase is due to some other mechanism is worthy of further investigation.

Autumn trial

Highest pyrethrins yield in treatments in the autumn trial were less than those observed in the spring trial. This may have been due to autumn treatments having been harvested to ground level in the previous summer, thereby reducing photosynthetic capacity. Since the spring and autumn trials were separate, statistically valid comparison of differences between the trials cannot be justified.

Differences in pyrethrins between treatments within the autumn trial were significant, with the April production being lower yield than all the other treatments. With a near complete failure of crop establishment in the April sowing, low plant density was responsible for low yield. Plants responded to the low density by increasing flowering tiller numbers, but there were no significant differences in flower number per tiller or mean flower weight. Flower maturity was found to be slightly higher, but this difference was small and therefore not considered to have influenced the pyrethrins yield results. No differences in pyrethrins concentration were found between treatments.

Apart from the low density April treatment, no yield component or overall pyrethrins yield differences were evident. It would appear that by the time of the second harvest, there had been adequate time for plant growth such that any differences between treatments due to sowing date had disappeared.

Calculated vs actual yields

Yields were calculated by the multiplication of individual plot dry matter yield components and then multiplying that figure by a common pyrethrins assay. The resultant 'plot products' were then subject to ANOVA. Calculated yields varied in a way that was consistent with actual yield, but calculated yields were consistently higher. The comparison provided confidence that yield component data were truly representative of the plants sampled.

Since some yield components are now known to vary with respect to time of sowing and density, whereas others remain relatively constant, more effective measures of yield for both trials and commercial crops may be developed. Evidence presented in the above trials indicate that plant density, tiller number per plant, and mean flower dry weight are yield components that vary significantly with time of sowing.

3.5.3 Summary

This work showed that choice of sowing time can have a profound influence on yield and its components. Evidence presented indicates that there would be little advantage in sowing crops earlier than mid-October. Opportunity for high yield in the following season may decline if sowings are attempted after mid-November. High yields in crops sown after mid-November would be initially limited by the small plant size. The decrease in flowering tillers per plant reported to be responsible for the decrease in yield in December is compensated to some extent by the increase in mean flower weight. Higher plant densities in these sowings may help maintain yields, although sowing as late as mid-January was found to be too late for high yields. Yield was limited both by insufficient plant growth and capacity to flower.



Plate 3.1 Trial site at FVRS shortly after the second autumn sowing. Note the micro-irrigation equipment.



Plate 3.2 A pyrethrum plant from an autumn sowing in flower, while those surrounding it remained vegetative.

Plate 3.3 November (left) and December treatments (right) immediately before the second season harvest. Note the lack of apparent yield difference between the plots.



Plate 3.4 Pyrethrum crowns remaining after harvest. Top (October) middle (November) and bottom (December). Note the tendency of decrease in crown size with sequentially later treatments.

Chapter 4: The effect of temperature on pyrethrum seed germination and emergence

4.1 Introduction

This chapter investigates the effect of temperature on pyrethrum seed germination and emergence. Until recently there was little interest in pyrethrum seed quality, since the crop was established vegetatively. Requirements for successful crop establishment from seed had not been determined. Attempts to establish commercial crops from seed resulted in low and erratic seedling emergence and the cause of those failures had not been investigated.

The influence of moisture, temperature and seed quality on germination is reviewed. The series of experiments conducted is diverse in that it investigates a range of aspects of seed and seedling characteristics. The trials are of an exploratory nature attempting to clearly describe germination and seedling behaviour. Trials focus on the influence of temperature and seed quality on germination and early seedling development.

Trial 1. Time of sowing

This trial aimed to investigate percentage and rate of seedling emergence when the seed was sown at different times of year. The purpose of the work was to investigate the importance of temperature, rather than moisture.

Trial 2. The effect of temperature on germination on one seed lot

The germination characteristics of a pyrethrum seed lot were investigated under a range of constant temperatures. Cardinal temperatures for germination were estimated, providing data on how this species compares with other sown crops. The implications of the germination results are discussed both from a seed testing and a sowing management perspective.

Trial 3. The effect of temperature on germination of six seed lots

This trial investigated germination parameters of six seed lots germinated at low, medium and high temperatures. Correlations among various germination parameters were investigated and an explanation for the observed differences between seed lot germination characteristics is proposed.

Trial 4. The germination behaviour of six seed lots after storage

The germination characteristics of Trial 3 seed lots were investigated after 18 months of storage. The primary aim was to identify whether there was an afterripening requirement and/or whether seed degradation occurred during storage. A second aim was to investigate correlations between seed lot weight characteristics and various germination parameters, within and between seed lots.

4.2 Literature review: The influence of moisture, temperature and seed quality on germination

A most hazardous time for an individual plant is the transition from quiescent seed to autotrophism. During this time, temperature and moisture have to be adequate for germination, seedling growth and development. Even if those requirements are met, the seedling is vulnerable to a wide range of other factors that limit the chance of establishment. Examples of such factors are: seed and seedling predation, losses through damping off, fluvial and burial losses, competition from weeds and same species seedlings, frost, and hail.

This review investigates key factors influencing crop establishment. The first section investigates physicochemical influences on germination and seedling establishment. Then the role of seed quality in crop establishment is examined. Finally, investigations into various aspects of seed quality in pyrethrum are reviewed.

4.2.1 Physicochemical factors influencing germination

In crops established from seed, the periods of germination and early crop establishment are particularly sensitive to the physicochemical environment. The importance of factors responsible for reductions in yield other than disease, insect damage, and weeds are significant. Nearly 70% of mean potential crop productivity is reported to be lost through unfavourable physicochemical environments (Boyer, 1982). Failure to germinate can occur for a variety of reasons which include flooding, drought, low temperature, surface crusting or poor seed soil contact (Matthews and Powell, 1986). Crops have specific moisture and temperature requirements for reorganisation of membranes, reassembly of metabolic pathways for catabolism of reserves, and to provide the driving force for cell expansion and for seedling development. This section focuses on the influence of moisture and temperature on germination and seedling development.

Moisture and germination

The water potential of the soil (ψ_{soil}) consists of; gravitational potential (ψ_g), osmotic potential (ψ_s), and matric potential (ψ_m). It is generally accepted that small changes in ψ_m have a significantly greater influence on seed water uptake and subsequent germination rate than changes in ψ_s of similar magnitude (Hadas and Russo, 1974). The greater influence of increasing ψ_m is due to soil moisture being confined to increasingly smaller soil pores resulting in smaller area of seed-water contact and reductions in conductivity within the soil.

In the steady state, rate of water uptake by the seed (R) is determined by the difference between ψ_{soil} and water potential of the seed (ψ_{seed}), i.e., $\Delta\psi$, and a conductivity constant (K) (Equation 1). The water potential of the seed, or more precisely, the ψ of cells in the seed, is determined by osmotic and matric potentials as well as a pressure potential associated with those cells. Pressure potential increases as the seed hydrates and opposes further hydration (Bewley and Black, 1994). K is determined by the soil/seed coat interface and by physical characteristics of the seed. The conductivity constant therefore acts as a resistance to water movement and, as stated in the previous paragraph, varies with ψ_m (Koller and Hadas, 1982).

$$R = K\Delta\psi \text{ (Equation 1)}$$

For orthodox seed under optimal moisture conditions, germination begins in dry, quiescent seed with initial rapid uptake of water. This uptake is a function of the seed matric forces described in the previous paragraph (phase 1). This is followed by phase 2, when there is little or no uptake of water. During this phase the influence of seed matric potential declines and moisture content is determined by opposing pressure and osmotic potentials (Bewley and Black, 1994). The final stage of water uptake (phase 3) defines the completion of germination and the beginning of seedling development (Bradford, 1990). The driving force of seedling development in this stage is reportedly due to the changing osmotic potential of cells owing to the production of osmotically active substances resulting from catabolism of stored reserves.

The effect of low moisture on germination percentage

Hadas and Russo (1974) stated that each species has a critical value of seed water content below which germination will not occur. Although this appears to be true, recently it has been shown that for seven important monocotyledonous and dicotyledonous species, the seed part which initiates growth is at a moisture content of 60 to 70% at the time of germination (Abdel-Aal and McDonald, 1998). Therefore, if

moisture of seed integument is not considered, a common water potential may be required for germination to proceed. Helms *et al.* (1996) demonstrated that low soil water contents reduced soybean emergence. At 0.07 kg/kg soil water content (the driest treatment) seed remained ungerminated although being fully imbibed. At 0.09 kg/kg soil water content, along with other higher moisture treatments, hypocotyl-root axis elongation was permitted. Further studies have recently been conducted investigating the influence of low soil moisture availability on corn, sunflower and soybean (Helms *et al.*, 1997).

The effect of high moisture on germination percentage

The metabolic implications of germination under flooding have been reviewed (Norton, 1986). When flooding occurs, the oxygen level in the soil declines and the seed undergoes a period of hypoxia before oxygen is fully depleted and anoxia is reached. Norton (1986) suggested that many previous works investigating the influence of flooding considered the effects of oxygen deprivation on germination failure, but had not investigated the possible physical impact of rapid rehydration on the seed. Norton (1986) commented that most researchers, realising that flooding generated an anoxic or hypoxic environment, have concentrated efforts on investigating anaerobic respiration of seed. The quantities and toxicity of products from various metabolic pathways were often discussed along with other possible causes of seed death including exhaustion through leaching or conversion of sucrose to alcohol (Norton, 1986). Recent reports on plant metabolism under low oxygen tension environments include a review by Richard *et al.* (1994) and a study on acclimatisation of corn seed to an anoxic environment (Cobb *et al.*, 1995).

Heydecker (1977) suggested high moisture was a potentially significant factor contributing to stress in germination. Heydecker (1977) cited numerous studies in which soaking of seed prior to sowing resulted in significant reductions in germination capacity, rate and uniformity. Interestingly, bubbling of oxygen through the water was found to decrease germination parameters even further. The way different species respond to excess water varies greatly but generally it may be argued that large seeds are particularly ill-suited to rapid hydration. Seed of most agronomically-important species are very dry when sown. Rapid hydration may cause physical damage to seed tissues. Little is known about the movement of water in the cells of seeds and what damage differential cell expansion may cause.

The influence of moisture on rate of germination

Wheeler and Ellis (1992) reported that soil moisture did not influence rate of emergence of onion seed above a critical level (7.6% w/w soil moisture). This water content was equivalent to -0.15 MPa., a water tension at which most of the seed failed to germinate and the few that did only did so slowly. High negative soil water potential (drier soil) reduces the rate of germination in lettuce (Bradford, 1990). This effect was due to a greater time required for weakening of the endosperm rather than a lower turgor pressure exerted by the embryo.

Gummerson (1986) in work with germination of sugar beet seed identified that successively later germinating cohorts of seed had increasingly higher base water potentials (ψ_b). This variation in ψ_b within a seed lot and ψ_b itself have been used as a basis for the development of further models. Bradford (1990) proposed that the rate of germination for a given seed lot may be determined for seed held at various osmotic potentials based only on three fundamental parameters of the seed lot. Those parameters were,

θ_H A hydrotime constant (MPa · h)

ψ_b Base or minimum water potential permitting germination

σ_{ψ_b} Standard deviation in base water potential within the seed population.

In a subsequent work Bradford and Haig (1994) presented the following (Equation 2),

$$\theta_H = (\psi - \psi_b(g)) t_g \quad (\text{Equation 2})$$

where $\psi_b(g)$ is the normal distribution of ψ_b in the population and t_g is the time to radical emergence of percentage 'g' of the population. Rearranging the above formula gives the germination rate for a given percentage 'g' of the population (GR_g) (Equation 3),

$$GR_g = 1/t_g = (\psi - \psi_b(g))/\theta_H \quad (\text{Equation 3})$$

Equation 3 describes a linear relationship ($y = mx$) where y is the germination rate (GR_g); x equals $(\psi - \psi_b(g))$; and m equals $1/\theta_H$.

Other factors relating to rate of germination have been investigated. Allowing seed moisture to gradually increase through exposure to humid atmosphere in the weeks prior to sowing has been reported to increase the rate of germination in a range of vegetable species (Heydecker, 1977). Seed moisture during storage may also influence rate of subsequent germination. Rate of germination was found to decline in seed stored at lower moisture contents in vicia, *Catharanthus roseus* (Carpenter and Boucher, 1992b) and annual phlox, *Phlox drummondii* (Carpenter *et al.*, 1993). However, this effect is suspected to be largely due to a greater loss in dormancy with higher seed moisture.

Temperature and germination

Temperature is recognised as the main environmental variable determining germination in moist soils. Heydecker (1977) suggested that soil temperature determines both the fraction of seeds in a sample that will germinate and the rate at which germination occurs.

The effect of low temperature on germination percentage

If soil water content is high enough for seeds to imbibe but temperatures are too low to allow embryo growth, seed will not germinate (Helms *et al.*, 1997). Each species has a critical temperature below which germination will not occur. Capacity to germinate at low temperature is considered a desirable seed trait and there has been considerable effort, with varying levels of success, to select for this characteristic in a range of vegetable species including; tomatoes (Smith and Millet, 1964; Ng and Tigchelaar, 1973), beans (Kooistra, 1971), and corn (Cal and Obendorf, 1972). Warm soil temperatures are necessary for rapid, even germination and seedling establishment in cotton. Kerby *et al.* (1989) recommended that cotton should not be sown if less than 10 heat units were expected in the subsequent five day period.

Low temperature can affect seed germination percentage in different ways: temperature may just be too low to allow metabolic activity, seeds and seedlings may be damaged, or seed may have an afterripening requirement that restricts germination. The former is discussed in the section investigating the influence of temperature on rate of germination. Seed damage and afterripening are discussed here.

Low temperature can affect germination percentage by damaging seed and seedlings. Steiner and Jacobsen (1992) cited earlier work by various authors who reported significant chilling injury of cotton seed in the period immediately after imbibition. Damage during this period was apparent as radical tip abortion, root cortex damage and slowness to resume normal seedling growth rates after the cool period. Damage to germinating seed due to low temperature is by no means restricted to cotton, but that seed is particularly sensitive.

Herner (1986) in reviewing germination under low soil temperatures proposed that plants may be divided into two groups depending on sensitivity to chilling injury. Non-chilling resistant seed i.e., seed that does not fail to germinate at temperatures below 10°C are from temperate species, whereas 'chilling sensitive seed', that is, seed that fails to germinate below 10°C-15°C are from tropical or subtropical species. Chilling sensitive species were divided into two broad categories, (1) species where seeds are sensitive to

low temperatures during imbibition and (2) species in which no damage occurs due to low temperatures during imbibition, but injury occurs if exposed once radical elongation has commenced. Regardless of chilling sensitivity of the species, low temperatures are renowned for being responsible for low population and slow, erratic crop establishment. Although Herner (1986) focused much discussion on 'chilling sensitive species' no evidence was presented to suggest that there is any physiological difference between chilling sensitive and tolerant species.

Another factor which may influence capacity to germinate at low temperature is dormancy. Allen and Meyer (1990) found that in various perennials and wildflowers, seed dormancy at low temperature is often responsible for poor or sporadic germination. A period of dry storage is often all that is required to release seed from dormancy and allow germination at the lower temperatures. Many vegetable and flower seeds exhibit this type of dormancy (Roos, 1980; Carpenter and Boucher, 1992a).

The effect of high temperature on germination percentage

Plant establishment from seed becomes extremely difficult at high temperatures (Cantillife, 1989). Sowing at high temperature often results in low populations of plants and non-uniform fruit maturity in tomato (Odell *et al.*, 1992) and poor stands in leek (Parera and Cantillife, 1992). If lettuce seed is permitted to go through the initial stage of germination under high temperatures (30°C) the seed enters thermodormancy (Grey, 1977). Thermodormancy is a type of secondary dormancy since it takes effect after maturation when the seed is placed in non-optimal conditions (Khan and Samimy, 1982).

The effect of temperature on rate of germination

Rapid seedling emergence has been a major goal of horticulturists in order to minimise the impact of soil crusting, damping-off and crop non-uniformity (Herner, 1986). At sub-optimal temperatures many vegetable species show a linear increase in germination rate as temperature rises to the optimum. The influence of temperature upon rate of germination in the absence of moisture stress can be predicted by heat sum in degree days (S) and the minimum temperature for germination (T_{\min}) (Wagenvoort and Bierhuizen, 1977). Bierhuizen and Wagenvoort (1974) suggested that the following relationship exists between soil temperature and germination rate;

$$t = S / (T - T_{\min}) \quad (\text{Equation 4})$$

Where,

t is the period in days to achieve 50% germination

S is the heat sum in degree days to achieve 50% germination

T_{\min} is the minimum temperature for germination (equivalent to T_b), and

T is the soil temperature

Bierhuizen and Wagenvoort (1974) identified the heat sum (S) and T_{\min} required for germination of a wide range of vegetable species. The predictive ability of this relationship for field germination may be reduced due to inconsistency in S between cultivars and seed lot quality. Variation in rate of germination due to field diurnal temperatures was investigated in further work (Wagenvoort and Bierhuizen 1977) and found to have little influence on rate of germination.

At a given temperature, progression of germination is characterised by an initial lag phase in which no germination occurs, followed by an approximately linear phase during which the rate of germination is constant, followed by a tailing off in germination rate as final germination is achieved (Dumur *et al.*, 1990).

For a given percentile of the seed lot, rate of germination increases from a base below which germination fails, to an optimum and then declines again at supra-optimal temperatures to a temperature above which germination again fails (Garcia-Huidobro *et al.*, 1982a). The cardinal temperatures T_b , T_o , and T_m represent the low temperature below which germination will not occur, the temperature at which maximum rate of germination is achieved and temperature above which germination is not permitted. Recent examples of studies where such cardinal temperatures have been calculated in various ways include investigations in sunflower, *Helianthus annuus* L. (Mwale *et al.*, 1994), in pearl millet, *Pennisetum typhoides* (Garcia-Huidobro *et al.*, 1982a), and in broad bean, *Vicia faba* L. (Dumur *et al.*, 1990) and the common bean, *Phaseolus vulgaris* L. (White and Montes, 1993).

Garcia-Huidobro *et al.* (1982a) proposed the following ways of calculating the thermal time for germination of a percentage (G) of the viable seed at; sub-optimal (Equation 5) and supra-optimal temperatures (Equation 6). The equations model the influence of temperature on the mean time to complete germination (t) for percentage (G) of the population.

$$R_1 = 1/\bar{t} = (T - T_b(G)) / \theta_1 \quad (\text{Equation 5})$$

$$R_2 = 1/\bar{t} = (T_m - T(G)) / \theta_2 \quad (\text{Equation 6})$$

Where,

θ_1 is a constant (where $T < T_o$)

θ_2 is a constant (where $T > T_o$)

T is a constant germination temperature

R rate of germination for percentage of population (G)

When used to describe the germination rate of a percentage of the population, Equation 5 assumes that T_b remains constant for different early and late germinating cohorts. If

this is actually so, differences in rate of germination within the population arise due to differences in thermal times required for germination rather than variation in base temperature of individuals within the population. The validity of this assumption was confirmed in that work (Garcia-Huidobro *et al.*, 1982a) and later by Finch-Savage (1995) who discussed similar findings in other species. In other work Garcia-Huidobro *et al.* (1982b) found that fluctuating temperature only slightly increased the predicted rate of germination.

Wheeler and Ellis (1992) identified soil temperature as the main determinant of rate of seedling emergence in all but the driest soils. In further work they determined that significant differences in rate of emergence were largely due to differing times taken to germinate, rather than variation in rate of seedling growth between seedlings (Wheeler and Ellis, 1994). Therefore emergence was found to be an indirect measure of time taken from sowing to germination. The importance of germination in the determination of time to field emergence has therefore been demonstrated.

Combined moisture and temperature effects on rate of germination

Vegetable crop seed germination varies in response to moisture together with temperature. Hegarty (1976) noted that brassica crops are less sensitive to dry, cool soil conditions than are carrots. Beetroot is more severely affected by moisture stress conditions than are most vegetable species (Hegarty, 1976). Finch-Savage and Phelps (1993) proposed that field emergence pattern is greatly influenced by soil water potential on initiation of phase three of germination, i.e., the start of radical growth. Above that base or minimum water potential, seedling emergence was largely determined by temperature.

Further work, to understand the combined requirement for moisture and temperature has been undertaken in the UK (Finch-Savage and Steckel, 1994). In these studies a method was devised for determining the optimum timing for a single irrigation in field trials on eight vegetable species including lettuce, carrots, broccoli, onions, leeks and parsnips. The method used 'thermal time' to indicate the progress of germination in the soil. Seeds were sown in a moist seed bed and the single irrigation (12.5 mm) was applied at various thermal times (degree days above a base temperature determined for each species in germination tests). In a separate field trial on onions, it was demonstrated that percentage seedling emergence was more predictable over a range of seed bed conditions with a single, timed irrigation than with pre-sowing or no irrigation. The recognition of the importance of moisture and temperature interaction, and the subsequent development of the concept of hydrothermal time has been attributed to Gummerson (1986). Finch-

Savage (1995) described hydrothermal time as "a combination of temperature above a base temperature, ψ above a base ψ (ψ_b) and time".

Bradford and Haig (1994) included the influence of temperature into the earlier equation (Equation 3) giving a relationship between germination rate and hydrothermal time (Equation 7) where T is the temperature of germination and T_b is base temperature and θ_{HT} is a hydrothermal time constant ($\text{MPa} \cdot ^\circ\text{h}$).

$$\text{GR}_g = 1/t_g = [(\psi - \psi_b(g))(T - T_b)] / \theta_{HT} \text{ (Equation 7)}$$

Dhal and Bradford (1994) report that this model does an adequate job of predicting radical emergence time courses across sub-optimal moisture and temperature conditions, although physiological changes affecting threshold values can limit the range of conditions in which it is accurate.

4.2.2 Seedling development after germination

Most vegetable crop yields and quality are dependent upon not only achieving high enough field populations, but also obtaining uniform emergence. The latter is critical in order to reduce cost and complexity of pesticide application and to allow simple once-over harvesting. In Tasmania, late autumn sowings of onions may take three to four weeks to completely emerge rather than the 10 days required at warmer times in the season. Carrots in Britain will take up to six weeks to completely emerge in early spring sowings but only 10 days in mid-summer (Fordham and Biggs, 1985).

Poor emergence may not be associated with failure to germinate, rather it is often associated with the failure to complete seedling establishment after germination (Doneen and McGillivray, 1943 cited in Matthews and Powell, 1986). In dry climates, rapid soil drying along with intense solar radiation can result in high soil strength which reduces the capacity of seedlings to emerge (Abrecht and Bristow, 1990). Heydecker (1977) suggested that at supra-optimal germination temperatures radicals often emerge within a short period after imbibition but then the seedling dies. Drought, where moisture is only found deeper in the profile than penetrated by the root system, causes significant seedling losses. Zobel (1995) cites numerous studies which have demonstrated significant genetic variability for depth of rooting in a wide variety of crops.

Young tender seedlings may also be damaged or killed if exposed to prolonged periods of cold, wet conditions. While it may be possible to compensate for low emergence by increasing the seeding rate, factors other than lower final population limit crop quality and quantity if sowings are conducted in cool conditions. Seedling emergence may be significantly delayed, irregular and patchy within the paddock. Many soil, seed and

environmental factors can contribute to variability between plants sown at the same time (Benjamin and Hardwick, 1986). Primary root elongation, function and branching are reported to be highly temperature dependent (Russell, 1977). The nutrient status of seedlings can be significantly influenced by temperature through effects on nutrient absorption and transport from the roots to the shoots (Leskovar and Stoffella, 1995).

4.2.3 Determination of seed quality

Seed quality is a complex concept since it can be influenced by many factors. In an attempt to measure quality, seed technologists generally ask two main questions. These questions are related to both seed lot purity and germination capacity. Seed lot purity investigates the amount of non-seed material in the sample and will not be discussed here. Seed germination capacity tests provide percentage germination values intended to inform the supplier, storer, or user of the seeds' ability to generate a normal plant under *favourable conditions*.

McDonald (1980) suggested that seed germination tests are inadequate for two main reasons. Firstly, *favourable conditions* are rarely encountered in the field and as a result plant populations are often lower than expected for a seed lot of a given germination capacity. Secondly, the test is not qualitative and therefore provides no information on likelihood of field establishment or how the seed will perform after storage. A single measure of germination capacity therefore does not necessarily provide reliable information on seed quality. Seed lots displaying equal germination capacity in standard tests may perform quite differently in poor field conditions (TeKrony and Egli, 1991, and Perry, 1982).

In an attempt to overcome limitations of dependence on 'germination capacity' for quality determination, seed vigour tests have been developed that are predictive of germination behaviour in the field. Seed vigour is defined in the following way:

"Seed vigour comprises of those seed properties which determine the potential for rapid, uniform emergence and development of normal seedlings under a wide range of field conditions" (TeKrony and Egli, 1991).

Seed vigour can therefore involve many variables, which may either individually or together, compromise successful field establishment. Vigour may be determined by investigating seed, germination, and seedling characteristics. Variability in vigour between seed lots may be due to genetic factors, environment and nutrition of the mother plant, stage of seed maturity at harvest, seed size, mechanical integrity, deterioration and pathogens (McDonald, 1980). Once critical factors for vigour for a particular crop have

been identified, then vigour tests can be developed. Development of tests that adequately describe the potential field performance of seed in a wide range of crops remains an area of intense research activity (McDonald, 1994). Current seed vigour tests are classified into the following groups (AOSA, 1983, cited by McDonald 1994):

- i. Seed and seedling growth and evaluation tests
For example, seedling growth rate, speed of germination
- ii. Stress tests
For example, cold test, controlled deterioration
- iii. Biochemical tests
For example, tetrazolium chloride, electrical conductivity

The above list is only intended to provide some examples of the more commonly used tests. In circumstances where tests are used for a particular crop, they firstly has to be developed. Evaluation of a candidate test is conducted by comparing results with actual field establishment. A vigour test for seed of any crop is therefore developed through a planned series of experiments which firstly identify factors limiting field performance and then evaluate appropriate candidate tests against actual field performance.

Two physiological reasons for low seed vigour have been recognised in vegetable species: seed ageing and imbibition damage (Matthews and Powell, 1986). Seed ageing is a series of irreversible, degradational changes which begin after physiological maturity. The germination capacity of a seed lot decreases little over the greater proportion of its storage life followed by a rapid decline in viability (Matthews and Powell, 1986). The position along this path of deterioration can be predicted using accelerated ageing, and electrolyte leakage assessment techniques.

Delouche and Baskin (1973) proposed a theoretical sequence of events which lead to increasing deterioration, culminating in the ultimate loss of seed germinability. Importantly, rate of germination and germination uniformity are two parameters lying in the early and middle stages, respectively in this sequence (McDonald, 1980). Damage during imbibition has been well documented in a range of leguminous species but its importance outside this group appears to be less than adequately explored. Upon imbibition seeds release electrolytes which may vary in type and concentration depending upon seed deterioration. Recent investigations associated with seed leachate include, interpretation of single seed conductivity data (Moore *et al.*, 1988), differential leakage of specific compounds (Priestley, 1986; Taylor *et al.*, 1988; Hill *et al.*, 1988); and solute leakage (Hill and Taylor, 1989).

In addition to types of seed vigour tests mentioned previously, larger seed size is generally regarded as being related to better field performance (TeKrony and Egli, 1991). Progress in vigour testing has been recently reviewed (Hampton and Coolbear, 1990). No reported research investigating appropriate ways to determine seed quality in pyrethrum were found. Since this is a relatively new area of research, even among many of the established vegetable and industrial crops, a wide range of techniques in many crops are still under investigation for seed quality assessment.

4.2.4 Seeding rate determination

To determine the seeding rate required to generate a given plant density, a 'field factor' is incorporated into the calculation as a constant. Field factor is the proportion of viable seed that is expected to emerge in a given environment. Fordham and Biggs (1985) suggested that a field factor of 0.4 may be expected when sowing is conducted in poor soil or climatic conditions. Under the best conditions, 0.8 is the highest that could be expected. Although field factor is a well known term, Bleasdale (1984) suggested that it should be eliminated from use due to inaccuracies in plant densities arising from its use. Bleasdale, 1984 suggested that rather than using a field factor term in the determination of an appropriate seeding rate, a constant percentage be subtracted from the laboratory test. Regardless of which of the above systems are used to determine seeding rate, both are empirically derived.

Field factor, irrespective of how it is calculated, is the combined influence of a wide range of factors on emergence. The mechanisms responsible for failure to germinate in the field appear to be less than adequately quantified but are reported to be due to non-ideal temperatures, moisture, soil type and preparation, depth of sowing, fertiliser ions, soil capping and seed quality parameters (Hegarty, 1976). Attaining the desired plant population in the field is largely determined through the 'operator experience' with the species, soil type, seed bed preparation, sowing equipment and environmental conditions.

4.2.5 Investigations into pyrethrum seed germination

Limited research has been reported on the germination characteristics of pyrethrum seed. Research has focused on seed storage, temperature regimes for maximum percentage germination and application of gibberellic acid (GA). A brief summary and some discussion of this work is presented below.

Germination percentage of pyrethrum seed is often found to be low, but this has been reported to be due to the presence of a high proportion of empty achenes (Barton,

1966). Past research has considered the achene to be the generative unit and percentage germination is typically expressed in terms of initial numbers of achenes.

Barton (1966) examined ungerminated achenes following an incubation period at 15°C and identified a close relationship between empty achenes and germination failure. Barton (1966) also showed that viability of pyrethrum seed could be maintained for up to 15 years by reducing seed moisture to 6.5 % and storage at or below 5°C. More recently Pandita (1984) demonstrated that dried seed of unreported percent moisture could be stored in oiled paper bags and held at room temperature for up to five years before viability fell to a commercially unacceptable level. Unfortunately no reasons were provided for this germination failure. Furthermore Pandita (1984) did not discuss the maturity of seed at harvest which is generally seen as an important factor in seed storability (Bewley and Black, 1994).

Pandita (1983) investigated the effects of temperature during germination on final germination percentage. Various combinations of alternating temperatures were tested and results compared with germination under constant temperature. Maximum germination (73%, 72%) was achieved with alternating temperature regimes of 25°C and 10°C for 12:12 and 18:6 hours. A constant temperature of 15°C resulted in a final germination of 70% indicating no significant differences between constant and alternating temperatures. Pandita (1983) observed that at high temperature (30°C) a lower final percentage germination resulted, but provided no explanation for the observation.

Mohandass and Sampath (1988) investigated the effect of application of GA on pyrethrum seed germination. Application of GA at 50 ppm resulted in a maximum final germination percentage, hastened commencement of germination and increased the coefficient of velocity of germination. Mohandass and Sampath (1988) stated that "the study conclusively proved that treatment of pyrethrum seed with GA not only enhances germination but also accelerates emergence". Although genotype, seed maturity at harvest, and general level of seed deterioration may influence the effectiveness of GA as a seed treatment, such issues were not addressed by Mohandass and Sampath (1988).

4.2.6 Summary

This literature review reported on a range of research investigating the influence of temperature, moisture and seed quality on germination and crop emergence. Recent advances in each of these are testament to both their importance to agricultural production and strong research interest in the disciplines. In discussing the failure or slowness to germinate, it becomes clear that a knowledge of seed quality, dormancy and

the influence of temperature and moisture, are interdependent in determining successful crop establishment. Furthermore, the capacity of a given soil to provide moisture may have a significant influence on establishment.

In laboratory conditions, at lower than optimal temperatures, it is well established that seeds take longer to germinate, germinate less evenly, and are more likely to fail to germinate. The reasons why seed responds to decreased temperature in this way are on the one hand simple in that seeds are living plants and therefore respond to temperature in a way common to more mature plants. On the other hand, slowness to germinate or failure to germinate at low temperature can be due to seed damage, seed immaturity, seed degradation, embryo size and different types of seed dormancy. Such topics are beyond the scope of the current review.

It is sufficient to conclude this section knowing that low temperature can have a significant impact on crop establishment and that this impact can be moderated. This can be achieved by choosing a warmer time to sow the crop and using high vigour seed. There is little reported information investigating temperature and moisture requirements for germination of pyrethrum seed. At the commencement of this study there were no reported successful commercial field sowings of this species. The following work focuses attention on seed germination behaviour under differing thermal environments while attempting to provide adequate moisture for germination and subsequent seedling growth.

4.3 Materials and methods

4.3.1 Trial 1. Time of sowing field trials

Two trials were established at Forthside Vegetable Research Station (FVRS): a spring trial when seed was sown in mid-September, October, November and December of 1995, and an autumn trial when seed was sown in January, February, March and April of 1996. Each trial was laid out in four randomised complete blocks. Seed was sown using a tractor mounted Ojoid cone drill (Plate 2.1). The drill was calibrated to sow 20 g of seed in four rows over a plot length of 18 metres (140 achenes per linear metre, equivalent to 5.5 kg/ha). Seed from the same seed lot was used for each monthly sowing. The seed was stored in a double plastic bag at 4.0°C and assessed for loss of germinability on two occasions. Prior to each sowing, plots were rotary-hoed to a fine tilth in order to generate a similar seed bed for each sowing. Sowing depth was approximately 10 mm.

Seedling emergence and seedling losses were assessed weekly over a two month period from within fixed quadrats. Seedlings were counted as having emerged when the cotyledons had opened and they were clearly discernible from weed seedlings, due to their lighter green colour of the pyrethrum seedlings (Plate 4.1). Pyrethrum seedlings were classified as dead when the whole seedling was brown or the newly emerged seedling shoot was brown and failed to support the weight of the cotyledons. When no further loss or newly emerged seedlings were evident (two months after sowing), final plant counts were conducted. Micro-irrigation equipment was used in an attempt to maintain low moisture stress on the most recently sown plots. Effective elimination of moisture availability as a variable was intended to allow results to be interpreted from temperature and seed quality perspectives.

Statistical differences between treatments with respect to final emergence and dead seedlings were determined by using embedded ANOVA analysis in Microsoft Excel 4.0™ and checked against Systat™ 5.2.1 software. Cumulative emergence profiles were then presented to display the relative rates of emergence for each of the seven sowing dates. Polynomial equations were fitted to each of the cumulative emergence profiles (Appendix 1.7). Using these equations time for 85% emergence was calculated and correlated against mean day degrees in the 17 days following sowing which was expected to be the period determining germination and emergence. Air temperature was assessed at a weather station located at 300 m from the trial site. Mean day degrees per day over the 17 days was calculated as described in Appendix 1.8. Surviving seedling numbers two months after sowing were compared against mean day degrees in the 17 days after sowing.

4.3.2 Trial 2: Germination of a single seed lot at a range of temperatures

This experiment was conducted to investigate the germination characteristics of a pyrethrum seed lot under a range of constant temperatures. The seed lot used in this trial was chosen since it reportedly had reasonable germination percentage and was intended to be used commercially. Parameters used to evaluate germination included % final germination (%FG), mean time to complete germination (t), coefficient of uniformity of germination (CUG) (Appendix 1.3) and examination of ungerminated seed which provided data on percentage dead (%D) and percentage fresh imbibed ungerminated seed (%FUG) in the sample (Appendix 1.3). Cardinal temperatures for germination were estimated by fitting a quadratic equation to the plot of rate of germination (R) (Appendix 1.3) versus constant temperature. This provided data on how this species compares with other sown crops.

The seed was harvested in January 1995 from a seed crop located at FVRS. Post-harvest seed treatment included cool air drying, threshing and cleaning. Final cleaning was achieved using a gravity table (Plate 1.13). Only the highest density fraction of seed was retained for germination trials. Less dense fractions were found to be mainly comprised of hollow achenes. High density seed was subsequently sealed in double plastic bags and stored at 4°C. The percentage moisture (10.6%) was determined in June 1995 before trials commenced using the low constant temperature oven method (ISTA, 1993).

Seed was germinated on double Whatman No.1 filter paper in 100 mm diameter lidded plastic petri dishes. Prior to imbibition the paper was treated with 3.0 ml of 2.0 gm/l of the dithiocarbamate fungicide Thiram 800WP® (thiram 80%). One hundred achenes were evenly distributed on the filter paper. Constant temperatures ($\pm 0.5^{\circ}\text{C}$) for incubation were achieved using separate incubators at 4, 10, 15, 20, 25 and 30°C. The requirement for water was evaluated on a daily basis and when needed, distilled water was added to point of paper saturation. The germination tests were conducted in the dark. Germination was evaluated at the same time every day for a period of 36 days. Individual seed germination was defined as being complete when the radical tip was observed to have emerged from the achene.

A completely randomised design with four replicates was used. The %FG data were subject to arcsine \sqrt{x} transformation prior to ANOVA. Due to confounding between replicates which resulted from all replicates of a treatment being assigned to the same growth cabinet (Gates, 1991), a P value of 0.01 was used in the determination of Fishers LSD between treatment means.

4.3.3 Trial 3. The effect of temperature on germination of six seed lots

This trial investigated the germination characteristics of six seed lots incubated at low, medium and high temperatures.

Six seed lots (A, B, C, D, E, F) were provided by Botanical Resources Australia (BRA). The seed was harvested in February 1996 from designated seed crops grown both on the north coast (Forth and Devonport) and in the south east (Derwent and Coal River Valleys). Harvest information and germination data provided with the seed lots by BRA along with a seed colour and achene weight data are presented in Table 4.1.

Post-harvest seed handling, % moisture determination and germination procedures were the same as those described in section 4.3.2.

Constant temperature incubators were set at 10°C, 20°C and 30°C (+/- 0.5°C). The choice of these temperatures was based on data obtained in the first germination trial (Section 4.3.2) where 10°C was found to be the temperature below which %FG became unacceptably low, 20°C was considered optimal, and 30°C was reported as the upper limit above which germination again declined to unacceptable levels.

Table 4.1 Data provided with seed lots and mean achene weight and colour observations

Data provided with seed lots						Assessed measures	
Seed lot	Name and code	Crop location	Harvest date	Moisture at harvest (%)	% germination	Av. achene DW (mg)	Seed colour
A	Pyper, 3A95F	Forthside	-	-	-	1.395	very brown
B	Python, C27A295CS	Coal River	17/2/96	20	84	1.146	green
C	Pygo, 365A95S	Derwent River	16/2/96	40	70	1.162	very green
D	Pyoneer, 3A295C	Coal River	22/2/96	11	80	1.363	brown
E	Pyrate, 365A195S	Derwent River	16/2/96	-	71	1.199	very green
F	Pyper, 3A2950	Devonport	-	-	85	1.420	very brown
G	Pyrte, 641A95C	Coal River	17/2/96	20	60	1.148	brown

Note (1) Crop location, harvest date, moisture at harvest, and germination data were provided by BRA with the seed samples. (2) Germination data refers to percentage of achenes germinating in tests conducted shortly after seed cleaning approximately two months after harvest. Hollow achenes were included as potential germinants. Tests were reported to be conducted at a constant temperature of 17°C in the dark. (3) Mean achene dry weight (DW) was assessed in the course of determining percentage moisture of the seed lots. (4) Seed colour refers to the arbitrary differences in colour of the different seed lots.

The trial consisted of six seed lots with each lot being evaluated at three different temperatures with four replications. ANOVA analysis was confined to within temperature comparisons since variation in germination parameters assessed between temperatures was generally large. Parameters used in describing germination characteristics were %FG, %FUG, %D, t and CUG (Appendix 1.3). Statistical analysis of the collected data was as described in Section 4.3.2.

4.3.4 Trial 4. The germination behaviour of six seed lots after storage

This work investigated the germination characteristics of seed lots from the previous trial (described in Section 4.3.3). Six seed lots (B, C, D, E, F and G in Table 4.1) stored in double plastic bags at 4°C for 18 months were removed from the cool room and sampled. Percentage moisture and mean seed dry weight were determined and seed was held at room temperature for 24 hrs prior to commencing germination trials.

The germination trial was a completely randomised design with four replicates. Each replicate was a petri-dish which contained two pieces of Whatman No.1. filter paper, pre-treated with 3.0 ml of 2.0 g/l Thiram 800 WP®. One hundred seeds were evenly placed on each petri-dish prior to incubation in the dark at 20°C. Water requirement was assessed on a daily basis and when required, applied to point of paper saturation.

Number of seeds germinating was assessed every second day for a 16 day period, by which time most of the seed had germinated. Upon germination, defined as when the radical tip emerged from the achene, the seed was transferred to slopes and identified by day of germination. The slope was a seedling development environment similar to those described by; Smith et al. (1973a and 1973b), Wurr and Fellows (1984), and Grey and Steckel (1983b).

Seedlings were maintained on the slopes until such time that they reached the cotyledons open stage (an angle of approximately 150° to 180° between cotyledons). Water requirement of seedlings was assessed on a daily basis and additional water was applied when necessary. Assessment of seedlings reaching cotyledons open, the number of seedlings, and the day on which those seedlings germinated was recorded every second day (Appendix 1.3).

Only five of the six seed lots tested were those used in the trial described in Chapter 4.2.3. A (2×5) factorial design with four replicates was used, factor 1 being assessment occasion (two levels, original assessment from trial 4.2.3 or the current assessment), and factor 2 being seed lot. A natural log transformation was used on the CUG data to increase normality prior to factorial analysis. Where differences between assessment dates were non-significant, data were presented with analysis of differences between seed lots pooled over assessment dates.

Seedlings and associated spent achenes were then placed in separate glass vials for each of three sequentially later germinating cohorts and for each replicate. These were dried at 70°C for 24 hours for assessment of mean seedling and spent achene dry weights. All data were subject to ANOVA.

4.4 Results

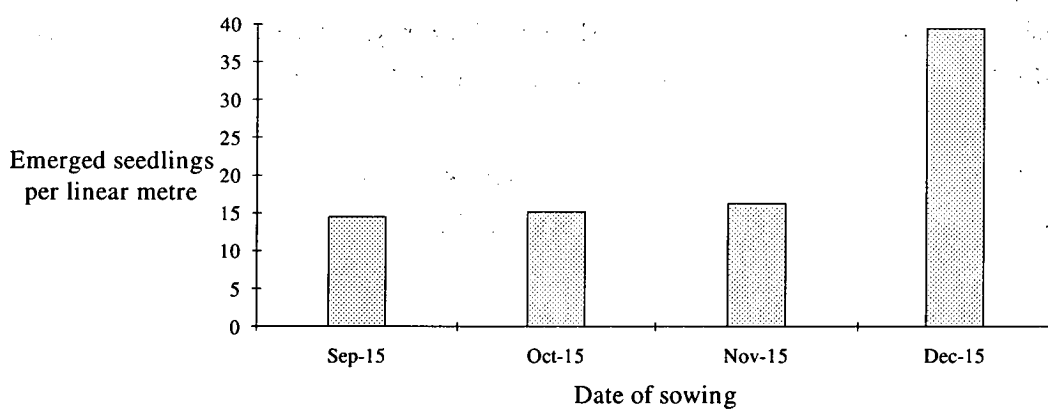
4.4.1 Trial 1. Time of sowing field trials

The final germination of seed used for this study was evaluated at 17°C prior to commencement of the first sowing and found to be 73%. When assessed again six months later, the germination percentage had not changed.

Significant differences in seedling emergence were noted among sowing dates in both the autumn and spring trials. It was calculated that 140 achenes were sown per metre and as 73% of those were viable, it follows that 102 seeds were sown per linear metre. Results are expressed as seedlings per linear metre and may be interpreted as percentage of viable seed sown. The proportion of viable seed emerged varied across plots from as little as 5%, to as much as 100% of the viable seed, but generally ranged between 15% to 80%. This percentage when expressed as a proportion of viable seed (i.e. 0.15 and 0.40) rather than a percentage, is known as the 'field factor' and is commonly used as a guide in determining the amount of seed to be sown in a given area.

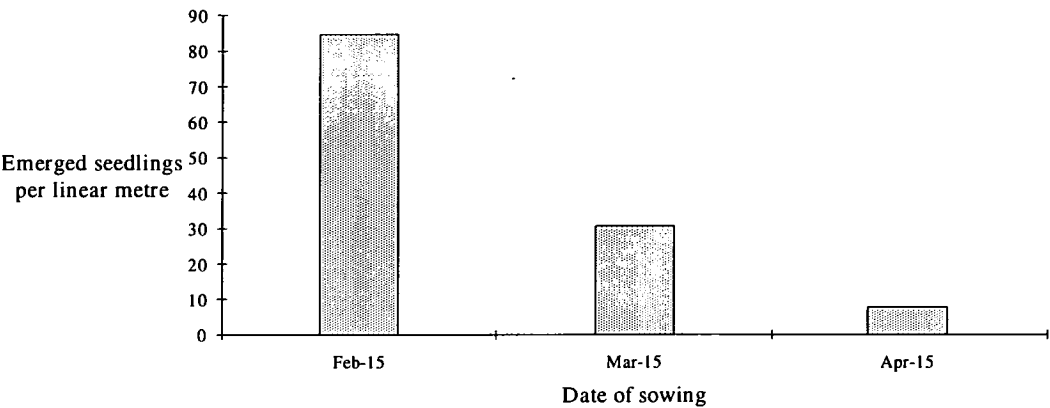
Mean total emergence for spring and summer sowings are presented in Figure 4.1 and Figure 4.2 respectively. Results from spring sowings indicate that the number of

Figure 4.1. Seedlings emerged per linear metre, assessed two months after sowing, spring trial



Note (1) Differences between some sowing date treatments are statistically significant at $P = 0.03$, LSD = 10.8 plants per linear metre

Figure 4.2 Seedlings emerged per linear metre, assessed two months after sowing, autumn trial

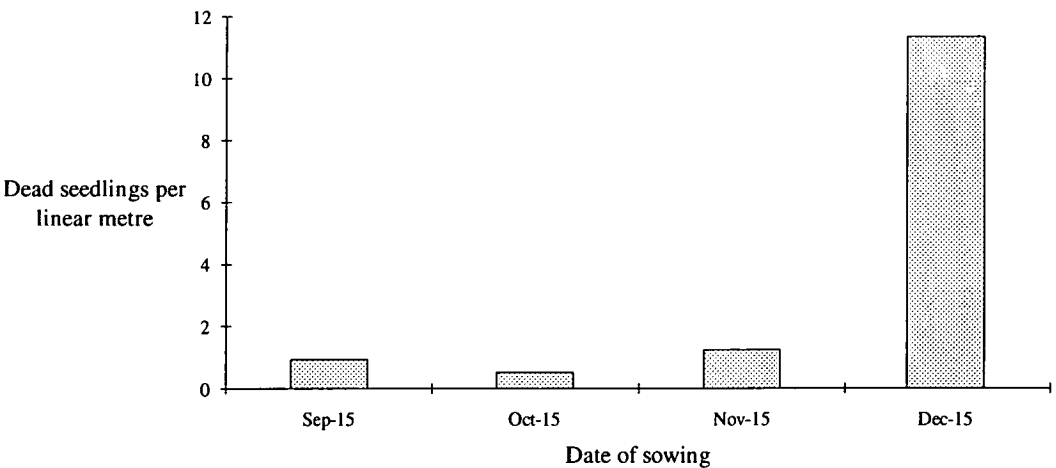


Note (1) Differences between some sowing date treatments are statistically significant at $P = 0.003$, $LSD = 33$ plants per linear metre

seedlings that emerged in the December sowing was higher than in earlier treatments. Figure 4.2 indicates that autumn seedling emergence decreased significantly from February to March and April.

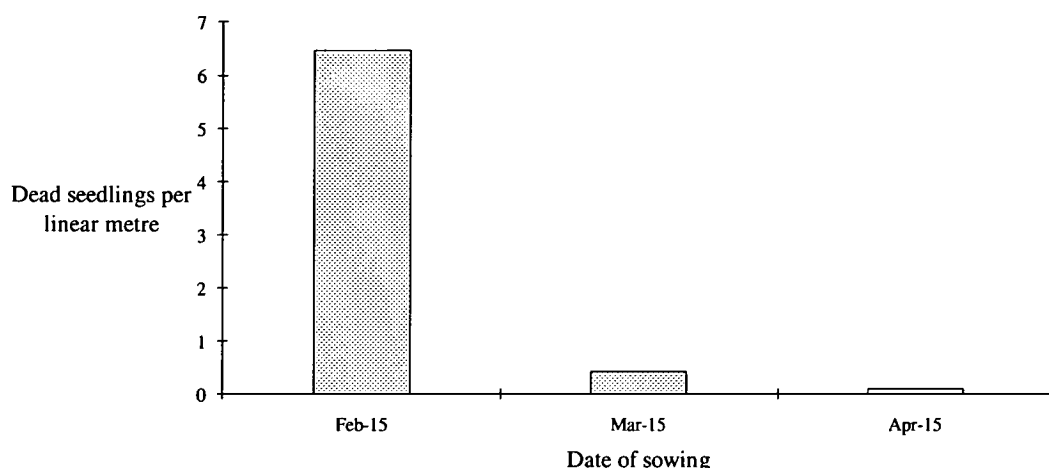
Although mean seedling emergence of 16 and 39 linear metre occurred in December and February sowings, more of those seedlings also died after emergence in these treatments (Figures 4.3 and 4.4).

Figure 4.3 Emerged seedlings that subsequently died, spring trial



Note (1) Differences between some sowing date treatments are statistically significant at $P = 0.0002$, $LSD = 4.75$ plants per linear metre

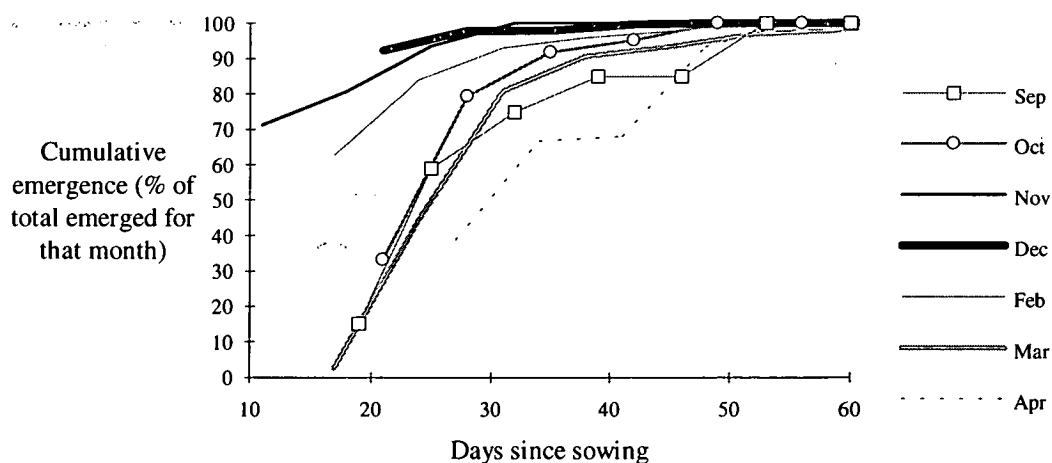
Figure 4.4 Emerged seedlings that subsequently died, autumn trial



Note (1) Differences between some sowing date treatments are statistically significant at $P = 0.023$, $LSD = 4.05$ plants per linear metre

Rate of emergence profiles (Figure 4.5) demonstrate variability in rate of emergence among sowing dates. Data indicate that by 20 days after sowing, most seedlings had emerged in the November, December, and February treatments, while emergence in other sowings was significantly slower.

Figure 4.5 Cumulative emergence vs days elapsed since sowing



Differences in rate of germination may have been largely due to variation in temperature between sowing dates. To investigate this, time taken to reach 85% of maximum final emergence (as estimated from polynomial equations in Appendix 1.7) was plotted against mean day degrees in the 17 days following sowing (Figure 4.6). Data indicate that in cooler sowings, emergence was delayed. Conversely, rate of emergence appeared to be optimal in sowings of between 6 and 8 mean degree days. February and March sowings

provide evidence indicating a decline in rate of emergence at above optimal temperatures.

Figure 4.6 Time taken to reach 85% of final emergence vs mean day degrees per day for the 17 days following sowing

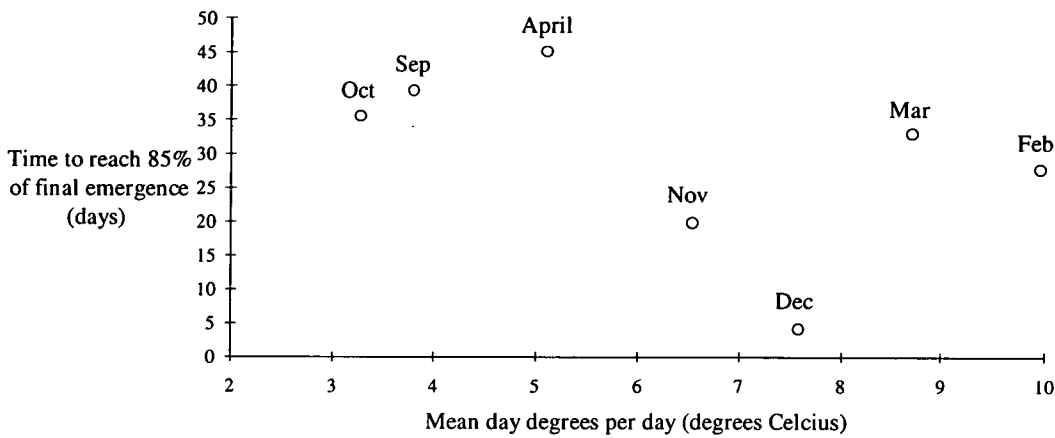
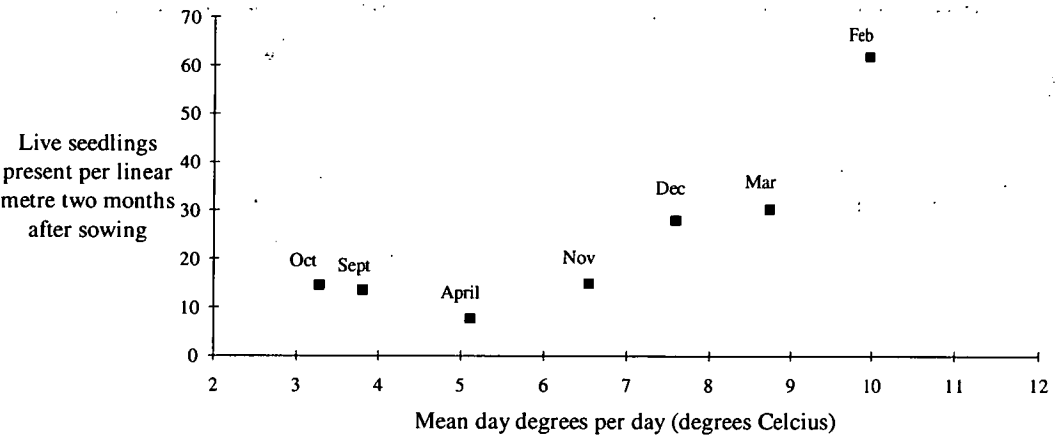


Figure 4.7 presents final seedling numbers two months after sowing in relation to the day degrees encountered in the 17 days after sowing. Data indicate that sowing in cold conditions resulted in low plant population.

Figure 4.7 Live seedling number per linear metre vs day degrees per day encountered in the first 17 days after sowing.



The emergence in Figure 4.8 and Figure 4.9 indicate the relative times of seedling emergence and seedling death for December and February respectively. The December data indicated a significant time interval between the main emergence time and subsequent seedling death. In contrast, the emergence and seedling loss data for February (Figure 4.9) indicate that seedling losses occurred throughout the whole emergence period.

Figure 4.8 Time after December sowing at which seedling emergence and seedling losses were observed

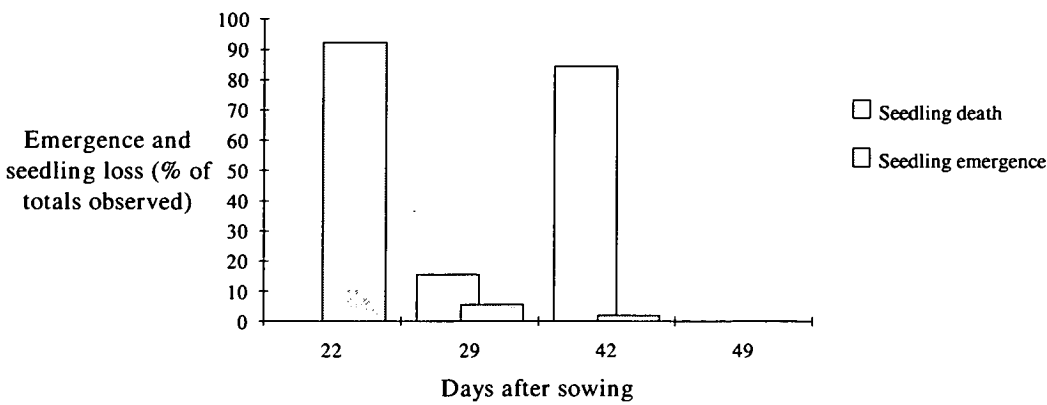
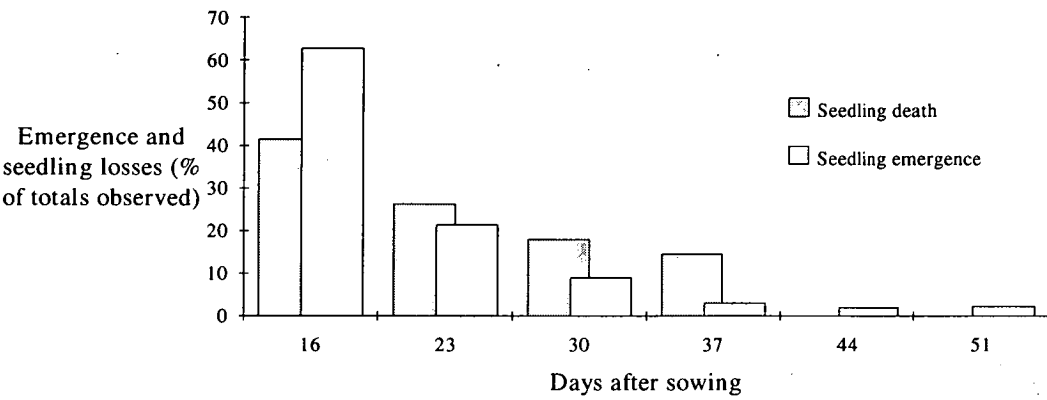


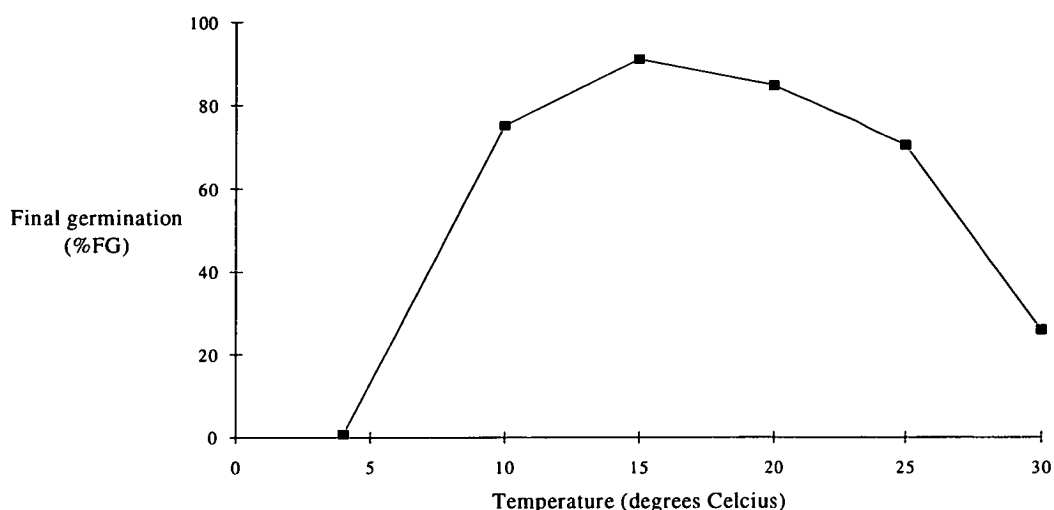
Figure 4.9 Time after February sowing at which seedling emergence and seedling losses were observed



4.4.2 Trial 2. Germination of a single seed lot at a range of temperatures

The %FG for seed at various temperatures is shown in Figure 4.10. Maximum germination percentage (89%) was achieved at 15°C. Percentage FG increased from 4°C to 15°C then decreased from 20°C to 30°C.

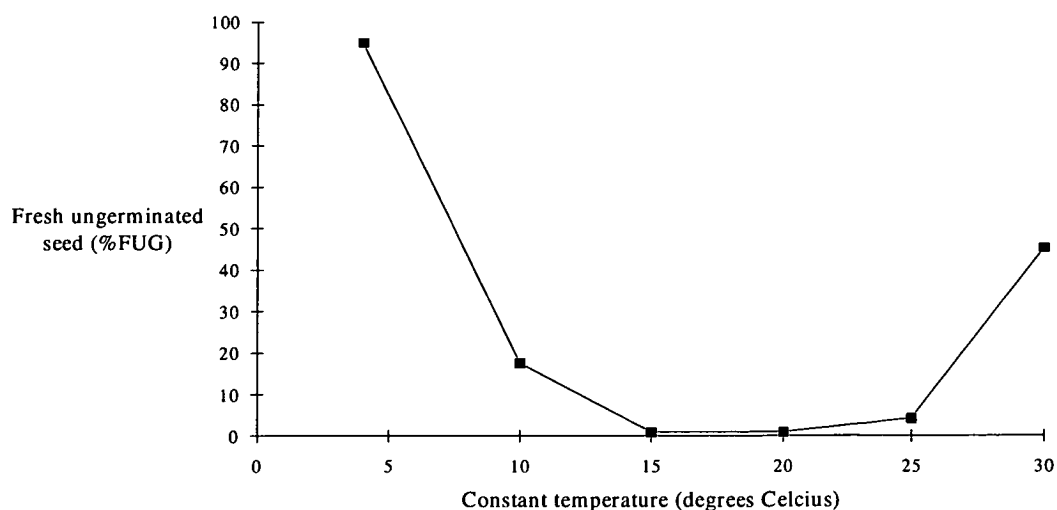
Figure 4.10. Final germination percentage (%FG) at various constant temperatures



Note.(1) Data were arcsine transformed prior to ANOVA. 4°C was not included in the analysis. Differences between some treatments are significant at $P < 0.001$. As an LSD for transformed data would be meaningless for the non-transformed data in this figure, standard error data is provided. SE's for increasing temperature treatments were: 0.66%, 0.70%, 1.68%, 3.87%, and 5.37%.

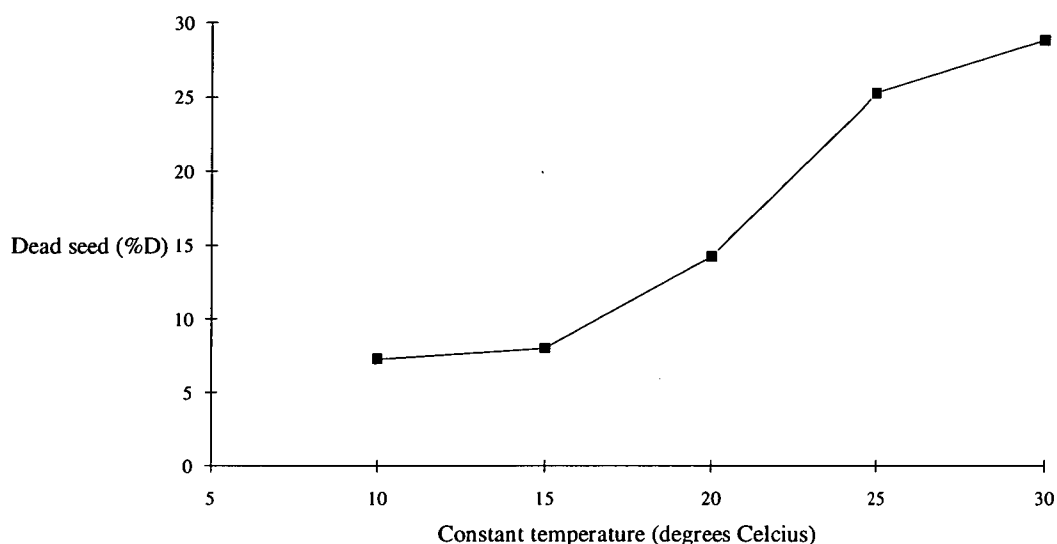
The %FUG data are presented in Figure 4.11. Means indicate a low percentage of fresh ungerminated seed at 15, 20 and 25°C. Percentage FUG is high at 10°C and falls as the temperature increases, indicating low temperature relative dormancy. The increase in %FUG from 25°C to 30°C also indicates seed dormancy. The %D is presented in Figure 4.12. The graph indicates that %D increases sigmoidally from 15°C to 30°C.

Figure 4.11. The percentage of seed found to be fresh and ungerminated (%FUG) after 36 days incubation



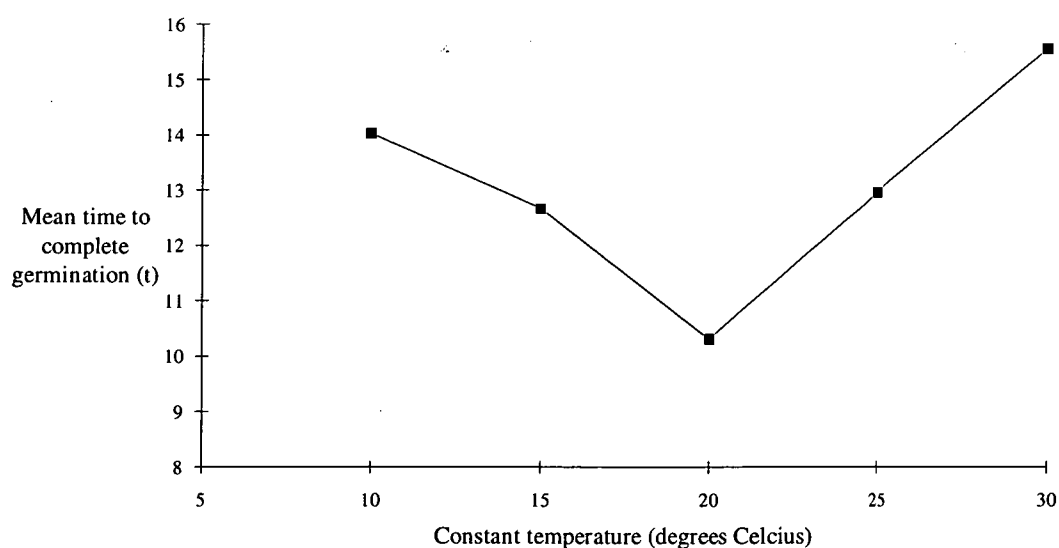
Note (1) Differences between treatments are significant at $P < 0.001$ $LSD(0.01) = 10.6\%$. (2).The 4°C FUG result is an estimate and therefore was not included in the analysis.

Figure 4.12. The percentage of seed found to be dead (%D) after 36 days incubation



Note (1) Differences between treatments are significant at $P < 0.001$. $LSD (0.01) = 7.76\%$. (2) The 4°C (%D) result was not included in the analysis due to near complete failure to germinate at that temperature.

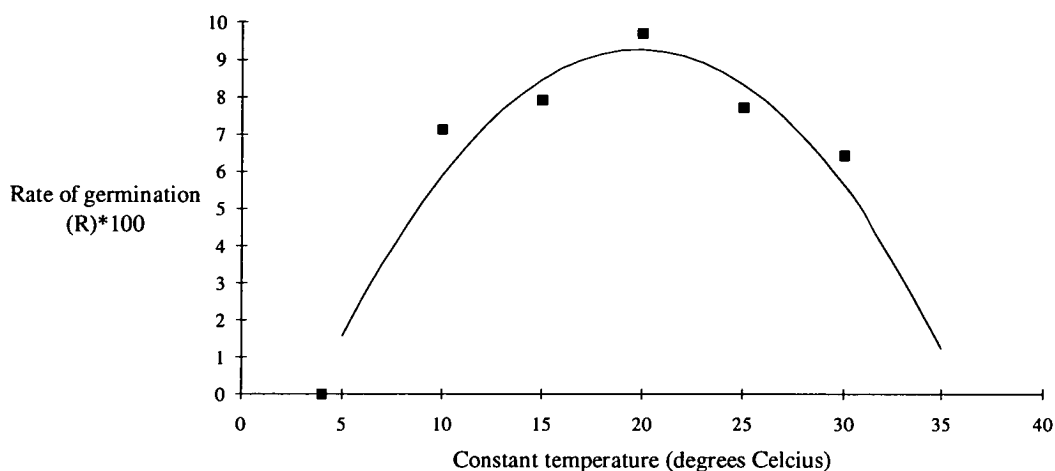
Mean time to complete germination (t) is shown in Figure 4.13. All means are statistically different except for the 15°C and 25°C treatments. Figure 4.13 shows t decreases from 10°C to 20°C and then increases again from 20°C to 30°C .

Figure 4.13. Mean time to complete germination (t)

Note.(1) Differences between treatments are significant at $P < 0.001$. $LSD (0.01) = 0.998$. (2) Data from the 4°C treatment was not included in the analysis due to failure to germinate at that temperature

Rate of germination data (Figure 4.14) allows for the estimation of cardinal temperatures for this seed lot.

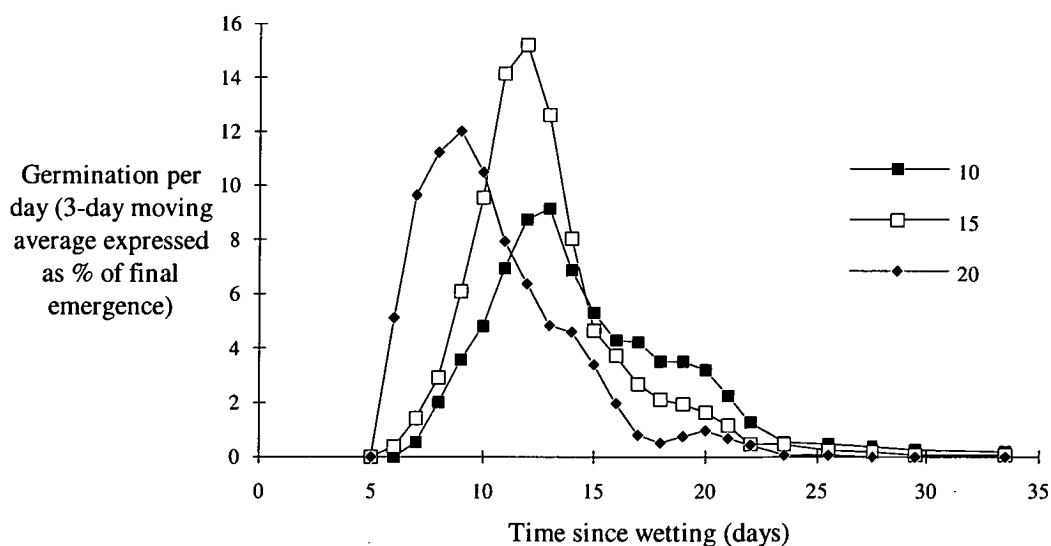
Figure 4.14 Rate of germination at various constant temperatures



Note (1) Equation for fitted curve; $y = -0.035x^2 + 1.388x - 4.487$, $r^2 = 0.945$. (2) Cardinal temperatures; $T_0 = 19.83^\circ\text{C}$; $T_m = 36.1^\circ\text{C}$; $T_b = 3.55^\circ\text{C}$.

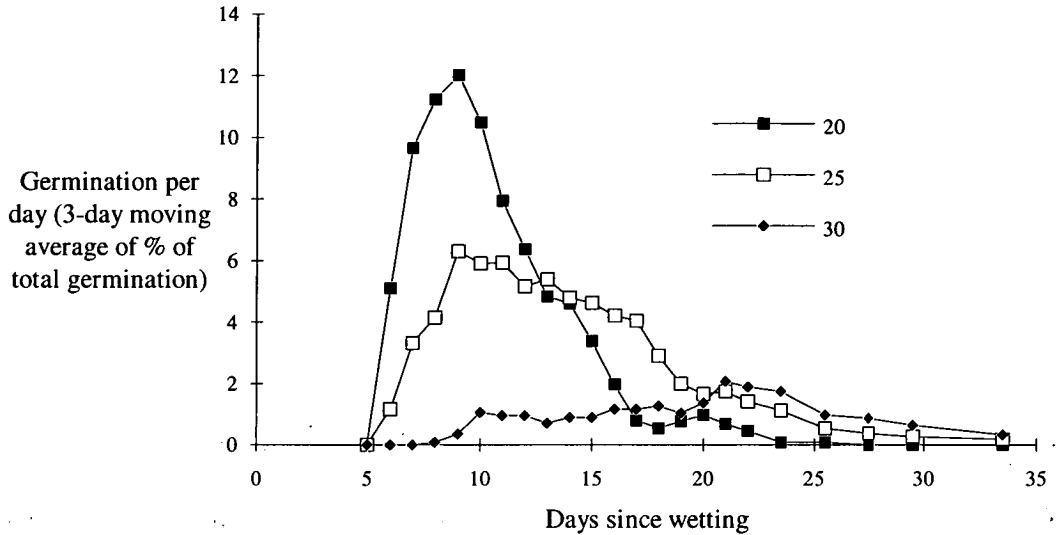
The relationship between percentage of germinated seed (as a percentage of final germination and time after wetting at 10°C , 15°C and 20°C are shown in Figure 4.15. Visible germination appears to have commenced at approximately the same time regardless of temperature. Although this was so, the lag phase was increasingly longer with the lower temperature treatments. Maximum rate of germination was achieved earlier at 20°C than at 15°C or 10°C . The highest germinations per day were achieved at 15°C .

Figure 4.15: Germination per day (3 DAYAV) at various temperatures.



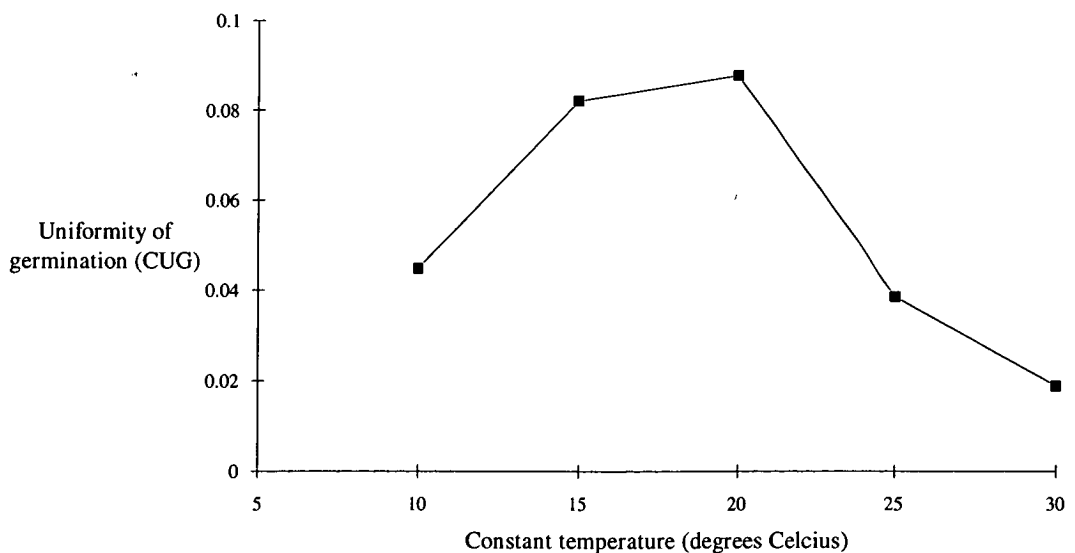
Germination per day profiles at 20°C, 25°C and 30°C are presented in Figure 4.16. The approximately normal distribution of the rate profiles of temperatures below 20°C appear to become increasingly skewed at higher temperatures.

Figure 4.16. Germination per day (3 DAYAV) at various temperatures



Uniformity of germination (CUG) data are presented in Figure 4.17. At 15°C and 20°C CUG is significantly greater than at 10°C and 25°C. Therefore the germination of this seed under non-optimal temperature conditions significantly increased spread in germination.

Figure 4.17. Uniformity of germination (CUG) at a range of temperatures.



Note.(1) Differences between some treatments are significant at $P < 0.001$. LSD (0.01)=0.029.

4.4.3 Trial 3. The effect of temperature on germination behaviour of six seed lots

Final germination (%FG)

Final germination at 10°C was high for all seed lots and ranged from 75% (seed lot A) to 89% (seed lot E) (Table 4.2). Increasing the temperature to 20°C resulted in increases in %FG in some seed lots but a substantial decrease in others. The most pronounced decrease was found in seed lot C, which declined from 84% to 52%. It was evident that

Table 4.2 Percentage final germination (%FG) for each seed lot

Seed lot	%FG at 10°C	%FG at 20°C	%FG at 30°C
B	88.0 ab	96.7 a	57.9 a
D	80.9 c	92.8 b	36.6 b
A	75.2 d	84.8 c	25.7 c
F	79.6 cd	88.0 c	25.1 c
E	89.1 a	78.0 d	10.4 d
C	83.9 bc	51.9 e	6.4 d
P = 0.0003, P < 0.0001, P < 0.0001, LSD = 5.3 LSD = 4.1 LSD = 8.2			

Note (1) Lettering denotes statistically different groups as determined by LSD.

only some seed lots were able to maintain a reasonable %FG at 30°C. The %FG ranking between seed lots corresponded closely in 20°C and 30°C treatments, whereas at 10°C ranking was inconsistent with the other temperatures.

Percentage fresh ungerminated (%FUG)

Table 4.3 depicts %FUG for seed lots at three temperatures. At 20°C %FUG was very low in all seed lots. At 10°C %FUG ranged from 5% (seed lot E) to 18% (seed lot A). At 30°C significant increases in %FUG, relative to 10°C treatments, were recorded in all seed lots except seed lot C. At 30°C, seed lots did not respond in a consistent manner with some seed lots (A and F) displaying high percentages, while seed lot C %FUG was only 10%.

Table 4.3 Percentage fresh ungerminated. (%FUG) for each seed lot

Seed lot	% FUG at 10°C	% FUG at 20°C	% FUG at 30°C
E	4.7 a	1.7 a	26.7 b
C	7.5 ab	2.0 a	10.0 a
B	9.8 bc	0.7 a	28.6 b
D	13.4 cd	0.5 a	28.3 b
F	14.0 cd	0.5 a	45.3 c
A	17.6 d	0.9 a	45.3 c
	P < 0.0001, LSD = 4.3	NS	P < 0.0001, LSD = 9.7

Note (1) Lettering denotes statistically different groups as determined by LSD.

Percentage dead (%D)

Data on the percentage dead seed (Table 4.4) indicate that seed death increases with higher temperatures. The relative level of seed death at 20°C appeared to parallel levels at 30°C, although at the higher temperature, levels were significantly greater.

Table 4.4 Percentage dead (%D) for each seed lot

Seed lot	%D at 10°C	%D at 20°C	%D at 30°C
B	2.2 a	2.5 a	13.5 a
D	5.8 b	6.7 b	35.1 b
A	7.3 bc	14.3 c	29.0 b
F	6.4 bc	11.5 c	29.6 b
E	6.2 bc	20.2 d	62.9 c
C	8.6 c	46.1 e	83.6 d
	P = 0.009, LSD = 3.1	P < 0.0001, LSD = 3.9	P < 0.0001, LSD = 10.5

Note (1) Lettering denotes statistically different groups as determined by LSD.

Mean time to complete germination (t)

Time required to complete germination was lowest at 20°C, increasing at both higher and lower temperatures (Table 4.5). At 10°C seed lots varied with mean times to complete germination ranging from 11.4 days to 16.2 days. At 20°C seed lots again varied, with following a similar ranking to that found at 10°C. Differences in t between seed lots at 30°C could not be usefully interpreted due to the low %FG in most treatments at that temperature.

Table 4.5 Mean time to complete germination (t) for each seed lot

Seed lot	t at 10°C (days)	t at 20°C (days)	t at 30°C (days)
B	11.45 a	8.15 a	20.25 ab
D	13.55 b	9.48 b	21.41 ab
F	14.28 bc	9.33 b	23.26 bc
A	14.96 cd	10.36 c	20.75 ab
E	15.87 de	11.54 d	17.95 a
C	16.17 e	12.37 e	25.24 c
P < 0.0001, LSD = 0.97			P = 0.011, LSD = 3.67

Note (1) Lettering denotes statistically different groups as determined by LSD.

Coefficient of uniformity of germination (CUG)

CUG generally increased from 10°C to 20°C, and decreased from 20°C to 30°C (Table 4.6). At 10°C CUG was low but still significant seed lot differences in uniformity were observed. Rankings of seed lots for CUG at 10°C and 20°C were similar but at 20°C CUG was higher and varied between seed lots more than at 10°C. At 30°C CUG was low, with no significant differences between seed lots.

Table 4.6 Coefficient of uniformity of germination.(CUG) for each seed lot

Seed lot	CUG at 10°C	CUG at 20°C	CUG at 30°C
B	0.158 a	0.166 a	0.0050 a
F	0.070 b	0.144 ab	0.0042 a
E	0.054 bc	0.093 bc	0.0047 a
D	0.053 bc	0.091 bc	0.0034 a
A	0.038 c	0.087 bc	0.0088 a
C	0.046 bc	0.074 c	0.0031 a
P < 0.0001, LSD = 0.032		P < 0.0001, LSD = 0.061	NS

Note (1) Lettering denotes statistically different groups as determined by LSD.

Relationships between germination parameters

Time to complete germination at both 10°C and 20°C was demonstrated to be negatively correlated with %FG at 30°C (Figure 4.18 and 4.19). The capacity of seed lots to germinate at high temperature was therefore positively associated with rate of germination at both 10°C and 20°C.

Figure 4.18 Final germination (%FG) at 30 degrees C. vs mean time to complete germination (t) at 10 degrees C

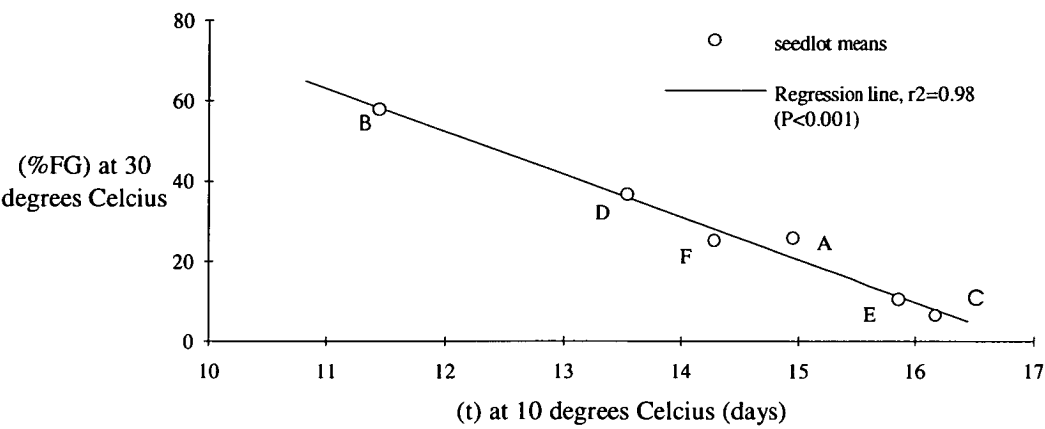
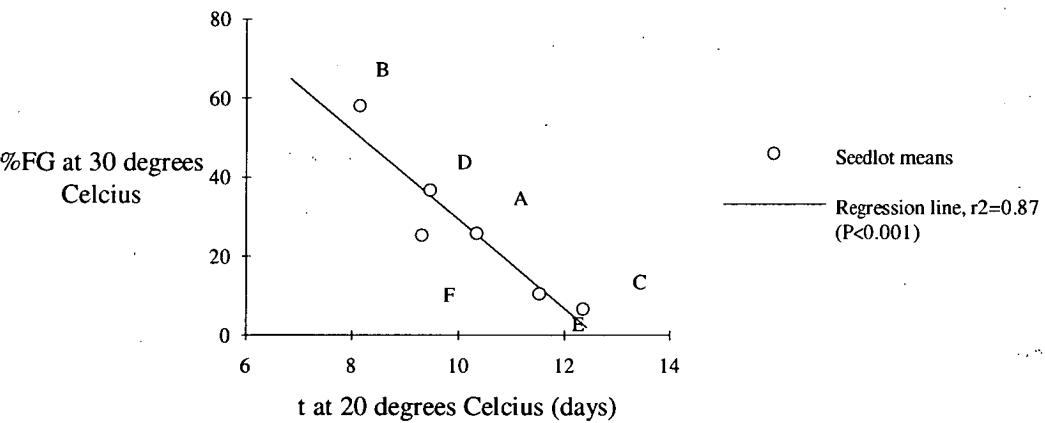


Figure 4.19 Final germination (%FG) at 30 degrees C. vs mean time to complete germination (t) at 20 degrees C



If t and %D are compared at 20°C, there appears to also be some correlation between these two parameters (Figure 4.20).

CUG at 20°C is compared with %FG at 30°C in Figure 4.21 and shows a close positive relationship between the two. Conversely seed lots with low uniformity had low %FG at 30°C. No such relationship was evident in seed lots displaying moderate uniformity (i.e., at 10°C or 30°C).

Figure 4.20 Dead seed (%D) after germination at 20 degrees C vs mean time to complete germination (t) at 20 degrees C

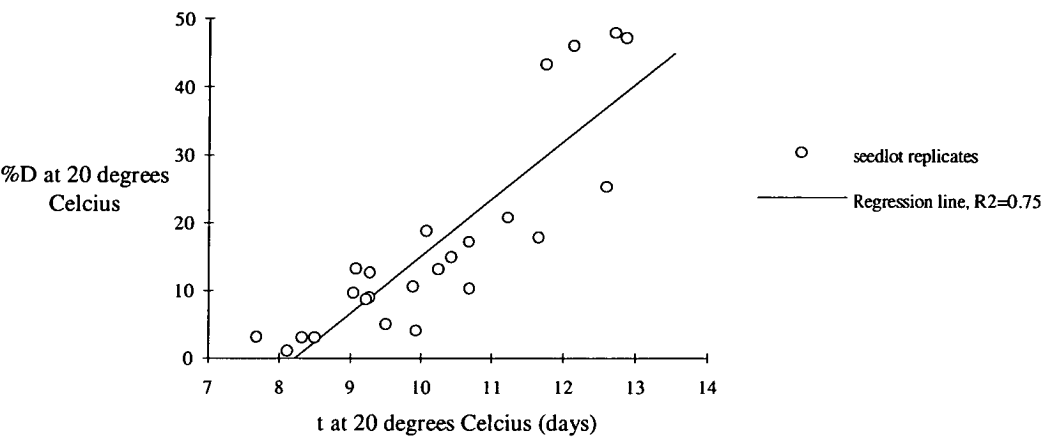
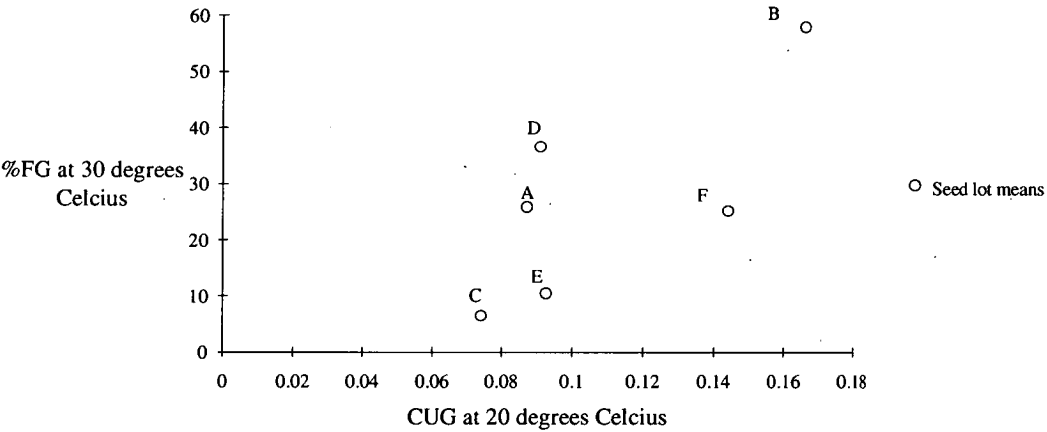


Figure 4.21 Final germination (%FG) at 30 degrees C vs uniformity of germination (CUG) at 20 degrees C

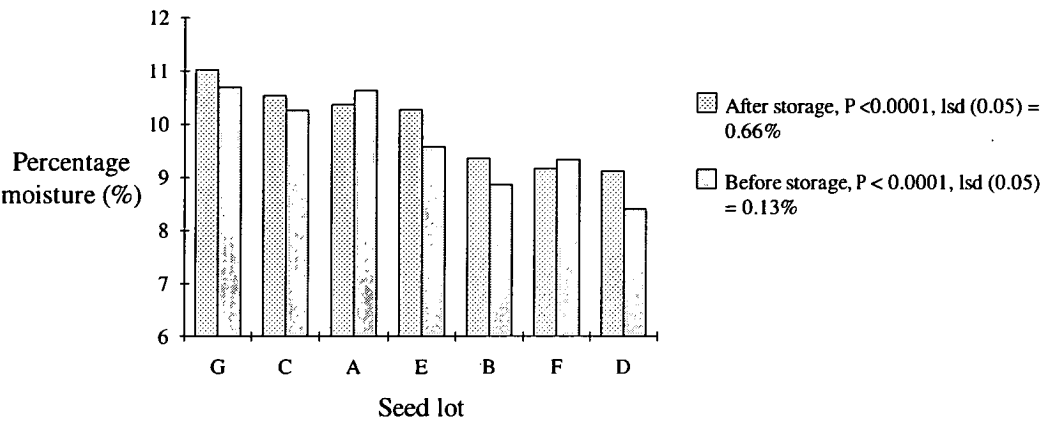


4.4.4 Trial 4. The germination behaviour of six seed lots after storage

Percentage moisture differed significantly between seed lots (Figure 4.22). Seed moisture did not change from during the storage period.

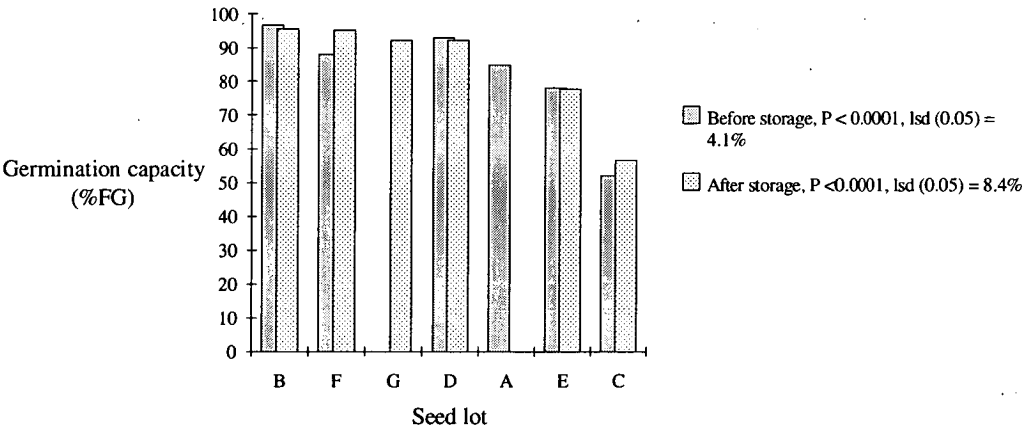
Final germination for seed lots is presented in Figure 4.23. Data indicate that little or no difference in germination capacity occurred over the 18 month storage period.

Figure 4.22 The percentage moisture in various seed lots before and after a storage period of 18 months



Note. As only small differences in percentage moisture were apparent between assessment occasions, no analysis of this factor was deemed necessary.

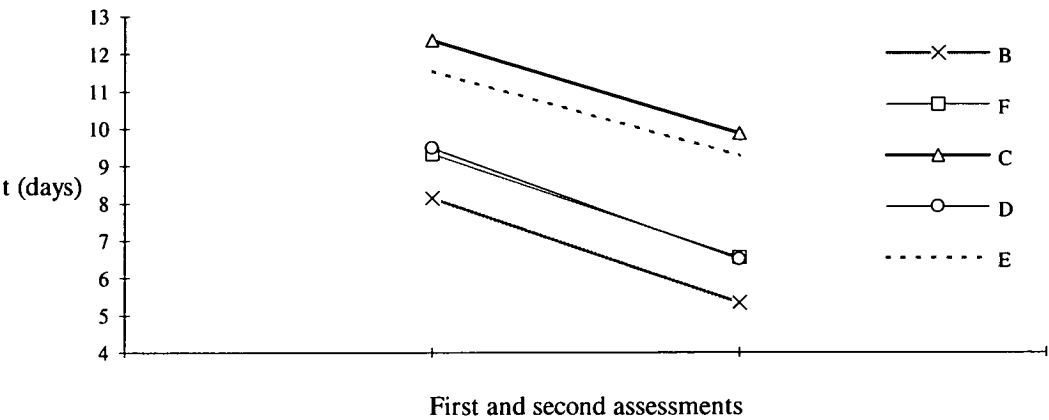
Figure 4.23 Germination capacity (%FG) of various seed lots before and after a period of storage



Note. As only small differences in germination capacity were apparent between assessment occasions, no analysis of this factor was deemed necessary.

The mean time to complete germination for seed lots prior to and after storage is presented in Figure 4.24. Ranking of seed lots for t was not affected by storage. A reduction in t in each seed lot of at least two days was evident after the storage period.

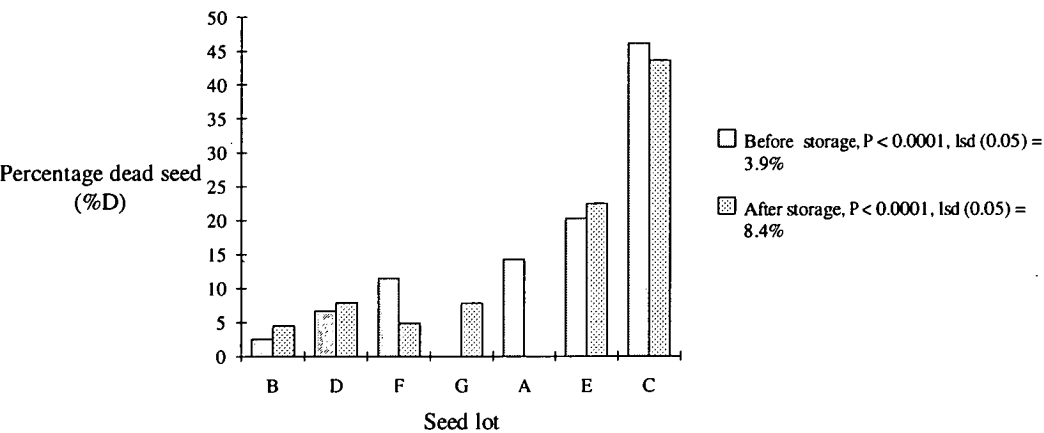
Figure 4.24 Mean time to complete germination (t) of various seed lots assessed before and after a period of storage



Note (1) Differences between first and second assessments were significantly different, $P < 0.0001$, $LSD = 0.295$ days. (2) Differences between seed lots were significantly different, $P < 0.0001$, $LSD = 0.47$ days. (3) Interaction between the two factors (seed lot and assessment occasion) was not statistically significant.

The percentage of dead seed for seed lots prior to and after storage is presented in Figure 4.25. Ranking of seed lots for %D prior to storage is similar to the ranking after the storage period. No appreciable changes in seed mortality after the storage period were evident.

Figure 4.25 Percentage dead (%D) after incubation in seed lots before and after a storage period

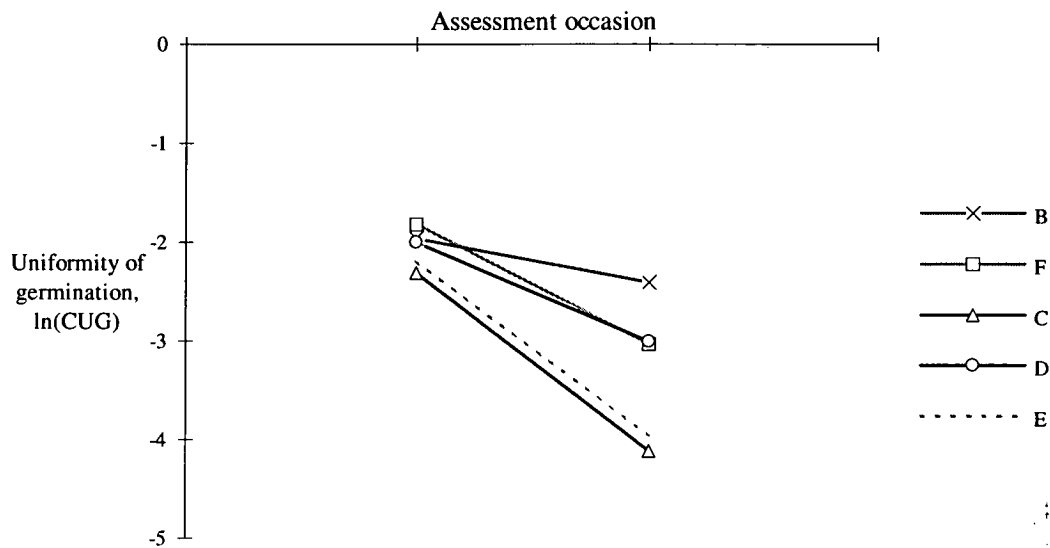


Note. As only small and inconsistent differences in %D were apparent between assessment occasions, no analysis of this factor was deemed necessary.

Uniformity of germination data are presented below (Figure 4.26). Data indicate that after a storage period, there is variability in loss of germination uniformity among seed

lots. Loss in uniformity was greatest in seed lots C and E; two seed lots suspected of being harvested early in their development.

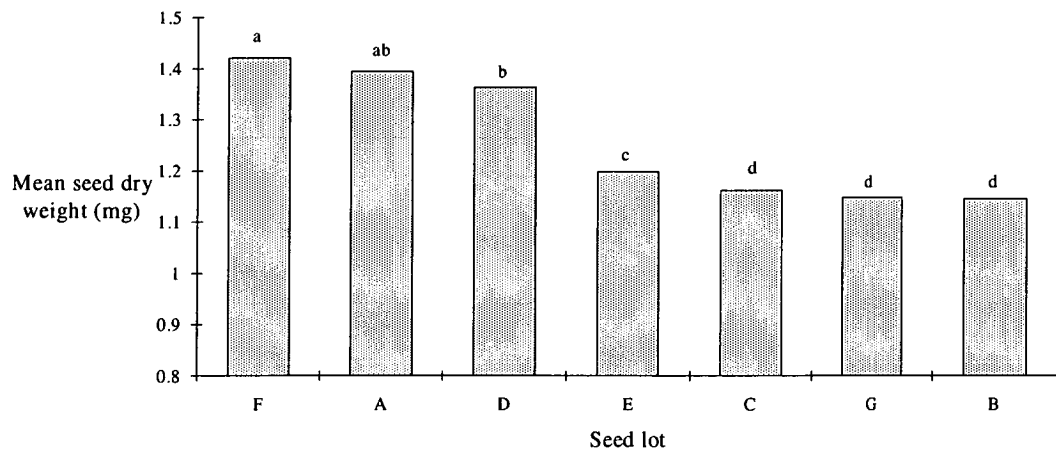
Figure 4.26. Uniformity of germination at 20°C in various seed lots prior to, and after, an 18 month period of storage



Note: (1) Natural log (ln) transformation significantly increased the normality of the data. (2) The interaction between treatments was significant at $P < 0.001$, $LSD = 0.263$

Data on mean dry weight/seed for different seed lots are presented in Figure 4.27. The three seed lots displaying heaviest mean seed weight demonstrated good germination characteristics. The four seed lots having low mean seed weight displayed poor germination

Figure 4.27 Mean seed dry weight for seed from different seed lots

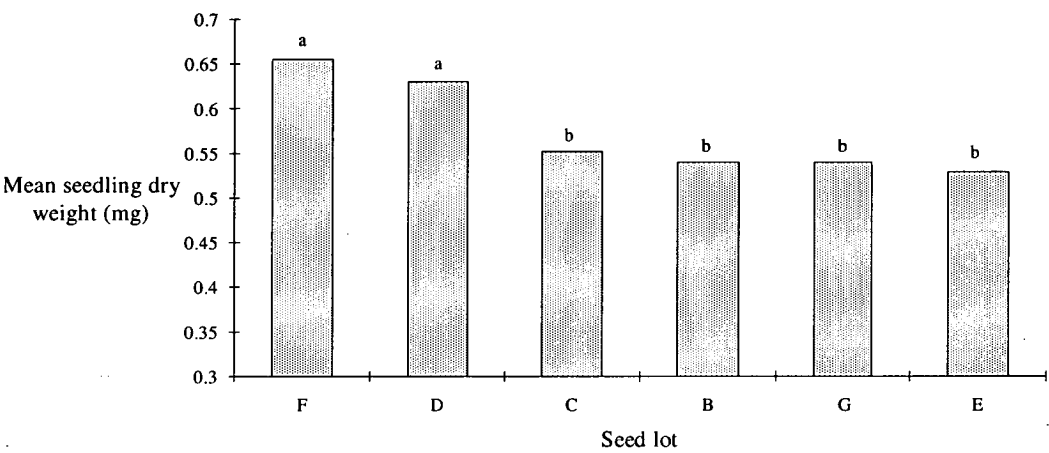


Note. (1) Differences between some seed lots are statistically significant at $P < 0.001$, $LSD = 0.0339$ mg. Statistically different mean dry weights ($P < 0.05$) are denoted by different letters

characteristics, except for seed lot B. Although mean seed dry weight and germination parameters are possibly correlated, seed lot B is a definite exception to this.

Mean seedling dry weight for seed lots is presented in Figure 4.28. Mean seedling weight of two seed lots were significantly greater than the other four seed lots. Those two seed lots demonstrated good germination characteristics, but, as with mean seed dry weight, (Figure 4.27) seed lot B (a seed lot of exceptionally high germination characteristics) was amongst the seed lots with the lightest mean seedling weights.

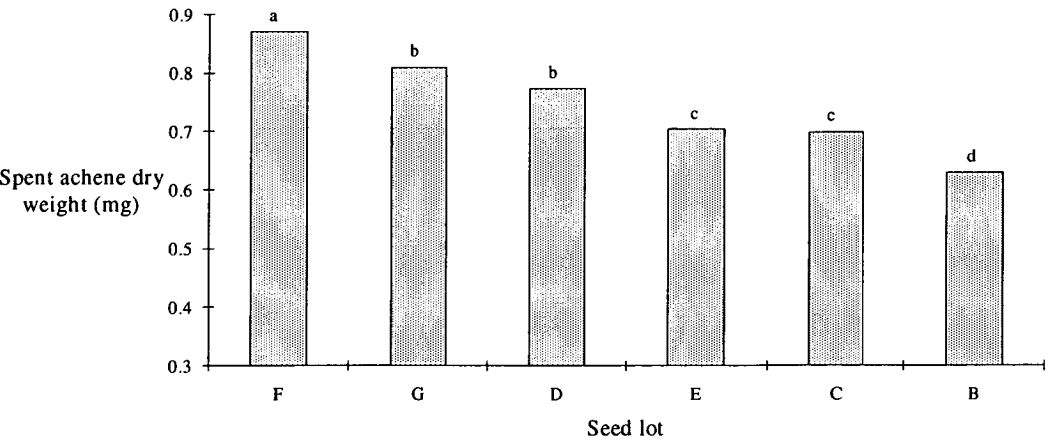
Figure 4.28 Mean seedling dry weight from seed in various seed lots



Note. (1) Differences between some seed lots are statistically significant at $P < 0.001$, $LSD (0.05) = 0.033$ mg. Statistically different ($P < 0.05$) mean dry weights are denoted by different letters.

Mean spent achene dry weights for seed lots are presented in Figure 4.29. Significant differences between seed lots were present. Those differences do not appear to be correlated to germination parameters.

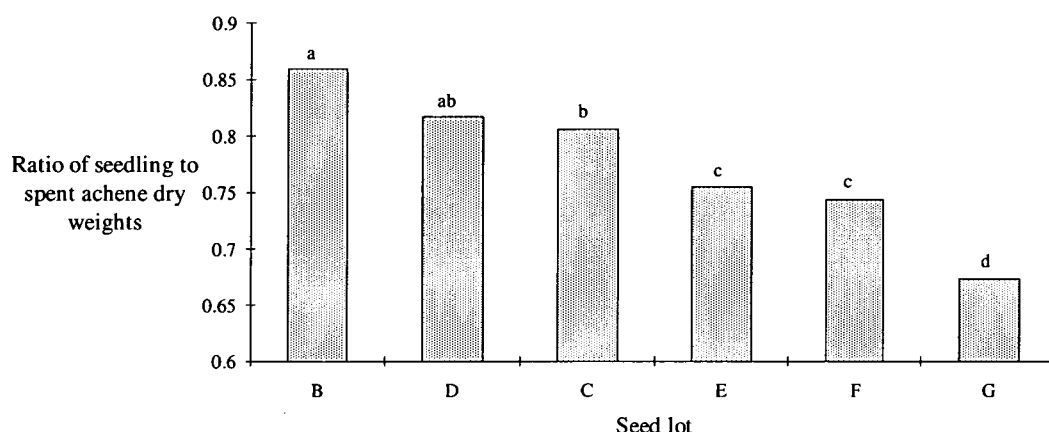
Figure 4.29 Mean spent achene dry weight for seed from various seed lots



Note (1) Differences between seed lots are statistically significant at $P < 0.001$, $LSD (0.05) = 0.049$ mg. Statistically different mean dry weights ($P < 0.05$) are denoted by different letters

Figure 4.30 demonstrates differences between seed lots with respect to seedling to spent achene ratio. This ratio was investigated as a measure of 'seed fill' in the seed that germinated.

Figure 4.30 Ratio of mean seedling dry weight to spent achene dry weight for various seed lots



Note (1) Differences between some seed lots are statistically significant at $P < 0.001$, $LSD (0.05) = 0.046$. Statistically different mean dry weights are denoted by different letters.

4.5 Discussion

4.5.1 Trial 1: Time of sowing field trials

Fordham and Biggs (1985) suggested that a field factor of 0.4 may be expected when sowing is conducted in poor soil or climatic conditions while under the best conditions, 0.8 is the highest that could be expected. Many of the sowings in this trial only achieved a field factor of 0.15. Therefore a markedly lower proportion of the viable seed sown in the current trial emerged than is generally expected with sowings of vegetable species in the poorest of conditions.

The number of dead seedlings varied significantly among sowing times in both the spring and autumn trials, with greatest mortality observed in the highest emergence sowings. Nearly 30% of the emerged seedlings in the December sowing subsequently died. Although data were collected relating time of emergence and subsequent death, they did not help identify likely causes of mortality. The findings are in agreement with Heydecker's observations that at supra-optimal temperatures seedlings emerge rapidly but subsequently died (Heydecker, 1977). A series of purpose designed trials would be required to fully explore this phenomenon.

Rate of seedling emergence varied considerably among sowing dates. Those differences were subsequently attributed to varying heat units (mean day degrees/day) in the 17 days

following sowing. Low day degrees were associated with slow rates of emergence. While warmer conditions after sowing appeared to increase rate of emergence, there was some evidence of a rate decline during the warmest sowing times. Live seedling numbers appeared to also be correlated to exposure to heat units, with low degree days associated with low emergence percentages.

This trial provided field emergence data which identified significant variation in seedling emergence with sowing time. Cool conditions after sowing were associated with less than acceptable seedling emergence (less than 15%) and slowness to emerge. Warmer conditions after sowing were associated with faster and more emergence and higher seedling mortality. If commercial sowing were conducted in warmer months, soil moisture rather than temperature may limit establishment success. An understanding of why pyrethrum seed fails to perform under cool conditions is required in order to provide recommendations to increase the success of crop establishment.

These results are generally in accordance with temperate vegetable species. Temperatures of between 15°C to 20°C are optimal for rate of germination for most vegetable species. At temperatures lower than this, the rate of development declines resulting in slower mean times to visible emergence in the field. Lower final populations often result from cool environmental conditions, since seed and seedlings spend longer in a state where they are susceptible to damping off organisms and insect attack.

4.5.2 Trial 2. Germination of a single seed lot at a range of temperatures

The seed lot appeared to germinate as a normally distributed population. Other Asteraceae species produce seed that varies greatly in germination requirements, which can alter the germination time distribution within the population markedly (Silvertown, 1984).

Data presented demonstrate that the seed lot achieved high %FG at between 10°C and 25°C. The seed appears to have a wide temperature range within which it will germinate. This result is consistent with previous findings in pyrethrum seed (Pandita, 1983). Germination percentage declined at temperatures approximately 5°C higher than the minimum required for reasonable germination percentage in most temperate vegetable species (Fordham and Biggs, 1985). At lower than optimal temperatures, increasingly more seed failed to germinate. At 10°C a significant proportion of seed was found as FUG, indicating the possibility of relative dormancy (as defined by Bewley and Black, 1994) while at higher than optimal temperatures, FUG seed together with dead seed accounted for the lower %FG.

Seed death increased sigmoidally with temperature. The greatest increase in seed death occurred between 15°C and 25°C, the temperature range in which rate of germination (R) was also greatest. From the data it could be speculated that the increasing occurrence of dead seed is associated with an ill fated attempt at rapid germination. Explanations for this correlation may involve uncoordinated reactivating of enzyme systems, incomplete membrane repair and solute leakage which are subjects beyond the scope of the current work. Regardless of actual mechanism(s), the appearance of an increasing proportion of dead seed at temperatures optimal for germination rate appears to be indicative of a significant flaw in the seed lot.

The temperature at which germination rate is maximal (T_0) was approximately 20°C. The evidence presented below for other species indicates that this assessment of T_0 may be applicable to other pyrethrum seed lots. In onions, *Allium cepa* L., T_0 does not appear to vary with priming treatment and seed lot viability (Ellis and Butcher, 1988). T_0 was found to vary only slightly but in a consistent way between early and late germinants within populations in pearl millet, *Pennisetum typhoides* S. & H., (Garcia-Huidobro *et al.*, 1982a), and only slightly with later germinants in dandelion, *Taraxacum officinale* Webber (Washitani, 1984). Conversely, in the warm climate species, common bean, *Phaseolus vulgaris* L., large differences in T_0 were identified both within and between gene pools (White and Montes, 1993). In general, it is evident that differences in T_0 occur within species, but those differences are small, particularly in temperate species. The actual variation in T_0 within, and between, seed lots and the influence of afterripening on T_0 in pyrethrum are yet to be determined. Such questions may be addressed in future studies.

The temperature below which rate of germination is zero, which is defined as base temperature (T_b) was estimated to be 3.55°C for the pyrethrum seed lot. This is a low T_b in comparison with warmer climate species such as tomato, *Lycopersicon esculentum* Mill., (Dahal *et al.*, 1990), cotton and pearl millet (Garcia-Huidobro *et al.*, 1982a), but similar to base temperatures for onions (Ellis and Butcher, 1988), dandelion (Washitani, 1984), faba bean, *Vicia faba* (Dumur *et al.*, 1990) and a range of other vegetable species (Bierhuizen and Wagenvoort, 1974). T_b varied little with later germinating cohorts in pearl millet, (Garcia-Huidobro *et al.*, 1982a), and only varied slightly with later germinants in dandelion (Washitani, 1984). As variation within seed lots in T_b at sub-optimal temperatures is generally found to be low, differences in rate of germination within a seed lot are largely due to variation between seeds in requirement for thermal time prior to germination (Finch-Savage, 1995). Although T_b may only vary to a minor degree between seed lots, research in a range of species has indicated that differences in

level of deterioration, genetic makeup, and possibly afterripening requirements may also vary T_b in a limited way. Further research on pyrethrum seed may identify and capitalise on any small but significant differences in T_b , but this is beyond the scope of the current work.

The estimated temperature above which rate of germination is zero (T_m) of 36.1°C suggests that this seed will germinate at higher temperatures than a range of temperate vegetable crops including onions *Allium cepa*, celery *Apium graveolens*, carrots *Daucus carota*, broad bean *Vicia faba* and common brassica species *Brassica oleracea* (Bierhuizen and Wagenvoort, 1974). There is significant variation in the optimum germination range of *Asteraceae* species and this most probably reflects the climate in which the particular species evolved (Baskin *et al.*, 1992a). A knowledge of cardinal temperatures for germination will aid interpretation of further pyrethrum seed quality research and contribute to identification of field conditions suitable for pyrethrum sowing.

Mean time to complete germination and CUG data at supra and sub-optimal temperatures may be confounded due to certain cohorts of seed failing to germinate. The cohorts which actually germinate may vary considerably in their behaviour, therefore care must be taken in the interpretation of these data. Although this may be so, the fact that t and CUG varied significantly even at temperatures at which final germination was constant and high suggests that temperature rather than non-random germination of different cohorts within the population was largely responsible for CUG and t differences.

It is likely that low germination uniformity results in low uniformity of emergence since Wheeler and Ellis (1994) identified that time of germination largely determined time of emergence. As discussed in an earlier section, uniformity of field emergence is an important seed quality characteristic. The influence of high or low temperature on this seed lot was to reduce uniformity. Mechanisms involved in determining germination time within pyrethrum seed lots require further investigation.

Coefficient of uniformity data demonstrate that with colder temperatures, uniformity of germination can be significantly reduced. In the field, where inter-seed environmental variability is encountered, along with lower than optimal temperatures, a further decrease in germination uniformity may be expected. Both the CUG and %FG results indicate that germination percentage would be low and non-uniform if sowings were conducted

during the months May, June, July, August and September when average maximum daily temperatures are 14°C, 12°C, 11°C, 11.5°C and 13.2 °C, respectively.

Several practical implications arise from the observation that high %D is found at just 5°C above the temperature at which maximum germination rate occurred. In the field, exposure of seed to high temperature could lead to poor germination. Highest maximum daily temperatures at FVRS occur in January (20.5°C) February (21.5°C) and March (19.5°C). Bare soil temperature at 10 mm may exceed maximum ambient temperature by more than 10°C on ferrosols (logger results, data not presented) which could increase the risk of germination failure during the hottest months. Irrigation may therefore have the dual role of wetting and cooling if sowing is to be attempted during this time.

The current work may be useful in determining various aspects of relative seed lot quality. If germination capacity were to be evaluated, 15°C would be the appropriate temperature. On the other hand if interested in other aspects of seed quality, then germinating seed at 20°C would be more appropriate. As capacity to germinate at low temperature is an important attribute in cool temperate regions, assessment of germination parameters at 10°C may also be of significant value. Further work is required to provide clear recommendations on germination test conditions.

Whilst limited to one seed lot, the current work provides basic information on parameters which may be used to describe the germination characteristics of pyrethrum seed. The parameters measured appear suitable for further germination studies aimed at the identification of quality parameters and the development of appropriate seed testing methods.

4.5.3 Trial 3. The effect of temperature on germination behaviour of six seed lots

The previous trial investigated the influence of a range of temperatures on a single seed lot. This work investigates the germination characteristics, at three temperatures, of six different seed lots harvested in that same year.

Percentage final germination (%FG)

Percentage final germination at 10°C was generally high due to the low seed death encountered at this temperature. The differences in %FG between seed lots at 10°C, although being significant, were relatively small. Seed lot rankings for germination capacity at this temperature were not consistent with other germination parameters at other temperatures. Consequently, %FG at 10°C cannot be regarded as a measure capable of separating seed lots for quality determination.

Increasing temperature to 20°C resulted in higher %FG in some seed lots but a significant decrease in others. Different seed lot responses resulted from two effects. Firstly, increasing the temperature released nearly all seed from relative dormancy. Since differences existed between seed lots in the proportion of seed dormant at 10°C, more or less seed was added to the 'germinable pool' at 20°C. Secondly, increasing the temperature from 10°C to 20°C resulted in a pronounced increase in %D in some seed lots and a minor increase in others. For example, seed lot C demonstrated a reduction in %FG at 10°C from 84% to 51% at 20°C. The increase in temperature to 20°C in this case resulted in seed death greater in magnitude than the corresponding release from dormancy. In most seed lots, germination at 20°C resulted in more seed being released from dormancy than seed being found to be dead, the combined effect being a higher %FG at 20°C.

The ranking of seed lots by %FG at 30°C followed the same pattern as at 20°C, although seed lots segregated more so at this higher temperature. Incubation at high temperature was clearly difficult or stressful for germination. At 30°C, the proportions of ungerminated seed found as %D or %FUG varied between seed lots. A possible explanation for these differences is proposed later in this discussion:

High %FG at 30°C of a seed lot was considered an indicator of high seed quality since 'in field' capacity to germinate at high temperature is a desirable characteristic in itself, but perhaps more importantly, %FG at 30°C reflects germination capacity, germination rate and CUG at lower temperatures.

Mean time to complete germination (t)

Time to complete germination readily separated seed lots at both 10°C and 20°C although differences between them were more pronounced at the lower temperature, presumably due to the longer time required for germination. Figures 4.18 and 4.19 demonstrate that the 'slow to germinate' seed lots also had unacceptably low %FG at 30°C. Time to complete germination at 10°C and 20°C are indicative of level of germination failure at high temperature. At 30°C *t* does not distinguish seed lots in an interpretable manner.

At 20°C the reason for germination failure was found to be seed death rather than dormancy. Therefore this was a convenient temperature to also investigate any relationship between seed death and rate of germination. Figure 4.20 demonstrated that a seed lot's capacity to germinate quickly was inversely related to its %D at 20°C. However, the relationship does not account for all the variability in rate of germination.

In summary, t at 10°C and t at 20°C adequately separate the behaviour of seed lots. In addition the measures are correlated to capacity to germinate at 30°C and seed death at 20°C. This provides some insight into understanding seed quality in the seed lots examined.

Correlations among various germination parameters

Relationships between seed lot %FG at 30°C versus t at 10°C, and between %FG at 30°C and t at 20°C account for much of the variability between the parameters, with r^2 at 0.98 and 0.87 respectively. The relationships are not intended to demonstrate that a single factor is influencing both parameters. Nor are they intended to imply any unidirectional change in seed quality among the seed lots. Rather, the relationships merely demonstrate that the two parameters are associated. Little evidence is available which can provide insight into what factor(s) may be responsible for observed differences between the seed lots. Furthermore, no data are available to identify whether the loss in seed quality occurred during seed development or after harvest. Even so, information generated may be used to formulate a hypothesis to explain some of the observed variations in quality. It may be proposed that much of the variation in germination behaviour observed between seed lots is due to differences in seed maturity at harvest. These ideas are developed further in the following paragraph.

An explanation for differences in germination behaviour between seed lots

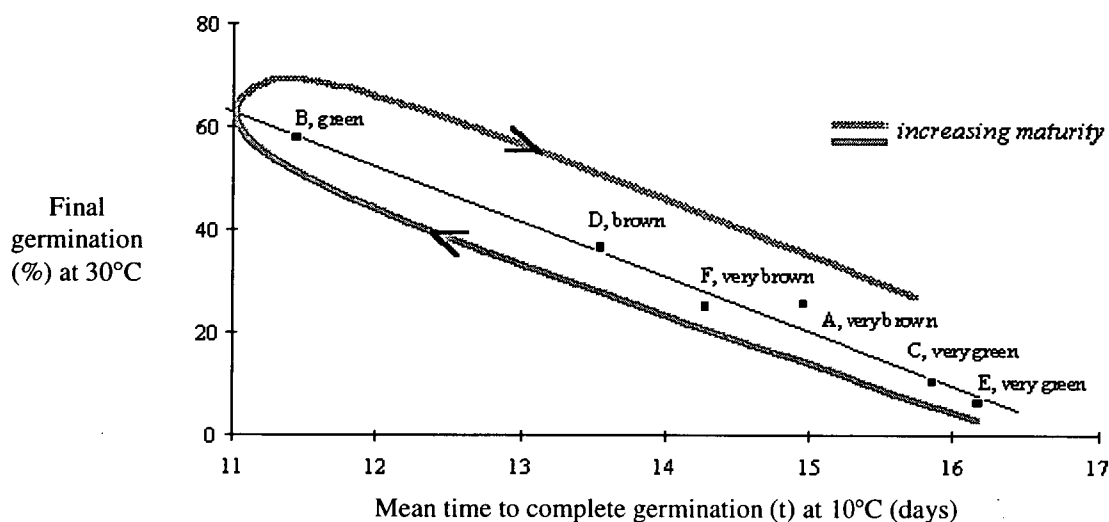
The following idea is proposed to explain the observed correlations between germination parameters on the basis of maturity at harvest:

- i. Seed harvested too early (very green) germinates slowly at 10°C and 20°C, fails to germinate to a high percentage at 30°C and a high proportion of the seed that fails to germinate is found as dead seed
- ii. Seed harvested when greenness has diminished (green) germinated rapidly at 10°C and 20°C, germinated to a high percentage at 30°C and very little of the ungerminated seed was found as dead seed.
- iii. Seed harvested when brown is slower to germinate at 10°C and 20°C than green seed, but faster than seed harvested too early (very green). A moderate percentage of this seed germinated at 30°C and most of the seed that failed to germinate at this temperature was found as FUG.

It is proposed that as pyrethrum seed matures its colour changes from very green to green to brown to very brown. Seed harvested relatively early in its development would therefore be very green and with increasing maturity become increasingly brown. With change in seed colour with maturity, percentage moisture of the fresh seed would also be

expected to decline, but information relating to this was not considered reliable. Figure 4.31 associates seed maturity, time taken to complete germination at 10°C and germination capacity at 30°C.

Figure 4.31. Final germination at 30°C vs mean time to complete germination (t) at 10°C (adapted from Figure 4.18)



The linear relationship proposed in Figure 4.31 appears simple but in fact at least two factors are acting to reduce both final germination and reduce the rate of germination. Investigating rate of germination, firstly, seed harvested when immature germinates slowly due to a range of factors, whereas seed harvested when 'over mature' (brown) is hypothesised to germinate slowly due to the development of an afterripening requirement during the later stages of seed development. Therefore both seed immaturity and seed dormancy are proposed as responsible for slow germination rate, and this depends on stage of seed development.

The hypothesis is that immature seed has a low final germination at 30°C due to seed failing to effectively complete germination. The result of this is that seed is found to be dead, or seedlings die by the end of the germination period. Furthermore, when seed was harvested when brown, the ungerminated seed at the end of the trial was predominantly dormant (FUG) rather than dead. The influence of harvest date on seed quality is the subject of a later study in this thesis, in which various aspects of the hypothesis are investigated.

Coefficient of uniformity of germination (CUG)

Germination uniformity within the seed lot is an important seed quality parameter since it may determine variability in time of field emergence. The observation that CUG was able to identify significant differences in uniformity between seed lots gives some credibility to

it as a measure of variability. Unlike t , CUG was only poorly correlated with capacity to germinate at high temperature (Figure 4.21). Therefore, factors other than those determining germination capacity at 30°C and rate of germination are influencing uniformity. Regardless of the mechanisms involved, factors determining CUG appear to vary from those which determine other germination parameters. Further work in this thesis investigates the influence of seed weight and position of origin on the plant as factors potentially influencing germination uniformity.

Some practical implications generated from the current research.

Temperature limitations for germination described in the trial on a single pyrethrum seed lot are consistent with the current trial results. Although there was a general consistency in germination behaviours between seed lots, they, seed lots varied considerably with respect to %FUG at low temperature. Seed lots also varied in their ability to germinate at higher temperatures. For consistently successful sowings, that is where an appropriate density of plants establish and develop synchronously, even under adverse germination conditions, seed of known high quality is necessary. With significant variability in behaviour demonstrated between seed lots, appropriate seed tests should be developed which account for poor seed performance. Such tests could be used along with other yet-to-be-investigated seed quality determinants.

Percentage final germination at 20°C may be useful as a commercial measure of seed quality. At 20°C obviously-flawed seed lots showed a reduction in %FG. At 15°C (the currently used assessment temperature) such defects may be less apparent since lower seed death levels were evident at this temperature in the trial on a single seed lot. Germination parameters at 20°C indicated how seed performed at both higher and lower temperatures. In addition, 20°C generated CUG data which may be an important indicator in uniformity of field establishment.

Germination as defined in the current work also marks the beginning of seedling development. Both germination and seedling development parameters will be found to influence seed vigour. "Seed quality is influenced throughout the life of the seed, from the time of fertilisation on the mother plant to the moment of sowing" (Pill, 1995). Regardless of when or how seed quality is influenced in pyrethrum seed, the current work indicates that the quality loss manifests itself in a consistent way. The further understanding of germination and what effects various seed characteristics have on subsequent seedling survival, growth and development parameters are to be investigated in ongoing trials

4.5.4 Trial 4. The germination behaviour of six seed lots after storage

Differences in moisture between samples can contribute to differential ageing or differential release from dormancy (Carpenter *et al.*, 1993). Data relating to percentage moisture of the seed indicate that little or no change in this parameter occurred over the storage period. Differences in germination parameters between seed lots across assessment dates were, therefore, not attributed to loss or acquisition of moisture.

Final germination data showed little change in germination percentage in any of the seed lots after the storage period. As expected at 20°C, no dormant seed was apparent and all the ungerminated seed remaining at the end of the experiment was categorised as dead. Although no differences in percentage germination were apparent, the time taken to complete germination decreased significantly in each of the seed lots. An afterripening requirement is therefore present which does not influence germination percentage at 20°C, but upon release allows faster germination.

Although seed deterioration may have occurred during storage, its impact, expected to present as an increase in time required for germination, may have been masked by the release from dormancy. That the percentage of seed found to be dead at the end of the trial was no different from the pre-storage assessment is further evidence that deterioration was not significant over the storage period. Delouche and Baskin (1973) suggested that uniformity of germination fell as a result of seed degradation. Although the current work demonstrated a decrease of CUG in seed lots after storage, this cannot be regarded as being due to degradation as this would have been inconsistent with %FG and *t*-data. Rather, a more plausible explanation for decrease in CUG involves seeds within seed lots varying in their response to, or requirement for, afterripening.

Although CUG decreased significantly in each seed lot after storage, the magnitude of the response varied. Loss of uniformity was greatest in seed lots thought to be harvested too early (C and E), moderate in seed lots harvested late (F and D), and least in (B) the seed lot thought to have been harvested at an appropriate time. The reasons why CUG of seed lots varied in response to storage may be due to a range of factors associated with differential release from dormancy, but such topics are beyond the scope of the current work.

Another aim of this work was to conduct a preliminary investigation into correlations between mean seed, seedling and spent achene dry weight and various germination parameters. This work was inconclusive, as no consistent trends between any of the weight and germination parameters were evident. Data did highlight significant

differences between seed lots in each of the mean weight parameters evaluated. Furthermore, differences in the contribution of various seed components to whole seed mass between seed lots were identified. The significance of such differences will be further investigated in a following chapter.



Plate 4.1 Pyrethrum seedlings at various stages of development (0.5 x magnification)

Chapter 5: The effect of seed mass on germination and early seedling development

5.1 Introduction

Previous investigations identified variation in germination characteristics among seed lots. Seed lots varied in germination capacity, rate of germination and uniformity of germination. Even among seed lots demonstrating high germination capacity, there was significant variation in germination rate and uniformity. The influence of seed mass in uniform, rapid crop establishment is generally recognised in the literature. The following study investigates seed mass as a factor that may contribute to variation in pyrethrum germination behaviour observed in previous work.

Initially, actual variability in seed mass was quantified in two seed lots displaying high germination capacity, but differing in uniformity and rate of germination. Germination and early seedling development characteristics in different seed mass classes were evaluated. Following this, a complementary study investigated seed and seedling mass of early, mid and late germinating seed in six seed lots. Results of both investigations provide valuable insight into the importance of seed mass in pyrethrum germination and seedling development.

5.2 Literature review: Seed mass and its variation within seed lots

The review firstly reports on the benefits of large seededness in seedling establishment. Following this, sources of variation in seed mass between populations is briefly discussed. Within population variation in seed mass is then introduced. Special reference is made to seed mass variation and germination behavioural differences within capitula in Asteraceae species. Finally, studies in Asteraceae species investigating the contribution of various seed parts to whole seed mass are discussed.

5.2.1 Benefits of large seededness

The influence of seed size or mass on various aspects of germination, seedling emergence, growth and final yield of many crops has been extensively researched. Examples of studies include; lettuce, *Lactuca sativa*, (Wurr and Fellows, 1983), carrot, *Daucus carota* (Grey and Steckel, 1983b), a range of vegetable crops (Taylor and Ten Broeck, 1988), and other non-crop species (Weis, 1982; Wulff, 1986a, 1986b and 1986c; and Milberg *et al.*, 1996b).

Seed mass is an easy physical parameter to evaluate and continued research into factors affecting it reflect the importance of this characteristic. Fenner (1992) indicated that even modest increases in seed mass can have important consequences for seedling establishment and competitive ability. Taylor and Ten Broeck (1988) demonstrated that both emergence force and total seed energy increased in proportion to seed size. Hegarty and Royle (1978) proposed that large seed size was beneficial where the soil acted as a physical barrier to seedling emergence. Heavier seed established from greater sowing depths than did smaller seed in; perennial ryegrass *Lolium perenne* (Naylor, 1980), pearl millet, *Pennisetum typhoides* (Lawan *et al.*, 1985), and wild radish, *Raphanus raphanistrum* (Stanton, 1985). Small seedling size has been correlated with increased probability of being grazed by molluscs (Boswel, in Fenner 1985; Hanley *et al.*, 1995) and reduced competitive ability in *Viola blanda* (Cook, 1980). Generally, larger seed has better field emergence and survival characteristics than smaller seed.

The advantages of using large seed other than for better seedling emergence percentage and uniformity has been reviewed (TeKrony and Egli, 1991). Generally, large size was found to enhance vegetative growth, but did not necessarily result in greater reproductive yield. The authors concluded that despite this, "the use of high vigour planting seed can be justified for all crops, ... to ensure adequate plant populations across the wide range of field conditions during emergence".

5.2.2 Variation in seed mass or size between populations

Seed size has been considered one of the least variable components of reproductive yield, particularly in plants with indeterminate growth (Harper *et al.*, 1970). Although it may be true that seed mass only varies within a given mass range, significant mean seed mass differences do exist. Variation in mean seed mass between sites may be due to differences in supply of assimilates (Lalonde, 1991) and trade-offs between seed size and number. Such relationships are addressed generally by Smith and Fretwell (1974), Winkler and Wallin (1987), McGinley and Charnov (1988) and Winn and Gross (1993). Trade-offs of this type have been identified in Asteraceae species including Californian thistle, *Cirsium arvensis* (Lalonde and Roitberg, 1989) and *Taragopogon dubius* (McGinley, 1989).

5.2.3 Within population variation in seed mass or size

Mean measures do not assess 'within-population' variability in seed size. Naylor (1980) reported that few studies had concentrated on the effects of different seed size 'within' rather than 'between' populations. Studies examining within-population variation in seed size and germination performance include Naylor (1980), Grey *et al.* (1983), Thompson (1984) and Milberg *et al.* (1996b). Seed size differences within a seed lot originate both

from within- and between-plant variation. Thompson (1984), working with an Umbeliferaceae species, found that seed mass varied 15.8 fold among even-aged plants grown under similar conditions, while within plants, up to 8.1 fold variation in seed mass was found. In many species, mean seed size is also found to decline through the growing season (Cavers and Steele, 1984).

There are numerous causes of variation in seed mass (Harper *et al.*, 1970; Pitelka *et al.*, 1983; and Fenner, 1992). Examples include variation in seed mass with position of the seed on the inflorescence (Wulff, 1986a), between capitula in Asteraceae, *Cirsium arvensis* (Lalonde and Roitberg, 1989), and the identity of the pollen donor (Mazer *et al.*, 1986; Marshall and Ellstrand, 1988).

General variation in seed morphology, behaviour and size within a plant is known as 'seed heteromorphism' and this has been described in more than 200 species of angiosperms (Flint and Palmblad, 1978). Such variation is sometimes associated with changes in seed mass (Imbert *et al.*, 1997).

5.2.4 Within capitula variation in seed mass or size in Asteraceae

Asteraceae species are well known for 'within-population' variability in seed size, morphology and germination behaviours. Some of the variation arises from positional differences within the capitulum. In ragwort, *Senecio jacobaea* L., the disc achenes are light and possess pappus, whereas ray achenes are larger and have no pappus (McEvoy, 1984). In most species centrally located achenes are smaller than peripheral ones, for example in; the telegraph plant, *Heterotheca grandiflora* (Flint and Palmblad, 1978), Californian thistle, *Cirsium arvensis* (Lalonde and Roitberg, 1989), and *Tragopogon dubius* (McGinley, 1989). However, in the forest understory herb, *Aster acuminatus*, Pitelka *et al.* (1983) found that inner and outer achenes had similar mass.

5.2.5 Variation in germination behaviour with respect to seed size in Asteraceae

In terms of germination behaviour, disc achenes were found to germinate more rapidly than ray achenes in camphor weed, *Heterotheca subaxillaris* (Awang and Monaco, 1978). Achene mass was found to have a small but significant effect on time to germinate in a *Taraxacum* sp. (Mogie *et al.*, 1990). Venable *et al.* (1987) while working with *Heterosperma pinnatum* Cav., an annual of Mexico, showed that peripheral achenes germinated over a slightly narrower temperature range than did central achenes.

The capitulum of pyrethrum, like that of many other species of Asteraceae, possesses two types of florets: disc florets, found in the centre of the receptacle and displaying

yellow corollas; and ray florets, located at the periphery and bearing white corollas (petals). Under the florets are single homomorphic achenes. Ray achenes, peripheral and central disc achenes appear to decrease in size in a continuous way with centralness of attachment to the peduncle. Variation in achene size is suspected to also occur among capitula and between plants.

5.2.6 Variation in seed components with respect to seed size in Asteraceae

The achene of pyrethrum is considered by commerce as 'the seed' since the seedling is only released from this structure after germination. Pyrethrum is similar to other Asteraceae species in that the endosperm, although being important during early seed development, is reduced to a few crushed cells in the mature achene and as such contributes little to mature seed mass (Gunn, 1972). The seed coat in pyrethrum seed is paper thin and remains inside the spent achene upon release of the germinating seedling. After germination, the empty achene containing the seed coat, and the seedling are separated. For seed of the same mass, the opportunity for different embryo mass therefore exists. Venable and Levin (1985), while investigating achene dimorphism in an annual weed *Heterotheca latifolia*, identified that although mass of the ray and disc achenes did not differ, disc embryos were 60% heavier than ray embryos. Fenner (1983) investigated the seeds of 24 Asteraceae species and revealed that larger seedlings may result from heavier embryos, more reserves, or both. Therefore, within Asteraceae, there is evidence to suggest that not only seed mass, but components of that mass may influence various quality characteristics of the seed.

5.2.7 Summary

This literature review cites numerous reports where the benefits of large seededness have been demonstrated. Generally it may be argued that larger seeds produce larger, more robust seedlings which are capable of rapid and continued radical and shoot elongation. Less time is spent in the vulnerable young seedling stage and the chance of successful plant establishment is increased. Mean seed mass varies between site and season due to varying maternal resources and trade-offs between seed size and number. Variation in seed mass within a population may also be significant and due to positional effects both between and within inflorescences. Capitula are often found to be a source of marked variation in seed mass in Asteraceae. Furthermore the capitulum allows for positional seed specialisation with respect to dispersal apparatus and level of dormancy. The possibility was raised that in some circumstances, seed mass may not parallel embryo mass.

There are no reported investigations on the significance of mass in pyrethrum seed. No reported research has investigated variation in seed mass in pyrethrum seed lots, the significance of variation in seed mass on germination parameters, the relationship between seed and seedling mass, nor the relationship between seedling mass and rate of seedling development upon germination. A knowledge of these factors will guide further research efforts aimed at improving quality of pyrethrum seed.

5.3 Materials and methods

Two approaches were used in assessing the influence of seed mass on germination of pyrethrum seed. Firstly, two seed lots with known high germination capacity were separated into mass classes and germination parameters assessed for each class along with seedling and spent achene mass. In the second trial, six seed lots were germinated at 20°C. Seedlings and spent achene dry weight were evaluated from cohorts of seed germinating on successive days.

5.3.1 Trial 1

Seed lots had been cleaned using a gravity table. The least dense fractions were evaluated and found to mainly comprise hollow achenes. Most of the true seed was retained in the densest fraction used in the germination studies. (Pers. comm. Brian Chung, Research Manager, BRA Pty. Ltd). The original 1.0 kg samples were stored in double plastic bags at 4.0°C prior to being sub-sampled to a working sample of 1.0g. Every seed from the working sample was weighed to an accuracy of +/-0.01 mg. Seeds were handled using air tweezers and separated into a range of seed mass classes, as shown in Table 5.1 and Plates 5.1 and 5.2.

Table 5.1 Seed mass classes

Container	Seed mass class (mg)
1	<0.8
2	0.8-0.99
3	1.0-1.19
4	1.2-1.39
5	1.4-1.59
6	1.6-1.79
7	1.8-1.99
8	2.0-2.19
9	>2.2

Seed in each class was divided into three equal lots (replicates) and germinated in petri dishes (Chapter 4.3.2). Germination was at 15°C and was assessed on a daily basis.

Germination parameters were subjected to ANOVA. Parameters used to evaluate germination were; final germination percentage (%FG), mean time to complete germination (t), and coefficient of uniformity of germination (CUG) (Appendix 1.3).

Air tweezers were used to transfer the germinated seed to slopes (slopes are described in Chapter 4.3.4). Transfer of germinated seed to slopes was conducted on a daily basis until the number of seeds germinating per day was very low or no seeds remained ungerminated. Slopes were maintained in the dark, in a growth cabinet (15°C +/- 0.5°C). Seedlings were then removed from the slopes once they had reached the cotyledons open stage. 'Cotyledons open' stage was defined as when cotyledons were approximately perpendicular to the stem. Seedlings were removed from the slope along with the empty achene and oven-dried (70°C for 3.5 hours) for dry weight (DW) determination.

The results of seedling tests were expressed as:

i. Percentage cotyledons open (%CO)

The percentage of germinated seeds achieving the cotyledon open stage

ii. Time interval from mean time to complete germination to mean time to complete cotyledons open ($\overline{co-t}$).

iii. Time interval from Mean time to complete cotyledons open (\overline{co}) gives an average measure of mean time elapsed for seedling cotyledons opening

$$\overline{co} = \frac{\sum (t_x \cdot co_x)}{\sum co}$$

Where

t_x = time in days starting from day zero as day of sowing

co_x = number of seeds reaching cotyledons open on day x

5.3.2 Trial 2

Seedlings and spent achenes evaluated in this trial were obtained from germinated seed of the 'after storage period' germination study reported in Chapter 4.

Rather than separating seed on a mass basis, seedlings and spent achenes were collected according to day of germination. This was achieved by positioning germinated seed on a slope such that seed germinating on a particular day could be recognised. Once the seedlings reached cotyledons open, seedlings and associated spent achenes were placed in glass vials and dried at 70°C for 24 hours. This allowed for the establishment of day of germination treatments for each of the seed lots examined. ANOVA analysis was conducted for each of the six seed lots. The variables examined were mean seedling and spent achene dry weight for the different germination period treatments. This trial therefore evaluated seedling variables originating from seed that germinated in

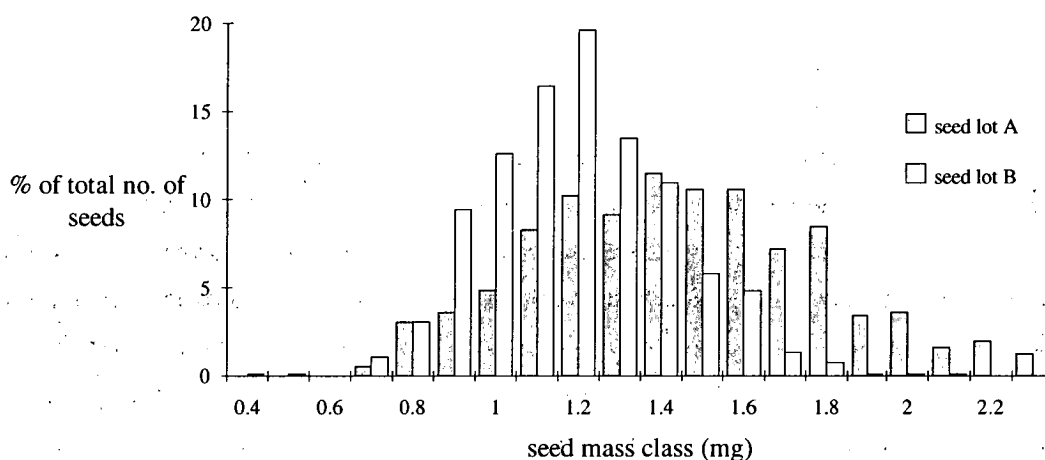
increasingly later periods (treatments). The time period chosen as a "treatment" was 48 hours.

5.4 Results

5.4.1 Trial 1

The frequency distributions of seed number in various seed mass classes for seed lots A and B are presented in Figure 5.1. Mean seed mass for seed lot A and B are 1.450 ± 0.083 mg and 1.197 ± 0.055 mg, respectively. The data show that both mean seed mass and variation in seed mass are greater in seed lot A than in seed lot B.

Figure 5.1 Seed mass distribution for two seed lots



Final germination data for seed mass classes of A and B are displayed in Table 5.2. The lightest seed of seed lot A had significantly lower germination capacity than the heavier seed mass classes. That trend was not apparent in seed lot B.

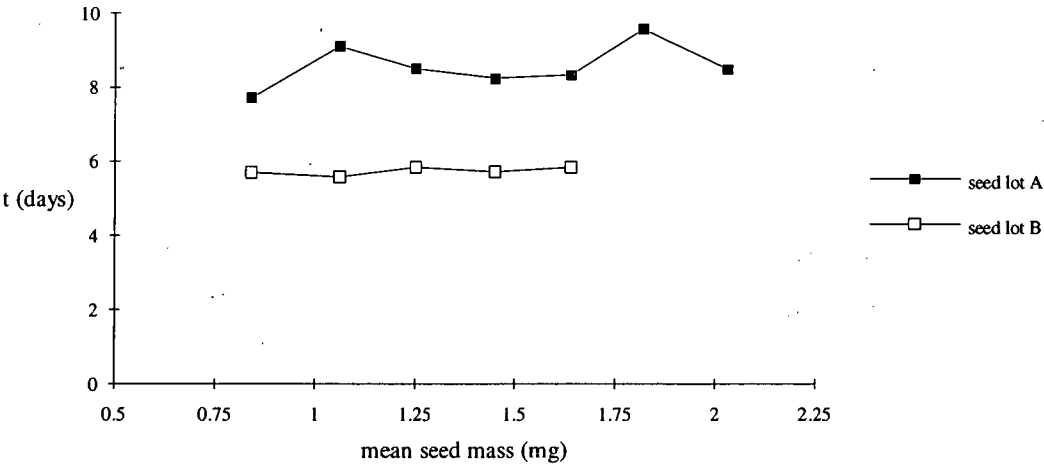
Mean time to complete germination is presented in Figure 5.2. Significant differences were found between seed lots but not among mass classes within seed lots. Mean time to complete germination for seed lot A was 8.57 days, while t for seed lot B was 5.74 days.

Table 5.2 Distribution and %FG in seed mass classes A and B

Seed lot class	Mean seed mass (mg)	No of seeds	% seeds per class (by number)	% Final germination (+/-SE)
A1, (<1.0)	0.84	40	7.19	61.5 (0.69) a
A2, (1.0-1.19)	1.06	93	13.13	87.0 (2.41) b
A3, (1.2-1.39)	1.24	154	19.42	90.6 (1.85) b
A4, (1.4-1.59)	1.45	174	22.12	95.8 (0.83) b
A5, (1.6-1.79)	1.64	158	17.81	92.7 (3.76) b
A6, (1.8-1.99)	1.83	106	11.87	95.4 (0.07) b
A7, (2.0-2.19)	2.03	48	5.22	96.7 (3.33) b
A8, (>2.2)	2.24	27	3.24	91.7 (8.33) b
P < 0.0001, LSD = 13.56				
B1, (<1.0)	0.85	126	13.82	95.2 (2.75)
B2, (1.0-1.19)	1.06	265	42.65	98.0 (0.43)
B3, (1.2-1.39)	1.24	302	33.11	98.3 (1.19)
B4, (1.4-1.59)	1.43	153	16.78	97.4 (1.32)
B5, (1.6-1.79)	1.62	56	6.14	100 (0)
A6, (1.8-1.99)	1.81	8	0.88	100 (0)
NS.				

Note. 1. Significantly different germination percentages ($P<0.05$) are denoted by different letters

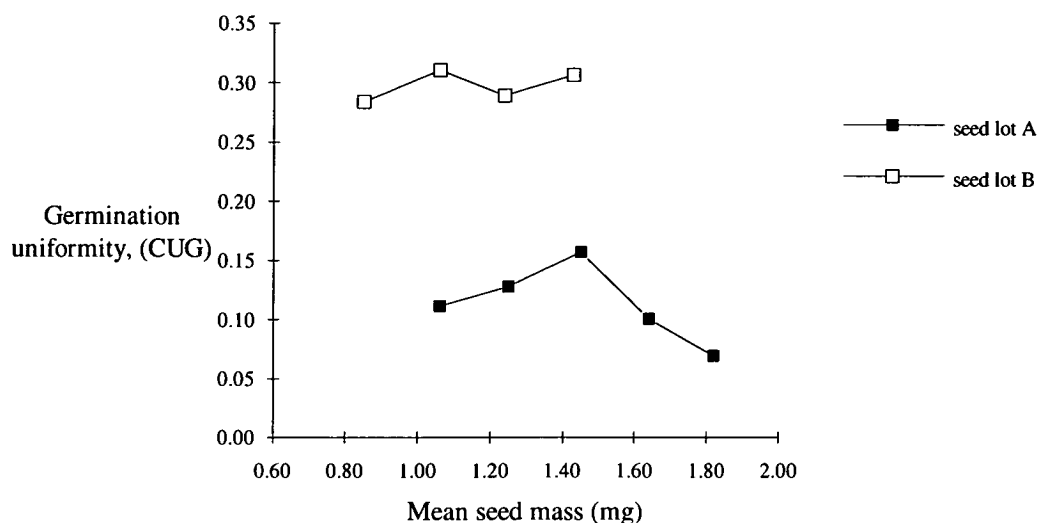
Figure 5.2 Mean time to complete germination vs seed mass class



Note (1) Differences between some treatments between seed lots are statistically significant ($P < 0.001$, $LSD(0.05) = 1.51$ days).

Uniformity of germination was evaluated in Figure 5.3 which demonstrates differences between seed lots but not between seed mass classes within seed lots.

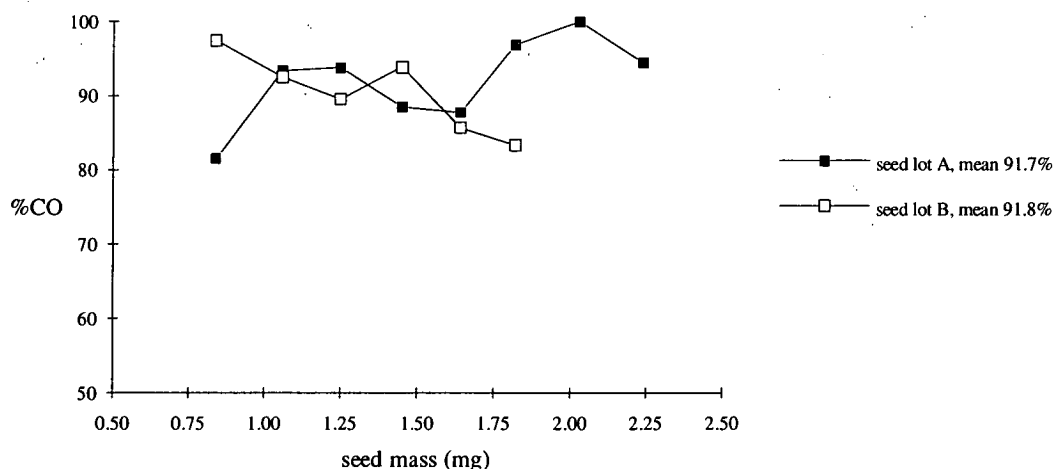
Figure 5.3 Uniformity of germination vs seed weight class



Note. (1) Only seed mass classes containing more than 30 seeds per replicate were included in this analysis. (2) Differences between seedlot treatments were statistically different only at $P = 0.06$ ($LSD = 0.19$). Square root transformation increased the level of significance to $P = 0.022$.

Figure 5.4 demonstrates that regardless of seed mass or seed lot, above 80% of germinating seeds achieved cotyledon open stage.

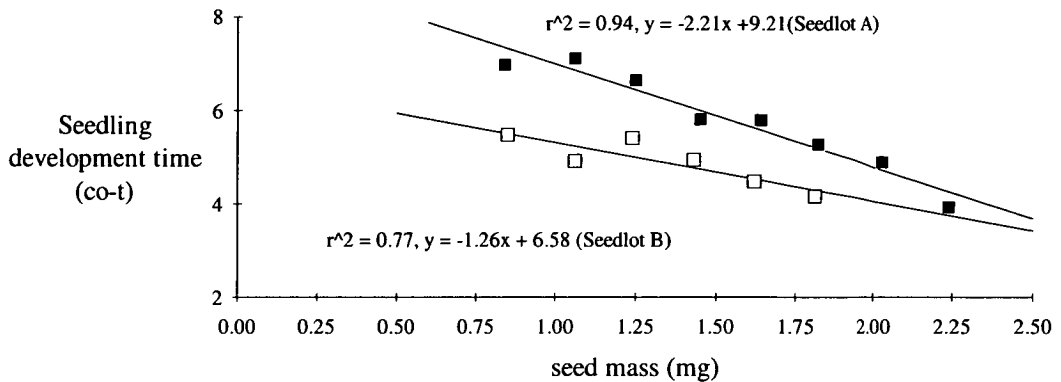
Figure 5.4 Percentage of germinated seed achieving cotyledons open



Note (1) Differences between treatments were not statistically different.

Seedling development data (Figure 5.5) showed a decrease in mean seedling development time with seed mass within seed lots. The decrease was more pronounced in seed lot A than B.

Figure 5.5 Seed mass vs time interval from complete germination to cotyledons open (co-t)

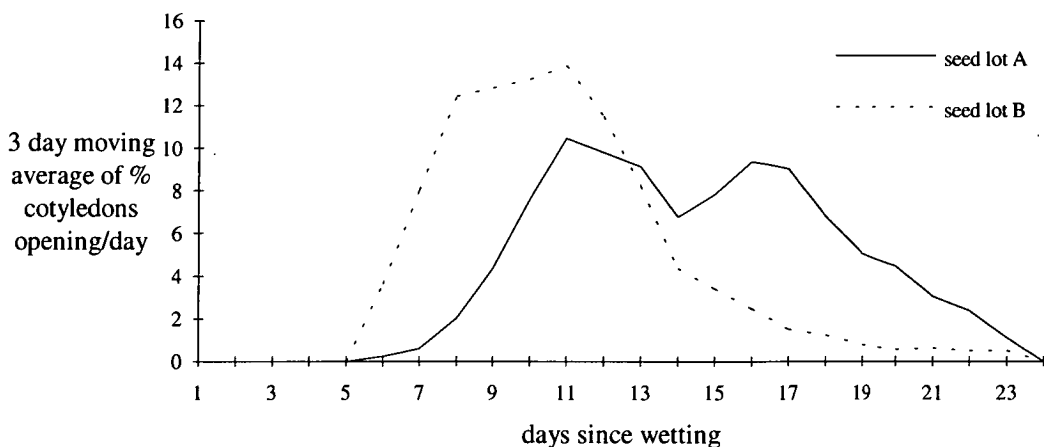


Note (1) The slopes of the regression lines for the two seed lots are significantly different ($P = 0.045$). (2) The decrease in development time with seed mass is significant for each seed lot separately ($P < 0.0001$). (3) The y intercepts for the two seed lots are significantly different ($P = 0.0013$). Therefore increases in seedling development with seed mass are greater in seed lot A.

In both seed lots, decrease in mean seedling development time may have been due to a faster development expressed in increasingly heavier individuals, or due to a shift in proportion of fast and slow developing seedlings with seed mass. Figures 5.6 to 5.10 investigate these possibilities.

The mean number of cotyledons opening per day expressed as weighted means for seed lots A and B are shown in Figure 5.6. The data indicate that cotyledons opened later in seed lot A than seed lot B. Of more significance is that seed lot A demonstrated a pronounced bimodal distribution, a trend not evident in seed lot B.

Figure 5.6 Cotyledons opening per day for two seed lots



Seedling development time for different seed mass classes of seed lots A and B appear in Figures 5.7 and 5.8 respectively. Figure 5.7 demonstrates that in seed lot A, bimodality is present among the profiles of all mass classes. Conversely, bimodality is not as apparent in seed lot B (Figure 5.8).

Figure 5.7 Cotyledons opening per day for mass classes of seed lot A

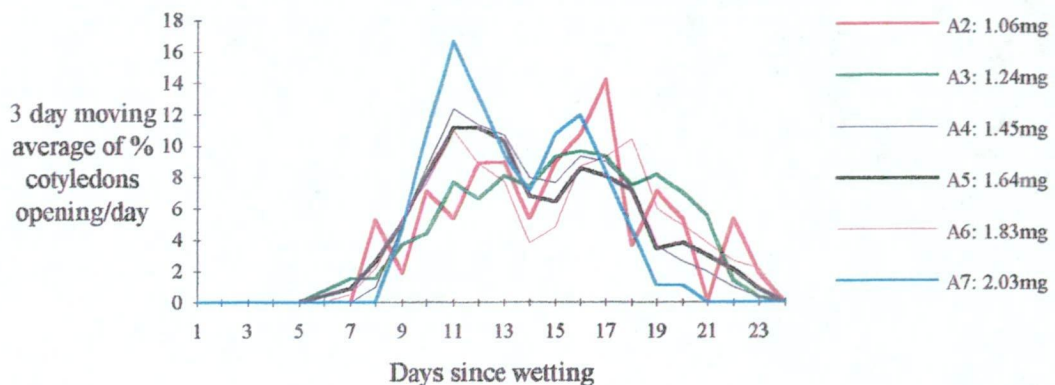
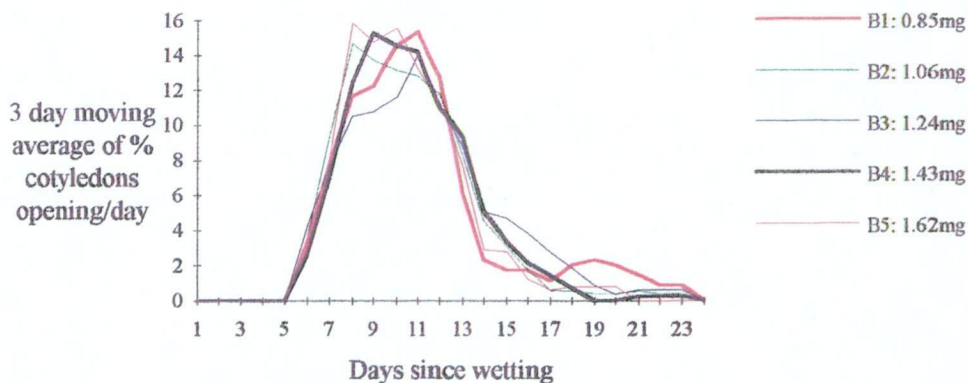


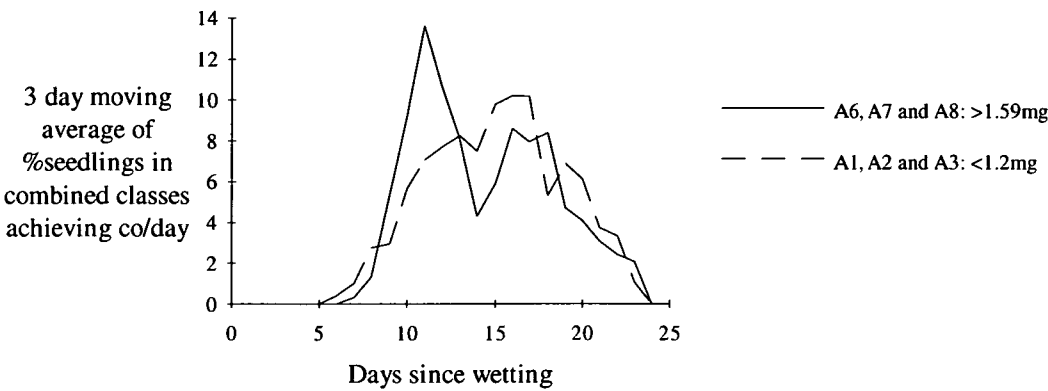
Figure 5.8 Cotyledons opening per day for mass classes of seed lot B



As seed lot A showed the greatest change in duration of seedling development with increased seed mass, and distinct bimodality in duration of seedling development, change in proportion of fast and slow developing seedlings with seed mass class was investigated.

The three heaviest seed mass classes of seed lot A were combined to give a weighted mean profile, and this is compared with the combined three lightest seed mass classes of the seed lot (Figure 5.9). In the combined heavy seed mass class, the earliest or first peak is much higher than the second peak. In the light seed mass class, the opposite trend is observed. With increasing seed mass class, the proportion of fast and slow developing seedlings changes, rather than all the seed in the population developing more rapidly.

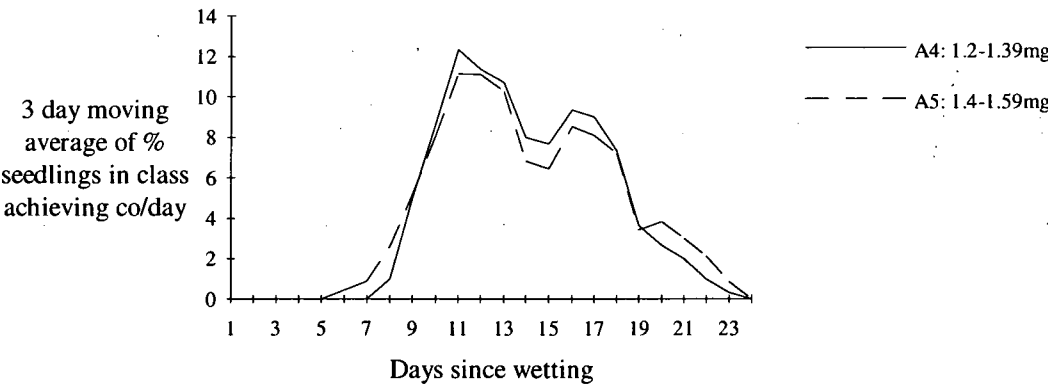
Figure 5.9 Cotyledons opening per day for high and low seed mass classes of seed lot A



Note (1) Combined class values are weighted averages of the three highest and the three lowest mass classes of the seed lot

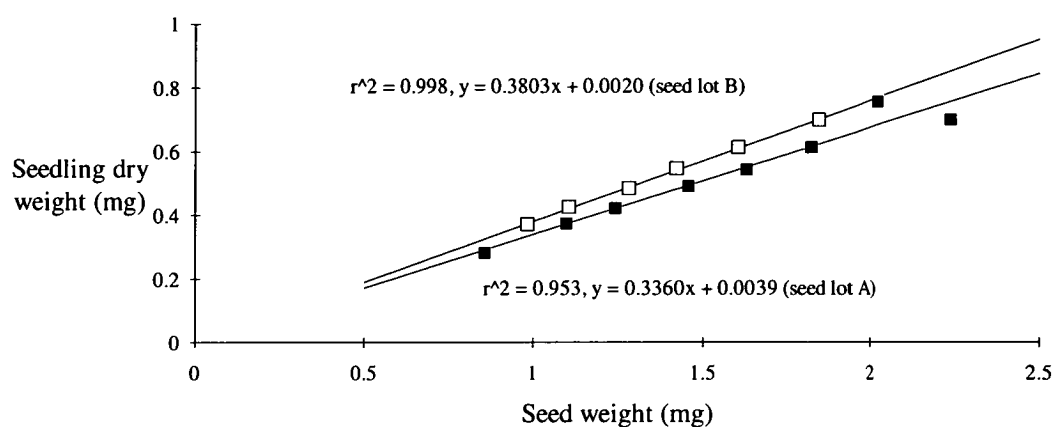
The cotyledon opening profiles of the two medium seed mass classes are presented in Figure 5.10. These two seed mass classes show similar profiles and display slightly more fast than slow developing seedlings.

Figure 5.10 Cotyledons opening per day for two medium seed mass classes of seed lot A



The above investigations described duration of seedling development. The following results presented in Figures 5.11, 5.12 and 5.13. describe changes in seedling and spent achene mass with increasing seed mass. A linear relationship was observed between seedling mass and whole seed mass, with seedling mass increasing with seed mass (Figure 5.11). Slope coefficients for each seed lot are not significantly different indicating that this proportion varied in a consistent way with seed mass between seed lots. Although this is so, the seedling as proportion of whole seed mass was greater in seed lot B than in seed lot A.

Figure 5.11 Seedling dry weight at cotyledons open vs seed weight



Note (1) The slopes of the regression lines for the two seed lots are not significantly different. (2) The increase in seedling dry mass with seed mass is significant for each seed lot separately ($P < 0.0001$). (3) The y intercepts for the two seed lots are significantly different ($P = 0.0033$).

The dry mass of empty achene versus seed mass for both seed lots is shown in Figure 5.12. Differences between empty achene means are highly statistically significant and treatment standard errors are generally less than two percent of the mean value. The figure demonstrates that spent achene mass increases at a constant rate with whole seed mass. The proportion of whole seed mass that can be accounted for by the spent achene is constant within seed lots but differs between seed lots.

Figure 5.12 Empty achene dry weight vs seed weight

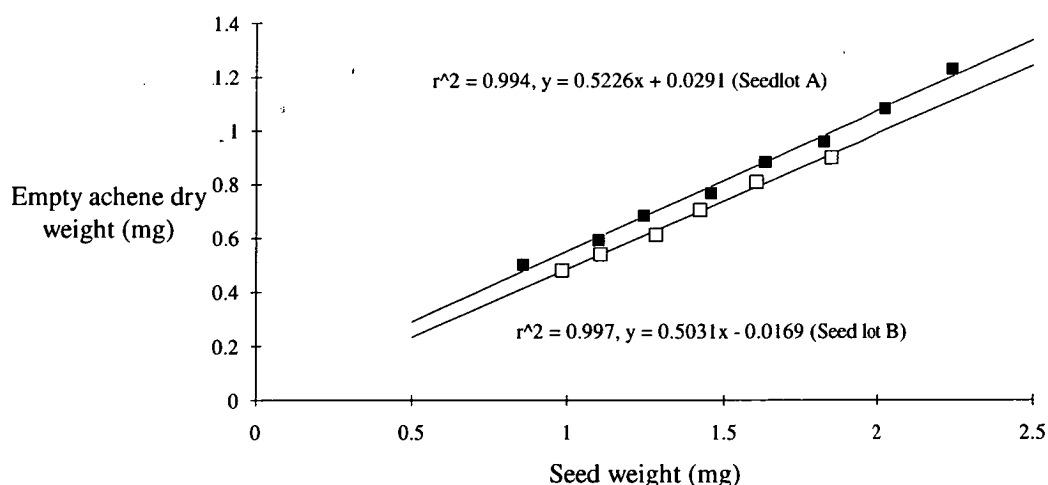
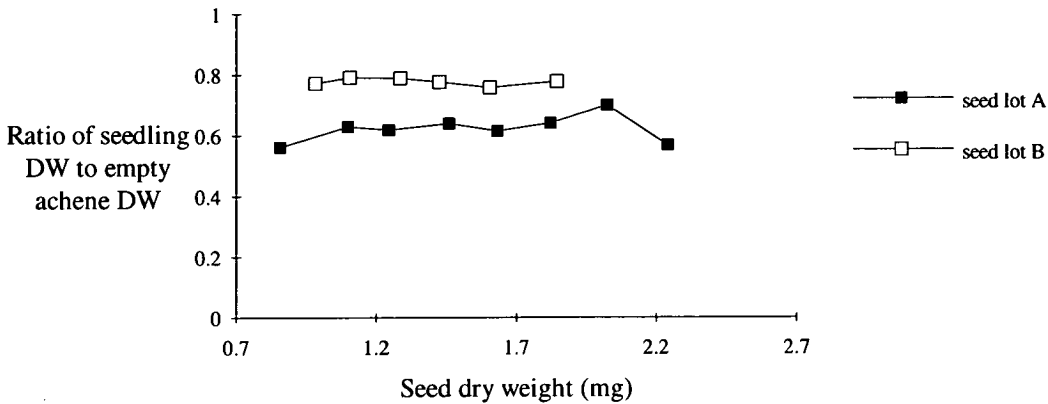


Figure 5.13 shows that the ratio of seedling DW to achene DW remains constant with respect to seed mass class but differs between seed lots A and B. Differences between

seed lot treatment means are again highly statistically significant and treatment standard errors are generally less than five percent of mean treatment values.

Figure 5.13 The ratio of seedling dry weight to empty achene dry weight



5.4.2 Trial 2

Figure 5.14 demonstrates that earlier germinating cohorts of seeds were from marginally heavier seed across all six seed lots. The seedling component of that total seed mass was found to decrease substantially (Figure 5.15). The empty achene component of the total seed mass did not change substantially with later germination (Figure 5.16). Therefore, a reduction in seedling mass was largely responsible for the lesser total seed mass associated with lateness of germination.

Figure 5.14 Mean seed weight vs lateness of germination in six seed lots

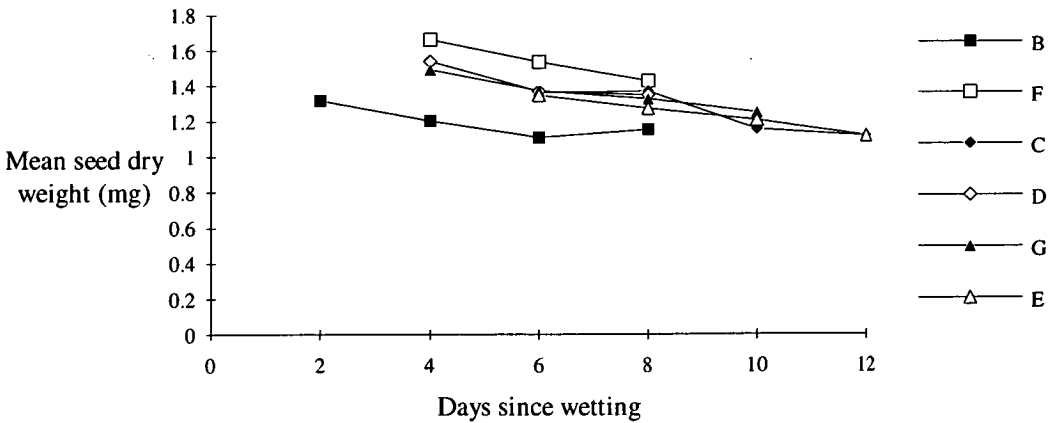
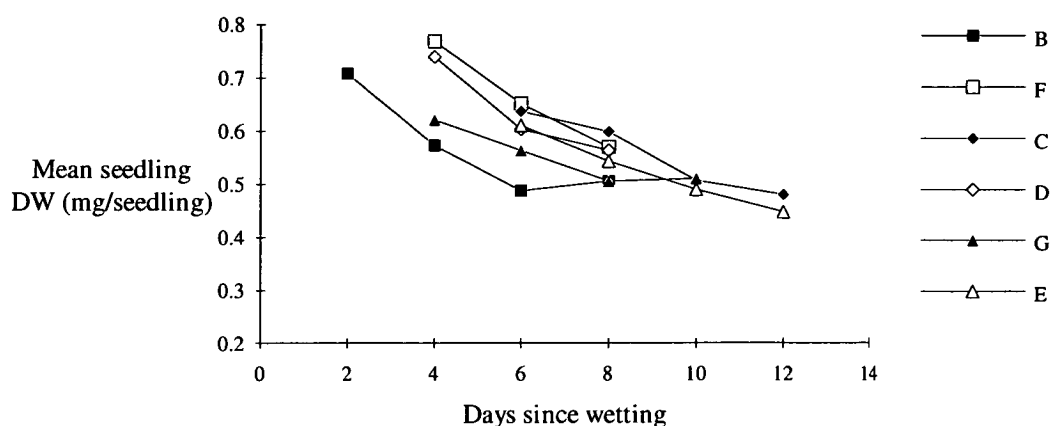
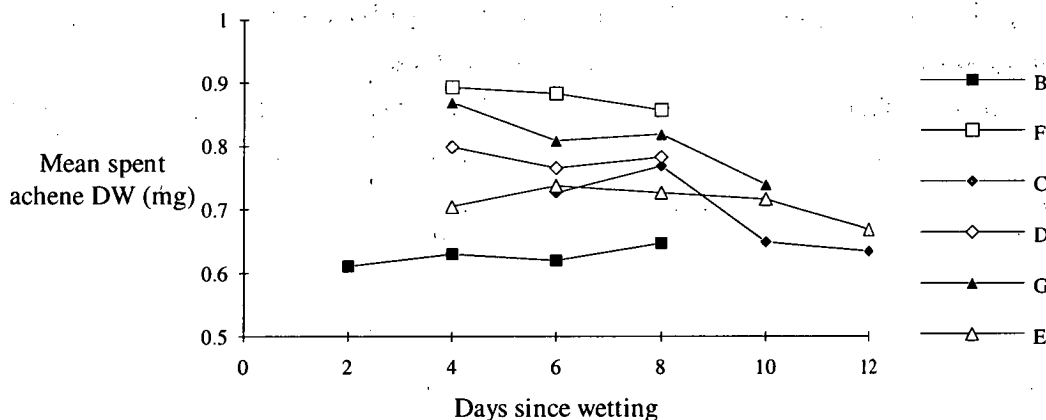


Figure 5.15 Mean seedling dry weight of seed germinating on various days after wetting



Note (1) Differences in mean seedling mass for seeds germinating on different days were significant; B, $P = 0.002$, $LSD = 0.097$ mg; F, $P = 0.0002$, $LSD = 0.047$ mg; C, $P = 0.001$, $LSD = 0.047$ mg; D, $P = 0.0025$, $LSD = 0.073$ mg; G, $P = 0.0087$, $LSD = 0.064$ mg; E, $P = 0.0004$, $LSD = 0.054$ mg.

Figure 5.16 Mean spent achene DW for seed of various seed lots vs day of germination



Note (1) Differences in spent achene DW not significantly different at $P = 0.05$ in seed lots; F, D and E. and G. (2) Differences were significant, $P = 0.01$, $LSD = 0.056$ mg, in seed lot C.

It may be argued that the decrease in mean seedling mass with lateness of germination was due to those later seeds having to respire for a longer period of time and therefore lose dry mass during germination. Table 5.3 investigates this possibility by comparing mean seed dry mass prior to germination with the sum of seedling and spent achene data collected after germination. If respiratory losses were responsible for the observed reduction in seedling mass, post germination seed mass (spent achene plus seedling) would be significantly less than the original mass of the seed.

Table 5.3 Comparison of mean dry mass per seed before and after seedling emergence

Seed lot	Before germination mean DW/seed (mg) (100°C, 24 hours)	After germination mean DW/(seedling plus spent achene) (mg) (70°C, 24 hours)	Percentage change in mean DW after germination (%)	Hollow achenes in ungerminated seed lots (% of total achenes)
F	1.420	1.538	+8.3	0.5 +/- 0.6
D	1.363	1.404	+3.06	0.75 +/- 0.9
E	1.199	1.247	+4.02	1.0 +/- 1.2
C	1.162	1.260	+8.46	2.8 +/- 1.7
G	1.148	1.358	+18.32	26.3 +/- 4.6
B	1.146	1.194	+4.21	0

Note (1) The observed increase of between 3 and 8 % in mean seed dry mass is presumed to be an artefact due to more moisture remaining in the samples dried at 70°C than at 100°C and ungerminated seed not contributing to after germination mean DW value. (2) The increase of 18.32% in seed lot G can be explained by the high proportion of hollow seed in that seed lot.

The percentage change in mean seed mass data in Table 5.3 indicate that no significant losses in dry mass occurred due to respiration during germination. Differences in seedling mass were present prior to initiation of germination and could not be attributed to respiratory losses.

5.5 Discussion

5.5.1 Variation in seed mass within seed lots

Variability in seed mass in seed lot A was greater than that found in seed lot B. Since variation in seed mass existed both within and between seed lots, it is reasonable to suggest that this may be a factor contributing to differences in germination rate and uniformity characteristics. Microscopic investigation revealed no great differences between seeds in surface architecture, shape, or seed colour within seed lots (Plates 5.1 and 5.2).

5.5.2 Germination capacity

Only the lightest seed mass classes in one seed lot (seed lot A) displayed decreased germination capacity. Gross (1984), while investigating the effects of seed size on seedling establishment, found that of six species of monocarpic perennials examined, only two (*Verbascum thapsus* and *Daucus carota*) showed significantly lower germination percentages in lower mass classes. Within Asteraceae, no consistent trend is observed, with examples where seed size has an influence on germination capacity, e.g., *Taraxacum hamatifforme* (Mogie *et al.*, 1990), and *Aster acuminatus* (Pitelka *et al.*, 1983), and where seed size had no effect on germination capacity, e.g., *Tragopogon dubius* (Maxwell *et al.*, 1994). The fact that seed lots used in the current trial had been

cleaned and density graded differentiates this study from previous research, but since low density fractions were found to mainly comprise of hollow achenes, the seed lots tested were considered as near complete samples of the total viable seed.

Investigation of ungerminated seed at the end of the experiment involved observation of achene external appearance and achene dissection. Low numbers of seed failing to germinate prevented legitimate statistical analysis of that seed. Nearly all the ungerminated seed was found to contain an incomplete or small embryo or if the achene appeared very narrow at the non calyx end, no embryo was found. The presence of small and incompletely formed embryos indicated that demands for seed development were seemingly not fully met. Likely reasons for incomplete seed development include early seed harvest and intra-plant competition.

Germination capacity differences between seed mass classes, although statistically significant, were small and classes affected only represented a small percentage of the seed lot. Consequently seed mass can be considered as having a small but significant effect on seed lot germination capacity in seed lot A, and no effect in seed lot B. Although the differences were minor, a significant body of evidence indicates that even small reductions in germination capacity can manifest themselves as large differences in seed vigour (TeKrony and Egli 1991; Fenner 1992).

5.5.3 Time to complete germination

Investigation of *t* revealed a difference of nearly three days between seed lots A and B. Seed mass did not appear to be associated with time to complete germination. Similarly, seed mass was not found to influence *t* between mass classes within seed lots. These are significant results since they reveal that seed mass failed to explain variability in seed lot *t*. Likewise, uniformity of germination was significantly higher in seed lot B, but no differences in uniformity were apparent within seed lots between seed mass classes.

Further evidence from the second trial supports the finding that seed mass had little influence on time taken to germinate. Only minor, but consistent reductions in whole seed mass were apparent. While no consistent reductions in spent achene mass were evident, substantial reductions in seedling dry masses of approximately 0.2 mg (30% of seedling DW) were identified between early and late germinants in each seed lot.

Although seed mass had little or no impact on time to complete germination, seedling mass did. The lack of influence of seed mass on germination time in both trials may have been due to the mass of the integument or achene masking the influence of the seedling.

Seeds containing lighter seedlings germinated later than seeds containing heavier seedlings.

There are many species of Asteraceae in which germination time was found to be associated with seed size; *Heterotheca subaxillaris* (Awang and Monaco, 1978), *Senecio jacobaea* L. (McEvoy, 1984), and *Taraxacum hamatiforme* (Mogie *et al.*, 1990). Conversely, other Asteraceae species also possessing a range of seed sizes displayed no variability in germination time with respect to this trait, e.g. *Tragopogon dubius* (Maxwell *et al.*, 1994).

5.5.4 Seedling development characteristics

Of the seed that did germinate, most reached 'cotyledons open' regardless of seed lot or mass class. Seedling development was found to be faster in seed lot B than in A. The fastest and most uniformly germinating seed lot (B), therefore, displayed another beneficial characteristic further differentiating it from seed lot A. Differences in time required for seedling development were also identified between seed mass classes within seed lots, with seedling development being faster for heavier seeds. Variability in speed of development was dependent on both seed lot and seed size (Figure 5.5).

Faster seedling development was explained by a shift in the proportion of distinctly different fast and slow developing seedlings in heavier seed mass classes. Graphical presentation of cotyledons opening per day revealed a bimodal distribution in seed lot A. It appeared that a higher proportion of slow developing seedlings existed in lighter than heavier seed mass classes. Furthermore, a similar, but far less pronounced trend appeared in seed lot B.

Overall the data indicate that in the least uniform seed lot there are two distinct seed development behaviours. Two types may be present in seed lot B also but in that seed lot behaviour is more homogeneous. Separating seed on a mass basis was only partly successful in separating seed with differing rates of seedling development.

5.5.5 Seedling contribution to whole seed mass

Both seedling mass and spent achene mass within seed lots retained the same proportional contribution to overall seed mass, regardless of seed mass class. However, seed lot B seedlings accounted for a greater proportion of whole seed mass than in the other seed lot. The ratio of seedling to empty achene mass was, therefore, constant between mass classes, but varied significantly between seed lots. The most rapid and

uniform germinating seed lot displayed a consistently higher ratio than did the other seed lot.

5.5.6 Summary

This work identified variation in seed mass within and between seed lots. Seed mass had little influence on germination capacity and did not account for differences in germination rate within, or between seed lots. However, larger seed produced larger seedlings. Faster seedling development observed in higher seed mass classes was explained by a shift in proportion of fast and slow developing seedlings. Finally, seed with larger embryos were found to develop more quickly than those with smaller embryos.

Further cleaning on a mass or possibly size basis would be beneficial in improving seed lot quality. An important advantage in performing further cleaning would be greater mass seedlings and possibly faster seedling development.



Plate 5.1 (top) Seed from seed lot A, displaying eight seed mass classes from 0.8-0.99 mg (far left) to >2.2 mg (far right) (10 × magnification)

Plate 5.2 (bottom) Seed from seed lot B, displaying seven seed mass classes from 0.8-0.99 mg (far left) to 2.0-2.19 mg (far right) (10 × magnification)

Chapter 6: The effect of harvest date on seed yield and germination

6.1 Introduction

In earlier work which investigated the germination characteristics of six seed lots, a hypothesis was proposed that much of the variability between seed lots might be explained by differences in seed maturity. The current study investigates the germination characteristics of seed harvested at weekly intervals during later stages of seed development.

Identifying the optimum time for harvest of pyrethrum seed is important for two main reasons. Firstly, if harvested too late, seed losses are likely to occur. Secondly, seed quality may be reduced if seed is harvested too late or too early. Harvested early, seed may not have reached its maximum weight and may not yet have developed quality germination characteristics. However, harvesting late may be associated with premature seed deterioration or may induce the seed dormancy. Complicating the determination of optimum harvest time for pyrethrum seed is the wide range of flower maturities that exist on the plant, resulting in seed being immature and other being lost or overmature, even at most appropriate harvest times.

This study aimed to identify factors which limit seed quality at various harvest times. It investigated changes in germination parameters from 'prior to' to 'past' the time at which seed had been harvested commercially.

The trial was not intended to investigate various stages of seed development such as desiccation tolerance or physiological maturity which are introduced in the literature review, but some of these stages were demonstrated. Another aim of the work was to provide information on seed losses from capitula with lateness of harvest.

6.2 Literature review: The influence of seed maturity on germination

The following literature overviews changes in germination characteristics with development from shortly after fertilisation to harvest or dehiscence of dry seed. Key stages of development such as acquisition of germinative capacity, desiccation tolerance and physiological maturity are introduced. Inception of dormancy, or the development of an afterripening requirement, is then discussed. This section includes some definitions for dormancy and discussion of inadequacies in current dormancy terminology. A classification system to describe various patterns of release from dormancy is explained

and factors influencing the level of dormancy in seed are introduced. Finally, release from dormancy through storage, chilling and light exposure is discussed.

6.2.1 Growth and development of seed after fertilisation

After fertilisation seed initially undergoes histodifferentiation leading to the formation of the embryonic axis and tissues for the accumulation of reserve materials (Kermode *et al.*, 1989). This is followed by a period of growth and development during which the seed acts as a significant sink for maternal assimilates. The seed is initially watery but is gradually loaded with reserves and water is increasingly displaced. As the seed increases in dry weight, it attains the capacity to germinate. A range of seed and germination quality parameters are acquired during this time. The change in soybean seed quality during development was investigated (Miles, 1985 in Dornbos, 1995) and it was found that as the dry weight increased, seeds firstly obtained the capacity to germinate, then they gained the capacity to produce normal seedlings before finally acquiring seed vigour characteristics. No information was found on the germination capacity during the period shortly after fertilisation in Asteraceae species.

6.2.2 Capacity to withstand dessication

At some stage during the accumulation of reserves, seed acquires the capacity to germinate, but not the capacity to be dried and maintain vitality. Further seed development is required if the seed is to survive dessication. If seed has developed enough to withstand drying it is said to have achieved dessication tolerance. Dessication tolerance is an important marker indicating stage of seed development.

Seeds generally have the capacity to germinate early in their development but are prevented from doing so. Prevention of precocious germination of immature seed is suggested to be due to high levels of ABA and osmotic constraints (Bewley and Black, 1994). Dessication tolerance is associated with gradual morphological and physiological changes with time. In some species the production of sucrose, certain oligosaccharides and late embryogenesis abundant (LEA) proteins are thought to have the protective function of improving the stability of membranes during drying, thereby increasing the viability of the resulting seed. Dessication tolerance has recently been extensively reviewed (Kermode, 1997).

6.2.3 Physiological maturity

Physiological maturity is described as the point at which maximum seed quality is acquired. As the seed grows and develops it is provided with assimilates via the fundiculus. Degradation of the fundiculus coincides with the seed achieving its maximum

dry weight. Time of detachment is also marked by maximum seed quality and maximum storage potential in soybeans, *Glycine max* (Dornbos, 1995) and a range of other cereal, vegetable and industrial crops harvested as dry seeds (TeKrony and Egli, 1997). This stage of development is defined as physiological maturity (PM). As the seed approaches PM, moisture is replaced by insoluble reserves. PM is generally achieved at around 35% moisture but this varies considerably between species.

Seeds harvested prior to PM are quite capable of germination but are thought to have compromised vigour and storability. It is generally accepted that harvest of seed should be conducted as soon as possible after PM (TeKrony and Egli, 1997). Stress such as drought is thought to have a much greater influence on seed quality if it occurs prior to rather than after PM. Dornbos (1995) suggested that there are at least two mechanisms by which environmental stress can prevent high seed quality from being attained by the time of PM. Firstly there may be an inadequacy in the supply of assimilates to the developing seed, and secondly there may be impaired physiological apparatus needed for the germination process.

6.2.4 Afterripening

During the growth of the seed and possibly well past PM, when the level of metabolism in the seed is reducing with dehydration, seed sometimes develops a resistance to germinate. Such seed is described as requiring a period of afterripening.

If the seed is dried, to less than 20% moisture, then placed into an environment conducive to germination, this sometimes has the effect of allowing germination to proceed, while non-dried seed may remain ungerminated for an extended period (Esashi *et al.*, 1993). Although dessication permits germination in some species, others still do not germinate. Seeds that require some period of time in a seemingly quiescent state before they will germinate are said to have dormancy; more precisely this is known as primary dormancy since it is imposed by the seed itself. In such seed a period of afterripening or some dormancy breaking treatment is required before germination can occur.

At this point it is important to define what is meant by dormancy and afterripening. Dormancy is defined by Copeland and McDonald (1995) as "a physical or physiological condition of viable seed that prevents germination even in the presence of otherwise favourable germination conditions" and afterripening as "the collective changes that occur in a dormant seed that make it capable of germination. It is usually considered to denote physiological changes". In the Penguin Dictionary of Biology, Abercrombie *et al.*

(1979) contribute further stating a requirement for afterripening is "...possibly associated with delay in production of required growth promoting hormones or with gradual breakdown of growth inhibitors".

Dormancy as defined only involves capacity to germinate, which is usually described in terms of germination percentage. Although germination capacity is of intrinsic importance, other behaviours may also be influenced by the same mechanism governing germination capacity. The window of capacity to germinate at supra- or sub-optimal temperatures may be significantly influenced by level or stage of dormancy (Vegis, 1964). So seed that germinates at an optimal temperature but fails to do so at temperatures above or below this, cannot be described as being dormant.

The term 'relative dormancy' (Bewley and Black, 1994) deals with germination capacity at non-ideal temperatures and thereby overcomes this inadequacy in nomenclature. Rate of germination at optimum, high, or low temperatures may similarly be influenced by mechanisms governing dormancy (Favier, 1995). Temperature range for both germination capacity and rate of germination may be significantly influenced by relative dormancy. The resultant effect on success of crop establishment may be significant. Dormancy, defined simply in terms of germination capacity, does not include these other important germination parameters, making description of such phenomena difficult. Some researchers, such as Dekker *et al.* (1996), choose to avoid the term completely.

Despite inadequacies in dormancy terminology, many workers have been successful in providing descriptions of dormancy-related changes in germination capacity of seed at different temperatures, along with other important germination parameters such as rate and uniformity. Recent examples of such works include; germination rate during dormancy loss in barley, *Hordeum vulgare* (Favier, 1995), germination rate and uniformity in *Bromus tectorum* L. (Allen *et al.*, 1995), and germination percentage under various temperature regimes in *Asteraceae* spp. (Baskin *et al.*, 1992b).

6.2.5 Factors influencing requirement for afterripening

Significant variation in afterripening requirement exists between species. Some species may germinate immediately after dehiscence. An example is the continuously flowering, desert living, perennial shrub, *Launaea aborescens* (Asteraceae) which is reported to have no dormancy mechanisms (Scheutz and Milberg, 1997). Other species remain dormant for up to 60 months, for example dock, *Rumex crispus* (Bewley and Black, 1994). The type and level of dormancy is species specific.

Inception of dormancy occurs at various stages of development in different species. Seed harvest time influences level of dormancy in bell peppers, *Capsicum annum* L (Sanchez *et al.*, 1993) and *Arabidopsis thaliana* (Derkx and Karssen, 1993). Dormancy may be detectable early in seed development, for example in some strains of oats, *Avena fatua*, or the onset of dormancy may become significant at PM and be associated more with the drying process. Bewley and Black (1994) cited two examples, *Sida spinosa* (unreferenced) and *Medicago lupulina* (Sindu and Cavers, 1977) where onset of dormancy occurred late in seed development or during seed drying. They comment that in species where inception of dormancy occurs during drying, changes in the seed coat may be responsible for initiating the dormant state.

The environment in which the seed develops has demonstrated impact on the level of dormancy. Examples include; photoperiodic effects in fat hen, *Chenopodium album* (Karssen, 1970), temperature on lettuce, *Lactuca sativa* (Grey *et al.*, 1988), light quality in a range of species, (Cresswell and Grime, 1981), and season on *Arabidopsis thaliana* (Cone and Spruit, 1983).

6.2.6 Typology for pattern of release from dormancy

Dormancy may limit capacity to germinate in a wide range of environmental situations. It may prevent germination completely for a period of time, prevent germination at high or low temperatures, under long or short days and under different light quality regimes. Capacity to germinate may fluctuate with the season (Baskin and Baskin, 1996; Baskin *et al.*, 1995) or the surrounding chemical environment (Derkx and Karssen, 1993; Strydom *et al.*, 1996). Often release from dormancy occurs in an orderly way with an increasing proportion of seed germinating under non-optimal conditions.

Vegis (1964) cited three types of response patterns describing release from dormancy:

- i. The maximum temperature at which seeds germinate increases during afterripening;
- ii. The minimum temperature decreases; and
- iii. Both the maximum increases and the minimum decreases.

The afterripening patterns of a range of Asteraceae possessing different life cycles were described in terms of the above typology and these are presented in Table 6.1.

Baskin *et al.* (1992a) suggested this information provides insight into the evolutionary histories of plant families. The data also provide some evidence to suggest that any seed

dormancy in pyrethrum, being a polycarpic perennial, may be expected to exhibit type two or three release.

Table 6.1 Type of afterripening pattern found in achenes of 32 species of Asteraceae with differing life cycles

Life cycle	No. of species	Type		
		i	ii	iii
Polycarpic perennials	20	0	15	5
Monocarpic perennials	2	0	2	0
Summer annuals	4	0	4	0
Winter annuals	6	3	1	2

Source: adapted from Baskin *et al.*, (1992a)

6.2.7 Release from dormancy

Breaking dormancy in seed has been and remains a popular area of investigation. As a result, a large body of information on this topic exists and is well documented in seed physiology texts. Much of the research also provides insight into mechanisms involved in dormancy release. Bewley and Black (1994) suggested that mechanisms involved in loss of dormancy are poorly understood but the efficacy of dormancy loss is dependent on a range of environmental conditions. The ensuing discussion aims to highlight three environmental factors associated with release from dormancy. After commenting on the influence of species, the effects of time, stratification and light on release from dormancy are discussed.

Level or depth of dormancy varies significantly between species. This same type of variation exists in the rate of dormancy loss which is also strongly species dependent. Within species there may also be large differences in level of dormancy due to cultivar and growing environment differences. Severity of treatment required to release different seed lots therefore may also vary significantly (for example, *Arabidopsis thaliana*, Derkx and Karssen, 1993).

Period of storage

Although time is used as the index of change in dormancy with storage, rate of dormancy loss is determined by temperature, seed moisture and oxygen levels during the storage period (Bewley and Black, 1994). Higher storage temperatures increase the rate of dormancy loss in wheat, barley and rice (Bewley and Black, 1994). Furthermore, Bewley and Black (1994) suggested that in low oxygen concentrations, and at seed moistures below 5%, rate of dormancy loss may decline. Recent articles on dry storage dormancy

release in *Asteraceae* include seed moisture in *Solidago* (Walck *et al.*, 1997b) and general dry storage in *Xanthium* (Esashi *et al.*, 1993).

Stratification

Subjecting rehydrated seed to low temperatures in order to overcome dormancy is known as chilling or more commonly as stratification. This technique is commonly applied to seed in forestry, ecological studies and horticultural crops for overcoming or investigating dormancy. Recent examples of studies investigating the various aspects of stratification include; efficacy in silver birch, *Betula pendula* (Vanhatalo *et al.*, 1996), mechanism of dormancy breaking in Douglas fir, *Pseudotsuga menziesii* (Jarvis *et al.*, 1997), relieving relative dormancy in sitka spruce, *Picea sitchensis* (Jones *et al.*, 1997), variation in response to chilling between habitats in blue flax, *Linum perenne* (Meyer and Kitchen, 1994), and chilling increasing the rate of germination in meadowfoam, *Limnanthes spp.* (Jolliff *et al.*, 1994).

The effects of stratification on germination characteristics of a range of *Asteraceae* species have recently been investigated. Bratcher *et al.* (1993) investigated the response to stratification of five wildflower species, namely: wild blue indigo, *Baptisia australis*, (Leguminosae); purple cone flower, *Echinacea purpurea*, (Asteraceae); maximillian sunflower, *Helianthus maximiliani* (Asteraceae); spike goldenrod, *Solidago petiolaris* (Asteraceae); and Missouri ironweed, *Vernonia missurica* (Asteraceae). Bratcher *et al.* (1993) identified that for each species, stratification at 5°C increased final germination of the seed, although the time of stratification required for maximum germination varied considerably between species. Days until first germination and range in germination times were also found to decrease with period of stratification. A more recent study on *Echinacea angustifolia* and *Echinacea purpurea* found that with increasing stratification time, there was no change in final germination, but that the rate of germination increased (Parmenter *et al.*, 1996). Baskin *et al.* (1992a) investigated the afterripening pattern of an *Echinacea angustifolia* variety and showed that minimum germination temperatures declined with release from dormancy. Baskin *et al.* (1992a) suggested that the high germination capacity, even prior to complete afterripening, contrasted *Echinacea angustifolia* to all other previously examined *Asteraceae* species displaying the same dormancy release pattern.

Light

The importance of light on release from dormancy has been demonstrated in a wide range of species (Bewley and Black, 1994). As with stratification and dry storage there is significant variation both between and within species in response to treatment with light.

Furthermore, release from dormancy is achieved in different species with a range of light treatments varying in wavelength, intensity, length of exposure and periodicity (Bewley and Black, 1994). Activity of light is frequently found to be dependent upon temperature of germination with examples of light treatments being required to allow germination at temperatures lower and higher than optimal for germination. Recent research on the effect of light on a range of Asteraceae species has been conducted. These include; dandelion, *Taraxicum officinale* (Milberg *et al.*, 1996a), a range of pasture species (Letchamo and Gosselin, 1996), and purple coneflower, *Echinacea purpurea*, (Wartidiningsih and Geneve, 1994). Overall, no consistent theme has yet emerged which could help predict whether pyrethrum seed would respond to light.

6.2.8 Summary

This literature review reports key stages during the development of seed. Those stages are generally marked by changes in germinative capacity under different conditions and are defined either by those changes, or in the case of PM, by the attainment of maximum seed dry weight. It is evident that seed harvested too early would be non-viable and therefore fail to germinate or display poor germination parameters such as low rate of germination and storage potential. Discussion on seed dormancy suggested that germination behaviour such as slowness to germinate and capacity to germinate under less than ideal conditions are important aspects of dormancy, are mediated by the same factors as dormancy, but are not satisfactorily described by dormancy terminology.

The literature on afterripening identified that germination behaviour of seed may be influenced both by factors operating during development on the plant and in storage afterwards. The level of dormancy in seed lots generally reduces with time. Any germination research conducted on dried stored seed is therefore contextual in that results may be altered not only by imposed treatments, but also by duration and conditions of seed storage and choice of germination conditions.

It is evident from the lack of information presented on pyrethrum seed that no research has provided insight into what factors influence germination parameters in this species. Asteraceae is a diverse and large family containing a wide range of plants in which a wide range of very different factors operate that may influence germination behaviour. Furthermore, in Asteraceae afterripening requirements vary significantly both between and within species. Little data were available which could provide insight into what mechanisms may be acting to modify the germination behaviour of pyrethrum seed.

6.3 Materials and methods

A seed pyrethrum crop, established in 1994, located at Forthside Vegetable Research Station (FVRS), was used as the source of seed for the trial. Capitula were collected after natural deterioration of the attractive ray florets, starting on February 2, 1997 on a weekly basis for four weeks. On each harvest occasion (Table 6.2), all the capitula from 10 randomly assigned plants in a given row were collected and bulked. Immediately following harvest, the fresh weight of the sample was assessed. Two sub-samples were taken from the fresh capitula, the first, a 200g sample for percentage moisture analysis; the second, a 200 flower fresh weight used to determine mean fresh weight per capitulum. Capitula were then dried on a well ventilated rack at 30°C for 7 days, carefully placed in a sealed plastic bag and stored at 4°C until further seed cleaning was conducted in November 1997.

Following the storage period, samples were weighed and the percentage moisture loss in relation to the fresh weight was re-evaluated. Samples of capitula were then sieved in order to remove florets and loose petals. Mean dry weight per floret-free capitulum was assessed using a sample of over 300 capitula.

Four sub-samples of 30 capitula were randomly selected from each flower harvest date. Capitula were separated into the following classes: small achenes, large achenes and non achene floral parts. Small achenes were those that passes through a 2 mm square sieve, while large achenes remained in or on that sieve. Seeds were subsequently weighed and counted in order to obtain mean dry weight/achene per harvest date. Percentage moisture of a sub-sample of approximately 750 mg of the small achenes was assessed using the low constant oven temperature method (110°C for 24 hours).

As only very few of the achenes remained on or in the 2 mm sieve, smaller numbers of large achenes were available for germination. Large (150) and small (250) achenes were counted out for each replicate and placed in pre-prepared petri-dishes for germination. Large and small achenes trials were germinated at 20°C in separate trials. Small achenes were also germinated at 10°C. Therefore, three separate trials were conducted, each trial having four replicates and arranged in completely randomised designs. Germination was carried out as described in Chapter 4.3.2 and evaluated every second day. Evaluations included assessment of the proportion of fertile achenes, and germination parameters including percent final germination (%FG), mean time to complete germination (t), percent dead (%D), percent fresh ungerminated (%FUG) and coefficient of uniformity of germination (CUG) (Appendix 1.3).

In the trials conducted at 20°C, germinated seed was removed from the petri-dish and placed on slopes until the seedling reached cotyledons open. Seedling and spent achenes from different germination days were collected and dried at 70°C for 24 hours. This allowed both overall mean seedling and spent achene dry weight data to be collected in addition to differentiation of early mid, and late germinants within each of the treatments.

Samples of flowers taken from the field on each harvest were not replicated. Actual capitulum losses could not therefore not be determined. Nevertheless, achene losses from within capitula provided some indication of overall field losses. This assessment documents a change in seed population with lateness of harvest which may in itself be responsible for variation in germination behaviour.

6.4 Results

6.4.1 Preliminary data

Flower harvest data in Table 6.2 indicate that mean flower moisture and dry weight per flower decreased with lateness of harvest.

Table 6.2 Data collected at flower harvest

Harvest date	No. of plants sampled	Fresh weight of flowers (g)	% moisture, 200g sample (70°C, 24 hours)	DW of harvested capitula (g)	DW per capitulum (g)
03/02/97	10	1832	41.9	1063	0.351
10/02/97	10	1194	24.3	904	0.295
17/02/97	10	884	14.7	754	0.289
24/02/97	10	900	2.5	878	0.263

Table 6.3 Data relating to sampled capitula after drying at 30°C for seven days and storage for eight months

Harvest date	Moisture loss (% of sample FW) (30°C, 7 days)	% of sample DW florets	% of sample DW capitula	Mean DW /capitulum (g) (30°C, 7 days)	No. of capitula harvested
03/02/97	40.7	19.1	80.9	0.283	2962
10/02/97	22.5	17.2	82.8	0.273	2535
17/02/97	13.2	17.5	82.4	0.258	2096
24/02/97	4.9	14.0	85.9	0.255	2446

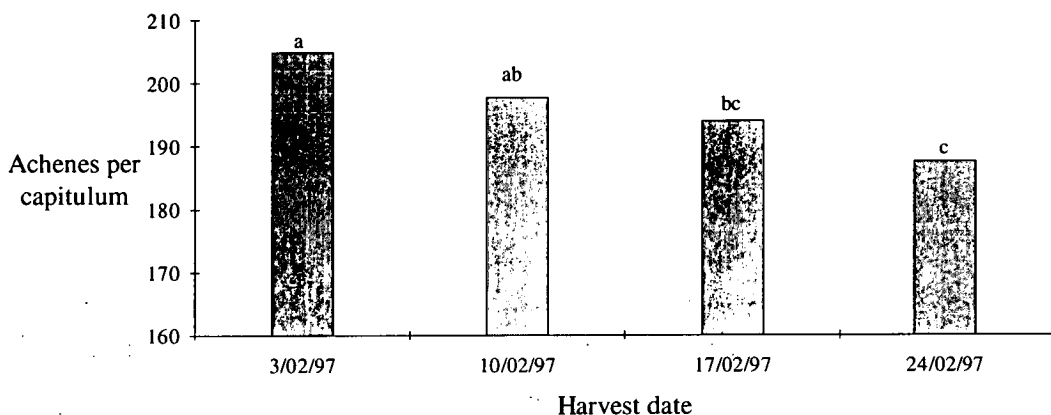
The after-storage weight assessment (Table 6.3) revealed that little change in moisture had occurred over the eight month period. The percentage of sample dry weight due to

contribution from disc and ray floret material decreased by about 5% over the period from the earliest to the latest harvest.

6.4.2 Achene and seed yield

Mean achene number per capitulum decreased with lateness of harvest (Figure 6.1). This small but significant decrease was thought to be due to achenes being lost from the capitula upon complete drying.

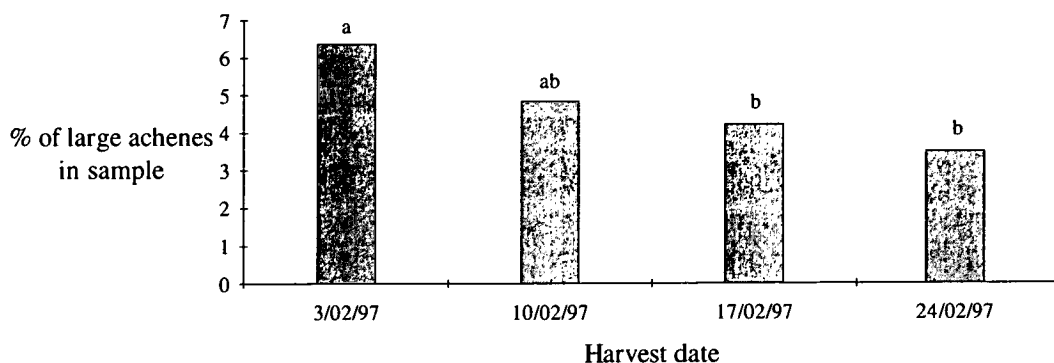
Figure 6.1 Mean achene number per capitulum vs harvest date



Note (1) Differences between some treatments are statistically significant, $P = 0.01$, $LSD (0.05) = 9.1$ achenes. Significantly different treatments ($P < 0.05$) are denoted by different letters.

The small reduction in proportion of large seed in later harvested flowers (Figure 6.2) indicates a significant change in achene population with harvest time due to achene dehiscence.

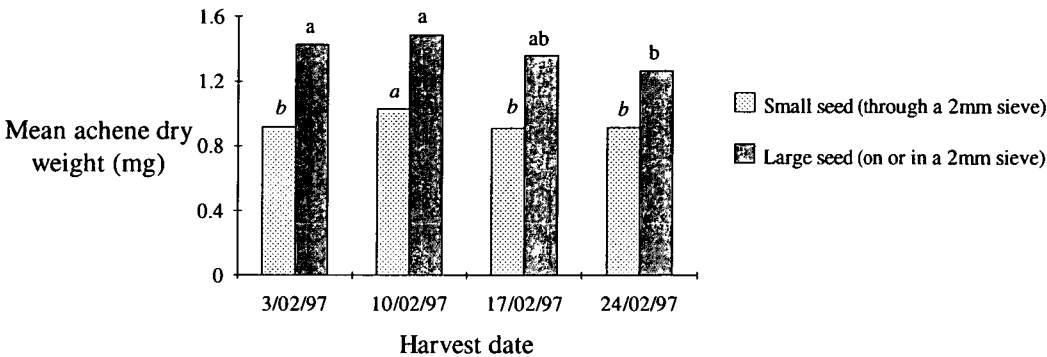
Figure 6.2 Percentage of large achenes in the sample vs harvested date



Note (1) Differences between some treatments are statistically significant, $P = 0.029$, $LSD (0.05) = 1.78\%$. Significantly different treatments ($P < 0.05$) are denoted by different letters.

Mean dry weights of achenes for different harvest times indicate that achenes from the second harvest are heavier or the same weight as those from harvest 1, and heavier than achenes from the latest harvest (Figure 6.3).

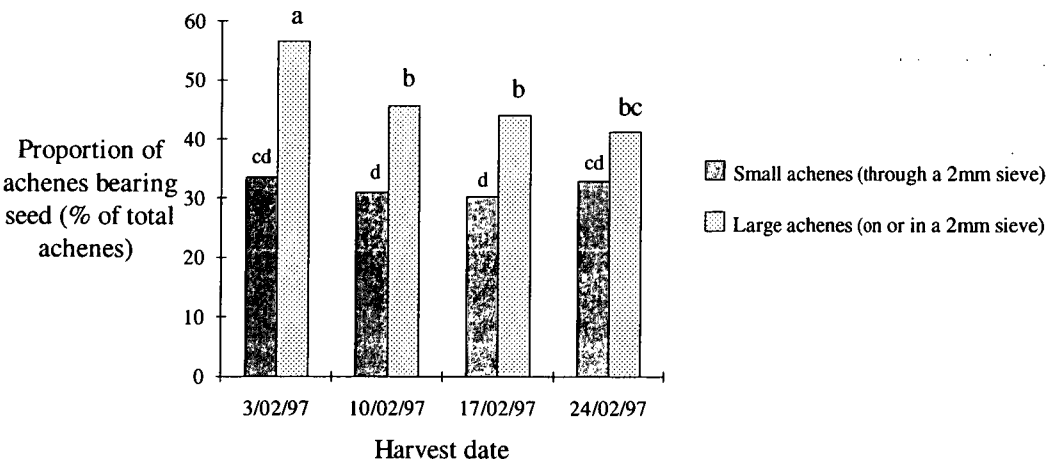
Figure 6.3 Mean achene dry weight of large and small achenes vs harvested date



Note (1) Large and small seed were analysed separately due to a greater mean variance originating from the large seed treatments. (2) Differences between some small seed treatments are statistically significant, $P = 0.003$, $LSD (0.05) = 0.06$ mg. (3) Differences between some large seed treatments are statistically significant, $P = 0.029$, $LSD (0.05) = 0.138$ mg.

Of the small achene sample (93% of the total) the proportion of hollow achenes did not change with respect to harvest date (Figure 6.4). A greater proportion of hollow achenes were found in the small, than in large achenes. In the sample of large seed, a lower proportion of hollow achenes was found in the earliest of the sequential harvests.

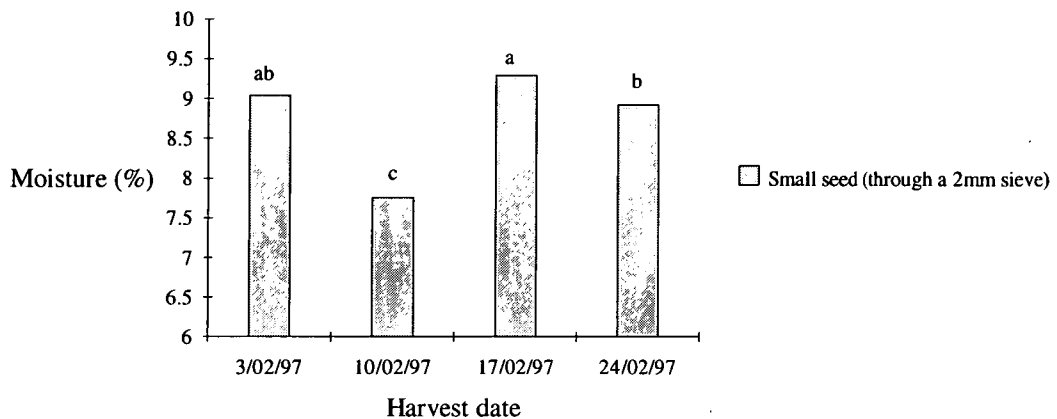
Figure 6.4 Percentage of achenes containing seed vs harvest date



Note (1) Differences between some treatments are statistically significant, $P = 0.001$, $LSD (0.05) = 9.1\%$. Significantly different treatments are denoted by different letters.

The percentage moisture of seed prior to germination studies is presented in Figure 6.5. The data indicate that although there are differences in seed moisture between seed lots, those differences are not directly related to harvest date.

Figure 6.5 Percentage moisture of achenes from different harvest times after rack drying and storage

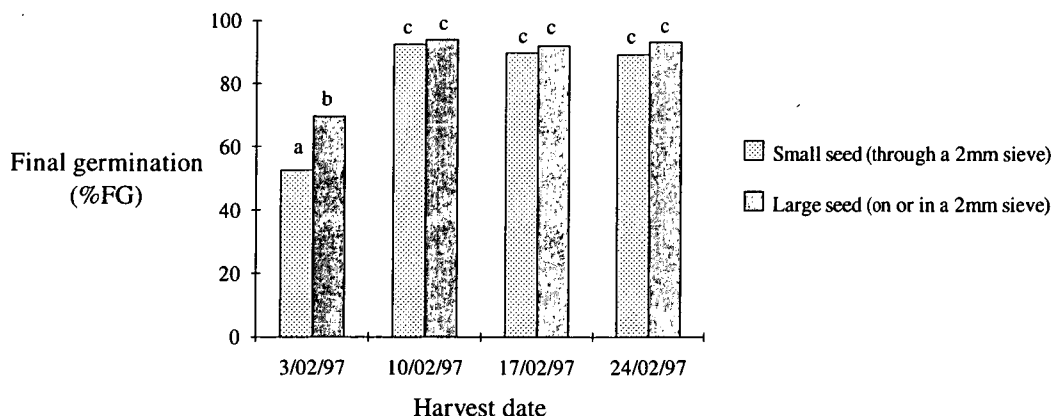


Note (1) Differences between some treatments are statistically significant, $P = 0.001$, $LSD (0.05) = 0.24\%$. Significantly different treatments ($P < 0.05$) are denoted by different letters (2) Percentage moisture was determined by drying at 110°C for 24 hours.

6.4.3 Germination characteristics

The germination capacity of seed harvested on the earliest of the four occasions was significantly lower than all later harvests in both large seed and small seed samples (Figure 6.6). Furthermore, the germination capacity of larger seed in the earliest harvest was significantly higher than for the smaller seed.

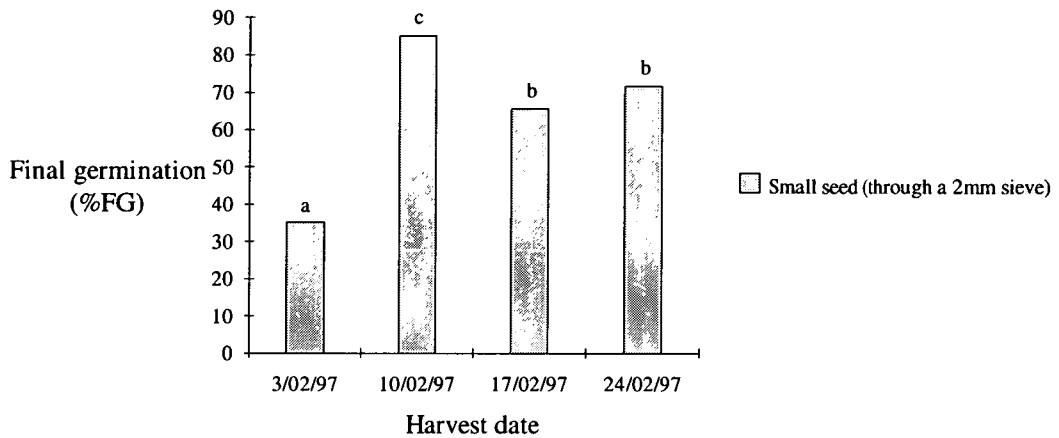
Figure 6.6 Final germination (%FG) at 20 degrees C vs harvest date



Note (1) Differences between some treatments are statistically significant, $P = 0.001$, $LSD (0.05) = 8.98\%$. (2) Significantly different treatments ($P < 0.05$) are denoted by different letters.

The germination capacity of small seed harvested on different dates was also evaluated at 10°C (Figure 6.7). As was observed at 20°C, germination at 10°C was low in the first harvest in comparison to all other harvest dates. Differing from 20°C, germination percentage at 10°C fell significantly in the last two harvests.

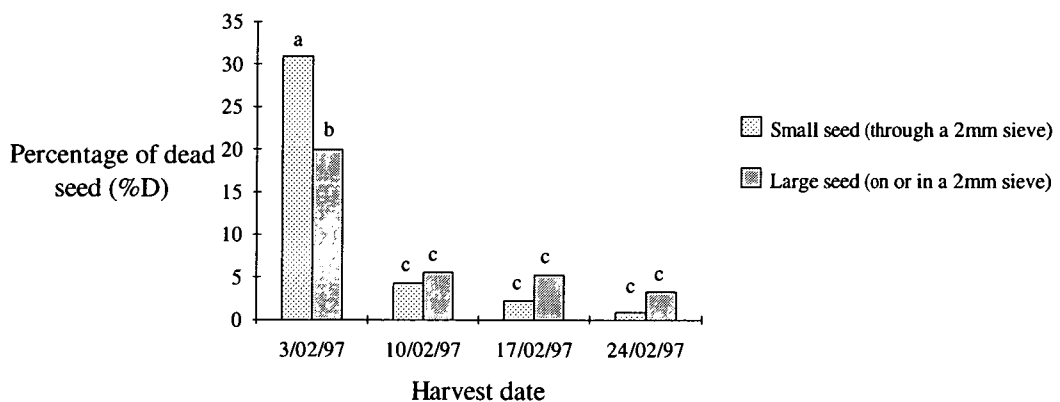
Figure 6.7 Final germination at 10 degrees C vs harvest date



Note (1) Differences between treatments are statistically significant, $P < 0.001$, $LSD (0.05) = 9.97\%$.
 (2) Significantly different treatments ($P < 0.05$) are denoted by different letters.

Of the seed that failed to germinate at 20°C, a significant proportion was found to be dead (Figure 6.8). The proportion of dead seed in the sample declined with lateness of harvest. More small than large dead seed was evident on the first harvest occasion, a trend that did not continue in subsequent harvests.

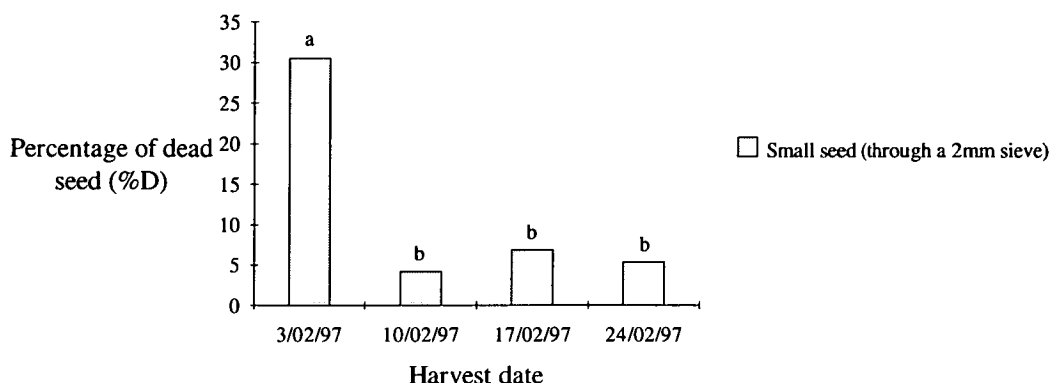
Figure 6.8 Percentage of dead seed after germination at 20 degrees C vs harvest date



Note (1) Differences between some treatments are significant, $P < 0.001$, $LSD (0.05) = 9.27\%$.
 (2) Significantly different treatments ($P < 0.05$) are denoted by different letters.

When dead seed was assessed after small seeds were germinated at 10°C, a similar pattern emerged, with a high percentage dead seed in the first harvest, reducing to a low percentage in subsequent harvests (Figure 6.9).

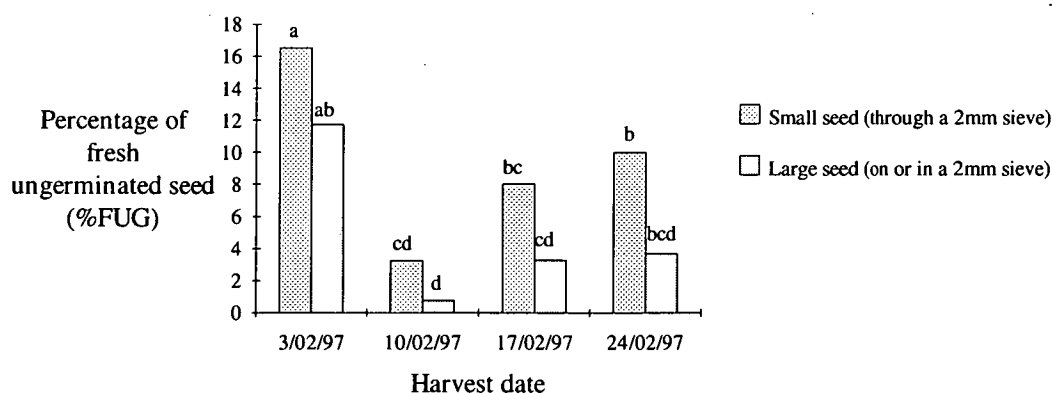
Figure 6.9 Percentage of small seed found to be dead after germination at 10 degrees C vs harvest date



Note (1) Differences between some treatments are significant, $P < 0.001$, $LSD (0.05) = 6.01\%$.
 (2) Significantly different treatments ($P < 0.05$) are denoted by different letters.

Fresh ungerminated seed (FUG) of both the large and small seed classes was found to be high in the first of the sequential harvests (Figure 6.10). There was also an increase in FUG from the second to the fourth harvest.

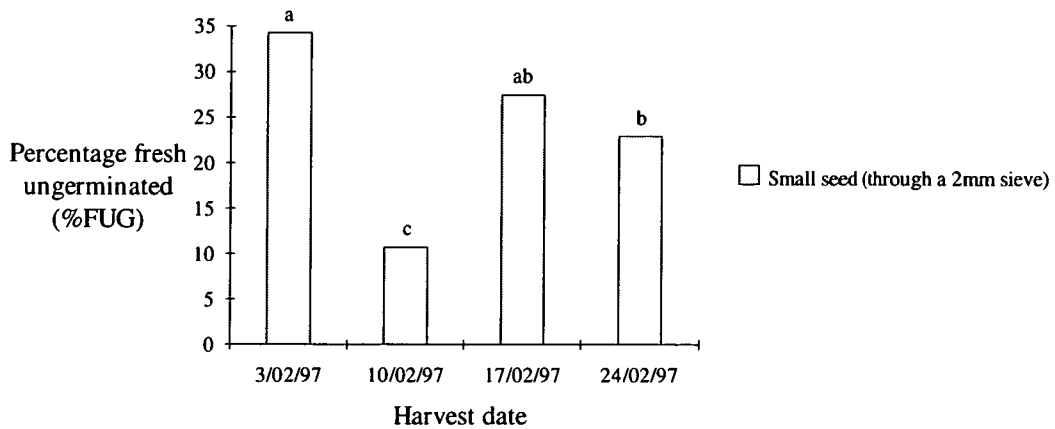
Figure 6.10 Percentage of fresh ungerminated seed at 20 degrees C vs harvest date



Note (1) Differences between some treatments are significant, $P < 0.001$, $LSD (0.05) = 6.39\%$.
 Significantly different treatments are denoted by different letters.

At 10°C a similar pattern of fresh ungerminated seed was evident, with %FUG being initially high, reaching a minimum at the second harvest and increasing again in harvest three and four (Figure 6.11). Although similar in pattern to FUG at 20°C, a greater proportion of seed was found as FUG at the lower temperature.

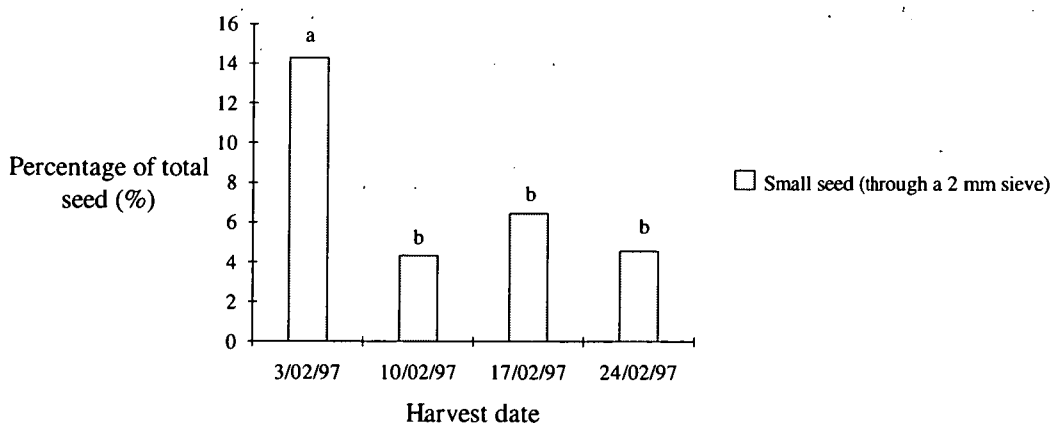
Figure 6.11 Percentage of small seed found to be fresh ungerminated after germination at 10 degrees C



Note (1) Differences between some treatments are statistically significant, $P = 0.002$, $LSD (0.05) = 9.84\%$. (2) Significantly different treatments ($P < 0.05$) are denoted by different letters.

Following germination at 10°C, ungerminated seed was incubated at 20°C in order to investigate the viability of the remaining seed (Figure 6.12). More seed from the first harvest responded to the higher germination temperature than did any subsequent harvest dates.

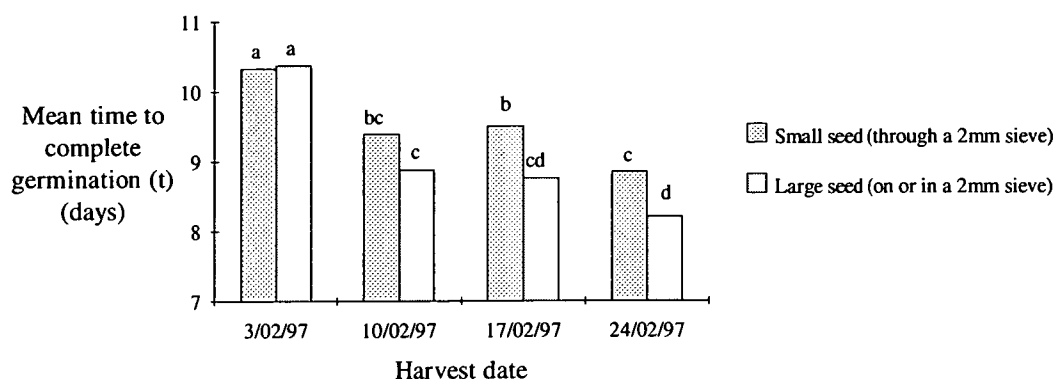
Figure 6.12 Small seed initially failing to germinate at 10 degrees C and subsequently germinating at 20 degrees C



Note (1) Differences between some treatments are significant, $P = 0.011$, $LSD (0.05) = 5.89\%$. (2) Significantly different treatments ($P < 0.05$) are denoted by different letters.

There was a significant decline in mean time to complete germination with lateness of harvest in both the large seed and small seed samples (Figure 6.13).

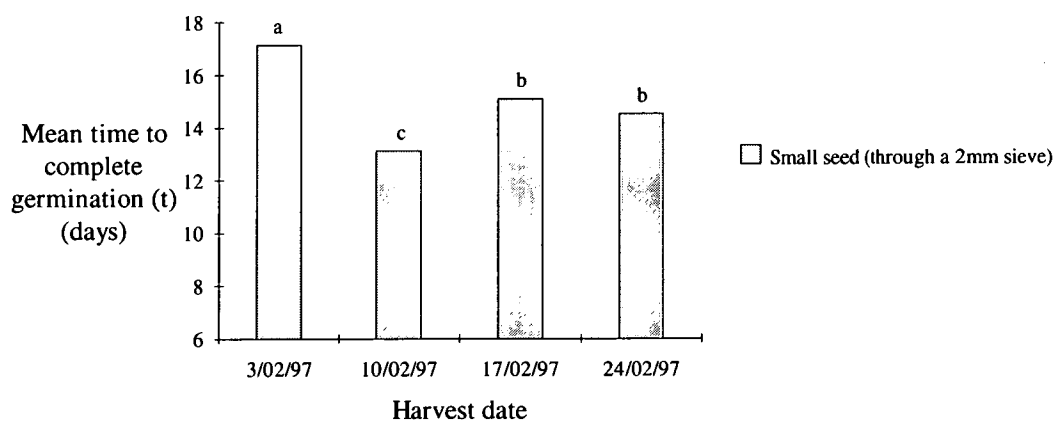
Figure 6.13 Mean time to complete germination at 20 degrees C vs harvest date



Note (1) Differences between some treatments are significant, $P = 0.001$, $LSD (0.05) = 0.55$ days.
 (2) Significantly different treatments ($P < 0.05$) are denoted by different letters.

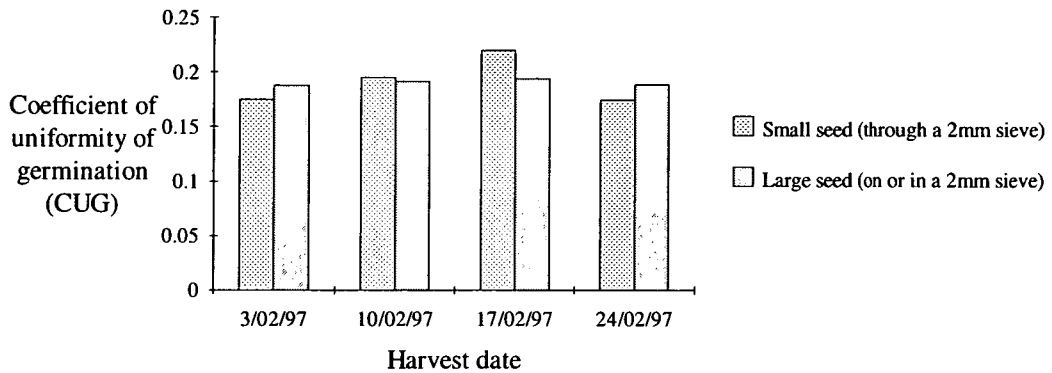
Investigation of mean time to complete germination at 10°C (Figure 6.14) revealed a similar slowness to germinate in seed from the first harvest. Mean time to complete germination then reached a minimum at the second harvest before rising again in the third and fourth harvests. Note that Figure 6.14 follows the same pattern as that of FUG at 10°C (Figure 6.11).

Figure 6.14 Mean time to complete germination for small seed germinated at 10 degrees C vs harvest date



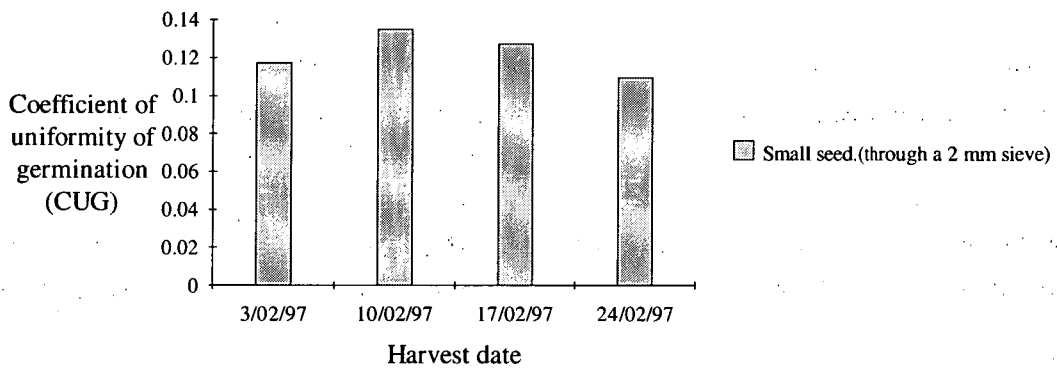
Note (1) Differences between some treatments are significant, $P < 0.001$, $LSD (0.05) = 1.15$ days.
 (2) Significantly different treatments ($P < 0.05$) are denoted by different letters.

Figure 6.15 Uniformity of germination at 20 degrees C vs harvest date



Note (1) No significant differences between treatments

Figure 6.16 Uniformity of germination in small seed germinated at 10 degrees C vs harvest date



Note (1) No significant differences between treatments

Uniformity of germination data for 20°C and 10°C are presented in Figures 6.15 and 6.16. Uniformity of germination at both temperatures was not influenced by date of harvest.

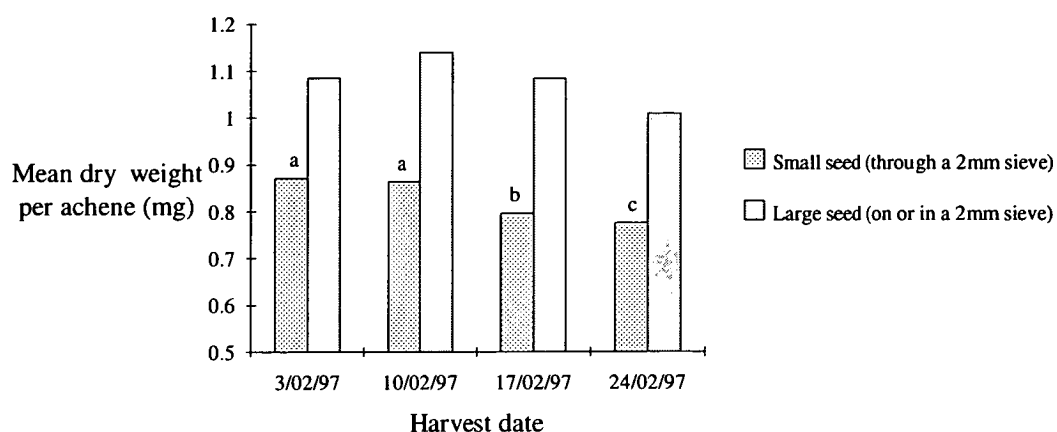
6.4.4 Seed components and seedling characteristics

Small, but statistically significant differences in mean spent achene weight were found between harvest dates in the small seed treatments. The mean dry weight per spent achene decreased after the second harvest (Figure 6.17). No significant differences between harvest date treatments were observed in the large seeds.

Mean seedling dry weight differed between harvest dates for the small seed, but no significant differences were observed between the large seed treatments (Figure 6.18). The trend from the first to the second harvest may be due to seed growth. The decrease

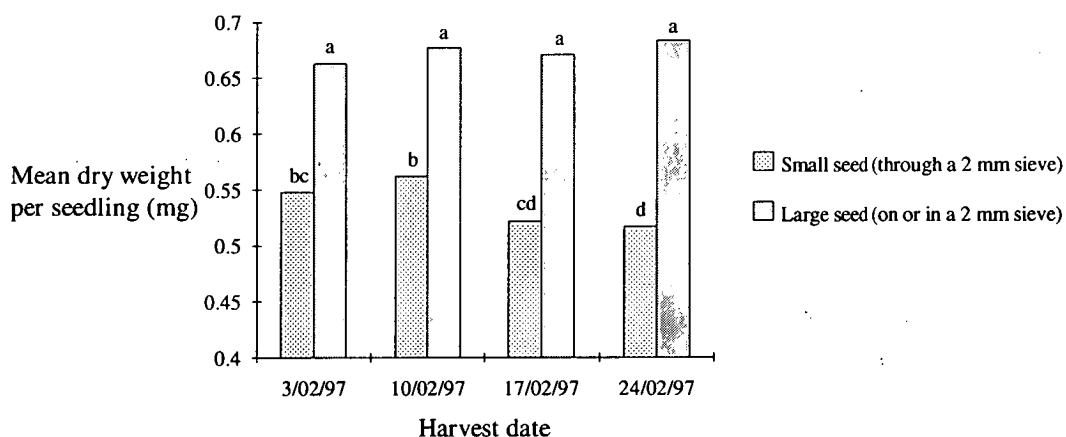
in dry weight per seedling after the second harvest may be explained by losses of larger achenes from the samples (see Figures 6.1 and 6.2).

Figure 6.17 Mean dry weight per spent achene vs harvest date



Note (1) Differences between some small seed treatments are significant, $P = 0.009$, $LSD (0.05) = 0.018$ mg. Significantly different treatments ($P < 0.05$) are denoted by different letters. (2) No significant differences between large seed treatments.

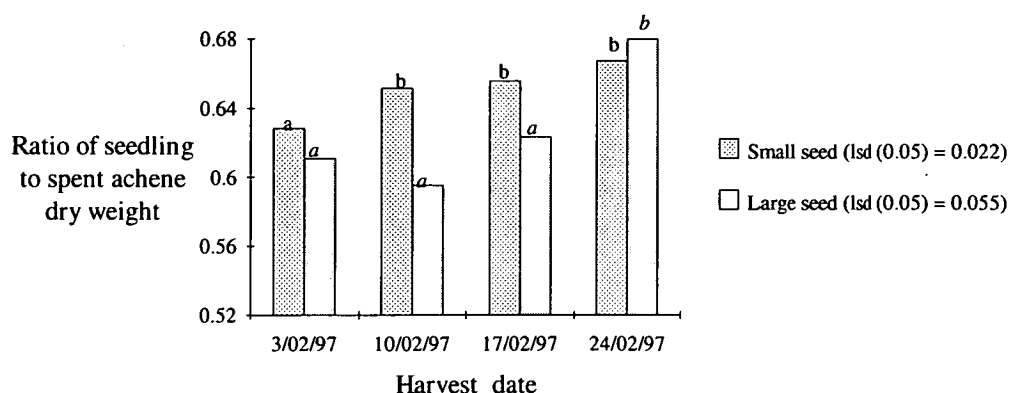
Figure 6.18 Mean dry weight per seedling vs harvest date



Note (1) Differences between some seed treatments are significant, $P = 0.001$, $LSD (0.05) = 0.03$ mg. (2) Significantly different treatments ($P < 0.05$) are denoted by different letters.

The increase in seedling to spent achene ratio in small seed from the first harvest to the second indicates that some embryos had not completely finished growing by the time of the first harvest (Figure 6.19). An increase in ratio in the large seed is apparent between the third to the fourth harvests. This increase may be explained by the loss of the largest achenes in which a lower ratio may have been present.

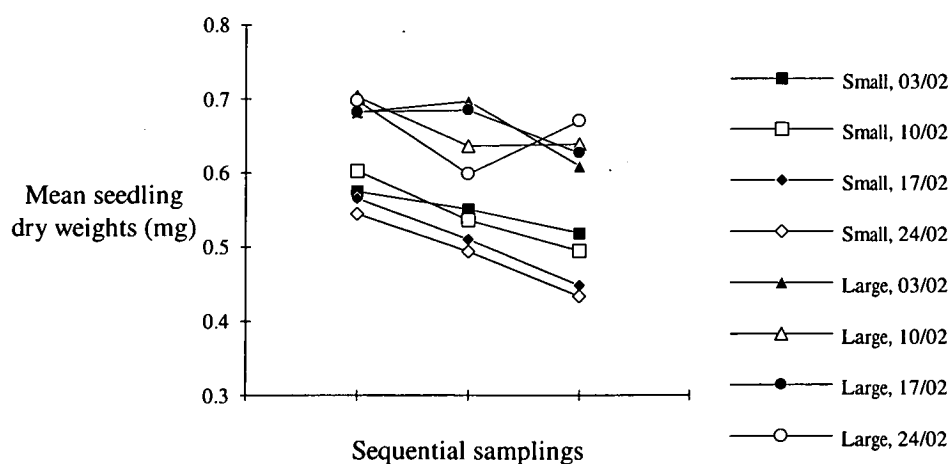
Figure 6.19 Ratio of seedling to spent achene dry weights vs harvest date



Note (1) Differences between some small seed treatments are significant, $P = 0.018$, $LSD (0.05) = 0.022$ units. Significantly different treatments ($P < 0.05$) are denoted by different letters. (2) Differences between some large seed treatments are significant, $P = 0.034$, $LSD (0.05) = 0.055$ units. Significantly different treatments ($P < 0.05$) of large seed are denoted by different italicised letters.

Figure 6.20 demonstrates that seedlings of lower weight emerged on sequentially later dates. This appears to be so in the small seed regardless of harvest time, but is not as clearly demonstrated in the large seed, where only the first harvest date displayed a statistically significant reduction.

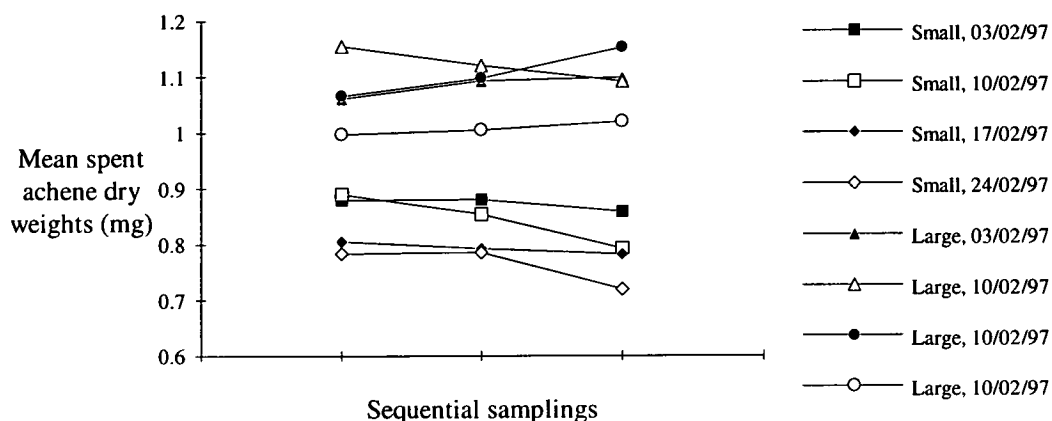
Figure 6.20 Mean seedling weight for early, mid and late germinations of seed harvested on sequential occasions



Note (1) Sequential sampling 1 includes seedlings germinated on days 4, 6, and 8. Sampling 2 includes seedlings from day 10, and sampling 3 contains germinants from days 12 and 14. Groupings were selected so similar numbers of seedlings were in each treatment (2) Statistics for differences in seedling dry weight with lateness of germination: Small, 03/02, NS. Small, 10/02, $P = 0.005$, $LSD = 0.044$ mg. Small, 17/02, $P = 0.0003$, $LSD = 0.029$ mg. Small, 24/02, $P = 0.01$, $LSD = 0.052$ mg. Large, 03/02, $P = 0.047$, $LSD = 0.063$ mg. Large, 10/02, 17/02, 24/02, all NS.

Mean spent achene dry weight remained relatively constant with lateness of germination (Figure 6.21). This was so in all the small seed regardless of harvest time, and in all but one of the large seed treatments in which there was an apparent increase in mean spent achene dry weight with lateness of germination.

Figure 6.21 Mean spent achene weights for early mid and late germinating seed



Note (1) Sequential sampling 1 includes spent achenes germinated on days, 4, 6, and 8. Sampling 2 includes spent achenes from day 10, and sampling 3 contains spent achenes from days 12 and 14. (2) Statistics for differences in spent achene DW with lateness of germination: Large, 17/02, $P = 0.008$, $LSD = 0.039$ mg. All others NS.

6.5 Discussion

The following discussion firstly investigates the influence of lateness of harvest on changes in seed population. Next, variation in proportion of achenes containing seed with lateness of harvest is discussed. The major topic of germination characteristics of seed is subsequently addressed, followed by aspects of changing seedling weight. Finally the rate of germination of seed possessing small seedlings is discussed.

6.5.1 Quantities of achenes and seed changing with harvest date

Flowers on the pyrethrum plant begin to visibly degenerate when all the disc florets have opened and their opportunity for fertilisation has passed. Firstly the attractive yellow disc florets become dry and turn brown, then the white ray florets dry and tend to coalesce with the involucre and the receptacle. Further external flower development is marked by ease of removal of disc florets which leaves the calyx of achenes exposed. Loss of disc florets is often accompanied by loss of fragile ray florets and this marks the later stages of seed desiccation. The whole capitulum continues to dry, but in particular the receptacle finally dries and shrinks which increases the chance of achene loss from the peduncle.

Sampling over the chosen four week period coincided with later seed development and drying of the flowers. Methods for assessment of seed maturity have been developed including time elapsed from flowering and changes in seed appearance. Both of these are reported to be unreliable indicators of maturity (Grey and Steckel, 1982). Conversely seed moisture has been associated with PM in onions, *Allium cepa* (Stiener and Akintobi, 1986) and percentage germination in carrots, *Daucus carota* (Steckel *et al.*, 1989).

During the four week period in this study, flower moisture decreased from 40% to a low level. Over this period, both a loss in florets and a decrease in cleaned capitulum dry weight occurred (Table 6.3). Loss of florets through abscission is a normal process in flower development. The decrease in mean weight per capitulum may have been due to a range of factors including losses of the most mature, heaviest capitula from the sample and/or losses of achenes from capitula. Further data from replicated sub-samples demonstrate small decreases in; achene number per capitulum, percentage of large achenes in the samples, and percentage of large achenes containing seed. Such findings are evidence of minor loss of achenes with lateness of harvest. Later harvested samples are not merely more mature, but also vary in composition to the previous sample. This may have had some influence on the observed changes in germination behaviour with harvest date thus this factor cannot be ignored in the interpretation of data.

Mean achene dry weight increased from the first to the second harvest then fell again in the later harvests. This was so with the small seed, which constituted about 95% of the achenes harvested. The increase in mean dry weight of around 12% occurred as larger seed were being lost from the sample which would have reduced the extent of the observed increase. The increase in mean dry weight is evidence of significant seed growth during that period. Therefore, at the time of first harvest, some seed had not yet achieved PM and was immature when harvested. The fall in mean seed weight after harvest two was suspected to be largely due to losses of large seed from the population.

6.5.2 Variation in the proportion of achenes containing seed

The proportion of fertile seed in the small seed sample did not alter with lateness of harvest. In the large seed, a small but statistically significant reduction in proportion of achenes containing seed is suspected to be due to losses of the largest, most mature achenes from the sample. A lower percentage of achenes containing seed was found in the smaller seed size regardless of harvest date. The proportion of achenes containing seed is investigated in Chapter 7.

6.5.3 Variation in % moisture of seed samples

Moisture content of the achene samples just prior to germination ranged from 7.75% to 9.29%, but did not follow any pattern that could relate it to maturity of the seed at harvest. Differences in seed moisture are known to influence rate of release from dormancy, seed lot deterioration (Bewley and Black, 1994) and rate of germination (Bradford, 1990). Since differences were small, this factor was considered to contribute little to differences in germination behaviour.

6.5.4 Germination characteristics of immature seed

The results demonstrate that poor seed performance on the first harvest occasion was due to seed immaturity. The increase in mean achene dry weight discussed in a previous section together with data presented below, associate seed immaturity with poor germination characteristics. Low final germination at 20°C of seed from the first harvest demonstrated significant germination failure. Subjecting ungerminated seed to a squash test (Appendix 1.3) revealed that approximately two thirds was D and one third was FUG. The percentage of both D and FUG seed fell dramatically in seed from the second harvest. Some immature seed was therefore apparent as D or FUG when germinated at 20°C.

Germinating early harvested seed at 10°C provided further insight into germination failure of this seed. As at 20°C, significantly more of the first harvest seed failed to germinate than seed from subsequent harvests. In addition, some of the initially ungerminated seed was able to germinate once placed in ideal germination conditions (20°C). Furthermore, germination was far slower in this early than in later-harvested seed, which may be considered as further evidence of seed immaturity (TeKrony and Egli, 1997). As seed was very slow to germinate, and a significantly higher proportion of seed germinated when placed in more conducive conditions (20°C), seed from the first harvest found as FUG may not have been dormant, rather it may have just been very slow to germinate. Of all the evidence presented indicating seed immaturity, the most convincing is the high proportion of dead seed. No visible pathogens were associated with seed necrosis. In summary, germination parameters associated with immature seed harvest were:

- i. Low germination capacity at both 10°C and 20°C
- ii. High proportion of dead seed at both 10°C and 20°C
- iii. Slow germination rate at both 10°C and 20°C

6.5.5 Germination characteristics of mature seed

Germination characteristics of seed from the last three harvest times differed significantly from the first harvest occasion. Data presented indicate that most of the seed harvested on the last three occasions had acquired desiccation tolerance, reached PM, and was becoming increasingly dormant with desiccation. Over this time, flower moisture decreased from 24 to 2.5 %, although 2.5 % seems unrealistically low.

At 20°C, final germination was consistently high in all but the first harvest. Investigation of the ungerminated seed revealed the percentage of dead seed was low and decreased from the second to the fourth harvest. Conversely, %FUG increased significantly making up most of the ungerminated seed by the last harvest. Inception of dormancy in pyrethrum seed appears to be associated with seed development during desiccation since in earlier harvests, less dormancy was apparent. Delay of onset in dormancy until this late stage in seed development has been demonstrated in *Medicago lupulina* (Sindu and Cavers, 1977).

A small decrease in mean time to complete germination at 20°C was noted from the second to the fourth harvests. A range of causes for this decrease are conceivable, including an increased proportion of seed reaching PM thereby being less compromised by seed immaturity, or more of the slower to germinate seed entering dormancy with lateness of harvest. Further research would be required to detail the mechanism by which this actually occurs.

Germination at 10°C for the last three harvests provided further evidence supporting the proposal that inception of dormancy predominantly occurred in the latter stages of seed development and desiccation. Final germination was significantly higher in harvest two than in harvests three and four. As no difference in the %D was observed in the latter three harvests, the decrease in germination percentage was largely due to FUG. This is a similar pattern of increase in %FUG as noted at 20°C, but the percentages of seed failing to germinate at 10°C were far greater. Unlike mean time to complete germination at 20°C, seed germinated faster in harvest two than in harvest three and four. Apparently, at this low temperature a 'dormancy type mechanism' not only increasingly reduced germination percentage but also decreased rate of germination.

In summary, once flower moisture fell to 24%, only a low percentage of ungerminated seed was found to be dead. Level of dormancy in the seed was low at 24% moisture and increased significantly with subsequent desiccation. While in the first harvest the major

influence on low germinability was immaturity, decreasing germinability over the last three harvests was due to dormancy.

Germination parameters indicating that seed was harvested prior to inception of dormancy include:

- i. High %FG at both 10°C and 20°C.
- ii. Fast germination at 10°C

Germination parameters indicating that harvest occurred after the onset of dormancy include:

- i. High germination at 20°C, but low germination at 10°C
- ii. A low proportion of dead seed in the remaining ungerminated seed at 10°C

6.5.6 Uniformity of germination

No differences in germination uniformity were observed at either 10°C or 20°C. Little evidence is available relating to the impact of harvest date on uniformity of germination in *Asteraceae*, although Bratcher *et al.* (1993) while investigating release from dormancy through stratification of a range of wildflowers found uniformity of germination increased with increasing period of stratification. Uniformity of germination may be influenced by harvest time, but this was not evident in the current work. Factors other than time of harvest are likely to influence uniformity of germination.

6.5.7 Seedling and spent achene dry weights

Investigation of mean seedling and spent achene dry weights was intended to identify any seed weight changes during the latter stages of development. Data presented earlier indicated that at the time of first harvest, much of the seed had not yet reached PM. Of most interest was the possibility that an increase in mean seedling weight could have been associated with improvement in germination characteristics occurring from the first to the second harvest. Focusing on this aspect of the data it was evident that no growth occurred in the spent achenes. There was also no statistically significant increase in seedling weight over this period. Although this was so, a significant increase in the seedling to spent achene ratio was apparent. The extent of embryo growth may have been masked by concurrent seed losses. Data indicate a minor increase in embryo dry weight from the first to the second harvest. This contributes to the already strong evidence in support of the proposal that seed growth was still underway at the time of the first harvest.

6.5.8 *Slow germination of small seedlings associated with harvest time*

In previous work it was demonstrated that large seedlings came from early germinants and seedlings became progressively smaller with later germination. This work investigated whether harvest date is a major factor influencing this characteristic. Within the sample of small seed all harvest date treatments displayed the same trend of smaller seedlings with lateness of germination (Figure 6.20). Even where seed was harvested on the final occasion the decrease in seedling weight with lateness of germination still existed. The presence of small, late germinating seedlings therefore did not appear to be associated with improper harvest time.

6.5.9 *Summary*

This study highlights some key opportunities for industry to improve the quality of seed. Although more focused studies are required to evaluate possible genotypic and year variation, the current study is the only information available on choice of harvest time. A preliminary recommendation for harvest of seed is to harvest flowers at 25% moisture.

Chapter 7: Seed quality and production associated with capitulum size and achene position

7.1 Introduction

Previous chapters in this thesis documented differences between seed lots. In an effort to understand why they behaved differently from one another, work was undertaken investigating variability within a seed lot. The current study examines the yield, weight and germination behaviour of seed borne on capitula of different size and borne either peripherally or centrally on the composite flower. Capitula evaluated in the trial were known to be mature at harvest.

The literature review aims to provide a broad theoretical framework upon which a range of questions associated with seed quality and quantity could be developed. The theory and the outcome of the current experimental work are expected to help direct further studies into the agronomy of pyrethrum seed production.

7.2 Literature review: Variation in seed within populations

This work overviews variation in seed quality and quantity produced in populations of genetically similar plants. Firstly the phenomenon of somatic polymorphism among the seeds of a plant is introduced. Then pollination, seed setting and consequences of stressful environmental conditions during seed development are discussed with respect to their potential influence on variation in seed quality and quantity. The review considers how and why differences arise rather than focusing on describing the influence of seed size on various aspects of germination and subsequent seedling establishment.

7.2.1 Somatic polymorphism

It is well established that in all species significant variation in seed quality and quantity occur between sites and years. Those differences are largely due to variations in environmental factors such as temperature, light, drought, nutrients, competition and interactions between such variables (Fenner, 1992). Within a single plant, in a given year, significant variation in seed quality is also common. A range in seed size is produced and often the smallest or least dense seed is considered to be of poor quality. 'Somatic seed polymorphism' and 'seed heteromorphism' are two analogous terms which may be defined as the occurrence of seeds of an individual plant that differ in form or behaviour (Venable, 1985a).

Species may produce seed that varies considerably in form, for example in *Heterotheca latifolia* (Venable and Levin, 1985), *Heterosperma pinnatum* (Venable *et al.*, 1987), *Heterotheca grandiflora* (Flint and Palmblad, 1978), *Senecio jacobaea* L. (McEvoy, 1984), or they may exhibit cryptic heteromorphism where seeds vary continuously in size, shape or germination time (Silvertown, 1984). An example of a cryptic heteromorphic species is *Tragopogon dubius* (Maxwell *et al.*, 1994).

Reasons why plants have evolved seed heteromorphism are complex and beyond the scope of the current work, but fall into the general framework of *bet hedging*, thus improving the chances of survival of the next generation. In many cases seed heteromorphism has been shown to result in effective variation in optimum germination conditions, dormancy and dispersal (McEvoy, 1984). Another model proposing explanations for seed heteromorphism suggests that sib-sib competition is decreased through separation in time. This delayed germination was found to be under the control of the mother plant through differential seed coat imposed dormancy (Ellner, 1986).

Significant variation is due to seeds being provided with differing levels of resources by the mother plant. Seed position can have the major influence on seed size. For example, maize kernels at the top of a cob are often smaller due to inadequate supply of assimilate (Hanft *et al.*, 1986). Such seed positional differences can have an impact on the germination and seedling growth characteristics of the resultant seed. Examples where this occurs include oats, *Avena sativa* (Brinkman, 1979). Positional differences can influence the chemical composition of seed. For example, the proportions of fatty acids in oil seed rape vary with position in the pod (Diepenbroke and Giesler, 1979). Nitrogen, phosphorus and potassium concentration vary with fruit position in an annual Malvaceae species (Benner and Bazzaz, 1985).

Studies investigating the relative sink strengths have been conducted and generally demonstrate complex relationships between seeds (Fenner, 1992). Techniques used for such studies generally involved: (+/-) removal of primary seeds; (+/-) fertilisation of seeds; addition or removal of nutritive resources; and (+/-) defoliation to limit assimilation.

Differential maternal supply of assimilates with respect to lateness of flowering has also been demonstrated to increase seed variability in a range of species (Cavers and Steel, 1984). Variation in seeds due to relative time of development has been described as an example of somatic heterochrony (Silvertown, 1984). In most cases it would appear that

the influence of 'position' and 'time of flowering' are not necessarily independent variables and may be simply describing the same phenomenon.

Paternal, maternal and embryo genetic material may also influence the variation in seed weight and germination behaviour. The relative importance of such factors has been investigated using an obligate apomict, *Taraxacum haematiforme* Dahlst. (Mogie *et al.*, 1990) and this general area of investigation has been reviewed by Roach and Wulff (1987).

Much of the variation in seed germination behaviour of cultivated plants has been significantly reduced through seed cleaning, well-developed seed-crop agronomy practices and plant breeding. Despite this, differences in germination behaviour still exist and can have a significant influence on the evenness of establishment in crops such as in carrot, *Daucus carota* (Grey, 1979; Grey and Steckel, 1983a). Variation among seeds from the same lot may be physiological rather than due to mere differences in weight (Fenner, 1992) and therefore impossible to separate and contend with.

7.2.2 Pollination

Seed production in many cultivated plants (McGregor, 1976) is limited by inadequate pollination. Although both gymnosperms and angiosperms produce a large number of ovules, most will not develop into mature seed. The percentage of ovules achieving seed set has been investigated and varies widely between species (Stephenson, 1981; Ehrlen, 1991). The first critical stage which determines the potential of the ovule for further development is pollination. This has been demonstrated through increased seed set via hand pollination. Bierzychudek (1981) while investigating the population biology of the forest understory perennial, *Arisaema triphyllum*, showed that even in the presence of high numbers of pollinating insects, upon hand pollination, the percentage seed set increased from one to 43 percent of the ovules. Bierzychudek (1981) cited numerous other examples of studies with a range of species that were found to be pollinator limited.

Flower size and number, and inflorescence design have all been associated with improving male function and increasing the chance of pollination (Fishbein and Venable, 1996). Intrinsic to this argument is the interaction between the plant and the pollinating insect. Plants expend significant resources and systems have evolved in order that plants make themselves more attractive and thus more likely to be pollinated by the action of insects. Willson and Agren (1989) investigated the pollination process from the insect's

perspective and suggested that male flowers more commonly offer insects more rewards than female flowers.

Problems with flowering plants having to devote a lot of resources to pollen production and facing potentially low insect visitation to small, awkwardly located individual flowers is overcome in a range of ways in different plants. In Asteraceae many perfect flowers are found enclosed in the one compound inflorescence. This overcomes problems associated with lack of visitation to the female flower. Advantages in having all the flowers in a capitulum are significant. The composite flower serves as a large attractive unit for pollinators as well as acting as a landing and traversing site. Investigating the evolution of Asteraceae capitulum types from the perspective of insect flower interactions, Leppik (1977) identified that all types of Asteraceae flowers imitated the floral pattern of common solitary flowers. Leppik (1977) recognised four capitulum classes in Asteraceae and that these are often visited by more or less specialised pollinating insects. Aspects of diversification and specialisation in Asteraceae such as the advent of self incompatibility and specialised function of inner and outer achenes have been thoroughly investigated (Burt, 1977).

Brewer (1968) reviewed various aspects of flowering and seed setting in pyrethrum. He observed that a single ring of florets open on a daily basis and the discharge of mature pollen occurs concomitantly with elongation of the style. The pollen mass is not released easily from the style because it is held together by a sticky substance. The mature pollen grain is found to germinate *in vitro* which is reportedly uncommon in similar tri-nucleate pollen grains (Delhay, 1956). Following anthesis, pollen loses viability rapidly and a greater proportion of it is often foraged by pollinating insects (Delhay, 1956). Only several hours following pollen release does the style become receptive, so the amount and viability of self pollen can be neglected. Self-fertilisation is reported to be limited by sporophytic self-incompatibility systems also found to be common in other closely related species (Brewer, 1968). As the ray florets are female only, and these are the first to open on the capitulum in the absence of self pollen, this may also be regarded as a means of limiting self fertility in pyrethrum. Although such mechanisms reportedly exist in pyrethrum, selfing still occurs in some clonal lines. Brewer (1968) suggests that this may be largely due to failure of sporophytic self-incompatibility in older unfertilised florets.

Changes in effectiveness of self incompatibility, and the influence of temperature and plant age on seed formation in witloof-chicory, *Cichorium intybus* L. (Asteraceae) have also been investigated (Eenink, 1981). Brewer (1968) indicates that rain and cloudy periods discourage insect pollination and that this factor is closely associated with low

seed set observed in such conditions in pyrethrum. In many species, low temperatures may reduce insect activity and may also prevent pollen tube growth thereby decreasing the proportion of ovules fertilised (Bleasdale, 1984).

7.2.3 Seed setting

Level of pollination alone does not necessarily determine the proportion of ovules set by the plant. Often a proportion of the fertilised ovules are aborted or fail to acquire enough resources to become viable seed. The proportion of pollinated flowers that set fruit generally decreases as the number of pollinated flowers increases. This has been demonstrated by many studies which show little or no increase in seed produced by the plant through hand pollination (Stephenson, 1981). Fenner (1985) suggested abortion is a mechanism through which the plant can regulate its reproductive effort in accordance with the resources available.

Stephenson (1981) in a review of fruit and flower abortion, stated that plant resources are divided for the purposes of growth, maintenance and reproduction. Furthermore, reproductive resources may be divided between male (pollen) and female (fruit and seed) allocation. Within the female function, there is also ballencing between seed number and seed weight (Harper *et al.*, 1970). The pool of resources available to the plant for partitioning can also vary both within and between seasons due to a wide range of plant and environmental factors. In most species it is recognised that plants produce far more ovules than they could potentially set into seed. From a resource allocation perspective this would appear wasteful, but Ehrlen (1991) proposed in a 'reserve-ovary model', that an oversupply of ovules allows for losses in the event of unpredictable external mortality.

Fertilisation promotes renewed growth of the ovary, and this phase termed 'fruit set' is often accompanied by wilting or abscission of the petals and stamens. The continued growth of the seed is reportedly under the control of hormones produced by the seed (Stephenson, 1981). Hormones play a leading role in mobilisation of resources from the mother plant. Tamas *et al.* (1979) demonstrated that inability to access adequate mother plant resources also promotes the production of growth inhibitors in the seed leading to abortion. Haig and Westoby (1986) proposed a theoretical model describing how both pollen limitation and maternal provisioning of ovules can limit seed number.

Investigations of which seeds on the plant survive and why, have been carried out by many workers in a wide range of species. It has been demonstrated that the probability of seed set decreases with lateness of flowering in a range of herbaceous species that flower over an extended period (Tamas *et al.*, 1979; Stephenson, 1981; Agren and Willson,

1992). Seed weight also declines with lateness of flowering (Cavers and Steel, 1984). Later flowering ovules are also often positioned differently on the plant, for example the last peas in a pod, seed from secondary umbles, or the last to develop corn on a cob.

Although timing of flowering appears to contribute significant support to the ideas discussed earlier relating to resource allocation and competition between fruits, other factors may also be involved. Marshall and Ellstrand (1988) while working with wild radish, *Raphanus raphanistrum*, identified several mechanisms which could be regarded as 'maternal mate selective seed abortion'. Seed setting is a complex area of study but one potentially worthy of further investigation in pyrethrum.

7.2.4 Consequences of stressful environmental conditions during seed development

Just as seed number can be determined by resources provided to the seed, continued seed growth, development, seed weight and quality can also be significantly determined by availability of maternal resources. Factors that influence the growth of all plants are temperature, moisture, light and nutrient availability. Lloyd (1980) proposed that plants serially readjust level of resources invested in offspring, the effects of which culminate in manipulation of seed number. As plant density will significantly modify these variables, this factor will be briefly discussed. Following this, the influence of varying other factors is investigated.

Plant density affects both the dry weight of individual plants and the proportions of various morphological parts. Grey *et al.* (1983) investigated the effects of plant density on the yield and seed size in carrot. They identified that with increasing density, the number of secondary umbles decreased considerably. Overall seed yield increased with increasing plant density in two of three trials. Seed was found to be heavier in low density treatments and from primary umbles, than at high densities and from secondary umbles. Since there was no difference in growth rate of seed from the various treatments, it was concluded that differences in seed weight originated at or prior to anthesis.

Examples of the influence of plant density on seed production are not restricted to horticultural crops. Baker and O'Dowd (1982) demonstrated that in *Hypochoeris glabra* L. a change in proportion of beaked and unbeaked achenes was produced with increasing plant density. This significantly affects the dispersal potential of the seed. Ackerly and Jasienski (1992) demonstrated a greater investment in male flowers in the tallest plants of a self thinned high density population of the monoecious annual, *Ambrosia artemisiifolia* (Asteraceae).

Generally it is evident that plant density influences overall seed yield, yield structure (position), and may influence quality parameters of the seed. It would appear that such differences arise from complex interactions associated with the timing and intensity of both between- and within-plant competition for limited environmental resources. Furthermore, those differences due to density may be determined during the growth and development of the plant, well before flowering takes place.

Drought stress that occurs prior to flowering, assuming that it is not prolonged and severe, will impact on seed quantity rather than quality. Conversely, drought occurring during the reproductive phase can have significant effect on both seed quantity and quality. The influence of drought on seed quality has been reviewed by Delouche (1980) and by Dornbos (1995), the latter focusing predominantly on soybean, *Glycine max*, and other legumes. Stress during the early reproductive phase may reduce seed number, while stress during the development of the seed is more likely to influence seed weight and seed quality. Research into the impact of drought is not limited to species of agronomic importance. While working with *Heterotheca latifolia* (Asteraceae), Venable (1985b) identified that under drought conditions maternal provisioning of disc and ray achenes varied, resulting in a change in seed size and germination time between the two achene types.

Delouche (1980) cited examples where high and low temperatures, including frost injury, during seed development were responsible for significant changes in seed quality and quantity. The influence of temperature on seed quality has been reviewed with examples mainly from soybean and other legumes by Dornbos (1995). In this detailed review Dornbos suggested that seed stress during seed fill, potentially caused by temperature, decreased seed quality by decreasing the availability of maternal reserves and secondly through the development of an impaired physiological apparatus required to drive germination.

A significant body of research exists which demonstrates that temperature during seed development influences seed weight, with higher temperatures resulting in smaller seed. In reviewing the influence of temperature, Fenner (1992) suggested the main influence of higher temperatures is an increase in rate of ripening of seed which reduces the time available for assimilation of resources. Gorecki *et al.* (1996) investigated the influence of temperature during seed maturation on seed weight and soluble carbohydrate composition in white lupins, *Lupinus albus* L. cv. Ultra. Seed maturing at high temperature (28°C) was found to be smaller in size and to produce plants that flowered later than for seed that matured at a lower temperatures (13°C). Although differences in

seed weight and subsequent plant behaviour were observed, only minor differences in seed soluble carbohydrate composition and concentration were identified.

The influence of temperature on seed development in lettuce, *Lactuca sativa* L. (Asteraceae) has been investigated (Grey *et al.*, 1988; Steiner and Opoku-Boateng, 1991). The first study indicated that with increasing day and night (16 hours day/ 8 hours night) temperatures (20/10°C, 25/15°C and 30/25°C) after flowering, the number of mature seed produced per plant was optimal with the middle temperature regime. This was due to an increase in the number of mature florets along with a decrease in seed number per floret. Mean seed weight and variation in seed weight decreased substantially at higher temperature, with the greatest differences being from the lowest temperature to the middle temperature regime. Greater seed weight was subsequently correlated with both increased root length, and ratio of root to hypocotyl length. The authors conceded that many of the differences reported to be due to varying temperature regimes, may have been better correlated to differential efficacy of pollination at the different temperatures, together with an increased growing period experienced at the lowest temperature. Although this was so, the important germination characteristic of capacity to germinate at 30°C did vary between treatments. The work confirmed earlier studies (Koller, 1962; Harrington and Thompson, 1952) which demonstrated that growing seed at high temperature alleviates or prevents high-temperature-induced dormancy.

A significant body of evidence indicates that seed grown at different temperatures vary in the composition and concentration of fats and proteins found in their lipid membranes (Dornbos, 1995; Fenner, 1992). This may influence the fluidity of membranes, particularly at low temperatures, but the significance of this on germination is yet to be determined.

The influence of limited mineral nutrition on seed crops has been investigated (Fenner, 1992). Delouche (1980), while discussing the work of Harrington (1960) who grew carrot, lettuce and peppers in very nutrient deficient sand culture, suggested that nutrient deficiencies in seed were rare. Even when the plants demonstrated severe symptoms of deficiency, the yield of seed decreased but the germination capacity remained relatively constant. Only calcium deficiency reduced germination capacity in some species. Delouche (1980) cited several cases in which large-seeded leguminous species demonstrated symptoms of calcium, boron and molybdenum deficiencies. Significant relationships have been demonstrated between drought and calcium deficiency (Smiciklas *et al.*, 1989).

7.2.5 Summary

A number of mechanisms may contribute to variation in seed productivity, seed size and seed behaviour within plants. These factors include somatic polymorphism, differential pollination, seed setting and environmental constraints. None of the potentially important factors influencing seed quality and quantity have been critically investigated for pyrethrum seed production. The current work investigated variation in pyrethrum seed production between capitula of different weight, and between seed positions on the capitulum.

7.3 Materials and methods

A population of seed was selected for investigation in which seed varied little with respect to seed maturity, was physiologically mature, and was not subject to significant seed losses. Capitula from the third harvest of the time of harvest trial (Chapter 6) were used in the current work. These flower heads were harvested on February 17, 1997 from 10 randomly selected plants in a pyrethrum seed crop located at Forthside Vegetable Research Station (FVRS). The capitula were in the process of self drying in the field (moisture at harvest; 14.7 %) when they were harvested. Capitula were dried at 30°C for one week and then stored at 4°C until the trial was conducted in January 1998.

Capitula were cleaned of any remaining floret material and a sub-sample of approximately 50g of capitula was taken using a cone and quarter technique. Observation of the dried flower sample revealed significant variation in capitula size. Over 200 randomly selected, whole capitula were used to identify a relationship between capitula diameter and dry weight.

The randomly selected sample of over 200 capitula was used to establish a capitulum weight frequency distribution (25 mg steps from 75 to 475 mg). Approximately one fifth of those capitula were removed because they had lost achenes. Three capitulum weight classes were established aiming to account for the heaviest, medium, and lightest 20% of capitula in the distribution. Generating sample classes in this way meant that the distribution defined what was heavy, medium and light, rather than imposing external categories which may have been less descriptive of the population. The mean capitulum weight was recorded for each of the weight classes.

Separation of approximately 50% of the outer achenes from those centrally located was conducted by hand. Achenes making up the outer 1/3 of the diameter of the capitulum were carefully eased away from the receptacle and remaining central achenes. The

achenes from 14, 20, and 17 capitula were selected for each of the four replicates of the light, medium and heavy capitulum classes. A factorial design with four replications was used in analysis of data. Factor one, capitulum dry weight, had three levels and the other factor had two levels; outer or inner achenes.

Non-achene material was removed firstly by sieving through a coarse (4 mm) wire sieve, and then by careful hand winnowing which successfully removed all debris without the loss of achenes. The mean number of achenes per capitulum and the proportion of achenes in the outer treatment were then analysed using single factor ANOVA.

Prior to the commencement of germination trials, the mean weight of achenes from various treatments was assessed. Achenes, only some of which possessed seed, were germinated at 20°C as described in Chapter 4.3.2. Germination was evaluated every second day and germinated seed was placed on slopes until the seedlings reached cotyledons open (Chapter 5.3.1). The percentage of fertile achenes in each treatment along with germination characteristics such as %FG, %FUG, t, and CUG (Appendix 1.3) were evaluated.

Samples of seedlings and spent achenes from each treatment were placed in glass vials and dried at 70°C for 24 hours. Mean seedling and spent achene dry weights were evaluated.

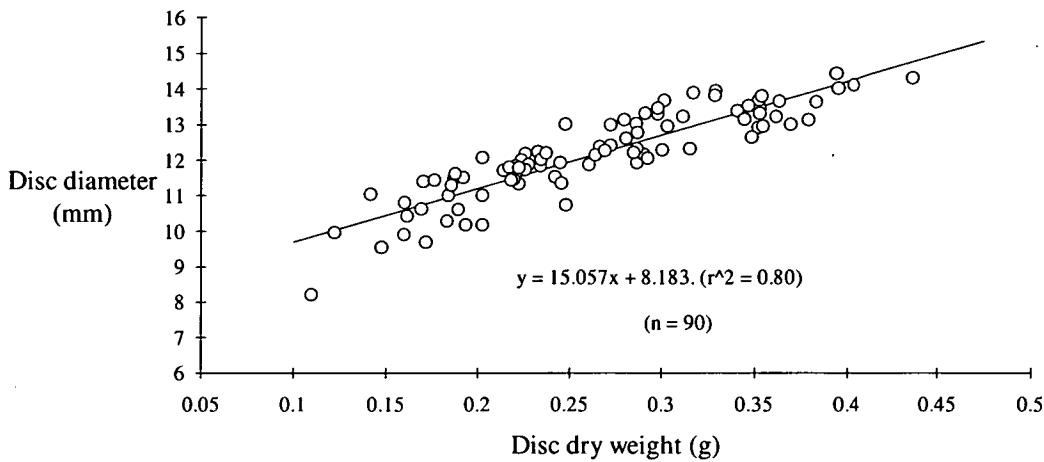
In previous work it was identified that the mean seedling dry weight decreased with lateness of germination. This work further investigated this observation by separating early, mid and late germinants in the heaviest capitulum class for both outer and inner achenes.

7.4 Results

7.4.1 Capitulum mass variation

The observed range in capitula diameters was correlated with dry weight (Figure 7.1); larger diameter capitula were significantly heavier than were smaller capitula.

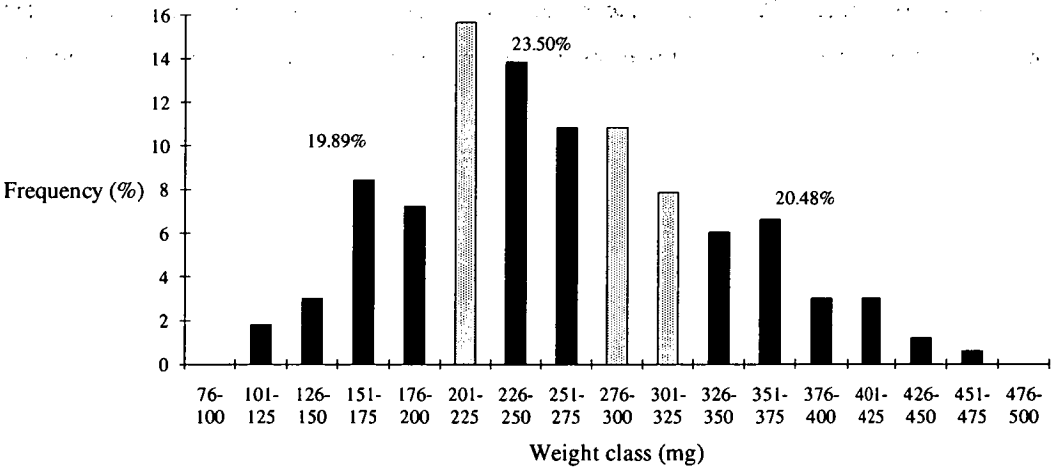
Figure 7.1 Capitulum disc dry weight vs disc diameter



Note (1) Capitulum moisture: 8.7 % moisture (considered as dry weight). (2) Coefficient statistics: slope, $P < 0.001$, 15.06 ± 0.08 ; intercept, $P < 0.001$, 8.18 ± 0.22 .

A histogram of capitulum weights after air drying (Figure 7.2) demonstrates that the distribution is positively skewed (0.38). Mean capitulum weight was 259.71 mg. The histogram demonstrates a wide range in capitula dry weights within the population. The darker bars with accompanying percentages of the population denote the capitulum weight classes selected for further studies.

Figure 7.2 Frequency distribution of mature capitulum dry weights



The mean number of achenes per capitula was analysed and the results are presented in Table 7.1. More achenes were found in heavier capitulum classes. The table also shows that a slightly higher proportion of achenes was taken from the large capitula than the two smaller capitula classes. This was unintended and potentially influenced following investigations.

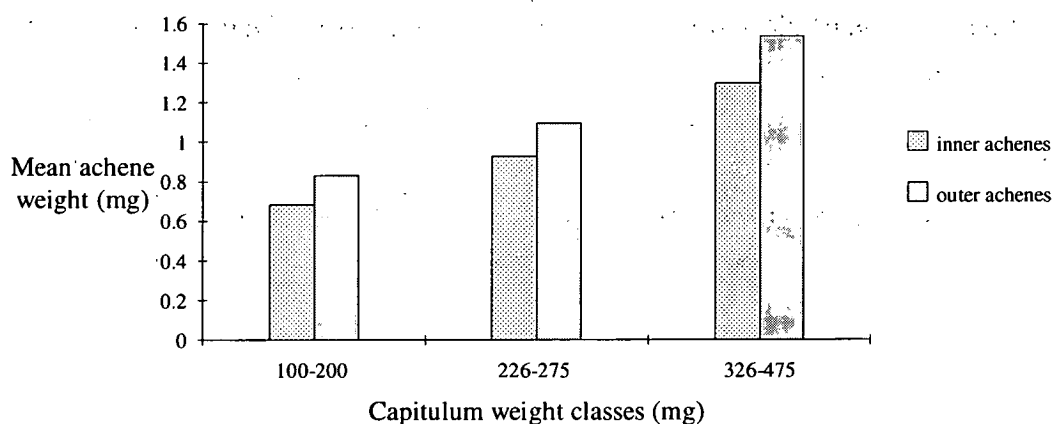
Table 7.1 Mean number of achenes vs capitula weight class

Capitulum weight class (mg)	Number of capitula per replicate	Mean weight per capitulum (mg)	Achenes per capitulum	% of achenes in outside treatment
100 - 200	17	163.9	165.69	41.17
226 - 275	20	250.2	201.45	43.54
326 - 475	14	372.3	222.60	48.03
			P < 0.001, LSD = 7.4	P = 0.003, LSD = 3.19%

7.4.2 Variability in achene yield

Analysis of mean achene dry weights revealed differences both between capitulum classes and between inside and outside achenes (Figure 7.3). Since no there was no interaction between these two factors, it can be stated that larger achenes were found on larger capitula, and on the outer achenes.

Figure 7.3 Outer and inner achenes of different capitula classes vs achene weight

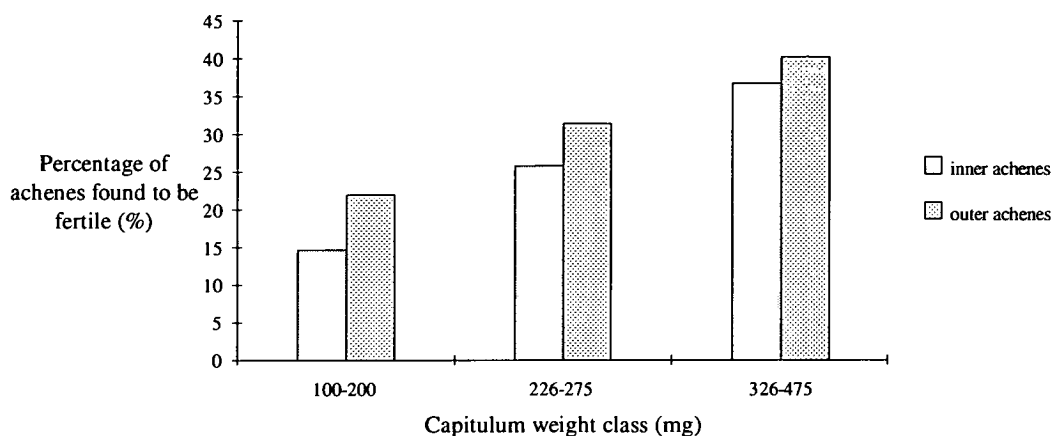


Note (1) Differences between capitulum weight classes are statistically significant at $P < 0.001$, $LSD = 0.05$ mg. Differences between achene position treatments are significant at $P < 0.001$, $LSD = 0.041$ mg. (2) Moisture content was 8.7% and assumed to not vary between treatments.

7.4.3 Variability in seed yield

The proportion of achenes found to contain seed varied both with respect to capitulum weight class and achene position (Figure 7.4). There were proportionally more achenes containing seed in larger capitula. Furthermore, outer achenes were also more likely to contain seed than more central achenes.

Figure 7.4 Outer and inner achenes of different capitula classes vs percentage of fertile achenes

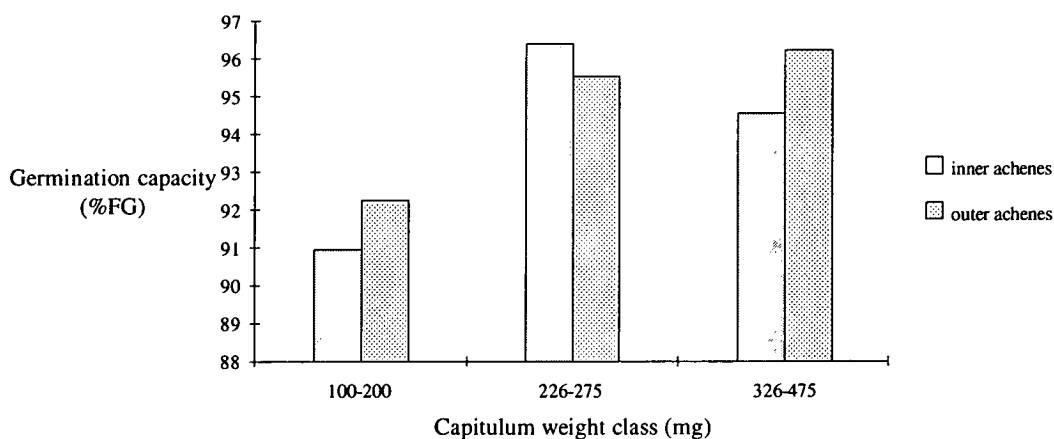


Note (1) Differences between capitulum weight classes are statistically significant at $P < 0.001$, $LSD = 3.6\%$. Differences between achene position treatments are significant at $P < 0.001$, $LSD = 2.9\%$. The interaction was not significant at $P = 0.05$.

7.4.4 Germination characteristics

Final germination was calculated after eliminating hollow or non-fertile achenes (Figure 7.5). Seed from the smallest capitulum weight class had a lower capacity to germinate than seed from heavier capitula. No differences between inner and outer seed germination capacity were identified. Squash testing the ungerminated seed after the germination period of 16 days identified that all the ungerminated seed was fresh rather than dead.

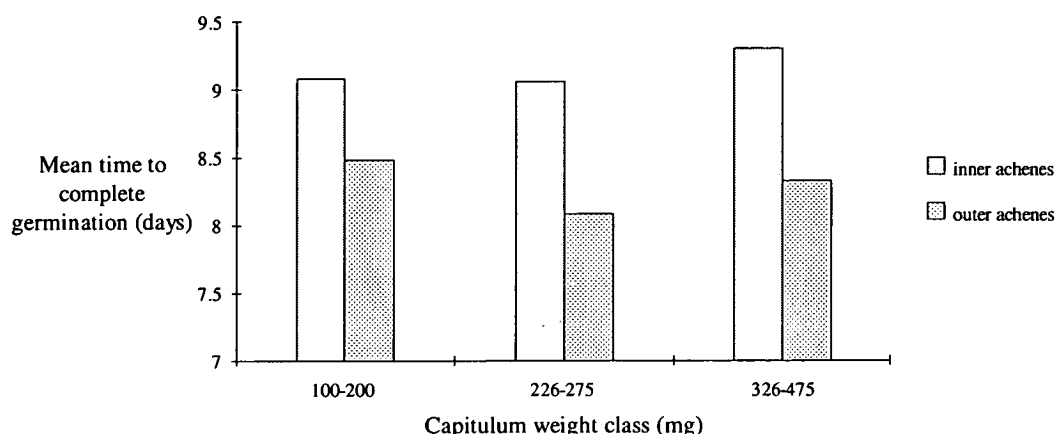
Figure 7.5 Outer and inner achenes of different capitula classes vs germination capacity



Note (1) Differences between capitulum weight classes are statistically significant at $P = 0.02$, $LSD = 3.19\%$. Differences between achene position treatments are not significant at $P = 0.05$.

Evaluation of rate of germination revealed that outer achenes germinated more rapidly than inner achenes while no differences were found between weight classes (Figure 7.6).

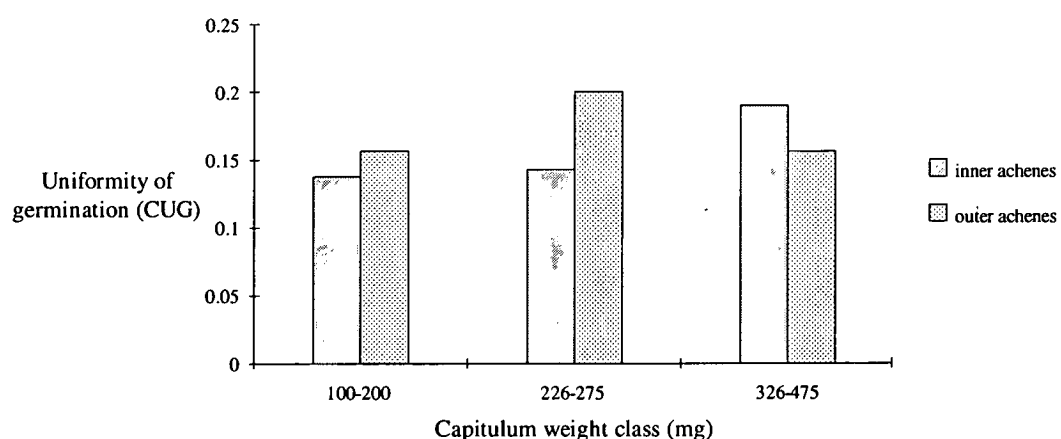
Figure 7.6 Mean time to complete germination of outer and inner achenes of capitula of different weights



Note (1) Differences between capitulum weight classes are not statistically significant at $P = 0.05$. Differences between achene position treatments are statistically significant at $P < 0.001$, $LSD = 0.19$ days.

Data presented in Figure 7.7 indicate that no differences in uniformity of germination were generated through the imposed treatments.

Figure 7.7 Uniformity of germination in various capitula size classes and achene positions

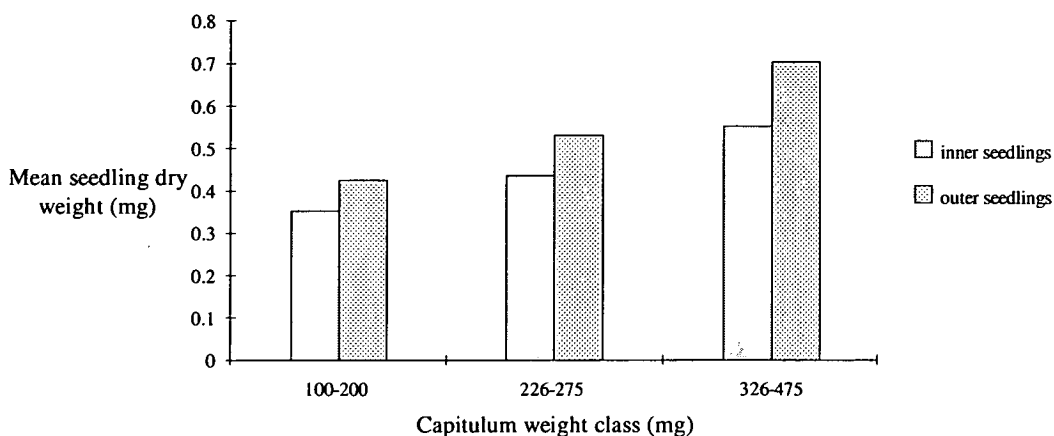


Note (1) Differences between all treatments were not statistically significant at $P = 0.05$.

7.4.5 Seed and seedling mass characteristics

Evaluation of mean seedling dry weights revealed significant differences between capitula classes and achene positions (Figure 7.8). Heavier seedlings were found to arise on seed from larger capitula, and in outer achenes.

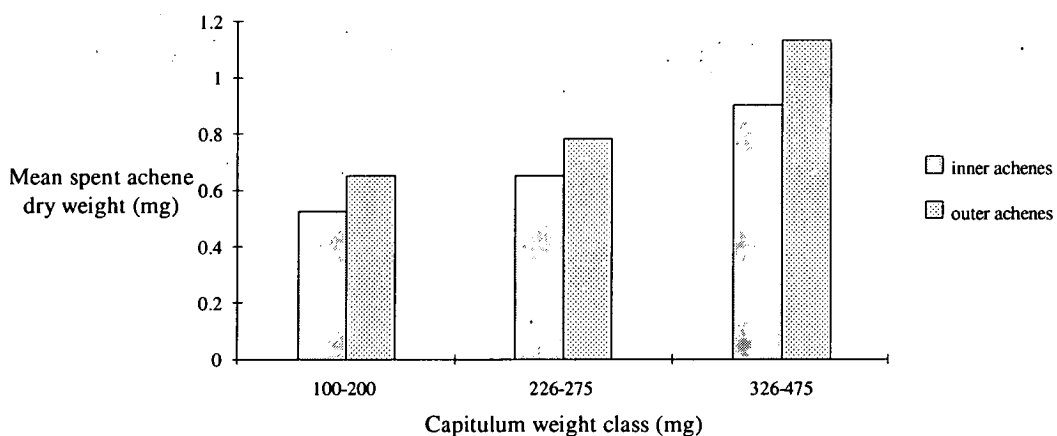
Figure 7.8 Seedling dry weights from various capitula weight classes and achene position treatments



Note (1) Differences between capitulum weight classes are statistically significant at $P < 0.001$, $LSD = 0.048$ mg. Differences between achene position treatments are significant at $P < 0.001$, $LSD = 0.038$ mg.

Heavier mean spent achene dry weights were found in heavier capitulum weight classes and in the outside achene position (Figure 7.9).

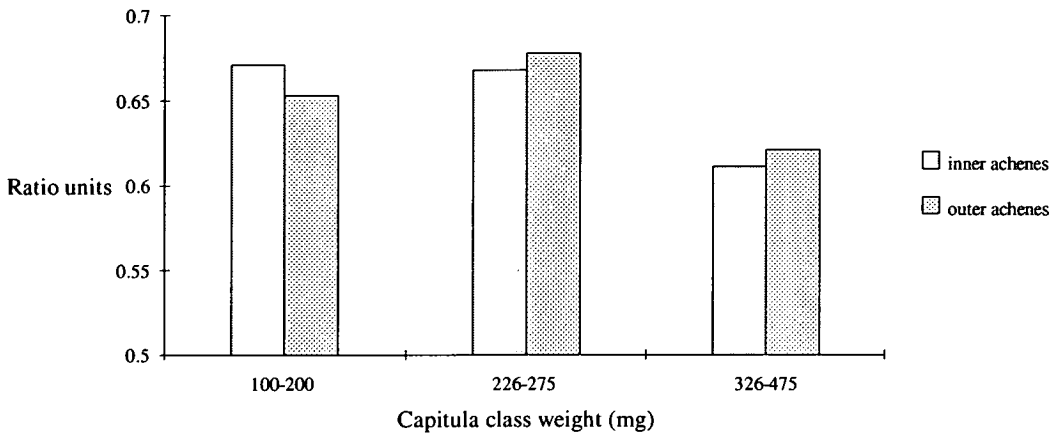
Figure 7.9 Spent achenes from various capitula weight classes and achene positions



Note (1) Differences between capitulum weight classes are statistically significant at $P < 0.001$, $LSD = 0.078$ mg. Differences between achene position treatments are significant at $P < 0.001$, $LSD = 0.063$ mg.

Seedling to spent achene ratio data demonstrate that achenes from heavier capitula possessed a slightly smaller embryo in proportion to the achene than found in smaller capitula (Figure 7.10).

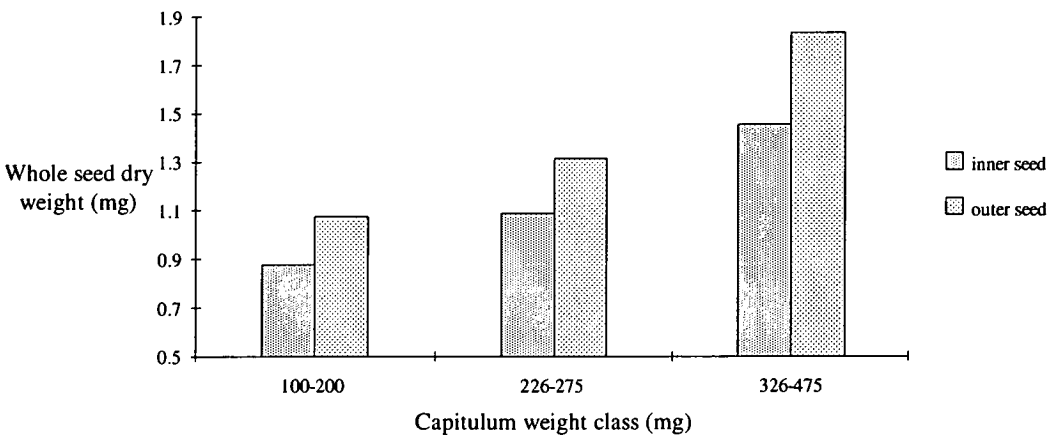
Figure 7.10 Ratio of seedling to spent achene dry weights for various capitula weight classes and achene positions.



Note (1) Differences between capitulum weight classes are statistically significant at $P < 0.001$, $LSD = 0.076$ units. (2) Differences between achene position treatments are not significant at $P < 0.05$.

Figure 7.3 presented data relating to mean achene weights. Samples in that evaluation contained both hollow achenes and seed. Figure 7.11 presents data where only seed that generated a seedling was included in the data set. Between 70% and 80% of the germinated seed from each treatment were therefore included. Seedlings not included in the analysis failed to develop to a stage which allowed easy separation from the spent achene by day 18 of germination.

Figure 7.11 Outer and inner seed of different capitula weight classes vs whole seed dry weights

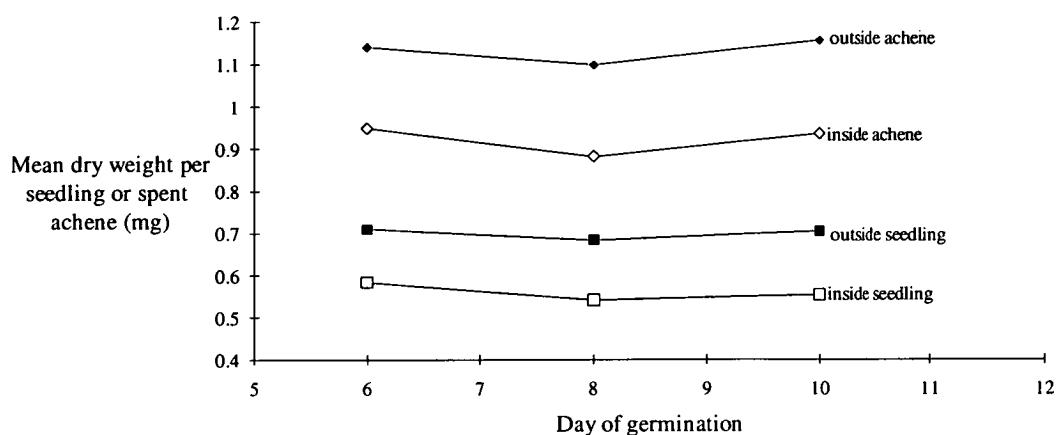


Note (1) Differences between capitulum weight classes are statistically significant at $P < 0.001$, $LSD = 0.124$ mg. Differences between achene position treatments are significant at $P < 0.001$, $LSD = 0.101$ mg.

7.4.6 Seedling weight and lateness of germination

Mean seedling and spent achene dry weights varying with lateness of germination in the heaviest capitulum class was investigated. Figure 7.12 reveals no differences in mean seedling or spent achene weights with seed germinating on different day treatments.

Figure 7.12 Mean seedling and spent achene dry weights of germinants from sequentially later days



Note (1) Differences in mean seedling dry weights between germination days were not statistically significant at $P < 0.05$.

7.5 Discussion

Many factors could have contributed to outcomes presented in the results. The purpose of the current work was not to investigate why differences appear, rather it was to demonstrate that differences exist and the extent of those differences. As the study was empirical, data may not be applicable to other seed production systems, seasons, locations or cultivars. This is the first study in which pyrethrum seed production has been reported. The results provide a base upon which further seed yield, seed quality and seed production investigations may be undertaken.

7.5.1 Variation in capitulum and achene mass

Variability in capitulum mass within the sample was closely correlated with capitulum diameter. The capitula population was found to approximate a normal distribution although it was skewed. Heavier capitula were found to have more achenes. In addition, heavier capitula possessed heavier achenes than lighter capitula. Research conducted in a wide range of species would suggest that observed differences in inflorescences are the result of different times of flowering (Cavers and Steel, 1984); and different positional effects (Fenner, 1992).

Within each capitulum class, centrally located achenes were found to be lighter. Although statistically significant, the difference between outer and inner achenes was small. Central achenes of large capitula were found to be still larger than the outside achenes of medium weight capitula. Variation in achene weight within capitula may be described as an example of cryptic heteromorphism, since there appears to be an orderly reduction in achene weight with centralness of the achenes within this highly organised structure, similar to *Tragopogon dubius* (Maxwell *et al*, 1994).

7.5.2 Proportion of fertile achenes and germination characteristics

The percentage of achenes containing seed almost doubled from the lightest to the heaviest capitulum class. Possible reasons why this occurred are, that there was more successful fertilisation occurring in the larger capitula, or that a significant proportion of the fertilised ovules aborted at some stage during development. If it can be assumed that much of the capitulum size difference is present prior to anthesis, then it is possible that in this crop, maternal provisioning of assimilates, rather than insufficient pollination was limiting seed set. Further studies are required to determine the mechanism by which reproductive effort is limited in capitula.

The smallest capitula demonstrated a significant, but minor, decrease from the higher germination capacity of larger capitula classes. The seed that failed to germinate did so under ideal germination conditions (20°C) and was found to be FUG. It is suspected that the lower germination percentage in the smallest capitula was due to inadequate maternal provisioning resulting in an impaired germination mechanism, but evidence to support this claim is merely circumstantial.

There were no significant differences between germination capacity of outer and inner seed in any of the capitulum classes. Although there may be evidence which suggests differences in maternal provisioning between capitula, there is none which indicates that inner achenes were less well provisioned than outer ones.

Rate of germination of outer seed was significantly greater than that of seed from the inner position. However, capitulum class had no significant influence on rate of germination. The evidence indicates that achene position in the capitulum, rather than seed weight or seedling size, *per se*, determined rate of germination. Previous differences in germination percentage, proportion of fertile achenes and achene dry weights, were tentatively explained through differential acquisition of maternal resources between capitula. In this case, rate of germination is being determined by position on the capitulum and is an example of behavioural somatic polymorphism. Further specifically

directed studies would be required to identify mechanisms involved in the observed germination delay and whether it can be lessened through some treatment to overcome the afterripening requirement. The current work indicates that variation in germination time within a seed lot may be largely due to differences within, rather than between, capitula. Although not examined, it is conceivable that in attempting to germinate seed in cold field conditions, inner achene seedlings would be slower to emerge than outer ones.

7.5.3 Seedling and spent achene characteristics

Seedling and spent achene dry weights increased with both capitulum weight and outer position. Data indicate that of the two variables, capitulum size limits seedling weight more than position on the capitulum. The benefits of large seededness are well documented in a wide range of crops. This work demonstrates that larger seed containing larger seedlings are obtained from heavier capitula.

Seedling to spent achene ratio data indicate that a greater proportion of maternal investment is committed to the achene in the largest capitulum than in smaller capitula classes. No such differences were noted between inside and outside achenes. The significance of this in pyrethrum is unclear, but in other species a greater investment in the integument is regarded as a greater protection for the seed (Maxwell *et al.*, 1994).

7.5.4 Germination time and seedling dry weight

In previous work it was identified that lateness of germination was related to seedling weight, with smaller seedlings emerging from the later germinants. In the current study, opportunity was taken to investigate whether this relationship still existed for within-capitula positions. Using the large capitula, it was demonstrated that from outside and inside positions, no decrease in seedling weight occurred with lateness of germination. The data demonstrate that within the variables studied, negligible variation in germination time due to seedling weight existed. The positional treatments accounted for most of the variation in germination time and seedling weight within the capitula class. Reductions in seedling weight with lateness of germination observed in prior studies may be explained by the positional differences demonstrated here.

7.5.5 Implications for seed production agronomy

This work has some immediate seed production implications. The weight of fertile achenes, the seedlings within them, and the percentage of achenes containing seed were closely associated with the capitulum weight. If small capitula could be eliminated from the sample, the result would be a seed sample containing larger seedlings. Furthermore, if a cleaning system could be developed which partitioned inner and outer achenes, the

result would be significantly more uniformly germinating seed lots. It is suspected that outer seeds, being quicker to germinate at 20°C than the inner seeds, may also perform better at low temperature, germinating to a higher percentage and doing so more quickly than inner seed. Clearly another study would be required to substantiate this suggestion.

Manipulating plant growing conditions such as plant density, climate, nutrient status and decreasing the number of seeds sharing limited maternal resources may all be ways of improving pyrethrum seed quality. The agronomy of seed production is clearly an area of study in which significant gains in seed quality are likely.

Chapter 8: General discussion: Agronomic and seed quality studies in pyrethrum

This final section summarises the main findings of the study in order to address the study objectives as outlined in the introduction (Chapter 1.6). In addition this work provides recommendations to industry based on the findings.

8.1.1 Density studies

The primary advantage perceived by industry in changing crop establishment practices was the dramatic reduction in costs from \$3 500/ha to an estimated cost of less than \$500/ha going from splits to sowing. Advantages in low establishment costs go beyond improving crop profitability. Less capital has to be allocated to crop establishment which increases flexibility for both the production company and the individual grower.

Beyond these advantages are the substantial increases in yield observed at higher plant densities. Data presented suggest that over a three season period, crops sown at high plant density will out-perform split-established crops at conventional densities. Harvested on two occasions, crops sown at high plant density may produce as much as 30% more yield than split-established vegetatively-propagated crops harvested on three occasions. Benefits to industry of high density sowings over vegetatively-propagated crops at conventional density are lower establishment costs, higher yields, decreased number of harvests, a shorter in-field time, and greater industry flexibility. The economic impact to the industry of establishment from seed is expected to be profound.

There were no plant density studies on pyrethrum in cool temperate environments prior to the current study. Differentiating the current work further from previous studies was that plants in this study were established from seed and a greater plant density range was examined than in previous studies.

The recommendation to industry is to aim to establish populations above 16 plants/m² to achieve maximum pyrethrins yields and below 35 plants/m² in order to minimise self thinning in the second season. If sowings are to be conducted in late spring on low fertility sites or on inland locations, plant densities towards the upper limit may be expected to produce higher first year yields.

The yield component investigations provided valuable insight into how pyrethrins yield varied with density. This information begins to provide some understanding of growth and development in the plant. The work is expected to help direct further agronomy and plant selection investigations aimed at increasing crop yields, and improving the

suitability of pyrethrum plants to this environment and the new crop establishment system.

8.1.2 Time of sowing

Even if conditions are suitable for germination and seedling establishment, yield may be limited by plant growth, or the extent to which it flowers. The time of sowing study revealed that a minimum time of approximately 15 months was required from sowing to first harvest. This equated to a mid-November sowing in Tasmania.

In the spring trial decreasing flowering tillers/plant in sequentially later sowings resulted in yield reductions. Data indicate that over the four sowings, some compensation for the reduced tiller number occurred through increases in mean flower weight. The January sowing, having been in the ground for 13 months prior to first flowering, demonstrated both a substantial increase in incidence of 'blind plants' and a reduction in extent of flowering per plant. Results indicate that January sowing was too late for a commercial yield.

Sowing in mid-September, with in-field time of 17 months, resulted in lower plant densities, making yield comparisons with other spring sowings difficult. The advantage, or otherwise, of September sowings over October and November remains unresolved.

With the current variety, mid-October to mid-November sowings are recommended. Earlier sowings may be conducted if high plant density can be achieved. Mid-October sowing is recommended to provide a long period for plant growth prior to first harvest.

8.1.3 Emergence and seed quality studies

Time of sowing trials indicated that both percentage and rate of emergence were influenced by temperature. Slow and very low percentage emergence of the seed during cooler times was considered a constraint to industry flexibility in sowing time and site selection. Investigation of the germination characteristics of a commercially used seed lot under a range of constant temperature conditions revealed that percentage germination was low at 10°C, as was rate and uniformity of germination. These were all considered to be important germination parameters. Seed failing to germinate at temperatures below 10°C were predominantly found to be fresh ungerminated rather than dead.

Further studies on six seed lots at 10°C revealed substantial reductions in germination capacity compared to that at 20°C. Furthermore there were significant differences between seed lots in several important germination characteristics at 10°C. Correlations

between various germination parameters and some limited information relating to maturity of the seed at harvest, led to the development of a hypothesis which explained differences between seed lots. The hypothesis related seed behaviour to seed maturity and this was tested in a further study.

As germination characteristics change with time due to both deterioration and satisfaction of afterripening requirements, seed lot performance was evaluated again after a storage period. The main findings were that there were no significant changes in germination capacity of the seed with storage, but the seed apparently overcame an afterripening requirement, allowing it to germinate in a shorter period of time.

Opportunity was also taken in this experiment to conduct preliminary investigations into mean seed and seedling weight differences between seed lots. Although large differences in weight were found between seed lots, they did not correspond to germination parameters in a consistent way. Further more detailed work was conducted to investigate various aspects of seed weight in pyrethrum seed.

8.1.4 *The effect of seed weight on germination and early seedling development*

Separating two seed lots into different seed weight classes revealed that weight had very little influence on germination capacity or rate of germination. Factors other than seed weight were predominantly responsible for differences both within and between seed lots. A further experiment examined the weight characteristics of successively later germinating cohorts of seed from six seed lots. Minor differences in seed weight with lateness of germination were identified. Those differences were subsequently attributed to substantial decreases in mean seedling weights with lateness of germination. Seeds with heavier seedlings demonstrated faster seedling development than did those with lighter seedlings.

When seedling development rate was investigated in a comparison between two seed lots, heavier seed classes were found to reach cotyledons open more rapidly than lighter classes. Data presented revealed that two groups, fast and slow developing seedlings, were present in different proportions in different weight classes. Separating seed on a weight basis was only partially successful in separating seed into two groups displaying differing rates of seedling development. Even so, within a given seed lot, heavier seedlings emerged from heavier seed, and as larger seedlings have a better chance of establishment than smaller seedlings, further cleaning of seed on a weight basis would be expected to increase seed quality.

8.1.5 Effect of harvest date on seed quality

The first series of germination studies proposed that seed maturity at harvest may be responsible for significant variation in germination parameters. This study demonstrated that significant differences in germination characteristics were indeed determined, to a significant degree, by seed maturity. The earliest harvest resulted in seed that failed to germinate and that was subsequently found to be dead. Seed harvested early was also found to be slow to germinate at both 10°C and 20°C.

Seed harvested latest was also slow to germinate at 10°C. Differentiating this from seed harvested too early was the observation that of the seed remaining ungerminated, none or very little was found to be dead. The work agreed with the hypothesis that differences in germination behaviour observed between the six seed lots in the initial studies were largely due to differences in maturity at harvest.

This trial also investigated whether small, slow-to-develop seedlings were associated with stage of maturity at harvest. Data revealed that substantial decreases in seedling weight occurred with lateness of germination regardless of harvest date. Therefore, low weight, slow to germinate seedlings existed in seed lots regardless of maturity. Factors other than maturity were suspected to be responsible for the presence of that seed. Subsequent studies provided insight into the occurrence and behaviour of late-germinating seed observed to containing small seedlings.

It was recommended that seed crops be harvested at 25% moisture. Harvesting pyrethrum seed crops at an appropriate stage of maturity will significantly improve seed quality. Further work to more clearly define optimum harvest time of pyrethrum seed is recommended to confirm the current findings.

8.1.6 Seed quality and production associated with capitulum size and achene position

This work showed that more seeds were produced in larger than smaller capitula. Furthermore, the seeds produced were heavier than those from smaller capitula. Variability in seed weight also existed within capitula with peripheral seed heavier than those which were more centrally located.

Investigation of the germination characteristics of differently positioned seed revealed that germination capacity was marginally lower in seed from small capitula. More important was the finding that centrally positioned achenes took significantly longer to germinate than outer achenes. Capitulum weight had no influence on germination rate.

The trial demonstrated that seed position on the capitulum rather than seed mass *per se* was responsible for variation in germination time observed within seed lots.

With significant differences in seed yield and germination behaviour demonstrated both between capitula and due to position on the capitulum, different seed harvesting and cleaning strategies should be considered by the industry. Techniques developed to firstly eliminate small capitula from the sample and secondly to enable partitioning of peripherally and centrally located achenes should be investigated as ways of improving seed quality.

8.1.7 Conclusion

Tasmania is the only cool temperate region currently growing commercial quantities of pyrethrum. Over the past 20 years the industry has developed high yielding cultivars, extraction and purification plants, nutrition, plant protection and irrigation strategies, developed relationships with land owners, governments, contractors, buyers, and local communities. Its continued capacity to provide work for Tasmanian people and contribute to the local community will depend on its commitment to improving current practices and maintain flexibility to meet continually changing market demands.

Field and seed quality studies provide industry with recommendations suitable for immediate implementation, as well as providing direction for further research. Agronomic studies demonstrated the benefits of sowing at high density and identified optimum sowing times. Seed studies quantified variation in germination behaviour both within and between seed lots, discussed what impact that variation may have, and investigated determinants of pyrethrum seed quality. Access to high quality seed is of critical importance to successful crop establishment.

No research has been undertaken investigating the agronomy of seed production in pyrethrum. Nevertheless, some research conducted in the current studies indicate that significant advances would be likely from such work. Results presented in both the density and time of sowing studies indicate that flower dry weight may be significantly manipulated through varying these agronomic practices. Furthermore, gains in seed quality may be made through similar attention to the agronomy of seed production.

Afterripening requirements of pyrethrum seed requires investigation in order to achieve rapid, uniform germination under low temperature conditions. Seed agronomy and afterripening studies will ultimately lead to improved seed performance and increased industry flexibility.

The Tasmanian pyrethrum industry is arguably the most efficient producer of pyrethrins in the world. Recommendations made in the current work, if implemented, will further increase profitability and crop acceptability in the local community. The challenges of recommendation implementation and future well directed research lie in the hands of BRA management. I thank the industry for the opportunity to conduct the current study and wish it a long, productive and profitable future.

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Appendix

1.1 Flower maturity

Table A.1 describes the developmental stages of pyrethrum flowers. These have been slightly adapted from Ikahu and Gnugi (1989) and are the maturity stages currently used to assess flower maturity by the Tasmanian pyrethrum industry.

Table A.1 Stages of flower development in pyrethrum flowers

Stage	Description
Button	Well developed, closed bud with no emergence of petal flowers (ray florets)
1	Well developed closed but with petal florets visible
2	Petal florets vertical
3	Petal florets horizontal with no more than one whorl of disc florets open
4	2-3 whorls of disc florets open
5	If r is the radius of disc, then r/2 florets open (i.e., 75% disc area with open florets).
6	Between 75% and 100% of the disc florets open i.e., middle disc florets just opening.
7	Petal florets intact, colour of disc florets diminishing.
8	Petal florets dried out, colour of disc florets diminishing.
9	Some disc florets fallen out. Stem drying out (brown <20 mm below the flower head)
10	No disc florets strongly held. Stem brown > 20 mm below the flower head

Mean flower maturity was calculated by sorting a sample of fresh flowers (usually approximately 200 g) into flower maturity stages as described in Table A.1. The number of flowers in each stage was counted. A Flower Maturity Index or (FMI) was calculated according to Equation 1.

$$FMI = \sum(x_i \times n_i) \times 100 / \sum n \text{ (Equation 1)}$$

Where

- x_i = stage of maturity,
- n_i = flower number in stage x_i .

Spread in maturity of flowers was assessed by calculating the standard deviation of a sampled population of flowers.

1.2 Pyrethrins assay

The percentage of total pyrethrins present in dried pyrethrum flowers can be assessed by several different methods including spectrophotometric analysis (Casida, 1973) and analysis by high performance liquid chromatography (HPLC) (McEldowney and Menary, 1988). Although the HPLC method provides further information about the sample in addition to total pyrethrins, such as the presence and quantities of individual pyrethrins and other components in the sample, the method requires expensive equipment and was therefore avoided. Spectrophotometric analysis is the method used for pyrethrins assay in

the current work and is the method used in most reported agronomic studies. Therefore data presented in the current work are comparable to percentage pyrethrins data reported in those studies. The following procedure (first described by Beckley, 1950) is currently used for initial screening of flowers for pyrethrins assay in the plant breeding programme at the University of Tasmania.

Spectrophotometric analysis

The sample was extracted with petroleum ether (40-60°C - boiling point). This solution was diluted in ethanol and its absorbance read at 227 nm.

Reagents

Petroleum ether (redistilled), non-contaminated ethanol and Pyrocite.

Method

Flowers which were previously dried at 50°C for 36 hours, sealed in plastic bags and stored at -18°C were removed from storage and subsampled. Grinding was achieved using a fine small-size grinding mesh and the grinder was thoroughly cleaned using compressed air between each sample. Approximately 15 to 20 flowers per sample were ground into a fine powder. Approximately 0.5 g of the powder was accurately weighed out into a 25 ml volumetric flask. Pet-ether was added, making the volume up to 25 ml. Extraction was allowed to proceed in the dark for two hours. Volumetric flasks were turned twice during the extraction and again during standing.

An aliquot of 0.5 ml of the extracted solution was placed in a 50 ml volumetric flask and the volume subsequently made up to 50 mls with ethanol. This sample was then ready for spectrophotometric analysis.

Determination of powder dry weight

Since dried flower samples may still contain some water and pyrethrins assay is calculated on a flower dry weight basis, the amount of water in the sample was determined. Approximately 2.0 g of each ground flower sample were placed in oven tins immediately after grinding and weighed. Samples were dried in an oven at 110°C for 2.5 hours, placed in anhydrous jars for 0.5 hours to cool, and reweighed. Percentage dry matter was then calculated as follows;

$$\%DM = (\text{dry weight}) \times 100 / (\text{wet weight}) \quad (\text{Equation 2})$$

Preparation of blank and standards

A blank was prepared by pipetting 0.5 ml of pet-ether into a 50 ml volumetric flask and filling to 50 mls with ethanol. The blank was used every three or four samples to zero the spectrophotometer for $\lambda=227$ nm.

Approximately 0.1 g pyrocidine was weighed into a 100 ml volumetric flask and the volume made up with 'blank' solution. This stock solution was subsequently diluted to approximately 0.05 mg/ml pyrethrins. Aliquots of between 1-20 ml of dilute stock solution were pipetted into 50 ml volumetric flasks making a series of solutions with concentrations of 0.1-2 mg pyrethrins/100 ml upon filling to 50 mls with blank solution. Absorbances of the dilution series were graphed. The points formed a straight line with a relationship of one absorbance unit approximately equal to 1 mg/100 ml pyrethrins.

Calculations

The formula of the standard curve is:-

$$\text{Absorbance (AU)} = a \times \text{concentration (mg/100 ml)} + b \quad (\text{Equation 3})$$

then for samples:-

$$\text{Concentration (mg/100 ml)} = (\text{absorbance} - b)/a \quad (\text{Equation 4})$$

To calculate the pyrethrins content as a percentage of dry matter:-

$$\% \text{ Py} = (\text{Absorbance} - b) / a \times 100 / \% \text{DM} \times 5 \quad (\text{Equation 5})$$

1.3 Germination parameters*Final germination percentage (%FG)*

%FG is a measure of germination capacity. Since some seed may germinate too late to be counted in a germination test, %FG can only be considered as an indicator of germinability.

$$\text{FG \%} = \frac{\text{no. of seeds germinated} \times 100}{\text{total no. achenes incubated} - \text{hollow achenes}} \quad (\text{Equation 6})$$

Mean time to complete germination (t) in days

Mean time to complete germination t gives an average measure of time elapsed for individual seed germination. t is the reciprocal of the mean rate of complete germination (R)

$$t = \frac{\sum (t_x \cdot n_x)}{\sum n} \quad (\text{Equation 7})$$

Where

t_x = time in days starting from day zero as day of wetting, and
 n_x = number of seeds germinating on day x

Three day moving average of germination rate.(3DAYAV)

Rate of germination may also be described by plotting % germination/day against time. Graphical representation of germination rate can provide information about the seed lot germination homogeneity. To reduce day-to-day variability it is useful to plot three day moving averages which are calculated as follows:

$$3 \text{ DAYAV} = \frac{n_{x-1} + n_x + n_{x+1}}{3} \quad (\text{Equation 8})$$

Where

n = % of seed germinating
 x = the day in question

Coefficient of uniformity of germination (CUG)

Coefficient of uniformity of germination (CUG) gives a measure of spread in germination and is expressed as a variance of individual time (t_x) around the mean time to complete germination (t).

$$(CUG) = \frac{\sum n}{\sum [(t - t_x)^2 \cdot n]} \quad (\text{Equation 9})$$

Percentage of seed fresh ungerminated (% FUG) or dead (%D)

Causes of non-germination may include; initial non-viability, viability loss as a treatment effect, random losses through accident or contamination, or premature termination of the test, e.g., dormancy (Scott *et al.*, 1984). Viability of the ungerminated seed remaining was determined using a 'squash' or 'pinch' test as described by Walck *et al.* (1997a).

After the incubation period, seed remaining ungerminated in each dish was examined. The seed was subject to a squash test, which resulted in achenes being grouped as fresh ungerminated, dead, or empty. To be assessed as fresh ungerminated (%FUG) the

embryo had to appear translucent and have no necrotic lesions. Dead seed (%D) included all other achenes containing seed. Empty achenes were not considered as seed and not included in germination data. The %FUG and %D values were therefore calculated as follows:

$$\%FUG \text{ or } \%D = \frac{100x}{\text{no. achenes incubated} - \text{hollow achenes}} \quad (\text{Equation 10})$$

Where

x = no of seed found to be healthy or dead (as determined by a squash test) at the end of the incubation period.

Seedling evaluation

Following germination, air tweezers were used to transfer the germinated seed to slopes similar to those described by; Smith et al (1973a and 1973b), Wurr and Fellows (1984), and Grey and Steckel (1983b). Seed transfer continued on a daily basis until the number of seeds germinating per day was very low or no seeds remained ungerminated. Slopes were maintained in a growth cabinet (15°C +/- 0.5°C). Seedlings were then removed from the slopes once they had reached cotyledons open. 'Cotyledons open' was defined as when cotyledons were approximately perpendicular to the stem. Seedlings were subsequently removed from the slope along with the empty achene, oven dried (70°C for 3.5 hours) and dry weights determined.

Data generated through the test were expressed as:

- i. Percentage cotyledons open (%CO): The percentage of germinated seeds achieving the cotyledon open stage
- ii. Time interval from mean time to complete germination to mean time to complete cotyledons open ($\overline{co-t}$).
- iii. Mean time to complete cotyledons open (\overline{co}) gives an average measure of time elapsed for seedling cotyledons opening

$$\overline{co} = \frac{\sum (t_x \cdot co_x)}{\sum co} \quad (\text{Equation 11})$$

Where

t_x = time in days starting from day zero as day of sowing

co_x = number of seeds reaching cotyledons open on day x

Appendix 1.4
Numbers of plants or areas harvested in the first flowering season

Code	Nominal Density (plants/m ²)	Plants Harvested			Area Harvested		
		H1	H2	H3	H1	H2	H3
A	2	1	1	2			
B	4	2	2	2			
C	8	4	4	4			
D	16	4	4	4			
E	24				0.2 m ²	0.2 m ²	0.2 m ²
F	32				0.2 m ²	0.2 m ²	0.2 m ²
G	64				0.2 m ²	0.2 m ²	0.2 m ²
H	100				0.2 m ²	0.2 m ²	0.2 m ²

Appendix 1.5 Number of tillers or plants assessed for flowers/tiller in each of three sequential harvests in the first season block trial

Treatment		H1 (5 reps)	H2 (6 reps)	H3 (6 reps)
Code	Density	Tiller no.	Tiller no.	Plant no.
A	2	6	6	2
B	4	12	12	2
C	8	18	18	4
D	16	18	18	6
E	24	18	18	approx.7
F	32	18	18	approx.7
G	64	18	18	approx.7
H	100	18	18	approx.7

Appendix 1.6 Numbers of plants or areas harvested in the second
flowering season, block trial

Code	Nominal	Plants	Area
	Density	Harvested	Harvested
	(plants/m ²)	H1	H1
A	2	4	
B	4	6	
C	8	8	
D	16	8	
E	24		0.5 m ²
F	32		0.5 m ²
G	64		0.5 m ²
H	100		0.5 m ²

Appendix 1.7

Polynomial equations for each of the cumulative emergence profiles

Sowing date	Polynomial equation	R ² value
Sept. 15	$y = -0.0532 x^2 + 6.0354 x - 70.121$	0.93
Oct. 15	$y = -0.0534 x^2 + 5.8667 x - 56.026$	0.89
Nov. 15	$y = -0.0245 x^2 + 2.2812 x + 49.429$	0.97
Dec. 15	$y = -0.0058 x^2 + 6.528 x + 82.389$	0.88
Feb. 15	$y = -0.0272 x^2 + 2.873 x + 26.235$	0.93
Mar. 15	$y = -0.0702 x^2 + 6.9879 x - 69.169$	0.95
April 15	$y = -0.0444 x^2 + 5.7047 x - 81.94$	0.96

Note. Equations are only intended to accurately reflect cumulative germination profiles within the 80%-100% of maximum cumulative germination range.

Appendix 1.8

Calculation of day degrees per day at FVRS

Date	Min. °C	Max. °C	Av. °C	Day Degrees
Sept. 15-Oct.1.	5.34	13.64	8.80	3.80
Oct. 15-31.	4.91	15.4	8.23	3.28
Nov.15-Dec.1	8.14	17.02	11.54	6.54
Dec.15-31.	9.46	19.02	12.59	7.59
Feb. 15-Mar.2.	10.62	19.93	14.96	9.96
Mar.15-31.	10.10	19.02	13.73	8.73
Apr.15-May 1.	5.59	15.37	10.11	5.11

Note (1) Day degrees were calculated as the average temperature (average maximum less average minimum over the 17 days) minus an estimate of a base temperature of 4°C.