

**REPRODUCTIVE POTENTIAL OF
SOLANUM MAURITIANUM SCOP. -
IMPLICATIONS FOR CONTROL**

by

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M.Sc. (Natal)**

**Submitted in partial fulfilment of the requirements
for the degree of**

DOCTOR OF PHILOSOPHY

in the

**Department of Botany, University of Natal,
Pietermaritzburg, Republic of South Africa**

December 1990

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PREFACE

The experimental work in this thesis was carried out under the supervision of Professor J. van Staden, Department of Botany, University of Natal, Pietermaritzburg, from March 1986 to April 1990.

These studies have not been submitted in any form to another University and, except where acknowledged in the text, are the results of my own work.

Peta Laurie Campbell

ACKNOWLEDGMENTS

I would like to thank the Plant Protection Research Institute for their financial support and for allowing me to enrol on a part-time basis for this degree. I am grateful for the support of Professor van Staden and for his belief that a student whose best quality is to work hard can accomplish a doctoral thesis. Thanks also to Dr Mike Smith for his expertise with various equipment and his open positive attitude to solving "slight technical hitches". I am grateful to Professor Drewes for his guidance and for making my work in the Chemistry Department a pleasure. Thanks to Mrs Barbara Herbert and Mr Dave Figenschau for their patience with a chemistry novice, reteaching me basics I had forgotten 15 years ago.

I greatly appreciated the excellent technical assistance from Mr Derek Tunnington, Mr Martin Hampton, Mr Doug Boddy, Mr Maurice Smith, the Mechanical Instrument Workshop, Mrs Linda Davies and Mr Anton Ndaba. I am also grateful for discussions with or assistance from my colleagues Mr Robin Denny, Mr Jeremy Goodall, Mrs Trudie Voordewind, Mrs Judy Greyling, Mr Rod Bell, Mr Alan Carser, Dr Peter Thomas, Mr Piers Whittle, Miss Lynet Khumalo and Mr Raphael Meriwa.

A big thank you to the very long-suffering librarians at Cedara, Mrs Mary Cummins, Trish Weston and Rhona van Niekerk - without a doubt they provide the best library service in the country. Mary Cummins also cheerfully proofread my references, probably the worst thing I ever asked her to do! I am indebted to the Agrometeorology Department at Cedara for relevant data, and to Jackie Webb from Ukulinga and Mr Atkinson followed by Mr Mansfield from Umdoni Park for their help with recording soil temperature fluctuations. Thanks to

relevant personnel for allowing me to do this work at the two aforementioned properties. Thanks to Mr Harry Haigh (Dept. of Environment Affairs) for discussions on practical aspects of weed control, and Mr Danny Jordaan for supporting the work done at Cedara State Forest. Thanks also to Dr Stefan Naser (PPRI, Pretoria) and Dr Johnny Hoffman (University of Cape Town) for providing bugweed seeds from Pretoria and Cape Town, respectively.

I appreciate the patience and expertise of Mrs Marie Smith, Mrs Cathy Stevens, and Mr Harvey Dicks for the biometrical analyses of results from this project, with special thanks to the data operators and to Mrs Margie Whitwell who carried the load extremely well when no biometrician was available at Cedara. I am very grateful to Professor Abbott, who has taught me a tremendous amount about good logical scientific writing, since bad English was something up with which he simply would not put. His scientific experience and literary expertise were invaluable in teaching me to handle this volume of work.

A big thank you to Carol de Beer, for all her willing effort in typing this manuscript. I am also indebted to Mrs Hannelie de Jager for teaching me Harvard Graphics, used for colour charts in this thesis. Thanks to Dr Hendrik Meyer, my benchmate at the Botany Dept. His sense of humour (and the occasional looted glass of wine) kept me going over several rough patches.

Thanks most of all to my parents who encouraged me to keep going (bribed me, actually) and to my friends Norman and the late great Connie Myers, all of whom didn't always understand what on earth I was talking about, but listened anyway.

ABSTRACT

Solanum mauritianum Scop. is rated the worst invader species in pine plantations throughout the Republic of South Africa. Control is costly and apparently ineffectual since the species is spreading in pine plantations at a rate of 16 % per annum. This is due to the high reproductive potential of the species.

S. mauritianum produces fruits throughout the year. Fruit and seed yield is related to tree size. *S. mauritianum* produced approximately 7.2 million viable seeds per hectare during 20 months when growing under conditions unfavourable for growth. Seeds are efficiently dispersed by animals and birds.

Although high seed or seedling mortality occurs, the initial prolific seed production and efficient dispersal ensures the rapid spread of this species in South Africa. Surviving seeds form the source for both further encroachment and reinfestation of areas in which *S. mauritianum* has been controlled.

S. mauritianum seeds require the presence of both light and alternating temperatures for optimum germination. Transfer of seeds from unfavourable to optimum conditions or the application of gibberellic acid (GA_3) can promote high germination percentages. However, the germination requirements of *S. mauritianum* are highly variable.

Germination is influenced by site, season and year of seed shed. Seeds varied in terms of primary dormancy; conditional dormancy; the response to transfer from unfavourable to favourable conditions; the response to application of GA_3 ; and

the occurrence of secondary dormancy. Germination requirements of seeds were also influenced by site, duration and depth of burial. All these factors contribute to a sporadic seedling emergence over a prolonged period, which results in current control operations being both costly and ineffective.

Alternative control methods were therefore considered. These included the application of herbicides or heat to kill seeds, application of various growth regulators to stimulate germination, and the chemical extraction of alkaloids from fruits and seeds for use in the pharmaceutical industry.

Two alkaloids (solasodine and a new molecule) were extracted from green bugweed fruits growing under unfavourable conditions. Although levels of solasodine extracted were very low compared with those from commercially grown species of this genus, *extraction of the second alkaloid raised the potential of the species for utilization purposes*. Utilization of the reproductive propagules could reduce the continual dispersal of seeds and thereby contribute to long-term control of this species.

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CHAPTER 1:

FRUIT AND SEED YIELD OF BUGWEED

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CHAPTER 1

FRUIT AND SEED YIELD OF BUGWEED

A. INTRODUCTION

(i) DESCRIPTION

Solanum mauritianum Scop. was known previously as *S. auriculatum* Ait. The name *Solanum* comes from the Latin "solacium" (solace/quieting) referring to the sedative properties of various glycoalkaloids commonly found in the genus.

Solanum mauritianum is a large shrub or small tree up to 3 or 4 m high (SYMON, 1981). All parts are densely pubescent with a tomentum of stellate hairs. Leaves are variable in size depending on the vigour of the plant, ranging from 10 x 4 to 30 x 12 cm and the shape is elliptic, acuminate and with bases cuneate. Leaves are sometimes slightly unequal with pubescence denser on the paler abaxial surface. The petiole is 3 to 9 cm long; and each leaf has one or two smaller auricle-like, sessile, rounded leaflets in their axils. These leaflets are absent on smaller twigs or weak growth.

SYMON (1981) goes on to describe the inflorescence as being a dichotomous, branched corymb with many flowers; the peduncle is up to 15 cm long to the first fork with pedicels 2 to 3 mm long. The anthers are 2 to 3 mm long, the ovary is densely pubescent with a 5 to **7 mm long**

pubescent style and green stigma. The fruits are 1 to 1.5 cm in diameter, globular, pubescent and yellow when ripe. There are between 100 and 250 seeds per fruit. Each seed is 1.5 to 2 mm wide, light brown in colour, with a finely reticulate surface. *Solanum mauritianum* is depicted in Figure 1.1.

(ii) **ORIGIN**

S. mauritianum shows considerable morphological variation in its countries of origin and this has contributed to the confusion about the exact identity and origin of the weedy entities. *S. mauritianum* appears to be part of a complex of closely related species eg. *S. granuloso - leprosum* Dun., *S. riparium* Pers., *S. erianthum* D. Don and *S. verbascifolium* L., which show considerable overlap in morphological characteristics. Some of these species share the same common names (WELLS & FOURIE, 1987; NESER, ZIMMERMANN, ERB & HOFFMANN, 1988).

S. mauritianum was first described in 1788 by Scopoli from material collected in Mauritius where it was thought to be indigenous. However, approximately 200 years later SYMON (1981) put forward the idea that the plant originated somewhere in tropical South America, and became naturalized in tropical and subtropical Africa, Asia, Australia and the Indian Islands. Common names for *S. mauritianum* in South Africa include bugberry, bugtree, bugweed, bugwood, wild tobacco (English); groot bitterappel, luisboom (Afrikaans) and bongabonga (Zulu). *S. mauritianum*, will be referred to by the common name bugweed in the rest of this thesis.



FIGURE 1.1: *Solanum mauritianum* Scop.

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(iii) **SPREAD OF BUGWEED IN SOUTH AFRICA**

FORCELLA (1985) commented on the rate of spread of alien invaders when introduced to a new continent. After introduction, alien weeds may spread widely or only very little and quickly or very slowly (SALISBURY, 1961). Some aliens may begin to spread immediately upon introduction or they may require repeated introductions or may go through a lag phase during which they build sizable but local populations with "export potential" (DAFNI & HELLER, 1982). Other aliens show a sudden increase in spread when changes occur in environmental conditions which facilitate their dispersal. For example, huge floods during 1955 caused a rapid spread of *Opuntia aurantiaca* Lindley in eastern New South Wales (AULD, HOSKING & MCFADYEN, 1982/83). Similarly, weed invasions may be blocked by environmental barriers such as length of drought or the presence of frost (FORCELLA & HARVEY, 1983).

In South Africa, large tracts of bushveld are very dry and inhospitable to mesic invaders such as bugweed (HENDERSON & MUSIL, 1984). Bugweed is, at present, not a problem on the coastal regions of Natal and the Cape, where salty coastal winds and warm moist conditions are more conducive to growth of other invader species. Instead, the large leaves of bugweed make the plant more suited to a milder, more mesic climate. Thus when bugweed was introduced, ideal conditions for its spread existed in the cool moist midland parts of the country.

WELLS & FOURIE (1987) reviewed the literature pertaining to the history of bugweed in South Africa. The earliest record of bugweed in

South Africa is a collection made in 1862 on the Natal Coast. In 1881 bugweed was restricted to the vicinity of towns along the Natal Coast (HARDIN, 1938) but it was noted that birds were eating the fruits. By 1877 bugweed was described as "frequent by roadsides and on the borders of woods" (BAKER, 1877). By 1890 bugweed had spread to the southwestern Cape, and in 1905 it was recorded in the Transvaal. By the 1930's it was abundant in Natal (RIPLEY & HEPBURN, 1935) the most infested districts being Pietermaritzburg, New Hanover, Camperdown, Durban and Umzinto (ANONYMOUS, 1937) where it occurred on the fringes of forests (PRINCIPAL BOTANIST, 1934).

Bugweed invades relatively undisturbed natural ecosystems and competes physically with indigenous plants. Other undesirable features of bugweed are that the fruit provides a winter feeding ground for the fruit fly *Pterandrus rosa* (RIPLEY & HEPBURN, 1935). The plant also acts as a host to certain strains of microbes such as *Pseudomonas solanacearum* (PEGG & MOFFETT, 1971). It has radically altered the feeding behaviour of the Rameron pigeon (*Columba arquatrix*) which could have serious consequences for indigenous forest trees such as *Olea*, *Ocotea* and *Prunus* species, whose seeds were formerly dispersed by this bird (OATLEY, 1980; WELLS & FOURIE, 1987). In tropical forests of Queensland, Australia, the presence of ripe bugweed fruits has altered the feeding behaviour of the brown pigeon (DREW, 1988). The fruits are infested with eggs and larvae of fruit fly during the breeding season of the pigeon. These infested fruits contain about twice as much amino acids as are found in uninfested fruits. DREW (1988) suggested that increased

amino acid levels enrich the "milk" the pigeon regurgitates from its crop for its young.

Bugweed is fast growing and can, at an early stage, compete vigorously with young pine trees, causing loss of increment and deformation of the stems (HINZE, 1983). In older pine stands it soon becomes troublesome in silvicultural and harvesting operations. Once the stand has been felled and the logs removed from the compartment, bugweed seeds germinate readily in the disturbed area and compete with the next rotation of planted pine seedlings (GOODALL, 1985). Vigorous multi-stemmed coppice growth can occur when the plant is slashed, and this increases the density of an infested area, further impeding access for silvicultural operations.

As the timber industry flourished in Natal, it increased the area suitable for mesic invaders such as bugweed (Plate 1). The forest habitat has proved to be so suited to bugweed that this weed has become a major problem in silvicultural areas. In a survey of pine plantations in both state forests and private lands, LE ROUX (1984) reported that a total of approximately 70 400 ha in the Republic of South Africa was infested with bugweed. This formed 27.6 % of the area in pine covered by all invader species (Table 1.1). *S. mauritianum* has, in recent years, made its appearance in pine stands on mountain slopes of the eastern Cape and covers an area of ca 124 ha (Table 1.1), and the author advised "... if steps are not taken immediately to eradicate this source, the plantations in the Cape Province will in the near future be invaded by this plant species". At present the



PLATE 1: Dense stands of bugweed impede access to silvicultural and harvesting operations in pine plantations.

TABLE 1.1: Area of pine plantations in South Africa covered by bugweed (LE ROUX, 1984).

| Area of infestation by weed invaders | Area infested by bugweed in different regions of South Africa (ha) | | | |
|---|--|--------------------|-----------|------------------------------------|
| | Cape | Natal and Zulaland | Transvaal | Total in R S A |
| Area covered by bugweed (a) | 124 | 25 275 | 45 018 | 70 417 |
| Area covered by all invader species (a) | 24 770 | 84 036 | 146 208 | 254 920 |
| % infested area covered by bugweed | 0.5 | 30.1 | 30.8 | 27.6 |
| Area covered by bugweed (b) | 105 | 4 115 | 12 913 | 17 123 (1983) (c) 87 700 (1993) |

- (a) Total area of infestation in state and private pine plantations.
- (b) Areas of infestation in state plantations only.
- (c) Estimated area infested after another 10 years.

main infestations however, occur in Natal and Zululand as well as the Transvaal (Table 1.1).

Based on a 16 % annual rate of increase, LE ROUX (1984) estimated the area covered by bugweed in state plantations would increase from 17 123 hectares in 1983 to approximately 87 700 hectares by 1993 (Table 1.1). Based on prices in 1983, the cost of controlling this increased area of infestation in 1993 would be R8.5 million, which is equivalent to the control costs for all invader species in state pine plantations in 1983 (calculated from LE ROUX, 1984).

Control of bugweed is thus of prime importance and it is considered the worst alien invader in pine plantations (LE ROUX, 1984). Bugweed was declared an invader weed in the Conservation of Agricultural Resources Act of 1983 (GOVERNMENT GAZETTE, 1984).

(iv) **FACTORS THAT AFFECT REPRODUCTIVE YIELD**

The question may be asked: what makes an alien species, such as bugweed, so successful in exploiting its new environment? Among the characteristics responsible for the wide spread and undesirable occurrence of a weed are a *high reproductive yield* and *efficient dispersal*. High reproductive yield refers to the production of numerous viable seeds. This is influenced in part by genetic factors and by both biotic and physical environmental factors. Genetic differences in reproductive strategy between species are well documented; thus even within the same environment, different species behave in different ways. In an alpine environment, for example, flower

and seed production differed radically among four pioneering alpine species (MARCHAND & ROACH, 1980).

Environmental factors such as planting density, competitive stress or plant size also influence yield. Increased planting density resulted in decreased seed production in a range of species including *Vicia faba* L. (HODGSON & BLACKMAN, 1956), *Agrostemma githago* L. (HARPER & GAJIC, 1961) and *Hordeum vulgare* L. (KIRBY, 1969). Yield of *Lycopersicon esculentum* L. was reduced by the competitive stress induced by continuous "weedy" conditions (LIPTAY & FRIESEN, 1982). The size of plants may also affect yield, for example, variation in *Pastinaca sativa* L. seed mass between plants was positively correlated with plant size. It is not only total seed yield that is affected by plant size, HENDRIX (1984) found that the larger the plant, the heavier the seeds it produced.

Physical environmental factors influencing yield include soil nutrient levels, water, temperature and radiation (mainly light). High soil nutrient levels increased seed production of *Plantago lanceolata* Hook. (PRIMACK & ANTONOVICS, 1981) and *Linum usitatissimum* Griseb. (BLACKMAN & BUNTING, 1954). Water stress reduced both the total number and number of viable seed produced in three strains of *Avena fatua* L. (PETERS, 1982).

Light gaps in a forest canopy, created by periodic disturbance, such as windfalls, lightning strikes or fires are characteristic of forest communities world wide (PICKETT, 1976; HARTSHORN, 1978). In natural forest

communities, light influences flowering, pollination, seed production, dispersal, herbivory and attack by fungal pathogens (PIPER, 1986a). In coniferous forests, bird-dispersed plants constitute a significant portion of the forest understorey and their life histories are influenced to a large extent by the distribution of light. Probability of flowering, frequency of visitation by pollinators, and timing of seed dispersal are all influenced by the amount of light that strikes individual plants. Furthermore, PIPER (1986b) records that the interaction between frugivorous birds and fruiting plants is often found to occur in patches within the forest understorey. In some species, removal rates of fruits within forests are higher in light gaps and along forest edges than under closed canopies (PIPER, 1986b).

Some species in the forest understorey produce larger fruit crops in light gaps than their conspecifics produce in the shade (PIPER, 1986b). For example, *Solanum ptycanthum* Dun. plants grown in 60, 80 and 92 % shade produced increasingly smaller berries with fewer seeds as shade levels increased (MYERS & STOLLER, 1984).

(v) **DISPERSAL POTENTIAL**

A second factor responsible for the success of a weed is an efficient seed dispersal mechanism. Dispersal of seed by wind is generally considered the most efficient method in terms of spread and survival of a species. At the other end of the scale would be seeds dispersed by gravity. These seeds would fall beneath the parent tree, and while this is not necessarily a bad event during favourable environmental conditions, it can be disastrous for the species during severe stress conditions. Somewhere along this arbitrary

dispersal efficiency "scale" would lie species whose seeds are dispersed by birds and animals.

Bugweed produces many fruits, which are eaten by birds and by animals (Plate 2) such as duiker and bush pig. It remains to be proved whether the seeds they contain, are destroyed or left intact during ingestion. If the seeds are actually digested (or rendered non-viable) then the animal may be said to be a predator of the seed. If the seed remains viable after passage through the gut, then the animal is an agent of dispersal.

ALLEN-ROWLANDSON, (1986) studied the feeding habits of bushbuck (*Tragelaphus scriptus*) and grey duiker (*Sylvicapra grimmia*) in Weza State Forest (Figure 1.6) and on neighbouring agricultural land. Much information was obtained pertaining to dispersal of bugweed by these antelope. The mean (\pm standard deviation of this mean) home range for bushbuck at Weza state forest was 100.8 ± 62.5 ha (Grey duiker movements and home range could not be determined due to problems with radio tracking). On an annual basis, fruits and seeds from several species constituted the larger portion (37.9 % by volume) of the diet eaten by duiker. In contrast, browse formed 89.5 % of the food eaten by bushbuck.

Fungi were almost twice as important as any other food item for duiker. The next most important food was *Rubus cuneifolius* Pursh (bramble)¹ particularly during spring, when predation of buds and leaves occurred. During summer, fruit became available and comprised up to 56 % of the

¹Bramble is another serious invader species of pine plantations in South Africa (LE ROUX, 1984).

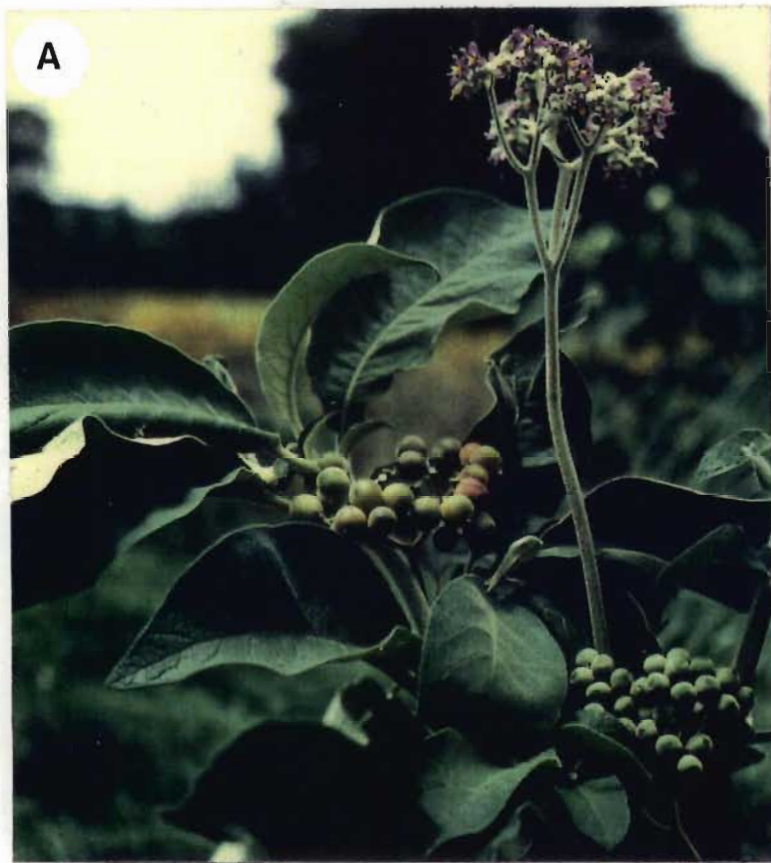


PLATE 2: Flowers and fruits of bugweed.

A. *In situ*

B. Half ripe and ripe fruits which have been partially eaten by a Rameron pigeon.

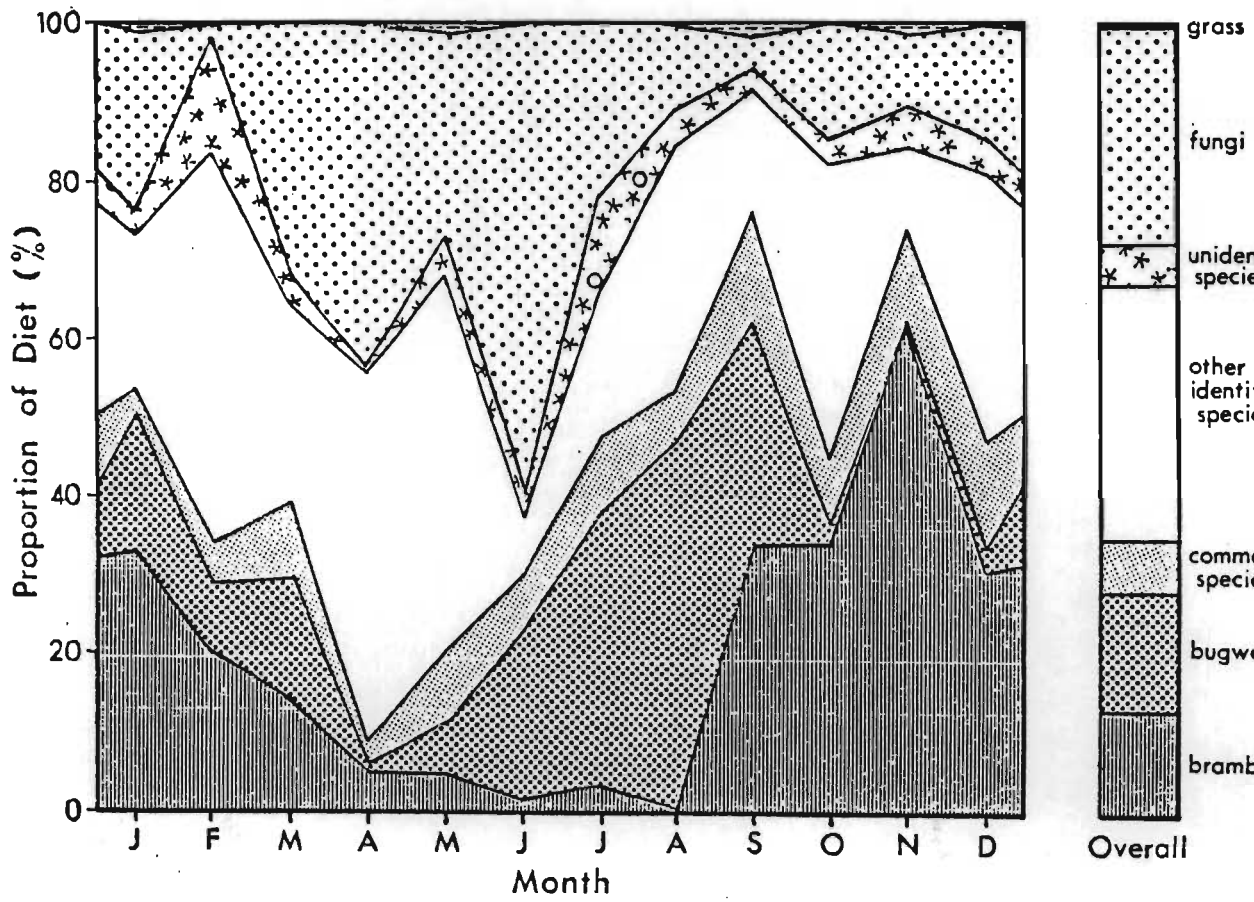


FIGURE 12: Monthly changes in the principal components of the diet of duiker in Weza State Forest (ALLEN-ROWLANDSON, 1986).

total volume of bramble eaten (Figure 1.2). During the winter months, however, bramble was of negligible importance, undoubtedly because the plants offered little green browse at this time (ALLEN-ROWLANDSON, 1986). Instead, during this time and in early spring, duiker consumed large quantities of the third most important food, bugweed fruits (Figure 1.2).

In contrast, although bugweed fruits were available throughout the year bushbuck rarely fed on these fruits except during winter months, and then only in small amounts (Figure 1.3). No other part of bugweed was apparently eaten by either antelope (ALLEN-ROWLANDSON, 1986).

The proportion of browse eaten by duiker on neighbouring farms was similar to that consumed by duiker at Weza State Forest during the winter months. However, duiker on neighbouring farms ate considerably more fruit and seeds and less fungi at this time (Figure 1.4). Bugweed fruits formed the most important component of this fruit preference of farmland duiker (ALLEN-ROWLANDSON, 1986). In contrast to this, similar proportions of fruits were eaten by bushbuck both on farmlands and in Weza State Forest (Figure 1.5).

From this it can be concluded that duiker, and to a lesser extent, bushbuck from forest and farm habitats can play a significant role in spreading bugweed seeds throughout their range. It remains to be proved, however, that these seeds retain their viability during passage through the gut of these animals.

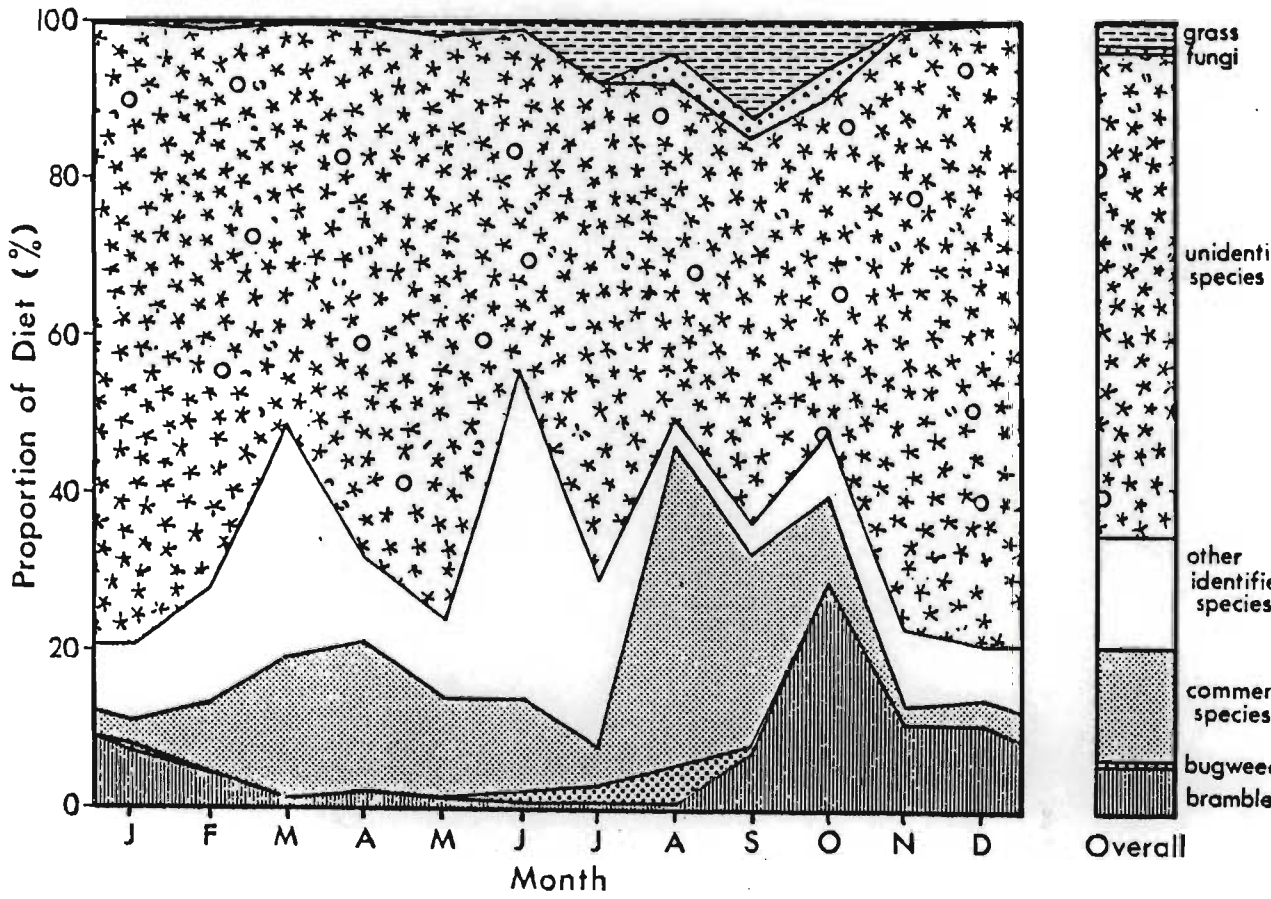


FIGURE 13: Monthly changes in the principal components of the diet of bushbuck in Weza State Forest (ALLEN-ROWLANDSON, 1986).

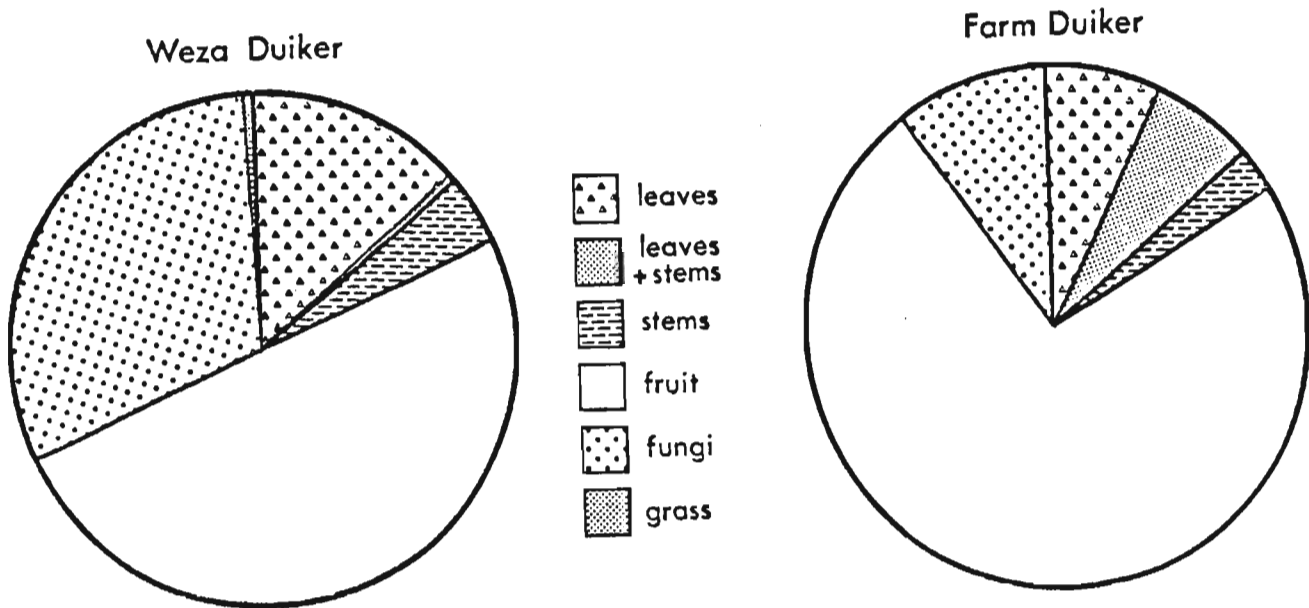


FIGURE 1.4: The proportion of plant parts eaten by duiker from Weza and from neighbouring farms during winter (ALLEN-ROWLANDSON, 1986).

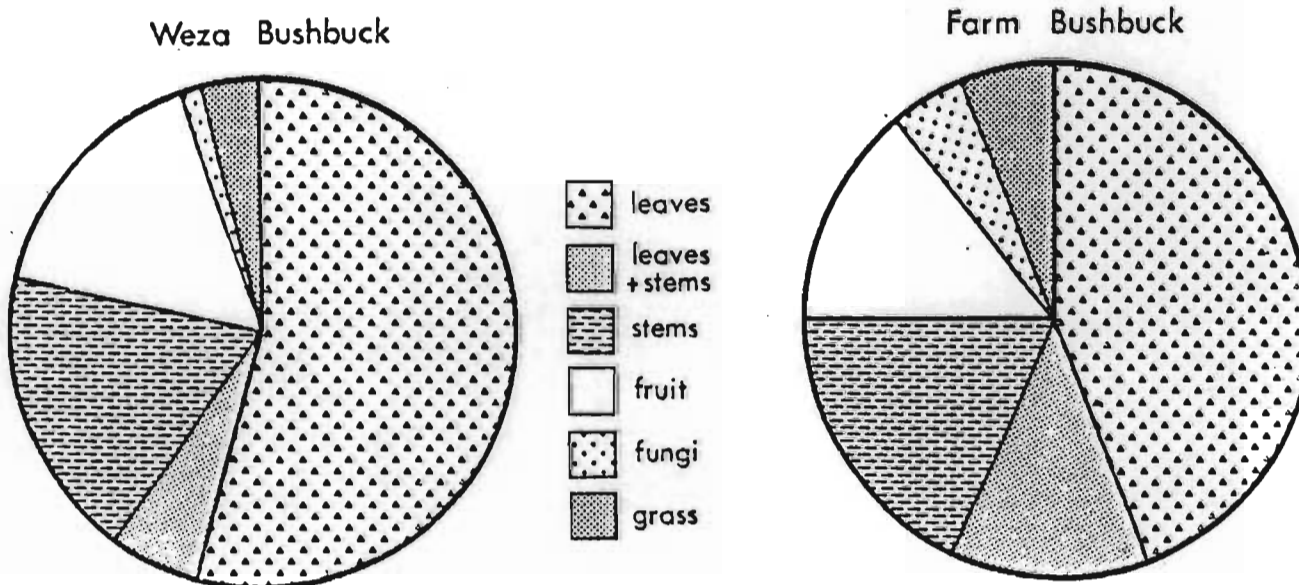


FIGURE 1.5: The proportion of plant parts eaten by bushbuck from Weza and from neighbouring farms during winter (ALLEN-ROWLANDSON, 1986).

Since fruits generally have high calorific values and are readily utilized by ungulates (JOHNSON & LANDERS, 1978; VANGILDER, TORGERSON & PORATH, 1982), ALLEN-ROWLANDSON (1986) suggested that these fruits provided duiker with a source rich in energy throughout much of the year but particularly in winter. Bramble fruits were readily consumed at all stages of ripeness by duiker as they become available in summer. It is interesting to note that regardless of the degree of ripeness of bramble (indicated by colouration of drupelets) the seeds are mature (ROTH, 1977). This also apparently occurs in bugweed, since most green fruits contained seeds which were fully developed, and which would probably not be damaged during passage through an animal (Plate 3). Only one fruit out of 50 contained seeds which were not fully developed (Plate 3). Ingestion of such fruits by animals at this immature stage would therefore probably constitute predation, and not dispersal.

Green fruits are probably not eaten by duiker due to their unpalatability and possible toxicity (ALLEN-ROWLANDSON, 1986). However, green fruits have been reported in the gut contents of a Rameron pigeon².

Food is retained in antelope for as little as 24 hours (HOPPE, 1984). Due to their strong endocarp, bramble seeds are unlikely to be affected by gut acids during this time period, and those that survive initial chewing and later rumination can survive so that dispersal, not predation, has taken place. Although bugweed seeds do not have hard endocarps, this short time period should allow survival during passage through the gut and thus

² (MR S KRÖEGER, Department of Forestry, Ciskei, *pers. comm.*, 1990).



PLATE 3: Green fruits of bugweed with fully (F) or partially (P) developed seeds.

successful dispersal. ALLEN-ROWLANDSON, (1986) speculated that duiker may act significantly in dispersing undamaged seeds of both bugweed and bramble.

Bushpig (*Potamochoerus porcus*) are also known to eat bugweed fruits since faeces have contained seeds (MELTON, COOPER & WHITTINGTON, 1989) The range of this species is increasing in South Africa, and there could be a simultaneous increase in the importance of this animal in spreading bugweed.

OATLEY (1980) noted that the Rameron pigeon eats bugweed fruits freely, with 94.5 % of the 109 birds examined having gut contents consisting solely of this source of food. Seedlings are reported to occur under fencelines where the birds perch. However, the viability of seeds passed through the gut of this species has still to be ascertained. Even if only a few seeds survive this predation, then it can be said with certainty that this species has spread via these agents and will continue to spread and infest new areas as further dispersal occurs.

The aim of this study was, firstly, to find whether significant predications of fruit and seed yield could be made from physical or biotic measurements, and secondly, to ascertain whether seeds could survive passage through animals and hence successfully be dispersed.

VAN DER KLOET & CABILO (1984) has commented that although periodicity in seed and fruit production has been described for many

species, most of this information is anecdotal. JANZEN (1976) suggested the reason for this paucity of reliable data was that "they take so long to accumulate, failure by observers to note precisely which plants were producing most of the seed, and few if any testable hypotheses". In this study, an attempt has been made to overcome most of these shortcomings.

B. MATERIALS AND METHODS

(i) DURATION OF STUDY

This study of the reproduction yield of bugweed was conducted over a period of 20 months. Initially, the trial was to be terminated at the end of 12 months. However, ripe fruits were produced in flushes so the study period was extended to 24 months to ascertain whether similar flushes would occur in the second 12 month period. Unfortunately the trial was prematurely terminated due to a serious staff shortage at this time and commitments to conduct other research priorities during the growing season.

(ii) STUDY AREA

The thirty bugweed trees used in this study were growing under a canopy of *Pinus patula* Schlechtd. & Cham. at Cedara State Forest (Figure 1.6). The area of the stand is 11.1 ha and the pine trees were planted in 1961, when there were 1 350 stems per hectare (Table 1.2). Three thinnings occurred since then, the first in 1974 when the pine trees were 13 years old; the second four years later and the final thinning in 1982, when the pine trees were 21 years old (Table 1.2).

During each thinning operation, all weed species were slashed. All single-stem, non-coppiced bugweed trees had therefore germinated from seed after the 1982 thinning, and at the commencement of this study in May 1987, the largest of these bugweed trees were therefore approximately five years old. According to the very coarse measuring system used in Forestry,

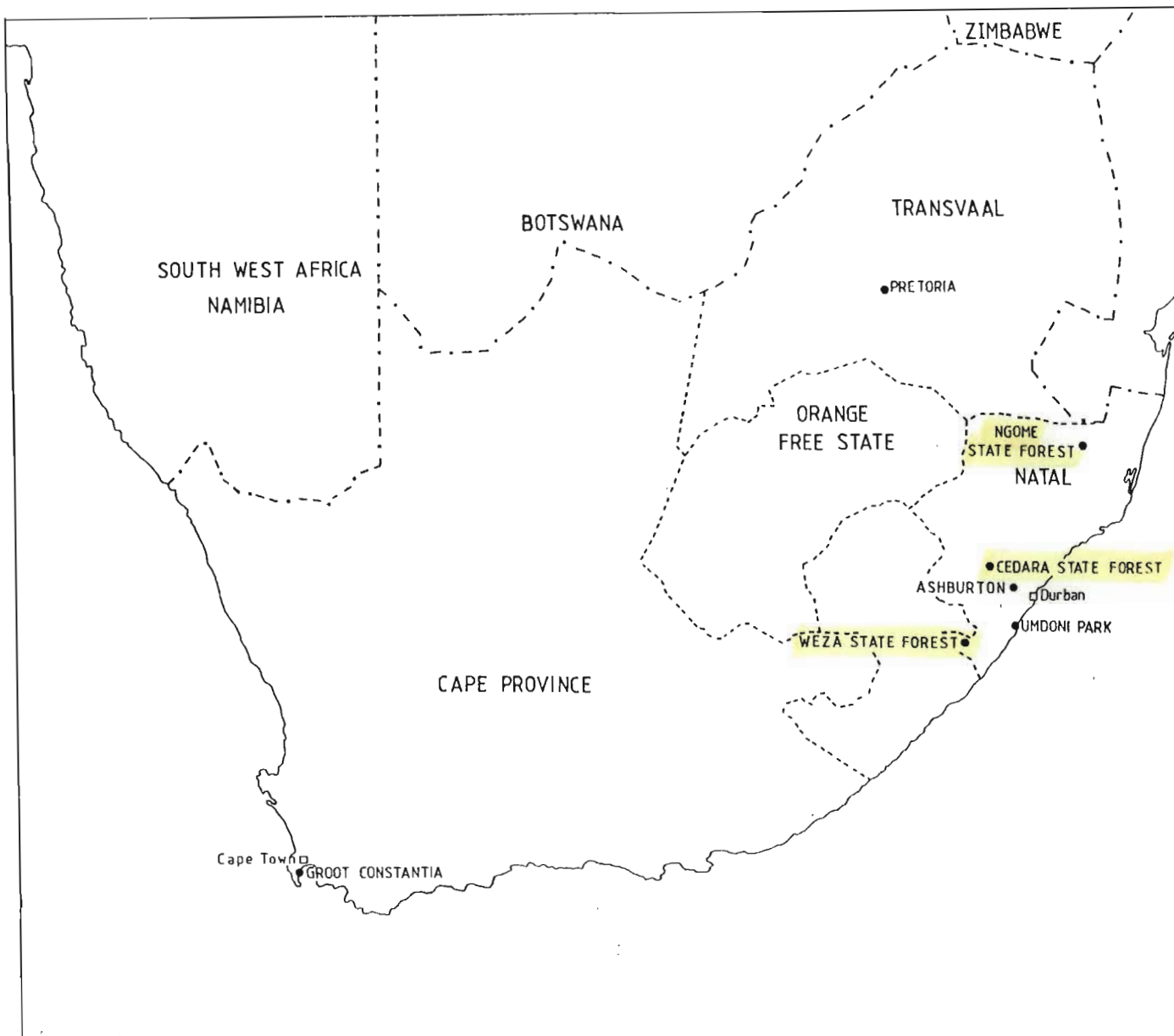


FIGURE 1.6: Map of the Republic of South Africa, indicating sites of bugweed seed and fruit collection at Weza State Forest (ALLAN-ROWLANDSON, 1986 study) Cedara State Forest and Ngome State Forest.

TABLE 1.2: Thinning regime of *Pinus patula* in the reproductive yield study area¹.

| Thinning | Year | Age of stand | Pine stems per ha |
|---------------|------|--------------|-------------------|
| Establishment | 1961 | 0 | 1 350 |
| 1 | 1974 | 13 | 597 |
| 2 | 1978 | 17 | 374 |
| 3 | 1982 | 21 | 267 |

¹Extract from working plan/Cedara November 6, 1986. Compas Program, Department of Environmental Affairs. Forestry Branch. Private Bag X447, Pretoria, 0001, RSA

spread and density of bugweed in the study area were essentially unchanged over the last ten years. A value of 2 was given for spread (approximately 50 %) and 1 was given for density (very sparse).

(iii) **COLLECTION OF FRUITS AND TREATMENT OF SEEDS**

In order to collect fully ripened fruits which would otherwise be lost, developing fruits were covered with grey gauze nylon bags, stapled together. These allowed free flow of air around the fruits (Plate 4). Each month ripe fruits from these bags were collected from each tree (Plate 4), counted, weighed, and opened up to remove the seeds. Seeds were cleaned with repeated washing in tap water and separated into float (apparently dead) and sink (apparently viable³) fractions. These were then counted and weighed. Float seeds were treated with an aqueous solution of 500 mg l⁻¹ GA₃ for up to 12 weeks. Any seeds germinating after application of GA₃ were obviously viable, and viability figures were adjusted accordingly.

Thus each month, the reproductive yield for that month of each of the thirty bugweed trees was ascertained in terms of fruit number, fruit mass, viable seed number, viable seed mass, dead seed number, dead seed mass, total seed number and total seed mass. At the end of the 20 month study period, each of these variables was totalled and related to various measurements of tree size and neighbouring plant species. Physical factors

³ROBERTS (1981) defined "apparently viable seeds" as those "which appear to be intact and which resist gentle pressure ..."



PLATE 4:

Grey nylon gauze bags used to collect ripe fruits of bugweed.

A. Bags were fastened around the fruits when they were unripe.

B. Ripe fruits were collected each month.

such as temperature, rainfall and hours of sunlight were related to fruit and viable seed yield of the whole sample on a monthly basis.

(iv) **ANALYSIS OF RESULTS**

Simple linear regressions for relationships between the different measurements of plant size and reproductive yield (RAYNER, 1967; Chapter 17) were calculated using the Genstat 4 program. Where applicable, analysis of variance was done using the Genstat 4 program and then the least significant differences (LSD) between means was calculated (RAYNER, 1967; Chapter 10). Alternatively, the correlation coefficient (r) was sufficient in some instances to test whether there was a correlation between two variables (PARKER, 1973; page 57). Each Figure or Table legend describes the type of analysis used.

(v) **MEASUREMENT OF PHYSICAL FACTORS**

Average monthly measurements of maximum temperature, minimum temperature, diurnal temperature range, rainfall and hours of sunlight were obtained from the Department of Agrometeorology at Cedara Agricultural College. As the crow flies, these measurements were taken approximately 5 km from the study area.

(vi) **MEASUREMENT OF BIOTIC FACTORS**

(a) **Plant size**

Diameter and circumference of stems were measured at 25, 50, 100 and 130 cm above the soil surface with calipers and a soft cloth tape measure, respectively. Diameter readings were taken across the widest axis of the

stem. Tree height was taken by placing an aluminium pole, marked off in 10 cm segments, vertically next to the base of each tree stem. A measure of canopy spread was obtained by placing two poles vertically under the outermost leaves of the tree and measuring the distance between them, then placing the poles at right angles to this direction and again measuring the distance between them. The canopy spread (m^2) was calculated by multiplying these two distances.

(b) **Photosynthetic capacity**

(aa) *Non-destructive measurements*

During the study, non-destructive measurements of photosynthetic potential of the trees took place by means of hemispherical photography. Here, the camera lens recorded each tree canopy photographically (Plate 5). This was done during June 1987, August 1987, November 1987, February 1988 and June 1989.

Photographs were printed (Plate 6) and a concentric circle grid⁴ superimposed on the prints. Each concentric circle represented the angle of elevation (α) above the horizon. Values of α were 5°, 15, 25, 35, 45, 55, 65, 75 and 85° respectively. Coverage was measured according to the area of grid covered by the tree leaves (Figure. 1.7). The scoring of each grid segment was determined by the following "scale":

⁴ Nine concentric circles, each composed of 48 segments, except the innermost, which had 24 segments (Figure 1.7).

0 = empty

1 = 25 % coverage by leaves

2 = 50 % coverage by leaves

3 = 75 % coverage by leaves

4 = 100 % coverage by leaves

(Figure 1.7)

These values were then added together for each angle, to give the sum of cover values, that is, SC (Figure 1.7). The sum of values for each angle was then corrected for distortion (discussed on page 43) by the following formula:

$$CSC = SC \cdot \cos \alpha \cdot \sin \alpha \quad (\text{Figure 1.7})$$

where α = the angle of elevation above the horizon

The relative percentage leaf cover was found according to the formula:

$$\frac{\Sigma CSC \times 100 \times \Pi}{4 \times 9 \times 48}$$

where ΣCSC = sum of the CSC values. The figures 9 and 48 refer to the number of angles of elevation in the grid and the number of segments within each angle, respectively (Figure 1.7). The figure 4 was used to normalize the formula (FUCHS, STANHILL & WAANDERS, 1972)⁵.

⁵This equation was derived from P.R. BERLINER (*pers. comm.*), Jacob Aoaustein Institute for Desert Research, Ben Gurion University of the Negev, Sede Bouquer Campus, Israel.

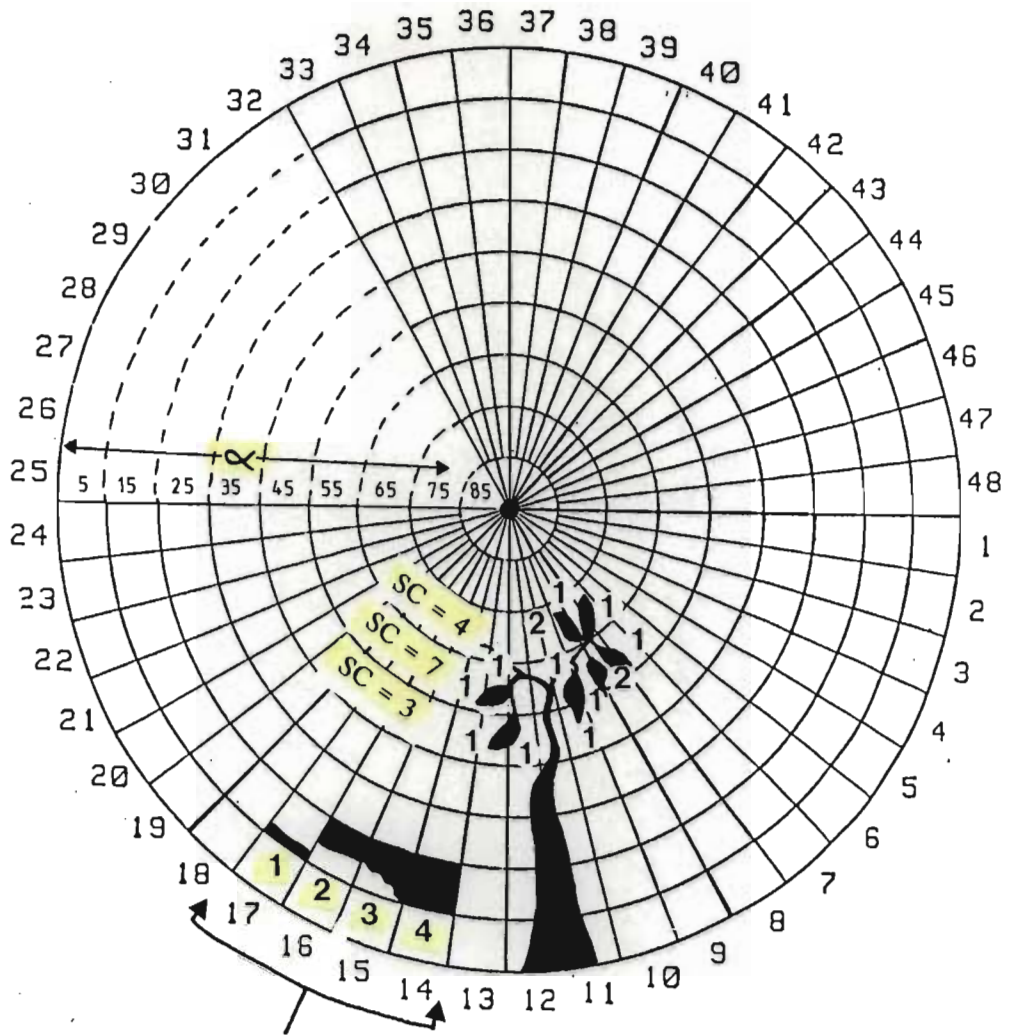


PLATE 5: Recording the leaf canopy of bugweed under a pine canopy by using a hemispherical lens.



PLATE 6:

An image disc of the leaf canopy of bugweed growing under a pine canopy as recorded by a hemispherical lens. The leaf canopy is outlined in black.



SCORING OF EACH GRID SQUARE

Relative % cover of bugweed leaves using hemispherical photography

| Angle of elevation (∞) above the horizon | Sum of cover values (SC) | Corrected sum of cover values (CSC) |
|---|--------------------------|-------------------------------------|
| 45 | 3 | 1.51 |
| 55 | 7 | 3.27 |
| 65 | 4 | 1.52 |
| | | Σ CSC 6.30 |

$$\text{Relative \% leaf cover} = \frac{\Sigma \text{CSC} \times 100 \times \pi}{4 \times 9 \times 48} = 1.145 \%$$

FIGURE 1.7: Calculation of relative percentage leaf cover of bugweed. For each angle of elevation (∞) above the horizon, the sum of cover values is found (SC). These values are corrected for distortion (CSC) and the relative percentage leaf cover is then calculated.

The computer programme⁶ developed at Cedara for these calculations is presented below

```
10 '---- program to calculate fish-eye .. P.P. Whitwell (Cedara) ----
20 GOTO 40
30 CLS:COLOR 15,0:PRINT "saving fish.bas";SAVE"fish",A:END
40 CLS:INPUT "Do you want to use previously inputed data? (y/n)";Y$
50 Y$=CHR$(ASC(Y$) AND 95)
60 IF Y$ <> "Y" AND Y$<>"N" THEN GOTO 40
70 IF Y$="Y" THEN GOTO 250
80 '----- create file -----
90 INPUT "Enter name of file to store data ";STORE$
100 A$="del "+STORE$
110 SHELL A$
120 OPEN "R",#1,STORE$,10:FIELD #1,5 AS ANGLE$,5 AS FREQUENCY$
130 CLS:PRINT "PLEASE ENTER ALL DATA ... (angle,frequency) ... 0,0 to end."
140 ANGLE=1:ISIZE=0
150 INPUT "angle,frequency";ANGLE,FREQUENCY
160 IF ANGLE=0 THEN GOTO 280
170 A$=RIGHT$(STR$(10^5+ANGLE),5)
180 LSET ANGLE$=A$
190 A$=RIGHT$(STR$(10^5+FREQUENCY),5)
200 LSET FREQUENCY$=A$
210 PUT #1
220 ISIZE=ISIZE+1
230 GOTO 150
240 '----- read file -----
250 INPUT "Enter name of file to read data from ";STORE$
260 OPEN "R",#1,STORE$,10:FIELD #1,5 AS ANGLE$,5 AS FREQUENCY$
270 ISIZE=LOF(1)/10
280 GT=0 ' grand total
290 NUMANGLES=0
300 FOR I=1 TO ISIZE
310 GET #1,I
320 ANGLE=VAL(ANGLE$)
330 FREQUENCY=VAL(FREQUENCY$)
340 NUMANGLES=NUMANGLES+1
350 RADIANS=ANGLE*3.1415927#/180!
360 GT=GT+FREQUENCY*SIN(RADIANS)*COS(RADIANS)
370 NEXT I
380 CLOSE #1
390 PRINT "grand total=";GT
400 PERCCOVER=GT*100!*3.141592654#/(4*9*48)
410 PRINT "PERCENTAGE COVER =";PERCCOVER
420 CLOSE #1
430 INPUT "Do you want to continue? (y/n)";Y$
440 Y$=CHR$(ASC(Y$) AND 95)
450 IF Y$ <> "Y" AND Y$<>"N" THEN GOTO 430
460 IF Y$="Y" THEN GOTO 40
470 CLS:END
```

⁶This program is in public domain (WHITWELL, *personal communication*), Private Bag X9059, Pietermaritzburg, 3200.

(bb) *Destructive measurements*

In June 1989 (at the end of this study), destructive sampling occurred, and all the leaves on the 30 sampled trees at that date were collected, passed through a leaf area meter, weighed, dried and reweighed to obtain dry mass values.

The area meter (model L1-3 100⁷) had an error of approximately 1 %, determined at the 99 % level with irregular shaped complex objects (Lambda Instruments Corporation Specifications).

(c) **Neighbouring plants/competition**

Competition was measured by counting separately, the members of each species of plant within a 2 m radius of each of the 30 sample bugweed tree stems. Each species within this area was given a subjective number of points, based on relative impact in terms of size and root spread, compared to the other species. These values are presented in Table 1.3. The degree of competition each bugweed was subjected to, was measured by totalling the products of the number of each species and its points score.

(vii) **DISPERSAL POTENTIAL**

To ascertain whether seeds ingested by birds and animals retain their viability, ripe fruits were fed to blue duiker under non-stressful conditions at the Zoology Department, University of Natal at Pietermaritzburg (UNP) (Figure 1.6). Seeds from faeces of bushpigs were obtained from the Zoology Department, UNP. These seeds had been kept in an air-tight

⁷ Lambda Instruments Corporation, P O Box 4425, Lincoln, Nebraska, 68504, USA.

TABLE 1.3: Subjective evaluation of degree of impact of neighbouring plants on fruit yield of bugweed.

| Neighbouring species | Description | Subjective score per plant |
|--|----------------------------|----------------------------|
| Bugweed seedlings | < 1 cm DBH; < 1.5 m high | 4 |
| Bugweed trees | > 1 cm DBH; > 1.5 m high | 10 |
| Pine seedlings | Natural regeneration | 4 |
| Pine trees | Trees planted in 1961 | 20 |
| Gum seedlings | Not coppice | 4 |
| Gum coppice | Multistemmed | 10 |
| Ferns, grass, sparse bramble and herbs | Low, shallow-rooted growth | 1 |
| Shrubs | Vigorous woody plants | 2 |
| Grass cover 100 % | - | 3 |
| Bramble cover > 25 % | - | 3 |

container in a laboratory for two years prior to the germination trials. Seeds taken from the gut of a Rameron pigeon from Ngome State Forest in Northern Natal (Figure 1.6) were examined for viability and germination.

C. RESULTS

In this study the reproductive (fruit and seed) yield of bugweed growing under pine was investigated. A number of factors may influence this potential. Once the factors that actually affect fruit yield are known, they can be used to predict reproductive yield under given conditions. Physical factors that might affect fruit yield are light, temperature and rainfall. Biotic factors that might affect fruit yield are tree size (in terms of stem diameter or circumference, tree height or branch spread) and competition from nearby plants. These factors were measured and each was tested for accuracy as indicators of fruit and seed yield in bugweed.

(i) MONTHLY FRUIT YIELD AND PREVAILING WEATHER CONDITIONS

In a sample of 30 trees (1.8 to 5.0 cm DBH⁸) growing under a *Pinus patula* canopy at Cedara State Forest, ripe bugweed fruits were produced periodically in flushes throughout the year (Figure 1.8). It was also evident that yield varied between years since, in October 1987 the 30 trees produced 448 ripe fruits, whereas in October 1988 a much higher yield of 1 918 ripe fruits was obtained (Figure 1.8).

To try and explain these differences, an attempt was made to relate these seasonal and yearly variations in yield to changes in the weather. The results are presented in Table 1.4. Rainfall, temperature minima and maxima, diurnal temperature range and the amount of daylight (sun hours)

⁸DBH = Diameter at Breast Height, defined as 1 300 cm above the soil surface.

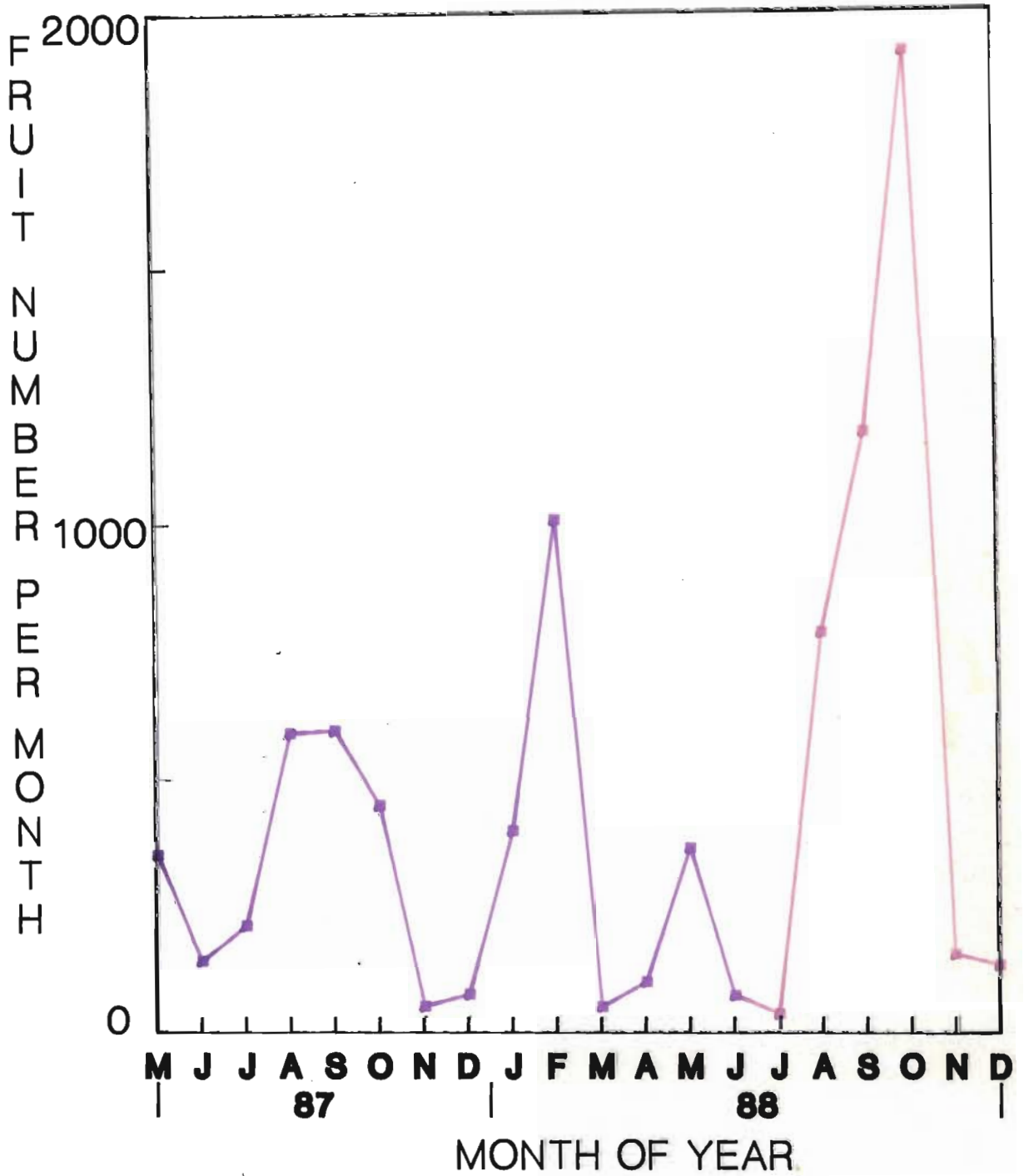


FIGURE 1.8: Monthly fruit yield (numbers) produced during 1987 and 1988 by a sample of 30 bugweed trees under a pine canopy at Cedara State Forest.

TABLE 1.4: The influence of environmental factors on monthly fruit production of bugweed growing under a pine canopy. The correlation coefficient (R), their standard errors (SE) and the significance of the relationship, as described by the student "t" test, are presented below.

| Environmental parameter | R | SE | t | Significance |
|--------------------------|---------|--------|--------|--------------|
| Rain (mm) | 0.0063 | 0.2582 | 0.0244 | NS* |
| Minimum temperature (°C) | 0.2763 | 0.2482 | 1.1132 | NS |
| Maximum temperature (°C) | 0.1922 | 0.2534 | 0.7585 | NS |
| Mean temperature (°C) | -0.2908 | 0.2470 | -1.177 | NS |
| Sun hours | -0.3520 | 0.2417 | -1.456 | NS |

*NS = No significant correlation.

did not significantly correlate with these growth flushes (Table 1.4).

It is clear therefore that no simple and direct relationship exists between fruit yield and these physical measurements. It is probable that if these factors are important in determining yield, then they must interact in some or other manner.

(ii) **REPRODUCTIVE YIELD AND TREE SIZE**

The diameter and circumference of the bugweed tree trunks were measured at 25 cm, 50 cm, 100 cm and DBH above soil surface. Results indicated that the thickness of the stem, as measured by diameter at any given height above soil level, had a highly significant regression with all the dependent variables; that is, both number and mass of fruits; viable seeds; non-viable⁹ seeds and total seed content (Table 1.5). Measurements of diameter at 100 cm above soil level were consistently the best indicator of reproductive yield, and except for non-viable seed data, accounted for 63.1 to 67.7 % of the variation in the dependent variables (Table 1.5).

If thickness of stems at a given height was considered in terms of circumference rather than diameter, similar results were obtained for the two parameters (Table 1.6). Regression coefficients were highly significant and therefore circumference measured at any given height on the stems of bugweed also served as an indicator of fruit and seed yield performance of the trees. The percentage variation accounted for in the fruit and seed

⁹Non-viable seeds are referred to as dead seeds in the rest of the Tables in Chapter 1, due to limited space.

TABLE 1.5: The influence of stem diameter on reproductive yield of bugweed. The regression coefficients (RC), their standard errors (SE) and the significance of these relationships in terms of % variation accounted for in each case (% V) and from T tables, are presented below (n = 29). All relationships are linear.

| Dependent variable | | Height above soil surface of diameter measurement (cm) | | | |
|--------------------|-----|--|-------|-------|-------|
| | | 25 | 50 | 100 | 130 |
| Fruit number | RC | 18.7 | 18.6 | 20.7 | 22.4 |
| | SE | 2.9 | 3.1 | 2.9 | 3.2 |
| | % V | 62.6 | 60.2 | 67.7 | 66.6 |
| | T | XXX | XXX | XXX | XXX |
| Fruit mass | RC | 9.2 | 9.0 | 10.3 | 11.2 |
| | SE | 1.6 | 1.7 | 1.6 | 1.7 |
| | % V | 57.0 | 53.4 | 63.1 | 63.0 |
| | T | XXX | XXX | XXX | XXX |
| Viable seed number | RC | 2 159 | 2 137 | 2 397 | 2 580 |
| | SE | 368 | 387 | 368 | 407 |
| | % V | 58.2 | 55.2 | 63.4 | 62.0 |
| | T | XXX | XXX | XXX | XXX |
| Viable seed mass | RC | 2.7 | 2.7 | 3.0 | 3.3 |
| | SE | 0.5 | 0.5 | 0.5 | 0.5 |
| | % V | 57.6 | 54.5 | 63.1 | 62.8 |
| | T | XXX | XXX | XXX | XXX |
| Dead seed number | RC | 147 | 151 | 178 | 185 |
| | SE | 36 | 36 | 34 | 39 |
| | % V | 40.0 | 41.4 | 53.0 | 47.5 |
| | T | XXX | XXX | XXX | XXX |
| Dead seed mass | RC | 0.3 | 0.3 | 0.4 | 0.4 |
| | SE | 0.1 | 0.1 | 0.1 | 0.1 |
| | % V | 40.7 | 40.5 | 49.3 | 47.6 |
| | T | XXX | XXX | XXX | XXX |
| Total seed number | RC | 2 306 | 2 288 | 2 576 | 2 765 |
| | SE | 395 | 413 | 390 | 435 |
| | % V | 58.0 | 55.3 | 63.9 | 62.2 |
| | T | XXX | XXX | XXX | XXX |
| Total seed mass | RC | 3.5 | 3.5 | 3.6 | 3.9 |
| | SE | 0.5 | 0.6 | 0.5 | 0.6 |
| | % V | 63.7 | 60.9 | 65.5 | 65.0 |
| | T | XXX | XXX | XXX | XXX |

XXX (P < 0.001)

TABLE 1.6: The influence of stem circumference on reproductive yield of bugweed. The regression coefficients (RC), their standard errors (SE) and the significance of these relationships in terms of % variation accounted for in each case (% V) and from T tables, are presented below (n = 29). All relationships are linear.

| Dependent variable | | Height above soil surface of circumference measurement (cm) | | | |
|--------------------|-----|---|------|------|------|
| | | 25 | 50 | 100 | 130 |
| Fruit number | RC | 5.4 | 5.5 | 6.7 | 6.6 |
| | SE | 0.9 | 0.9 | 1.1 | 0.9 |
| | % V | 54.4 | 55.6 | 62.4 | 64.1 |
| | T | XXX | XXX | XXX | XXX |
| Fruit mass | RC | 2.6 | 2.7 | 3.4 | 3.3 |
| | SE | 0.5 | 0.5 | 0.5 | 0.5 |
| | % V | 49.0 | 50.1 | 60.9 | 60.7 |
| | T | XXX | XXX | XXX | XXX |
| Viable seed number | RC | 619 | 629 | 777 | 767 |
| | SE | 124 | 124 | 130 | 123 |
| | % V | 49.0 | 50.7 | 59.0 | 61.2 |
| | T | XXX | XXX | XXX | XXX |
| Viable seed mass | RC | 0.8 | 0.8 | 0.9 | 0.9 |
| | SE | 0.2 | 0.2 | 0.2 | 0.2 |
| | % V | 49.0 | 50.0 | 58.6 | 60.6 |
| | T | XXX | XXX | XXX | XXX |
| Dead seed number | RC | 41.5 | 43.2 | 57.0 | 55.7 |
| | SE | 11.6 | 11.5 | 11.9 | 11.5 |
| | % V | 33.1 | 35.5 | 47.8 | 48.4 |
| | T | XX | XXX | XXX | XXX |
| Dead seed mass | RC | 0.1 | 0.1 | 0.1 | 0.1 |
| | SE | 0.0 | 0.0 | 0.0 | 0.0 |
| | % V | 32.9 | 35.2 | 42.6 | 47.2 |
| | T | XX | XXX | XXX | XXX |
| Total seed number | RC | 660 | 672 | 834 | 823 |
| | SE | 133 | 133 | 139 | 131 |
| | % V | 49.6 | 50.6 | 59.4 | 61.5 |
| | T | XXX | XXX | XXX | XXX |
| Total seed mass | RC | 1.0 | 1.0 | 1.1 | 1.1 |
| | SE | 0.2 | 0.2 | 0.2 | 0.2 |
| | % V | 56.6 | 57.2 | 59.3 | 61.6 |
| | T | XXX | XXX | XXX | XXX |

XX (P < 0.005)

XXX (P < 0.001)

yield data by circumference measurements was, however, 1 to 10 % less reliable than diameter measurements (Tables 1.5 and 1.6). This is probably because bugweed stems are not truly cylindrical and therefore circumference measurements incorporated a certain degree of error. Diameter measurements, on the other hand, were always taken across the widest axis of the stem, and thus a higher level of consistency was achieved.

Canopy spread as a size parameter was measured four times during this study, at the beginning of the trial (May 1987) during January 1988, in May 1988 and finally in May 1989. It was found that canopy spread had a highly significant relationship with fruit and both viable and total seed yield over the whole period when measured during May each year (Table 1.7).

Measurements of canopy spread during May at the beginning of the study (1987) accounted for 73.3 to 81.7 % of the variation in all the dependent variables except for non-viable seed numbers and mass (Table 1.7). However, the variation accounted for declined when measurements taken in May 1988 and in May 1989 were compared. In 1988, it ranged from 68.0 to 74.8 % and in 1989 it decreased to a range from 58.1 to 61.9 % (Table 1.7). This suggests that as the tree increases in size, with an increasing canopy spread (vegetative growth), then other measures of growth may play an increasing role in influencing reproductive yield. Again, non-viable seed numbers and mass were less influenced by canopy spread measurements in the autumn of 1988 and 1989 (Table 1.7).

In contrast to the autumn measurements, canopy spread was not such a

TABLE 1.7: The influence of canopy spread on reproductive yield of bugweed. The regression coefficients (RC), their standard errors (SE) and the significance of these relationships in terms of % variation accounted for in each case (% V) and from T tables, are presented below (n = 29). All relationships are linear.

| Dependent variable | | Year of canopy spread measurement | | | |
|--------------------|-----|-----------------------------------|--------------|----------|----------|
| | | May 1987 | January 1988 | May 1988 | May 1989 |
| Fruit number | RC | 77.6 | 69.4 | 82.5 | 89.2 |
| | SE | 7.5 | 12.3 | 11.3 | 15.2 |
| | % V | 81.7 | 56.2 | 68.6 | 58.2 |
| | T | XXX | XXX | XXX | XXX |
| Fruit mass | RC | 37.8 | 37.5 | 44.1 | 46.5 |
| | SE | 4.6 | 5.8 | 5.2 | 7.6 |
| | % V | 73.3 | 63.0 | 74.8 | 60.3 |
| | T | XXX | XXX | XXX | XXX |
| Viable seed number | RC | 9 038 | 8 264 | 9 996 | 10 879 |
| | SE | 985 | 1 475 | 1 300 | 1 753 |
| | % V | 77.6 | 55.9 | 70.8 | 61.0 |
| | T | XXX | XXX | XXX | XXX |
| Viable seed mass | RC | 11.4 | 10.7 | 12.6 | 13.5 |
| | SE | 1.3 | 1.8 | 1.7 | 2.3 |
| | % V | 76.9 | 57.8 | 70.3 | 58.1 |
| | T | XXX | XXX | XXX | XXX |
| Dead seed number | RC | 668 | 531 | 646 | 844 |
| | SE | 101 | 146 | 146 | 151 |
| | % V | 64.2 | 33.7 | 43.7 | 55.9 |
| | T | XXX | XX | XXX | XXX |
| Dead seed mass | RC | 1.5 | 1.2 | 1.4 | 1.8 |
| | SE | 0.2 | 0.3 | 0.3 | 0.4 |
| | % V | 67.2 | 33.7 | 40.7 | 51.6 |
| | T | XXX | XX | XXX | XXX |
| Total seed number | RC | 9 706 | 8 795 | 10 642 | 11 723 |
| | SE | 1 040 | 1 590 | 1 409 | 1 853 |
| | % V | 78.2 | 55.2 | 70.0 | 61.9 |
| | T | XXX | XXX | XXX | XXX |
| Total seed mass | RC | 13.4 | 11.9 | 14.1 | 15.6 |
| | SE | 1.4 | 2.2 | 1.9 | 2.6 |
| | % V | 79.8 | 56.3 | 68.0 | 60.2 |
| | T | XXX | XXX | XXX | XXX |

X (P < 0.2)
 XX (P < 0.005)
 XXX (P < 0.001)

good indicator of fruit and seed yield when taken during the active growing season (January, 1988). Here, although canopy spread still had a highly significant relationship with reproductive yield, somewhat less variation was accounted for, ranging from 55.2 to 63.0 % (Table 1.7). Thus predictions of fruit and seed yield based on canopy spread should be made during times when vegetative growth is less vigorous. The relationship became less significant ($P < 0.2$) with non-viable seed numbers and mass when canopy spread was measured in January (Table 1.7).

When considering the influence of tree height on reproductive yield of bugweed (Table 1.8), it was apparent that the regression coefficients were generally significant ($P < 0.005$ or $P < 0.050$). However, the percentage variation accounted for in fruit and seed yield by measuring height was generally low; from 27.8 to 30.0 % in May 1987, 32.3 to 35.2 % in January 1988 and 13.0 to 17.9 % in May 1989 (Table 1.8). Once again, non-viable seed numbers and mass were less influenced by tree height than the other dependent variables. The only period during which tree height measurements accurately indicated yield over the 20 months of this study was in May 1988. Here, regression coefficients were highly significant ($P < 0.001$) with percentage variation accounted for in fruit and seed yield ranging from 57.0 to 60.8 % (Table 1.8). Thus the somewhat lower percentage variation accounted for by canopy spread in May 1988 might be due to the increased influence of tree height during this time. However, it may conclude that in general, tree height is a very poor indicator of reproductive potential in bugweed. This is not really surprising since growth of bugweed trees under pine is somewhat abnormal, being

TABLE 1.8: The influence of tree height on reproductive yield of bugweed. The regression coefficients (RC), their standard errors (SE) and the significance of these relationships in terms of % variation accounted for in each case (% V) and from T tables, are presented below (n = 29). All relationships are linear.

| Dependent variable | | Year of tree height measurement | | | |
|--------------------|-----|---------------------------------|--------------|----------|----------|
| | | May 1987 | January 1988 | May 1988 | May 1989 |
| Fruit number | RC | 184.5 | 153.8 | 141.1 | 69.4 |
| | SE | 55.0 | 41.9 | 24.3 | 31.6 |
| | % V | 30.0 | 34.2 | 57.7 | 13.8 |
| | T | XX | XXX | XXX | X |
| Fruit mass | RC | 92.4 | 78.9 | 74.2 | 34.8 |
| | SE | 28.5 | 21.5 | 12.0 | 16.3 |
| | % V | 28.4 | 34.3 | 60.8 | 13.0 |
| | T | XX | XXX | XXX | X |
| Viable seed number | RC | 21 647 | 18 600 | 16 993 | 9 010 |
| | SE | 6 612 | 4 962 | 2 862 | 3 697 |
| | % V | 28.8 | 35.2 | 58.8 | 17.1 |
| | T | XX | XXX | XXX | X |
| Viable seed mass | RC | 27.3 | 23.1 | 21.3 | 10.4 |
| | SE | 8.4 | 6.4 | 3.7 | 4.8 |
| | % V | 28.3 | 33.6 | 57.0 | 13.2 |
| | T | XX | XX | XXX | X |
| Dead seed number | RC | 1 668 | 1 310 | 1 240 | 811 |
| | SE | 546 | 430 | 262 | 289 |
| | % V | 25.8 | 25.7 | 47.1 | 22.2 |
| | T | XX | XX | XXX | X |
| Dead seed mass | RC | 3.2 | 2.6 | 2.6 | 1.4 |
| | SE | 1.3 | 1.0 | 0.6 | 0.7 |
| | % V | 17.7 | 18.2 | 39.0 | 12.3 |
| | T | X | X | XXX | X |
| Total seed number | RC | 23 316 | 19 910 | 18 233 | 9 822 |
| | SE | 7 054 | 5 309 | 3 050 | 3 937 |
| | % V | 29.3 | 35.2 | 59.1 | 17.9 |
| | T | XX | XXX | XXX | X |
| Total seed mass | RC | 30.7 | 25.7 | 24.5 | 13.2 |
| | SE | 9.8 | 7.4 | 4.3 | 5.7 |
| | % V | 27.8 | 32.3 | 58.0 | 16.0 |
| | T | XX | XX | XXX | X |

X (P < 0.050)

XX (P < 0.005)

XXX (P < 0.001)

considered very tall and "spindly" as the trees grow towards the light.

Of the total of 30 trees in the original sample, ten were physiologically changed during the 20 month study period. One tree was partially broken in error during September 1987, and its fruit yield was drastically reduced for the remainder of the study period. This tree was therefore omitted from the regression analyses, thus $n=29$. There was dieback of four trees in June and July of 1987, and a further five trees succumbed in June/July of 1988. This was probably caused by cold winter temperatures each June and July, and the weakened trees were attacked by borer. All the affected trees recovered and produced coppice growth lower down on the stem (Plate 7). Diameter or circumference measurements were, however, taken from the main tree stem, and would therefore not be altered by this dieback. However, canopy spread and height measurements were always obtained from living tissue, thus dieback in trees during winter would alter these readings and might affect the regression analysis against reproductive yield. To test for this, coppiced trees were omitted from the regressions. Results obtained indicated that omission of these affected trees did not influence the relationship between any of the measured parameters and reproductive yield. The degree of variation remained virtually the same.

Thus both coppiced and non-coppiced trees fit into these linear relationships between vegetative and reproductive parameters of bugweed.

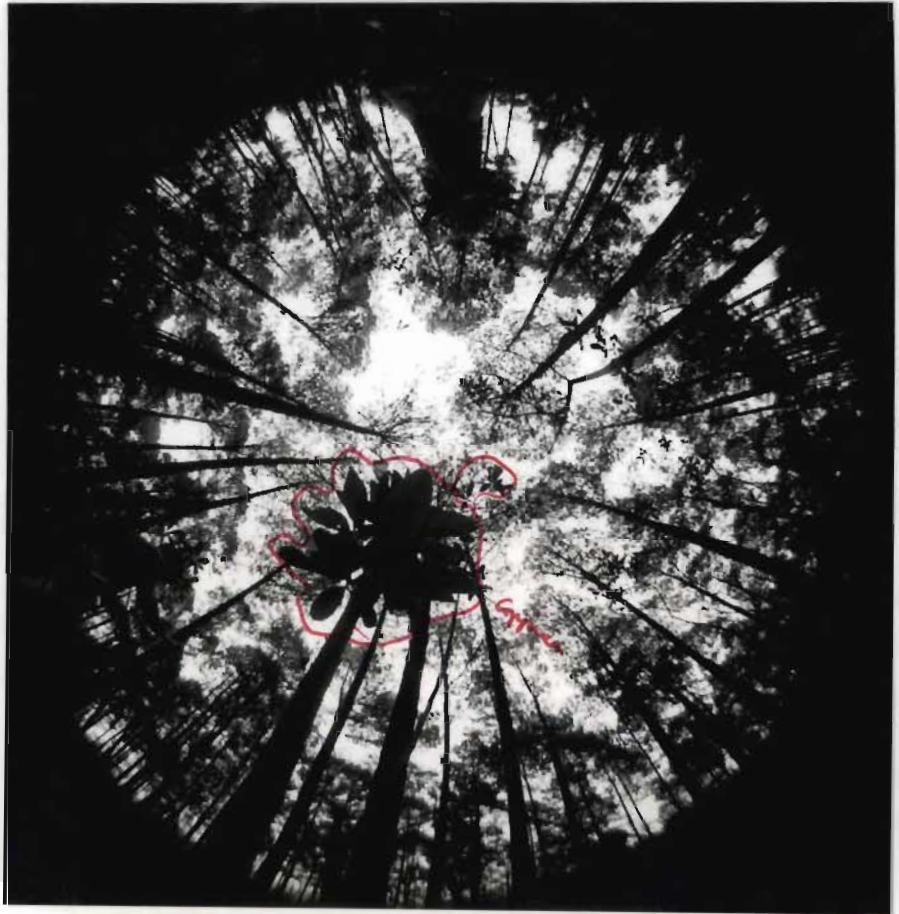


PLATE 7: Die-back of bugweed during winter induces coppice growth near the base of stems. Coppice growth is outlined in black.

(iii) **REPRODUCTIVE YIELD AND PHOTOSYNTHETIC CAPACITY**

Because canopy spread appeared to be the most significant indicator of fruit yield, a more detailed study was made of leaf output on the trees.

(a) *Non-destructive measurements with a hemispherical camera*

Hemispherical photography was used to record the leaf canopy of each tree. Relative percentage leaf areas were calculated and these values were related to fruit and viable seed yield, these being the facets of reproductive capacity of greatest significance to the spread of the species.

The regression coefficients proved highly significant when the trees were photographed in June or August, 1987 (Table 1.9). During this winter season, the four trees which died back produced coppice growth lower down on their stems and this greatly affected scoring of their photographs. Percentage cover values for these trees as recorded by the camera were then inaccurate, being far too high, because the leaves on these coppiced trees were nearer the camera than those on a normal tree, and thus appeared larger on the photographs. As a result the significance of regression coefficients was low when photographed in November that year (Table 1.9). Nonetheless, regression coefficients became highly significant after the four trees were omitted from the analysis (Table 1.9).

During February, when the peak of summer growth occurred, a much higher leaf output was evident (Plate 8). The regression coefficients then proved less significant ($P < 0.050$) than other recorded values, probably due

TABLE 1.9: The influence of relative percentage leaf area on reproductive yield of bugweed. The regression coefficients (RC), their standard errors (SE), and the significance of these relationships in terms of % variation accounted for in each case (% V) and from T tables, are presented below (n = 29). All relationships are linear.

| DATE WHEN PHOTOGRAPHS RECORDED THE LEAF CANOPY | | | FRUIT NUMBERS | | | | VIABLE SEED NUMBERS | | | |
|--|-----|----------|---------------|------|------|------|---------------------|-------|------|------|
| | | %f (n-2) | RC | SE | % V | T | RC | SE | % V | T |
| June 1987 | (a) | 27 | 149.2 | 24.5 | 56.4 | XXXX | 14 748 | 2 341 | 58.0 | XXXX |
| August 1987 | (a) | 27 | 192.5 | 39.1 | 45.4 | XXXX | 18 595 | 3 843 | 44.5 | XXXX |
| November 1987 | (a) | 27 | 102.0 | 37.4 | 18.7 | XX | 10 202 | 3 621 | 19.9 | XXX |
| | (b) | 23 | 231.2 | 33.6 | 65.9 | XXXX | 22 354 | 3 413 | 63.6 | XXXX |
| February 1988 | (a) | 27 | 14.5 | 21.8 | < 1 | NS | 1 692 | 2 116 | < 1 | NS |
| | (b) | 20 | 82.1 | 32.8 | 20.1 | X | > 505 | 3 233 | 17.3 | X |
| June 1989 | (a) | 27 | 28.4 | 36.9 | < 1 | NS | 2 659 | 3 598 | < 1 | NS |
| | (b) | 18 | 277.1 | 77.6 | 38.2 | XXX | 27 001 | 7 774 | 36.8 | XXX |

a = regressions include coppiced trees
b = regressions exclude coppiced trees
X (P < 0.050)
XX (P < 0.020)
XXX (P < 0.010)
XXXX (P < 0.001)
NS not significant

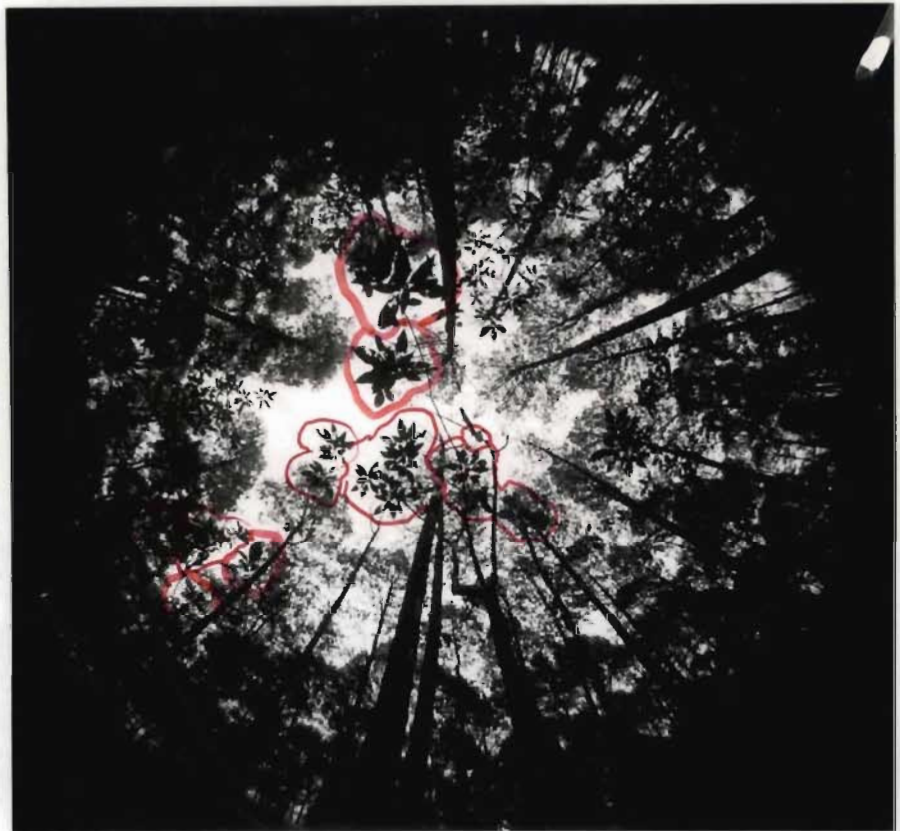


PLATE 8: Overlapping leaves in the canopy of bugweed during summer. The leaf canopy is outlined in black.

to overlapping leaf growth affecting relative percentage cover readings (Table 1.9).

The final set of photos were taken six months after the end of the study. During the second winter of the study (July 1988) a further five trees had died back and coppiced. Here, inclusion of all trees once again produced unreliable results, but exclusion of these nine coppiced trees from the regression analysis showed a significance of $P < 0.01$ (Table 1.9). Thus any predictions based on this relationship between relative percentage leaf cover and fruit or viable seed yield must exclude coppiced trees.

(b) *Destructive harvesting of leaves*

At the end of this study, all leaves were removed from each of the trees; weighed, measured to determine area, dried and then reweighed. Data were subsequently related to fruit and viable seed yield. Results are presented in Table 1.10. It is evident that leaf area and both wet and dry leaf mass had a highly significant relationship with fruit and viable seed numbers (Table 1.10). To test the accuracy of using hemispherical photography to estimate relative percentage leaf area, the percentage cover values measured in June 1989 were correlated with leaf area, fresh mass and dry mass. For $n=29$ trees, correlation coefficients were not significant for these values. When the nine coppiced trees were omitted from the analysis, however, (Figure 1.9) the correlation coefficients were highly significant ($P < 0.001$). It is therefore evident that the coppicing effect itself did not affect the regression analysis since leaves from these trees were included in the regressions with fruit and viable seed numbers when

TABLE 1.10: The influence of area, fresh mass and dry mass of leaves on reproductive yield of bugweed. The regression coefficients (RC), their standard errors (SE) and the significance of these relationships in terms of % variation accounted for in each case (% V) and from T tables are presented below (n = 29). All relationships are linear.

| Leaf measurements of bugweed under pine (June 1989) | Dependent variable | | | | | | | |
|---|--------------------|-------|------|-----|--------------------|-------|------|-----|
| | Fruit number | | | | Viable seed number | | | |
| | RC | SE | % V | T | RC | SE | % V | T |
| Leaf area (cm ²) | 18.56 | 3.00 | 57.2 | XXX | 0.190 | 0.030 | 57.1 | XXX |
| Leaf fresh mass (g) | 0.333 | 0.048 | 62.5 | XXX | 0.003 | 0.001 | 59.4 | XXX |
| Leaf dry mass (g) | 0.096 | 0.014 | 62.1 | XXX | 0.001 | 0.000 | 58.6 | XXX |

XXX Highly significant relationship (P < 0.001)

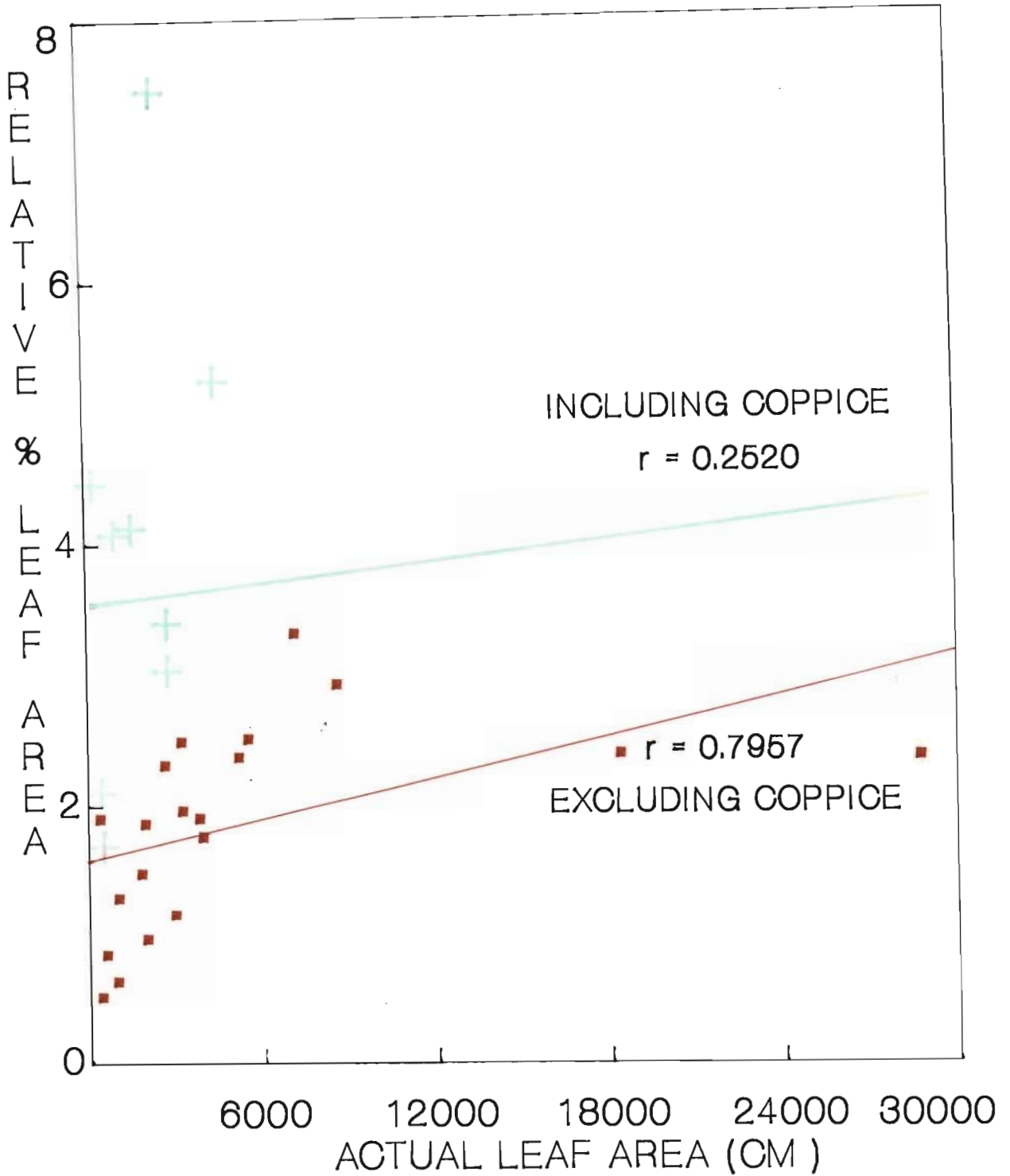


FIGURE 1.9: The relationship between relative % leaf area of bugweed and actual leaf area. The former was recorded by a hemispherical lens and actual leaf area was measured by means of a leaf area recorder. The correlation coefficient (r) is given to describe the relationship when excluding (\rightarrow) and including (\leftarrow) leaves from coppiced trees.

destructive techniques were used (Table 1.10). It is only when the measurement of this coppice growth is indirect i.e. by photography rather than by direct means (leaf area, fresh or dry mass) that the relationship is affected.

(iv) **REPRODUCTIVE YIELD AS INFLUENCED BY NEIGHBOURING SPECIES/COMPETITION**

Another biotic component of the system measured was the effect of neighbouring plants on fruit and seed yield of bugweed under a pine canopy. Here, however, correlation coefficients between fruit or viable seed numbers and neighbouring plants were not significant (Table 1.11). Thus trees, shrubs and herbaceous plants growing within a 2 m radius of each bugweed tree did not influence fruit yield.

(v) **PREDICTED REPRODUCTIVE YIELD OF AN "AVERAGE-SIZED" BUGWEED TREE UNDER A PINE CANOPY**

Based on the results from this sample, an "average-sized" bugweed tree which is not more than 5 years old due to the thinning operations in this pine stand, has a stem diameter of 32 mm and a circumference of 98 mm at 100 cm above the soil surface. This tree would have a canopy spread of 3.99 m², and a height of 3.68 m (Table 1.12). Based on the relationships between tree size and fruit yield (see Section (ii) above), such a tree could be expected to produce 157 ± 12 fruits and $18\,095 \pm 1\,342$ viable seeds in 20 months (Table 1.12). Each fruit on average would contain 115 ± 3 viable seeds ($86 \pm 12\%$ of the total number of seeds).

An average-sized tree with a canopy spread of 3.99 m² has a relative leaf

TABLE 1.11: The influence of neighbouring plants/competition on reproductive yield of bugweed. The correlation coefficient (R), their standard errors (SE) and significance as described by the student "t" test are presented below.

| DEPENDENT VARIABLE | TOTAL VALUES FOR NEIGHBOURING PLANTS | | | |
|---------------------|--------------------------------------|-------|--------|--------------|
| | R | SE | t | Significance |
| Fruit numbers | -0.311 | 0.183 | -1.702 | NS* |
| Viable seed numbers | -0.234 | 0.187 | -1.252 | NS |

* NS = no significant correlation.

TABLE 1.12: A comparison of using several vegetative growth measurements of an average-sized bugweed tree under a pine canopy to predict fruit and seed yield over 20 months. All relationships are linear and highly significant ($P < 0.001$).

| Vegetative growth measurements of an average-sized tree | Dependent variable | | | | |
|---|--|--|--|---|----------------------------|
| | Predicted fruit number ^a | Predicted total seed number ^a | Predicted viable seed number ^a | Viable seed number per fruit ^b | % viable seed ^c |
| Stem diameter at 1 m above soil = 32 mm (June 1987) | 159 (67.7) ^d $y = 21x - 513$ ^e | 19 226 (63.9) $y = 2\ 576x - 63\ 206$ | 17 630 (63.4) $y = 2\ 397x - 59\ 074$ | 111 | 92 |
| Stem circumference at 1 m above soil = 98 mm (June 1987) | 158 (62.4) $y = 6.68x - 497$ | 20 110 (59.4) $y = 834x - 61\ 622$ | 18 472 (59.0) $y = 777x - 57\ 674$ | 117 | 92 |
| Branch spread or canopy area = 3.99 m ² (May 1987) | 170 (81.7) $y = 78x - 141$ | 28 398 (78.2) $y = 8\ 795x - 6\ 694$ | 19 726 (77.6) $y = 9\ 038x - 16\ 336$ | 116 | 69 |
| Tree height = 3.68 m (May 1988) | 141 (57.7) $y = 141x - 378$ | 18 045 (59.1) $y = 18\ 233x - 49\ 052$ | 16 550 (58.8) $y = 16\ 993x - 45\ 984$ | 117 | 92 |
| Mean \pm SE | 157 \pm 12 | 21 445 \pm 4 712 | 18 095 \pm 1 342 | 115 \pm 3 | 86 \pm 12 |
| Relative % leaf area = 1.79 % (June 1987) | 227 (56.4) $y = 147x - 36$ | 18 457 (56.5) $y = 15\ 527x - 9\ 336$ | 17 124 (58.0) $y = 13\ 147x - 6\ 409$ | 75 | 93 |

^a Based on raw data of results presented in Tables 1.5 to 1.9

^b Calculated by $\frac{\text{Predicted viable seed number}}{\text{Predicted fruit number}}$

^c Calculated by $\frac{\text{Predicted viable seed number} \times 100}{\text{Predicted total seed number}}$

^d % variation in the dependent variable accounted for by the vegetative growth measurement

^e slope of the linear relationships

area of 1.79 %. Based on the relationship between relative percentage leaf area and fruit yield (see Section (iii) above), this tree would probably produce 227 fruits and 17 124 viable seeds in 20 months (Table 1.12). Each fruit on average would contain 75 viable seeds (Table 1.12).

There is therefore discrepancy in reproductive yield of an average-sized tree as measured by different tree size parameters or by leaf measurements. To ascertain which measurement is the most accurate indicator of reproductive yield, total fruit number over the 20 month study period was related to total viable seed number. The relationship of this is $y = 76x + 5$, with a T value of 15.06 and the percentage variation accounted for is 82.8 % (Figure 1.10). From this, the percentage error of the various measurements for predictions of fruit and seed yield can be ascertained. These are presented in Table 1.13.

It is apparent that the degree of error in viable seed yield prediction using gross tree size measurements is fairly large, approximately 35 % (Table 1.13). It was also evident that predictions from stem diameter measurements ranging from 28mm to 72mm retained a similar % error (Table 1.13). In contrast to this, indirect recording of leaf area during winter with a hemispherical lens and subsequent measurements of relative percentage leaf area with a two-dimensional grid is highly accurate with an error of 0.8 % (Table 1.13).

The relationship between tree size and fruit size was then investigated *i.e* do large trees mostly produce large fruits? Conversely, do small trees

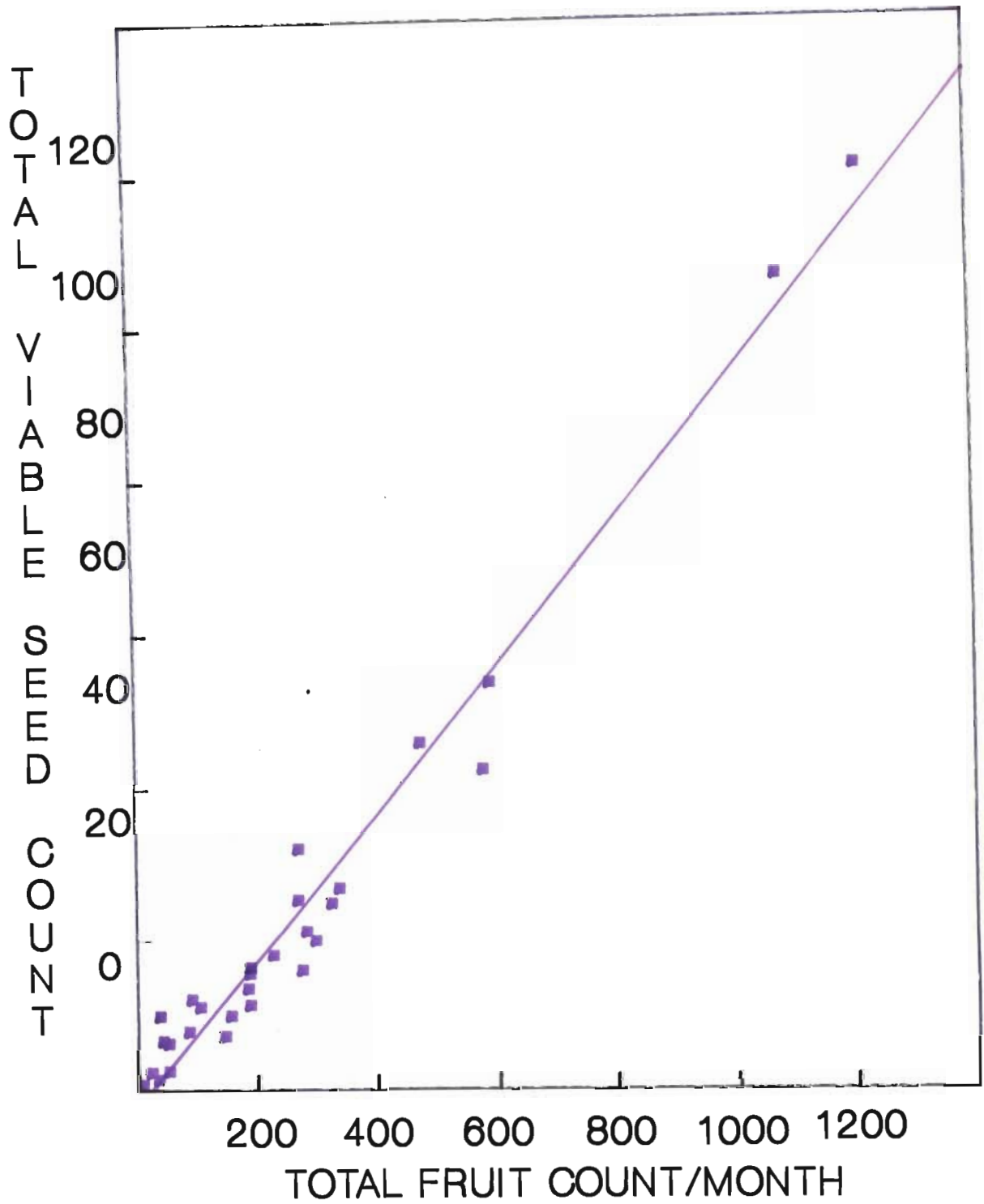


FIGURE 1.10: The relationship between total fruit number of bugweed and total viable seed number produced over 20 months. This is described by $y = 76x + 5$, where a T value of 15.06 is highly significant ($P < 0.001$). The % variation in total viable seed number accounted for by total fruit numbers is 82.8 %.

TABLE 1.13: A comparison of using different measurements of tree size to predict viable seed yield in bugweed under a pine canopy. The percentage error in viable seed numbers from each measurement of tree size is given.

| Growth parameter measured | Actual fruit numbers ^a | Total viable seed number ^a | Expected viable seed number ^b | % error ^c |
|--|-----------------------------------|---------------------------------------|--|----------------------|
| Stem diameter (32 mm) ^d June 1987 | 159 | 17 630 | 12 089 | 31.4 |
| Stem circumference (98 mm) (June 1987) | 158 | 18 472 | 12 013 | 34.9 |
| Canopy spread (3.99 m ²) (May 1987) | 170 | 19 726 | 12 925 | 34.5 |
| Tree height (3.68 m) (May 1988) | 141 | 16 550 | 10 721 | 35.2 |
| Relative % leaf area (1.79 %) (June 1987) | 227 | 17 124 | 17 257 | 0.8 |

^a Calculated from linear relationships presented in Table 1.12.

^b Calculated from the linear relationship presented in Figure 1.10.

^c $\frac{\text{Difference between viable seed number}^a \text{ and expected viable seed number}^b \times 100}{\text{viable seed number}^a}$

^d Percentage error for stem diameter 28 mm is 29.1 %; for 53 mm is 32.9 % and for 72 mm is 33.0 %, respectively.

mostly produce small fruits? To ascertain this relationship, ripe fruits were collected and then divided into three categories based on fruit mass:

1. small fruits (< 1.5 000 g)
2. medium fruits (1.5 000 to 2.1 999 g)
3. large fruits (>2.2 000 g)

Results presented in Tables 1.14 to 1.17 showed that tree size, whether measured by stem diameter or circumference, or tree height or canopy spread, did not influence the proportion of small fruits produced. Thus although large trees produced more fruits than small trees, *a similar proportion of small fruits was found on all trees in the population*. In addition to this, seed size is not affected by tree size. Small and large bugweed trees, as measured by stem girth, canopy spread, tree height and relative percentage canopy cover, have *similar-sized viable seeds* (Table 1.18).

(vi) **SUPPRESSION OF BUGWEED FRUIT YIELD UNDER A PINE CANOPY**

When observing bugweed trees growing under a pine canopy, it is evident that their growth has been suppressed, since their habit is spindly compared with that of trees growing in the open. This raised the question whether reproductive yield was also suppressed?

A comparison was made between the fruit production of this sample of 30 trees under a pine canopy and that of four trees growing in an open sunny situation in the Plant Protection Research Institute (P P R I) weed garden

TABLE 1.14: Small fruit proportion as a dependent variable of bugweed stem diameter. Each measurement was taken at 25, 50, 100 and 130 cm above soil level. The regression coefficients (RC), their standard errors (SE) and the significance of these relationships in terms of % variation accounted for in each case (% V) and from T tables, are presented below (n = 29).

| Proportion small (< 1.5 g) fruits | Height above soil of diameter measurement (cm) | | | |
|-----------------------------------|--|------|------|------|
| | 25 | 50 | 100 | 130 |
| RC | -0.2 | -0.2 | -0.2 | -0.3 |
| SE | 0.3 | 0.3 | 0.3 | 0.3 |
| % V | 0 | 0 | 0 | 0.3 |
| T | NS | NS | NS | NS |

NS = relationship not significant

TABLE 1.15: Small fruit proportion as a dependent variable of bugweed stem circumference. Each measurement was taken at 25, 50, 100 and 130 cm above soil level. The regression coefficients (RC), their standard errors (SE) and the significance of these relationships in terms of % variation accounted for in each case (% V) and from T tables, are presented below (n = 29).

| Proportion small (< 1.5 g) fruits | Height above soil of circumference measurement (cm) | | | |
|-----------------------------------|---|------|------|------|
| | 25 | 50 | 100 | 130 |
| RC | -0.1 | -0.1 | -0.1 | -0.1 |
| SE | 0.1 | 0.1 | 0.1 | 0.1 |
| % V | 0 | 0.6 | 0.9 | 3.7 |
| T | NS | NS | NS | NS |

NS = relationship not significant

TABLE 1.16: Small fruit proportion as a dependent variable of bugweed tree height. The regression coefficients (RC), their standard errors (SE) and the significance of these relationships in terms of % variation accounted for in each case (% V) and from T tables, are presented below (n = 29).

| Proportion small (< 1.5 g) fruits | Year of height measurement | | | |
|-----------------------------------|----------------------------|--------------|----------|----------|
| | May 1987 | January 1988 | May 1988 | May 1989 |
| RC | -6.4 | -3.2 | -2.2 | -1.9 |
| SE | 3.6 | 2.9 | 2.2 | 2.0 |
| % V | 8.5 | 0.7 | 0.2 | 0 |
| T | NS | NS | NS | NS |

NS = relationship not significant

TABLE 1.17: Small fruit proportion as a dependent variable of bugweed canopy spread. The regression coefficients (RC), their standard errors (SE) and the significance of these relationships in terms of % variation accounted for in each case (% V) and from T tables, are presented below (n = 29).

| Proportion small (< 1.5 g) fruits | Year of branch spread measurement | | | |
|-----------------------------------|-----------------------------------|--------------|----------|----------|
| | May 1987 | January 1988 | May 1988 | May 1989 |
| RC | -0.3 | -1.4 | -1.1 | -1.7 |
| SE | 1.0 | 1.1 | 1.2 | 1.3 |
| % V | 0 | 3.3 | 0 | 3.0 |
| T | NS | NS | NS | NS |

NS = relationship not significant

TABLE 1.18: The relationship between viable seed size and various tree size parameters in bugweed. Tree size parameters were measured by stem diameter and circumference, canopy spread, tree height and relative percentage leaf cover. The regression coefficients (RC), their standard errors (SE) and the significance of these relationships in terms of % variation accounted for in each case (% V) and from T tables, are presented below (n = 29).

| Viable seed size | Tree size parameter | | | | |
|------------------|---------------------|---------------|---------------|-------------|-----------------------|
| | Diameter | Circumference | Canopy spread | Tree height | Relative % leaf cover |
| RC | 0.0003 | 0.0002 | 0.0028 | 0.0030 | 0.0002 |
| SE | 0.0007 | 0.0003 | 0.0014 | 0.0050 | 0.0020 |
| % V | 40.7 | 40.0 | 35.0 | 44.1 | 32.3 |
| T | NS | NS | NS | NS | NS |

NS = relationship not significant

at Cedara¹⁰. These four trees were five years old (DENNY & GOODALL, *pers. comm.*). Height and spread of the latter trees were similar to those of the trees under a pine canopy, although the trunks were thicker (Table 1.19).

A far higher fruit yield ranging from 3 194 to 5 169 fruits was produced by the four trees growing in sunny conditions (Table 1.20). Viable seed yield per tree was also very high when compared with yield of bugweed under a pine canopy, ranging from 221 879 to 429 077 seeds in one year (Table 1.20). If fruit and viable seed yield from trees of a similar size at both sites are compared (*i.e.* smallest tree in sun *vs.* largest tree under a pine canopy), the fruit yield of a bugweed tree in the sun is 3.89 times as high as that of similar-size tree growing under a pine canopy, while its viable seed yield is 2.50 times as high (Table 1.20). It would appear then that fruit and viable seed production of bugweed growing under a pine canopy can be described as being suppressed or reduced. This idea was further substantiated when considering fruit size in the whole bugweed sample, since 85 % of the fruits produced by bugweed under a pine canopy were in the small fruit category, < 1.5 000 g (Table 1.20) each containing approximately 66 seeds (Table 1.21). Only 32 % of the fruits produced by bugweed in the sun were in this category, the majority being medium-sized fruits, 1.5 000 to 2.1999 g (Table 1.20), each containing approximately 172 seeds (Table 1.21). Most of the seeds were viable, regardless of fruit size

¹⁰This area of land, roughly 0.1 hectare, was established by P P R I for educational purposes and contains many Natal invader species, including four bugweed trees.

TABLE 1.19: Size description of bugweed trees used to compare fruit and viable seed yield between trees growing under a pine canopy and in the sun.

| Fruit collection site (m ²) | Sample number (n) | Diameter range at 25 cm above soil surface (cm) | Circumference range at 25 cm above soil surface (cm) | Tree height range (m) | Canopy spread range (m) (May 1987) |
|---|-------------------|---|--|-----------------------|------------------------------------|
| Trees in a pine stand | 29 | 2.2 to 5.6 | 6.5 to 16.8 | 2.0 to 5.0 | 1.04 to 12.75 |
| Trees in sunny conditions | 4 | 5.7 to 9.4 | 16.4 to 26.5 | 3.2 to 3.4 | 9.52 to 15.91 |

TABLE 1.20: Fruit and viable seed yield in bugweed growing under a pine canopy and in the sun. Yields were presented as the range produced during the period of May 1987 to May 1988.

| Collection site | Sample number (n) | Number of fruits per tree in one year (range) | Number of viable seeds per tree in one year (range) | Proportion (%) fruits \pm SE | | |
|---|-------------------|---|---|--------------------------------|--------------|------------|
| | | | | < 1.5 g | 1.5 to 2.1 g | > 2.2 g |
| Bugweed under pine | 29 | 7 to 822 | 775 to 88 583 | 85 \pm 12 | 14 \pm 12 | 1 \pm 1 |
| Bugweed in sun | 4 | 3 194 to 5 169 | 221 879 to 429 077 | 32 \pm 3 | 51 \pm 2 | 17 \pm 3 |
| Smallest bugweed in sun \div Largest bugweed under pine | | 3.89 x more | 2.50 x more | 2.66 x less | 3.64 x more | 17.0 more |

TABLE 1.21: A comparison between the seed composition of different-sized ripe bugweed fruits. Fruits were separated into three categories; < 1.5 000 g; 1.5 to 2.1 999 g and > 2.2 000 g. The percentage coefficient of variation (% CV) and LSD at the 5 % level are presented below.

| Fruit size (g) | Total seed number per fruit | Viable seed number per fruit | Dead seed number per fruit | % viable seeds | Mass per dead seed* (mg) | | Mass per viable seed* (mg) |
|----------------|-----------------------------|------------------------------|----------------------------|----------------|--------------------------|-------|----------------------------|
| | | | | | Small | Large | |
| < 1.5 000 | 66 | 57 | 9 | 86 | 0.19 | 0.58 | 1.45 |
| 1.5 - 2.1 999 | 172 | 161 | 11 | 94 | 0.14 | 0.45 | 1.35 |
| > 2.2 000 | 201 | 188 | 13 | 94 | 0.16 | 0.45 | 1.32 |
| % CV | 6.8 | 3.8 | 52.2 | 4.7 | 19.5 | 5.3 | 6.7 |
| LSD 5 % | 15.9 | 8.1 | 9.2 | 6.9 | 0.05 | 0.04 | 0.15 |

* Calculated from total mass of viable (or dead) seeds per total viable (or dead) seed numbers.

(Table 1.21). Viable seeds *are the same size*, approximately 1.35 mg (Table 1.21).

(vii) **VIABLE SEED YIELD PER HECTARE UNDER A PINE CANOPY**

A transect of 300 x 4 m² was laid down in the study area. Stem diameter of all bugweed trees within this area were measured at a height of 100 cm above the soil surface, as this has earlier been established as a reliable indicator of viable seed production (Table 1.5). It was also the most convenient way of obtaining an indication of tree size. The frequency of stem diameter present in this transect is presented in Table 1.22. This was converted to tree number per hectare. Viable seed numbers per tree in each diameter class were then calculated, using the equation $y = 2\,397x - 59\,074$, based on the linear regression between stem diameter and viable seed number (Table 1.12). These values were converted to seed number per hectare for each diameter class, and the product gave the total expected viable seed number by this population over a 20 month period (Table 1.22).

From these data, approximately 10.5 million viable seeds per hectare can be produced in 20 months by bugweed growing under a pine canopy (Table 1.22). Since predictions of viable seed numbers from stem diameter measurements are apparently an overestimate by approximately 32 % in a tree size range of 28 mm to 72 mm (Table 1.13), this is recalculated as 7.2 million viable seeds per hectare which can be produced in 20 months. It is therefore clear that reproductive yield in bugweed on a per hectare

TABLE 1.22: Viable seed yield in 20 months of bugweed growing under a pine canopy. The frequency of trees in different size classes is presented below. Based on this, tree number per hectare, viable seed number for each tree size class and for this population on a per hectare basis are calculated.

| | Seedlings | Frequency of stem diameter measured at 100 cm above soil level | | | | | | | | | | | | | |
|--------------------------------------|-----------|--|------|--------|--------|---------|-----------|-----------|-----------|-----------|---------|---------|-----------|---------|---------------------------|
| | | <1 cm | 1 cm | 1.5 cm | 2.0 cm | 2.5 cm | 3.0 cm | 3.5 cm | 4.0 cm | 4.5 cm | 5.0 cm | 5.5 cm | 6.0 cm | 6.5 cm | Total |
| Tree no. in transect ^a | 116 | 38 | 74 | 48 | 47 | 16 | 11 | 9 | 6 | 4 | 1 | 1 | 3 | 1 | 375 |
| Percentage total tree no. | 30.9 % | 10 % | 20 % | 13 % | 12.5 % | 4.1 % | 3 % | 2 % | 1.5 % | 1.1 % | 0.3 % | 0.3 % | 1 % | 0.3 % | 100 % |
| Tree no./ha | 967 | 317 | 617 | 400 | 392 | 133 | 92 | 75 | 50 | 33 | 8 | 8 | 25 | 8 | 3 125 |
| Seed no./tree/20 months ^b | 0 | 0 | 0 | 0 | 0 | 851 | 12 836 | 24 821 | 36 806 | 48 791 | 60 776 | 72 761 | 84 746 | 96 731 | 439 119 |
| Seed no./ha/20 months ^c | 0 | 0 | 0 | 0 | 0 | 113 183 | 1 180 912 | 1 861 575 | 1 840 300 | 1 610 103 | 486 208 | 582 088 | 2 118 650 | 773 848 | 10.5 million ^d |

^a The transect direction was 165° and covered 0.12 ha.

^b Calculated using the equation $y = 2.397x - 59.074$ for viable seed number where x = tree diameter (in cm) at a height on the stem of 100 cm above soil level.

^c Calculated by tree number/ha x seed number/tree/20 months.

^d Later adjusted to 7.2 million to account for the 31.4 % error in the prediction when using the equation $y = 2.397x - 59.074$ (refer to text).

basis can be very high. No doubt this contributes to the success of the weed.

(viii) **DISPERSAL POTENTIAL**

Seeds within ripe fruits eaten by animals may have one of five responses after passage through the gut. First, they may be rendered non-viable; secondly, they may become scarified via action of gut acids; thirdly, they may become freed from any soluble inhibitors which might be present (VAN DER PIJL, 1972; TYAGI & SHARMA, 1982); fourthly their response to environmental conditions such as light may change (VÁZQUEZ-YANES & OROZCO-SEGOVIA, 1986) and lastly, they may be totally unaffected in terms of germination response.

Seeds collected from faeces of a blue duiker (Plate 9) were incubated in the presence of faecal material at optimum conditions (15/30°C in the light). The germination response was found to be high (Figure 1.11). Thus seeds retain their viability during ingestion by duiker and germinated readily under favourable environmental conditions. This action is not thought to be one of scarification, since notched (mechanical scarification) seeds and intact control seeds germinated equally well (Figure 1.11).

Seeds immersed in aqueous solution containing 33 %, 50 % or 100 % volumes of fruit pulp from ripe fruits did not germinate at optimum conditions. However, washing these seeds well in running tap water after 12 weeks incubation resulted in some germination (Figure 1.11). Thus passage through the gut of an animal separates seeds from the maternal fruit tissue and may well also simultaneously remove any inhibitors from



PLATE 9:

- Animals used in the feeding experiment to test germinability of bugweed.
- (A) A captive blue duiker at University of Natal. Bugweed fruits were fed to this animal and seeds collected from faeces.
 - (B) Gut contents of a Rameron pigeon indicating the ingestion of bugweed seeds.

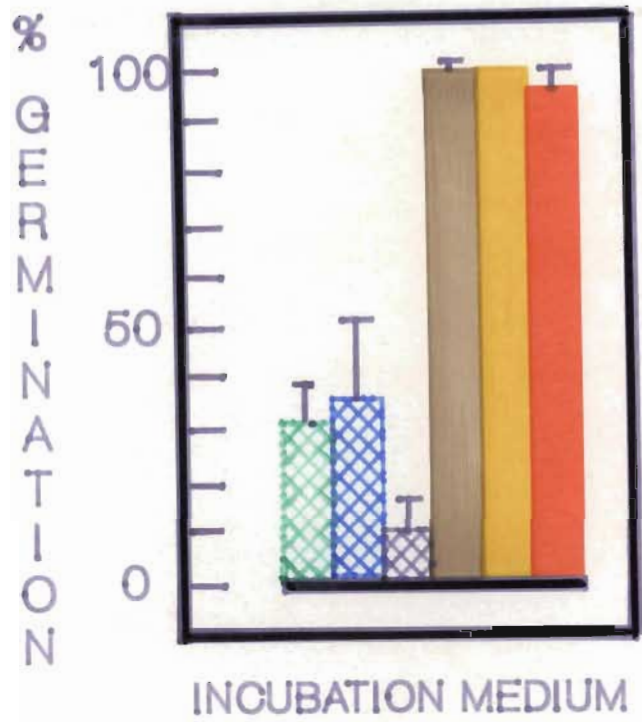


FIGURE 1.11: Germination of bugweed seeds under optimum conditions (15/30°C in the light) after ingestion by a blue duiker (—). Control treatments are intact (—) or mechanically notched (—) seeds incubated in distilled water; or intact seeds incubated in 33 % (—), 50 % (—) or 100 % (—) fruit pulp. Solid bars represent germination after 12 weeks. Hatched bars represent germination after 12 weeks when seeds were subsequently washed free of fruit pulp. Vertical bars represent the standard errors of the means.

the seed. Non-germinated seeds washed free of fruit pulp retained their viability and apparently had become dormant, possibly due to inhibitors from fruit material entering into the seeds. A secondary fungal infection eventually killed the non-germinated seeds.

Dissection of a wild Rameron pigeon showed the ingestion of approximately 700 seeds during one feed (Plate 9). Of these, from 89 to 100 % sank in water and were considered viable (Figure 1.12). The germination responses of seeds from different portions of the gut of this pigeon to optimum conditions was variable (Figure 1.12).

None of the seeds from the crop and upper intestines germinated. Only 6.5 % of the seeds recovered from the stomach of the bird germinated (Figure 1.12). Seeds positioned lower in the intestines were covered in faecal material. These showed a 57 % seed germination response (Figure 1.12). Seeds collected from bird droppings on a bugweed plant near where the pigeon was killed showed a lower percentage viability than those collected from the bird but a much higher germination response (Figure 1.12). Fruit fibre material was mixed with seeds in the crop and stomach, and this may have caused inhibition of seed germination (Figure 1.12) even though seeds were cleaned before the 12 weeks of incubation at optimum conditions. Passage through the intestines removed this fruit material and a higher germination response was seen. These results suggest that seeds retain their viability when eaten by a Rameron pigeon and therefore dispersal, not predation, occurs. Seeds from faeces of bush pig showed a


| MOUTH |  | Number of sink seeds | Number of float seeds | Percentage viability | Percentage germination |
|---|---|----------------------|-----------------------|----------------------|------------------------|
| CROP | | 76 | 0 | 100 | 0 |
| STOMACH (WITH FRUIT FIBRES) | | 605 | 8 | 99 | 7 |
| UPPER INTESTINE (NO FAECES YET) | | 24 | 1 | 96 | 0 |
| LOWER INTESTINE (WITH FAECAL MATTER) | | 16 | 2 | 89 | 57 |
| FAECES FROM LEAF | | 42 | 14 | 75 | 100 |

FIGURE 1.12: Schematic representation of a Rameron pigeon gut containing seeds of bugweed. Seed number, percentage viability and the germination response of seeds from each part of the gut are presented above.

high mortality ($83 \pm 7 \%$)¹¹ since these seeds floated in water and would not respond to the application of $500 \text{ mg l}^{-1} \text{ GA}_3$. This is probably due to their storage conditions, as they were kept in an air-tight container for two years prior to being tested¹². Nonetheless, the remainder of the seeds germinated ($17 \pm 8 \%$) under optimum conditions, indicating these animals can spread bugweed throughout their home range. From these experiments it can be concluded that ingestion of seeds by these animals constitutes dispersal and not predation.

(ix) **THE FATE OF SEEDS AFTER DISPERSAL**

The fruits of bugweed are either eaten and dispersed by birds and animals or dropped nearby the parent plant. Seed numbers present in the soil vary with site and depth (DENNY & GOODALL, *pers. comm.*). Under large bugweed trees growing under close to ideal conditions in a valley at Cedara, most of the seeds counted in soil samples were located between 0 cm and 10 cm but seed numbers declined rapidly deeper in the soil (Table 1.23a). This pattern was also seen at a site where bugweed was growing under a pine canopy where bugweed had been slashed twice. However, far lower seed numbers were found at this site (Table 1.23b).

Although more than 90 % of the seeds at each site were dead, at both sites, the proportion of viable seeds was numerically sufficiently high to germinate and reinfest a cleared area. At the valley site an estimated 350 million viable seeds per hectare occurred, whereas 100 fold fewer seeds;

¹¹ Mean \pm SE

¹²The seed sample was obtained from Dr Melton, Zoology Department, UNP.

TABLE 1.23(a):

The distribution of viable and non-viable bugweed seeds in a soil profile from an "ideal site" (GOODALL & DENNY, *pers. comm.*). Results are presented as mean \pm SE. Sample size = 0.14 m x 0.15 m, thus 1 seed = 476 190 seeds per hectare.

| Depth below soil surface (cm) | Viable seed number | | Dead and damaged fraction | | Estimated total seed number |
|-------------------------------|--------------------|-------------|---------------------------|-------------|-----------------------------|
| | Total | % | Total | % | |
| litter | 67 \pm 47 | 3 \pm 2 | 1 964 \pm 1 014 | 97 \pm 2 | 2 031 \pm 1 046 |
| 0 - 5 | 310 \pm 117 | 7 \pm 6 | 5 846 \pm 3 222 | 93 \pm 6 | 6 157 \pm 3 275 |
| 5 - 10 | 282 \pm 245 | 9 \pm 4 | 2 950 \pm 2 824 | 91 \pm 4 | 3 232 \pm 3 058 |
| 10 - 15 | 52 \pm 19 | 13 \pm 4 | 366 \pm 159 | 87 \pm 4 | 418 \pm 168 |
| 15 - 20 | 24 \pm 22 | 16 \pm 14 | 442 \pm 723 | 84 \pm 14 | 466 \pm 721 |
| overall | *735 \pm 320 | 6 \pm 2 | 11 569 \pm 2 289 | 94 \pm 2 | 12 304 \pm 2 423 |

* 735 x 476 190 = 350 million viable seeds/ha

TABLE 1.23(b):

The distribution of viable and non-viable bugweed seeds in a soil profile under a pine canopy. Bugweed trees were slashed in 1985 and again in 1986 (GOODALL & DENNY, *pers. comm.*). Results are presented as mean \pm SE. Sample size = 0.14 m x 0.15 m, thus 1 seed = 476 190 seeds per hectare.

| Depth below soil surface (cm) | Viable seed number | | Dead and damaged fraction | | Estimated total seed number |
|-------------------------------|--------------------|-------------|---------------------------|-------------|-----------------------------|
| | Total | % | Total | % | |
| litter | 1 \pm 1 | 1 \pm 2 | 12 \pm 13 | 99 \pm 3 | 12 \pm 13 |
| 0 - 5 | 3 \pm 5 | 2 \pm 3 | 121 \pm 39 | 98 \pm 3 | 124 \pm 41 |
| 5 - 10 | 3 \pm 3 | 6 \pm 9 | 77 \pm 138 | 94 \pm 9 | 79 \pm 141 |
| 10 - 15 | 1 \pm 2 | 11 \pm 22 | 16 \pm 32 | 89 \pm 22 | 17 \pm 34 |
| 15 - 20 | 0 \pm 1 | 2 \pm 3 | 7 \pm 10 | 79 \pm 44 | 8 \pm 11 |
| overall | *8 \pm 10 | 3 \pm 4 | 233 \pm 193 | 97 \pm 4 | 241 \pm 200 |

* 8 x 476 190 = 3.8 million viable seeds/ha

3.8 million viable seeds per hectare, was found in soil from under the bugweed-infested pine stand (Tables 1.23a and b).

Fruit production at the second site was effectively halted due to slashing in 1985 and 1986, and consequently the continual input of seed to the soil bank was interrupted. In addition to this, the bugweed trees in this second site were growing under suppressed conditions. Much lower numbers of seeds were therefore found in the sample area, although the proportion of viable seeds was similar to that produced by trees growing under ideal conditions at the valley site. Nonetheless, sufficient viable seed numbers occur at this site to reinfest an area for many years to come.

In the study area described in this chapter this 90 % mortality would mean that out of 7.2 million viable seeds produced over a 20 month period in one hectare, only around 720 000 would remain viable in the soil.

In a separate study, DENNY & GOODALL (*pers. comm.*) found that a massive seedling mortality of 94 to 100 % occurred under a pure bugweed canopy. This was not due to inter-seedling competition, since similar trends were found in light, medium and dense seedling emergence (Figure 1.13). Rather, seedlings blackened and seemed to rot, due probably to moist conditions under the leaf litter. If this data is used for this study, then out of 720 000 viable seeds in the soil seed bank, only 6 % would survive as small seedlings, thus 43 200 seedlings per hectare could potentially emerge in the study site. In a transect through the study site, however, there were only 3 125 trees per hectare (Table 1.22). This

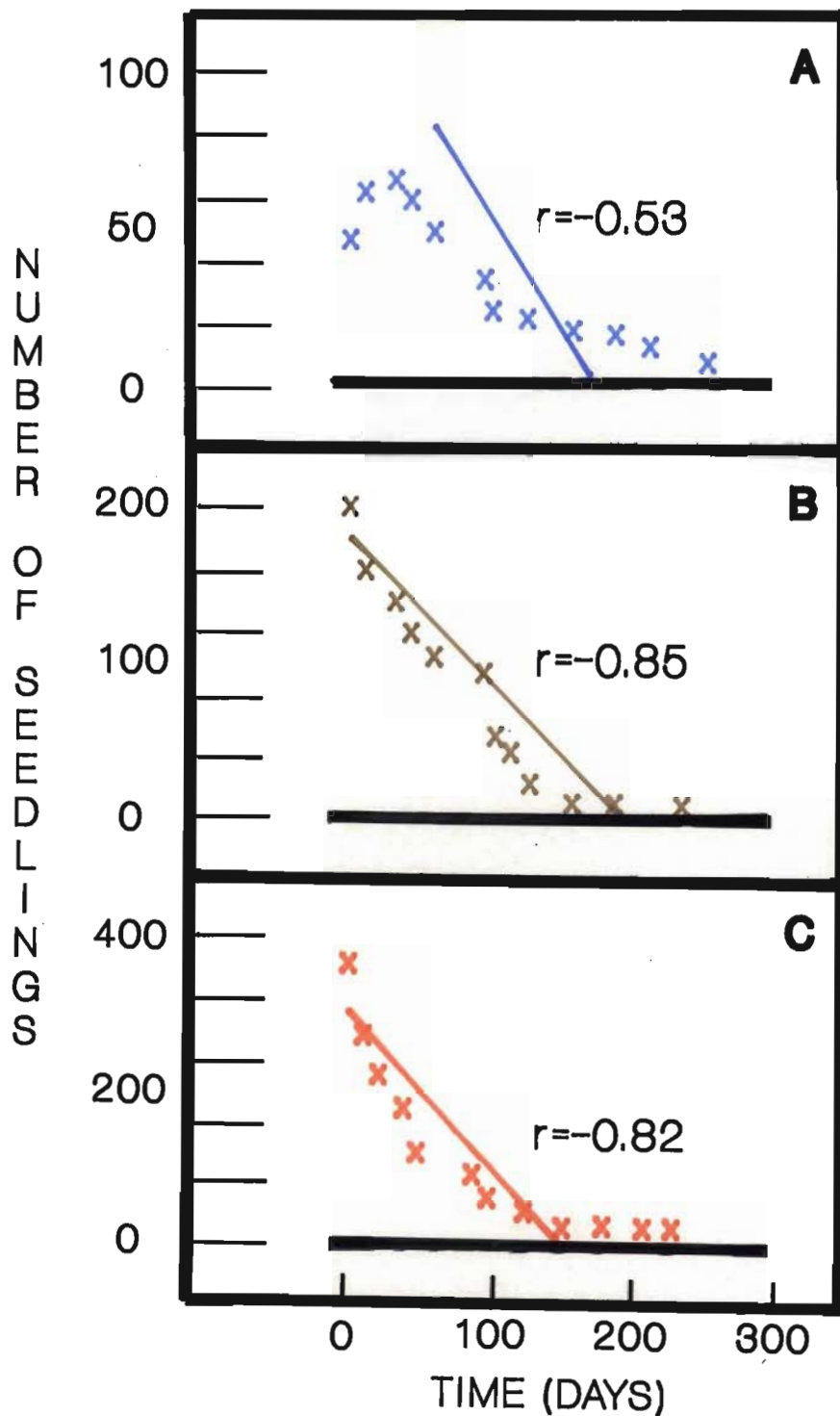


FIGURE 1.13: Seedling mortality in bugweed (GOODALL & DENNY, *pers. comm.*)

- (A) Light infestation of seedlings
- (B) Medium infestation of seedlings
- (C) Heavy infestation of seedlings

The number of live bugweed seedlings per 0.25 m² quadrant was recorded for up to 250 days. The correlation coefficient (r) was used to describe these relationships.

represents 7.2 % of the potentially emerging seedlings. This may be partially explained in terms of prolonged seedling emergence.

In a transect under pine where bugweed parent trees had been removed DENNY & GOODALL, (*pers. comm.*) found an initially massive germination occurred after removal of the parent trees, and seedlings were still emerging five years later, with the vast majority being produced after the summer months (Figure 1.14). Thus it is clear that although most seeds which fall from bugweed trees die, and seedling mortality is very high, the infestation problem arises from the much reduced but still significant numbers of viable seed, whose changing levels of dormancy lead to sporadic emergence. This plus the input of new seed from other trees has rendered current control operations aimed at parent trees ineffective in the long term.

The further theoretical fate of these seedlings was found by measuring transects of bugweed in stands where the pine trees (*Pinus patula*) had been given their third thinning 7 or 15 years previously. During thinning, bugweed trees were slashed and poisoned. Thus bugweed trees used in transects were less than or equal to 7 or 15 years old. For each stand, the number of bugweed trees in each diameter class was plotted on a log scale against a linear scale of diameter class. The correlation coefficient for each stem diameter/plant number distribution was then calculated, and found to be highly significant (Table 1.24). From prior regression analysis (Table 1.22), plants with stem diameter less than or equal to 2.5 cm do not produce fruits. In practice, however, it was found that trees of greater than

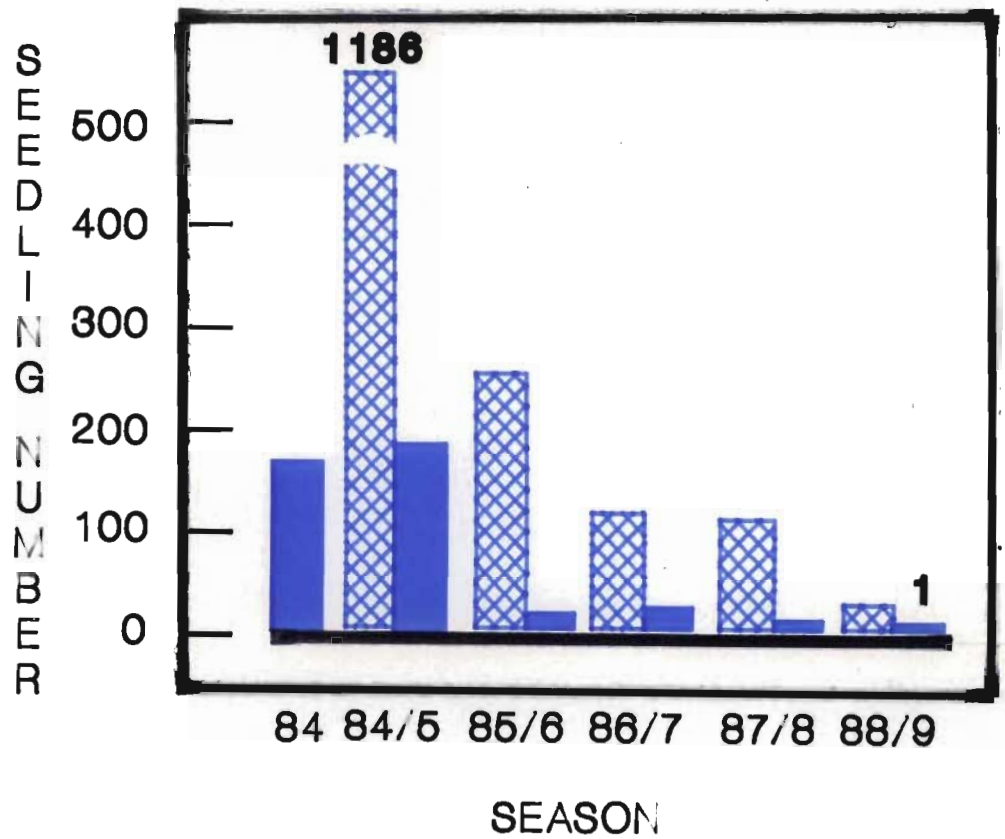


FIGURE 1.14: Seasonal seedling numbers of bugweed produced under a pine canopy (GOODALL & DENNY, *pers. comm.*). Seedling numbers were monitored bi-annually during winter (solid bars) and summer (hatched bars) months during the years 1984 to 1989.

TABLE 1.24: The relationship between plant numbers and stem diameter of bugweed under a pine canopy which had been thinned 7 or 15 years previously. The correlation coefficients (r) and significance as described by the student "t" test are presented below. All relationships are linear. Stems per hectare and the proportion (%) of reproductively immature trees (< 2 cm) are also presented below.

| Time of thinning | Transect | Significance of linear relationship (n = 13) | | | Total stem number per hectare | % trees < 2 cm diameter |
|------------------|----------|--|--------|--------------|-------------------------------|-------------------------|
| | | r | t | significance | | |
| 7 years | 1 | -0.971 | -13.46 | p < 0.001 | 3 125 | 74 |
| | 2 | -0.924 | - 7.99 | p < 0.001 | 6 004 | 60 |
| | 3 | -0.955 | -10.62 | p < 0.001 | 9 624 | 87 |
| Mean ± SE | | | | | 4 000 ± 1 800 | 74 ± 14 |
| 15 years | 1 | -0.862 | -5.64 | p < 0.001 | 970 | 80 |
| | 2 | -0.958 | -11.06 | p < 0.001 | 2 291 | 72 |
| | 3 | -0.952 | -10.26 | p < 0.001 | 1 848 | 62 |
| Mean ± SE | | | | | 1 700 ± 650 | 71 ± 9 |

or equal to 2 cm diameter could produce fruit. Approximately 70 % of the plants in these transects had not reached 2 cm stem diameter and had therefore not reached reproductive maturity, *irrespective of time since thinning* (Table 1.24).

Although there are potentially 43 200 seedlings per hectare (page 37), only approximately 4 000 stems per hectare were found (Table 1.24) in three 7 year stands of bugweed. This discrepancy between potential and actual stem numbers per hectare is due either to seed dormancy and/or to further intraspecific competition between older seedlings or suppression by the pine trees. Whatever the cause, sporadic germination over several years (Figure 1.14) will continually replenish this population, at a rate which maintains a proportion of 70 % non-reproductive plants.

In a 15 year stand of bugweed, the number of stems per hectare tended to decline to approximately 1 700 (Table 1.24) probably due to factors such as increased shading and competition from the larger pine trees. Sporadic germination replenishes this population *apparently at the same rate* to that occurring in 7 year old stands, since once again 70 % of these populations were not reproductively mature. Implications of this are that once conditions for growth of bugweed improve, for example, after clear-felling, windfall or lightning strikes which result in reduced competition for light and other factors, a rapid establishment of *reproductively mature* trees can occur.

From the results it was evident that fruits are produced periodically in flushes throughout the year. Fruit and seed yield is influenced by tree size. Measurements of stem diameter or circumference; tree height; canopy spread; relative percentage leaf area and actual leaf area are indicators for predictions of reproductive yield. Bugweed growing under a pine canopy apparently has a suppressed fruit and seed yield. However, approximately 7.2 million viable seeds per hectare over a 20 month period are produced by bugweed trees under these conditions. Seeds are dispersed by birds and animals and show high viability and germination percentages after ingestion. Although a high viable seed production occurs, most of the seeds found in soil beneath bugweed trees are dead, and a high seedling mortality occurs. Germination is apparently sporadic and the population is replenished at a rate which maintains a proportion of 70 % non-reproductive plants.

D. DISCUSSION

(i) PHYSICAL FACTORS AND PREDICTIONS OF REPRODUCTIVE YIELD

Many attempts to correlate crop yield measurements with corresponding weather, usually expressed in terms of monthly averages of temperature, rainfall or radiation during the growing season have proved unsuccessful (MONTEITH & SCOTT, 1982). A study of wheat yields and weather recorded over nearly a hundred years produced an immense amount of arithmetic but very little in terms of meaningful results. MONTEITH & SCOTT (1982) comment that the main reason for the lack of progress in studies of this kind is the complexity of the dynamic interaction between crops and their environment. The authors state that "little confidence can be placed on predictions from a computer model of the relation between crop yield and a specific element of weather." This is probably true also in the case of bugweed, where yield is determined by no one single environmental factor, but rather by an interaction of many variables.

(ii) BIOTIC FACTORS AND PREDICTIONS OF REPRODUCTIVE YIELD

The reproductive potential of a bugweed tree can, however, be related to tree size. The larger the tree the more fruits and seeds it produces. However, bugweed tree size is not related to size of fruits or seeds. Both small and large trees therefore produce similar-sized fruits and seeds. In addition to this, the size of viable seeds remains constant irrespective of fruit size.

This result contrasts with those reported in the literature, where seed weight within a species or even an individual plant can vary greatly, for example, in *Lomatium grayi* (= *Peucedanum grayi* Coulc. & F.N. Rose) (THOMPSON, 1984), *Lupinus texensis* Hook. (SCHAAL, 1980), *Silene alba* (Miller) Krause (GROSS & SOULE, 1981), *Pastinaca sativa* L. (HENDRIX, 1984), and *Castanea mollissima* Bl. (SHEPARD, MILLER & MILLER, 1989). Such variation in seed weight within a species might affect subsequent germination and/or seedling characteristics and therefore also population recruitment. Large seeds frequently have a greater germination response or emergence than small seeds and produce larger more vigorous seedlings (HENDRIX, 1984; SHEPARD, MILLER & MILLER, 1989). HENDRIX (1984) commented that, on the other hand, small seeds might germinate more rapidly than large seeds and thus gain a competitive advantage.

In the case of bugweed under a pine canopy, all seeds appear to have an equal emergence potential, whether they are from small or large fruits or from small or large trees.

Although no single physical factor, among those studied, apparently affected reproductive potential of bugweed, it is probable that light plays the most important role in determining seed yield, since it is the area of leaves, the organs which intercept light to produce chemical energy, which, to a large degree, determines yield. The use of hemispherical photography for recording leaf area of canopies has provided a good indicator of reproductive potential in bugweed. Advantages of this technique are that

the resulting photographs contain a great deal of information on the foliage and its distribution, they offer high resolution, the recording medium is inexpensive, the process of recording is rapid and the photographs can be stored for analysis at leisure (CLARK & FOLLIN, 1988). However, despite several reports on the use of hemispherical photography to study vegetation (CLARK & FOLLIN, 1988) the technique has not received widespread application (CLARK & FOLLIN, 1988). This is, in part, because manual analysis of the resulting photographs is slow and laborious, a problem which has led to attempts to automate analysis (BONHOMME & CHARTIER, 1972). In addition to this, analysis of photographs is complicated by the requirement for calibrations to compensate for errors caused by image distortion due to unavoidable limitations of lens design (HERBERT, 1986, 1987).

Neither of these problems seemed insurmountable in the case of bugweed. Using the grid system and computerizing the analysis, provided a rapid method of determining relative percentage leaf area. Errors in estimated areas caused by image distortion occur particularly towards the edge of the image disc (HERBERT, 1987). In this study of bugweed, the image distortion due to lens design was corrected by using the equation:

$$CSC = SC \cdot \cos \alpha \cdot \sin \alpha$$

where CSC is the sum of cover values, corrected for distortion; α is the angle of elevation above the horizon, and SC the sum of cover values for each angle (Figure 1.7).

This proved adequate for all non-coppiced trees, since their foliage was in the centre of each disc, where distortion is relatively low (Plate 6). In the case of coppiced trees, however, leaves appeared near the edge of the image disc where distortion was very high and the correction equation did not compensate for this error (Plate 7).

The use in this study of hemispherical photography for measuring indirectly the relative percentage leaf area has therefore proved excellent for non-coppiced trees. Its quick, easy to carry out and to evaluate, and accurate for bugweed in this situation.

Its applicability for other species is, however, limited. Consideration of the major weeds in Natal (MACDONALD & JARMAN, 1985), suggests that the method could not be used generally. *Acacia mearnsii* De Wild., *A. dealbata* Link, *Psidium guajava* L., *Melia azedarach* L. and *Sesbania punicea* (Cav.) Benth. have too great a degree of leaf overlap (Plate 10) whereas the dense, shrubby growth habit of *Chromolaena odorata* (L.) R.M KING & H. ROBINSON, *Lantana camara* L., *Caesalpinea decapetala* (Roth) Alston and *Rubus cuneifolius* would preclude the use of this technique (Plate 10). These latter four species tend to grow in thickets which greatly increases leaf area overlap and which would therefore mean scoring of photographs would be inaccurate. The other species in this list is bugweed; here, although photography might give accurate results for trees growing under a pine canopy, it is not a suitable method for those growing in the sun. Firstly the leaf overlap is high, and secondly, the leaves are not horizontal (Plate 10). In a pine stand where the light is low, leaves are

PLATE 10: The use of hemispherical photography to record the leaf canopies of different plants.

(A) *Psidium guajava*

(B) *Lantana camara*

horizontal and spread over as large an area as possible *i.e.* with as low a degree of overlap as possible. These leaves therefore are orientated to receive the maximum amount of light possible, and relative percentage leaf areas are easy to record photographically.

A consideration of the neighbouring species of each bugweed tree within a 2 m radius indicated that such plants apparently do not influence fruit and seed yield. This might be due to the weed clearance operations during the third tree thinning (Table 1.1). The studied pine stand will be felled in about 2 026 A.D. During this intervening period no more thinnings will occur. The effect of neighbouring species might then conceivably be greater due to an increase in their size and density and consequently they may, in future, play a greater role in affecting the reproductive yield of bugweed.

To calculate the reproductive yield of many trees in a large area, diameter at 100 cm above the soil surface was employed. Other measurements were not considered for the following reasons:

1. Circumference is a less reliable indicator than diameter since stems are not truly cylindrical.
2. Tree height is not a consistently good indicator of reproductive potential.
3. Reliability of canopy spread measurements as an indicator of yield varies between years and declines during the active growing season. Also trees close together would have overlapping canopies which

would confuse measurements.

4. Relative percentage leaf area has the same disadvantages as canopy spread measurements and in addition, cannot be used for coppiced trees.
5. Destructive sampling is time consuming and destroys the experimental material.

(iii) **THE FATE OF SEEDS AFTER DISPERSAL**

The feeding pattern of ungulates at Weza State Forest indicated that most bugweed seeds are ingested during winter (Figure 1.2). Assuming that this is also true of ungulates at Cedara State Forest, then this corresponds to times of the year when ripe fruit production is very low (Figure 1.7). It is reasonable to conclude from this that the fate of most bugweed seeds is not to be eaten, but rather, to fall to the forest floor beneath or near to the parent tree. As regards the Rameron pigeon, feeding on bugweed fruits seems to occur throughout the year (OATLEY, 1980). However, so many fruits are produced during October, for example, that the population of pigeons would effectively transport only a small proportion of the total seed yield away from the parent tree. In addition to this, fructiverous birds tend to choose trees on the edge of a canopy (PIPER, 1986a). Thus once again, most bugweed seeds produced under a pine canopy probably are not eaten but reach the forest floor near the parent tree. Seedling mortality in bugweed after 37 weeks of observations is known to be from 94 to 100 % irrespective of seedling density (DENNY & GOODALL, *pers. comm.*). Thus of the 7.2 million seeds per hectare produced in 20 months, only about 0 to 6 % will survive as seedlings. However, 7.2 million seeds

is such a high figure that 432 000 seeds per hectare could potentially reach reproductive maturity.

This agrees with the literature, where FROUD-WILLIAMS (1987) commented that "although the size of a seed bank may be considerable, comparatively few seeds contribute to the annual seedling recruitment." The transect through the study area showed approximately 3 125 bugweed stems of varying diameters per hectare (Table 1.22). This is only approximately 0.7 % of the potential seedling survival. The large discrepancy could be due to one of four factors:

- Either: (i) 99.3 % of the seeds die in the soil
- or (ii) 99.3 % of seeds are dormant in the soil
- or (iii) 99.3 % of the seeds produced seedlings which died and were therefore not seen in the transect.
- or (iv) a combination of these, where 0.7 % of the total potential tree density represents an optimum, with rate of plant and seed mortality being balanced by rate of seedling emergence from seeds arising from a continually replenished seed bank.

In the literature, where similar discrepancies have been found, this was attributed to the third option, *i.e.* to post-germination mortality of seed in the soil, not resulting in successful seedling emergence (FROUD-WILLIAMS, 1987). In his review article, FROUD-WILLIAMS (1987) states that confirmation of such a fate is provided by SCHAFER &

CHILCOTE (1970) who reported 85 % seed mortality of *Lolium perenne* from germination of buried seeds, whereas as comparative figure of 49 % mortality was obtained for *Lolium multiflorum*.

However, in the case of bugweed, results from the transect study (Table 1.24) suggests that option (iv) above is correct, and that the system is dynamic:

1. Once conditions become more favourable, for example, after clear felling, rapid vigorous growth can be expected and a far higher proportion of fruit-producing trees will be seen, derived from the large proportion (70 %) of reproductively immature trees. This potential gives this species a highly competitive advantage over many other species and could explain why this is the major weed in pine plantations.
2. If, on the other hand, pine trees are not felled for several years, such as in the present situation, then the population will slowly reach a balance of variously-aged trees which can be described as a "climax" or "optimum" community. The balance within the system will be determined by environmental constraints. Here a proportion of fruit-bearing trees (approximately 30 % of the population) will be continually maintained, hence dispersal potential of fruits via animals to other areas remains constant.
3. If the environment becomes unfavourable, then there are sufficient viable seeds in the soil to replenish the population when favourable conditions return (DENNY & GOODALL, *pers. comm.*). Thus the

threat of bugweed is continuous and any control measures must take into account this fruit and seed yield. Any meaningful control strategy must include prevention of fruiting by treating trees < 2 cm diameter and causing exhaustion of the seed bank in the soil.

SUMMARY AND CONCLUSIONS

1. Ripe fruit production of bugweed is periodic in nature, but this could not be related to simple measurements of prevailing weather conditions.
2. Fruit and seed production is related to tree size, but
 - (a) the most accurate indicator of potential reproductive yield is relative percentage leaf cover.
 - (b) the most convenient indicator of reproductive yield is tree stem diameter at a height of 100 cm above the soil surface.
3. Reproductive yield of bugweed appears not to be influenced by competition from neighbouring species.
4. An "average-sized" bugweed tree under a pine canopy produces approximately 17 000 viable seeds in 20 months.
5. A large tree in sunny open conditions produces 2.5 times as many viable seeds as similar-sized tree under a pine canopy, therefore bugweed growing under a pine canopy is suppressed.
6. Bugweed produces approximately 7.2 million viable seeds per hectare in 20 months under suppressed conditions, for example, under a pine canopy.
7. Seeds ingested by animals and birds survive and are capable of germination, however, most seeds are apparently not dispersed in this

manner but fall beneath the parent tree.

8. Seed mortality is high, in excess of 90 %.
9. Seedling mortality is high, in excess of 94 %.
10. Although approximately 70 % of plants in transects were reproductively immature there were sufficient larger trees so that
 - (a) if conditions become favourable, an explosion of vegetative growth from these smaller trees can be expected, which will lead to rapid seed production;
 - (b) if conditions become unfavourable, the population can be replenished by viable seed reserves in the soil produced by the reproductively mature trees;
 - (c) if conditions remain the same, a fruit-bearing population (approximately 30 %) will be maintained with a continual seed dispersal potential.
11. Any meaningful control strategy for bugweed must include prevention of fruiting and exhaustion of the seed bank in the soil.

CHAPTER 2

GERMINATION CHARACTERISTICS OF BUGWEED SEED

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CHAPTER 2

GERMINATION CHARACTERISTICS OF BUGWEED SEED

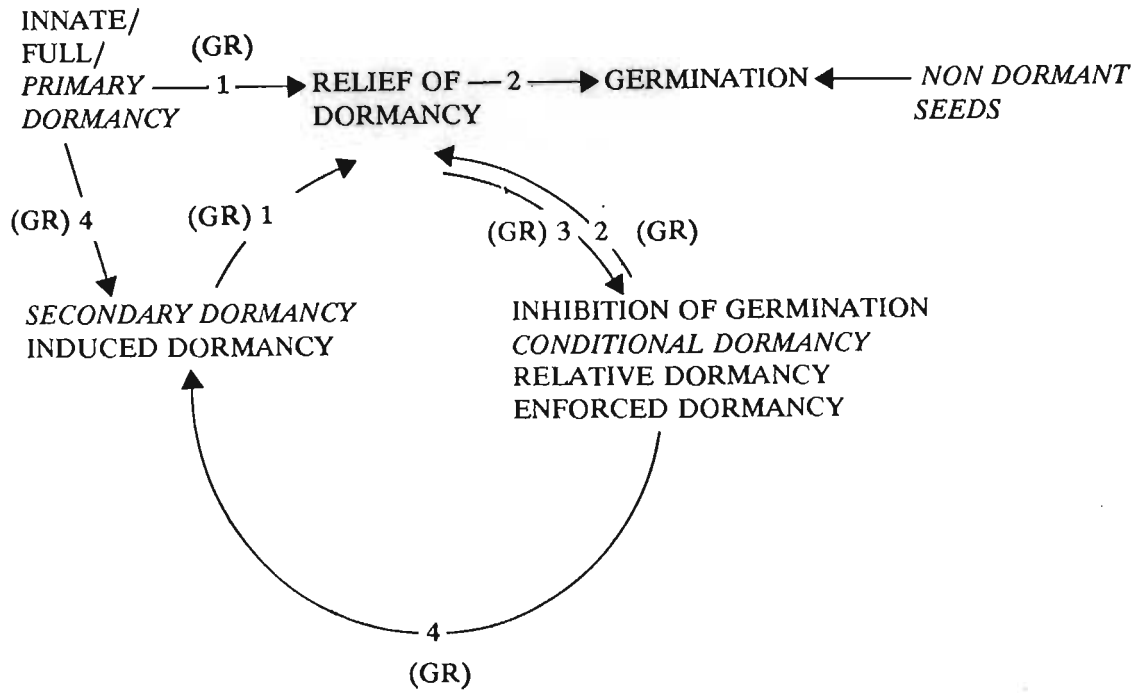
A. INTRODUCTION

This study was motivated by a need to understand the success of bugweed, with reference to its spread by seed. Only by understanding mechanisms governing seed germination and dormancy can it be hoped to achieve manipulation of the massive seed bank (Chapter 1), and consequently achieve any measure of successful control of this weed.

(i) DORMANCY TERMINOLOGY

A discussion of seed dormancy is needed in order to introduce the concept of the survival strategy of bugweed. Dormancy of seeds has been neatly divided up into "innate", "enforced" and "induced" by HARPER (1957) who adapted a well-known Shakespearean quotation to read "some seeds are born dormant, some achieve dormancy, and some have dormancy thrust upon them". *Innate dormancy* is also called *primary dormancy* (KARSSSEN, 1980/81a) or *full dormancy* (VEGIS, 1964, HARPER, 1982). It is present when the seed is shed; it may be genetically based but environmental conditions undoubtedly play a very important part in determining the expression of dormancy. This relationship is described in Figure 2.1.

Primary dormancy prevents germination during development and maturation on the mother plant and usually also for some time after



- 1 Breaking dormancy
 - 2 Coinciding with favourable conditions for germination
 - 3 Different from conditions for germination and/or factors inhibiting germination
 - 4 Inducing dormancy
- (GR) Growth regulators may break dormancy or prevent seeds entering dormancy when they substitute for favourable environmental conditions

FIGURE 2.1: Model for seed dormancy (after KARSSSEN 1980/81a; EGLEY, 1986)

shedding or harvesting of seeds (KARSSEN, 1980/81a). In this state, they show little or no germination even when conditions normally favourable for germination occur (BASKIN & BASKIN 1984b). Many species display little or no dormancy and so will germinate in the same year as shedding. Two creeping grasses, *Agropyron repens* (L.) Beauv and *Agrostis gigantea* Roth., have no primary dormancy and germination of most seeds occurs in the first autumn (WILLIAMS, 1978). CHANCELLOR (1982) concludes that the major part played by vegetative regeneration in the spread of these species has possibly resulted in dormancy not being developed because reproduction by seed is only of secondary importance. These species have *non-dormant* seeds which germinate to high percentages over a wide range of temperatures (BASKIN & BASKIN 1984b) *i.e.* they do not exhibit primary dormancy.

Other species with little or no primary dormancy are two thistles, *Carduus pynoccephalus* L. and *C. tenuiflorus* Curt. (EVANS, YOUNG & HAWKES, 1979), and both *Tussilago farfara* L. and *Taraxacum officinale* Weber (BOSTOCK, 1978). Many weeds appear to have only a short period of primary dormancy. *Pistia stratiotes* L. is recorded as having 1.5 months dormancy (PIETERSE, LANGE & VERHAGEN 1981); *Amaranthus retroflexus* L. when stored at 24 -28°C started losing dormancy within two months after shedding (SCHONBECK & EGLEY, 1980); *Pennisetum pedicellatum* Trin. lost most of its dormancy in the field in three months (MOTT, 1980), as did *Panicum dichotomiflorum* Michx. (BRECKE & DUKE, 1980).

Alternatively, seeds may lose their primary dormancy but due to unfavourable environmental conditions they do not germinate. Thus germination is inhibited. This lasts as long as the imposing conditions exist. Germination ensues when the unfavourable conditions are removed. Thus these seeds germinate only over a narrow range of temperatures (BASKIN & BASKIN 1984b) and are therefore different from non-dormant seeds. These seeds are described as being in a state of *relative dormancy* or, alternatively, *conditional dormancy* or *enforced dormancy* (KARSSSEN, 1980/81a; BASKIN & BASKIN, 1984b; Figure 2.1).

Seeds in the soil may cycle between dormancy and non-dormancy and their germination or continued dormancy will depend upon soil environmental conditions (EGLEY, 1986). Primary dormancy may be alleviated when environmental conditions allow physiological changes to occur in seeds which result in germination under a wide range of conditions (Figure 2.1).

If the inhibiting conditions are not removed *by a critical time* (EGLEY, 1986), the seeds may pass from this state of inhibited germination (*relative/conditional/enforced dormancy*) into a *secondary dormancy/induced dormancy*. Alternatively, seeds may pass straight from primary dormancy into secondary dormancy.

In this state, seeds do not germinate under those favourable conditions which normally allow germination of non-dormant seeds or seeds which have had their primary dormancy or *relative/ conditional/ enforced dormancy* broken (Figure 2.1). Seeds with secondary dormancy require

another dormancy-breaking condition before germination can occur (EGLEY, 1986).

Seeds may break off from the cycle fairly rapidly and germinate, or else continue through the cycle several times and thus exhibit delayed emergence. Many weed seeds exhibit cycles of dormancy. A few have just one cycle a year but others, probably a majority of species, have two (CHANCELLOR, 1984). ROBERTS & LOCKETT (1978a) showed that seeds of *Veronica hederifolia* L. were initially dormant when shed but they soon lost this dormancy and later entered secondary dormancy, then lost it again. A similar situation was reported for *Stellaria media* Cyrill., *Valerianella umbilicata* Wood and *Phacelia Purshii* Buckl. (BASKIN & BASKIN, 1976).

Growth regulators may substitute for environmental conditions and can manipulate or modify this cycle. Thus application of GA₃, for example, may break primary or conditional dormancy or prevent primary or conditionally dormant seeds entering secondary dormancy (Figure 2.1).

Recently it was suggested that dormancy terminology be changed. In an attempt to find a "new universal terminology" and thus do away with confusing and conflicting terms LANG, EARLY, MARTIN & DARNELL (1987) have reclassified the terminology for dormancy to embrace three broad groupings: *Endodormancy*, which is regulated by physiological factors inside the affected structure this includes both primary and secondary dormancy as well as after-ripening. *Paradormancy* is regulated by

physiological factors outside the affected structure and encompasses, for example, relative dormancy. *Ecodormancy* is regulated by environmental factors which are usually non-specific in their effect on overall metabolism, for example, temperature extremes, nutrient deficiency and water stress.

JUNTILLA (1988) found difficulty with the new terminology mainly because dormancy under environmental control and that under physiological or anatomical control have been "partially mixed", whereas they should each be as precise a description of dormancy as possible. JUNTILLA (1988) concludes that "although the proposed terminology sounds attractive and simple, it is difficult to adapt to physiological descriptions of dormancy. In most cases, dormancy is probably a complex combination of eco-, para and endodormancy, and the use of the new terminology to describe it confuses rather than clarifies the issue."

This author is in full agreement with JUNTILLA'S (1988) comments and chooses for the remainder of this text, to use the terms primary, secondary, and conditional dormancy in describing dormancy of bugweed seeds.

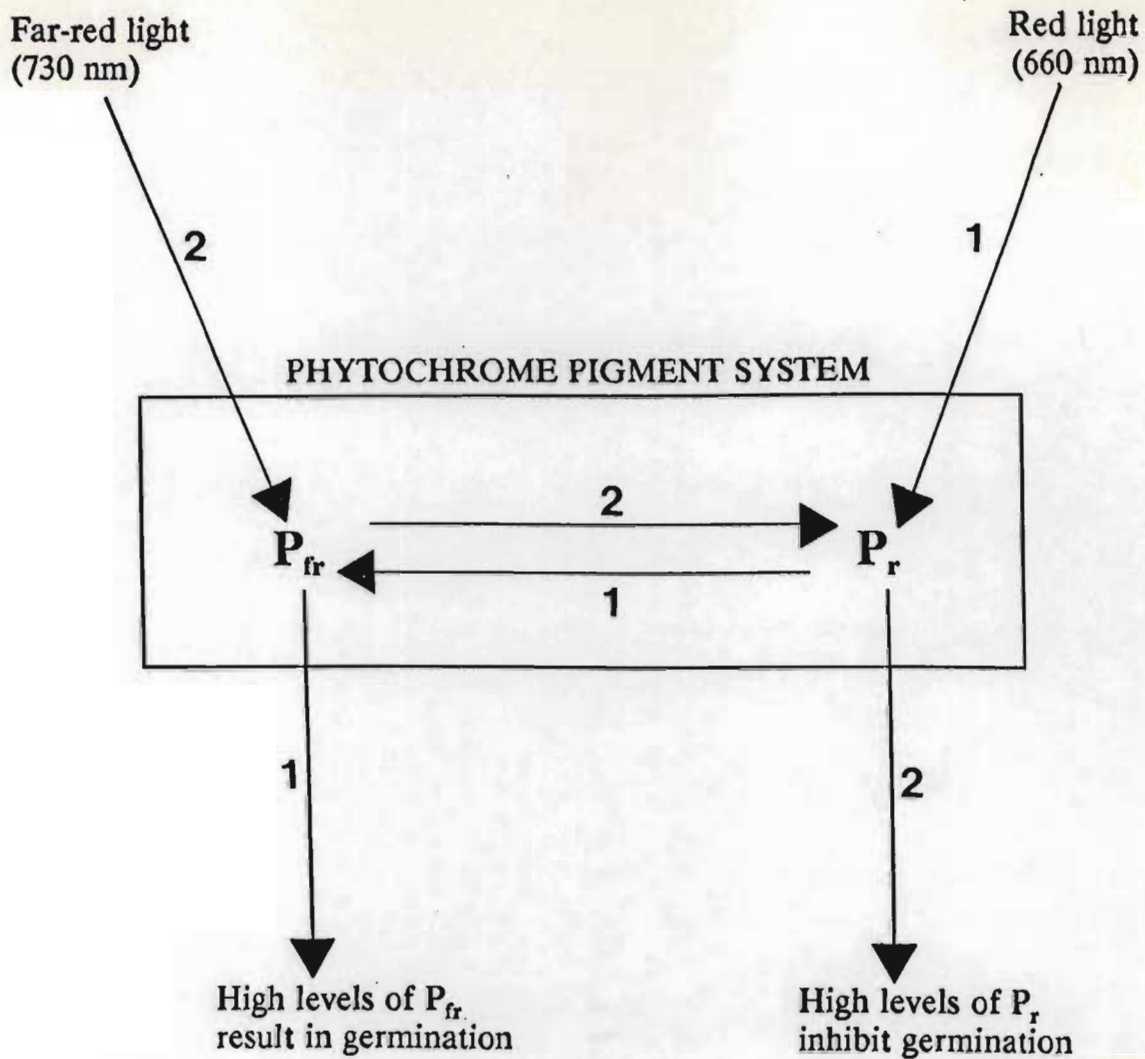
(ii) **GENERAL GERMINATION RESPONSES OF *SOLANUM* SPECIES**

In the case of solanaceous species, incubation at constant temperatures is not conducive to germination. Alternating temperatures, or stratification followed by a higher subsequent incubation temperature, have been shown to be beneficial for this process (WAKHLOO, 1964; PORTER & GILMORE, 1976; ROBERTS & LOCKETT, 1977, 1978b; CAMPBELL & VAN STADEN, 1983). In a number of cases, improved germination in

the family Solanaceae has also been reported following treatment with different gibberellins (KOSIKOVA, 1960; NAKAMURA, WATANABE & ICHIHARA, 1960; PORTER & GILMORE, 1976; ROBERTS & LOCKETT, 1977; CAMPBELL & VAN STADEN, 1983). The application of various gibberellins replaced a fluctuating temperature requirement for germination in *S. laciniatum* Ait. and *S. aviculare* Forst. (PORTER & GILMORE, 1976), *S. dulcamara* L. (ROBERTS & LOCKETT, 1977) and *S. mauritianum* (CAMPBELL & VAN STADEN, 1983). Germination of several solanaceous species is enhanced in the presence of light, for example, in *S. nigrum* L. (HOROWITZ & GIVELBERG, 1982) and *S. ptycanthum* Dun. (LE & ILNICKI, 1983). A discussion of the effects of light, temperature and of various gibberellins is therefore presented to understand more fully the germination characteristics of bugweed.

(a) **Light and germination**

The majority of responses to light can be understood on the basis of one photoreceptor system, that of the pigment phytochrome, which was named by BUTLER, NORRIS, SIEGELMAN & HENDRICKS (1959) who isolated it from dark-grown *Zea mays* L. seedlings. The absorption spectrum of purified phytochrome has been determined both by HARTMANN (1966) and by HANKE, HARTMANN & MOHR, (1969), who found that there are two photoconvertible forms of phytochrome. P_{fr} absorbs the longer wavelengths of the spectrum with a maximum absorbance at 730 nm while P_r absorbs shorter wavelengths of 660 nm (Figure 2.2).



The ratio far-red:red light determines whether seeds will germinate.

FIGURE 2.2: Schematic and simplified representation of the effects of the phytochrome pigment system in light-requiring seeds

It is generally considered that P_{fr} is the active form of the photoreceptor and it is the presence of high levels of this form which normally promotes germination (Figure 2.2). There is presumably a threshold level of this form which leads to germination. This threshold level will vary in a population of seeds because of the different levels of phytochrome present in the individual seeds. Seeds which germinate in darkness and do not require the formation of P_{fr} by light apparently have high endogenous levels of P_{fr} (HILTON, 1985a). Conversely, seeds which require light have low levels of P_{fr} and shorter wavelength light is necessary to convert P_r to the higher levels of P_{fr} needed for germination to occur. Thus the two forms of phytochrome, P_r and P_{fr} , are interconvertible, the ratio between the two forms being dependent on the wavelength of light reaching the seeds (Figure 2.2).

When sunlight is filtered by leaves, the wavelengths corresponding to the red region of the spectrum (660 nm) are absorbed more strongly than those in the far-red region (730 nm). This results in the transmitted light having a high far-red/red ratio (TAYLORSON & BORTHWICK, 1969; SMITH, 1972) which results in a decreased germination of seeds below the leaf canopy. For example, in the vast majority of 240 species tested, there was a secondary requirement for light before seed germination developed beneath a leaf canopy (GORSKI, GORSKA & RYBICKI, 1978).

KARSSSEN (1980/81a) suggested that seeds can be divided into three categories, those germinating equally well in the light and darkness (light independent), those germinating only in the light (light requiring) and

those germinating neither in the light nor in darkness (light insensitive). However, EVENARI (1965) proposed a different term, photoblastism, to describe the response of seeds in which germination is governed by light. Positively photoblastic seeds require light for germination while the germination of negatively photoblastic seeds is inhibited by light and these will germinate only in the dark.

P_{fr} can revert to P_r in darkness. P_r may develop during prolonged incubation in darkness at germination temperatures in light-requiring *Lactuca sativa* L. (BORTHWICK, HENDRICKS, TOOLE & TOOLE, 1954; IKUMA & THIMANN, 1964), in *Rumex crispus* L. and *Portulaca oleracea* L. (DUKE, EGLEY & REGER, 1977) and in *Chenopodium album* L. (KARSSSEN, 1970). This type of inhibition of germination is often referred to as secondary skotodormancy because it develops in darkness.

Seeds may alter in their requirement for light. For example, some species may acquire a requirement for light during burial (WESSON & WAREING, 1969) with consequent changes in dormancy and light-sensitivity. Other species may exhibit a cyclical pattern of change in their sensitivity to light: seeds of *Barbarea vulgaris* R.Br. are dormant and require light when they are freshly shed, but they lose light-sensitivity over the first six months of burial at 2.5 cm, re-acquire it after a further six months and lose it again over the succeeding months (TAYLORSON, 1972).

(b) **Growth regulators and germination**

Many studies have attempted to clarify the role of growth regulators in seed dormancy. Early studies showed that the presence of certain inhibitors in seeds or seed parts correlated with the degree of dormancy (in most cases primary dormancy). This gave rise to the inhibitor concept of dormancy (WAREING, 1964). However the presence or absence of inhibitors is not apparent in dormant seeds of many species. The discovery of gibberellins and their ability to break the dormancy of many seeds prompted studies relating gibberellin levels to the degree of seed dormancy. It was suggested that an inhibitor-promoter balance with gibberellin as the major class of promoters might regulate dormancy (WAREING & SAUNDERS, 1971). Other growth regulators such as cytokinins had little effect on germination by themselves, but acted synergistically with light (MILLER, 1958) and gibberellin (SKINNER & SHIVE, 1958). The observation that gibberellin-induced dark germination in lettuce seeds could be blocked by ascorbic acid and reversed by cytokinins, but not by excess gibberellin, led to a working hypothesis that gibberellin, cytokinins and inhibitors play primary, preventative and permissive roles in the regulation of seed dormancy and germination (KHAN, 1980/1981). Similar interactions between these three growth regulators were shown in lettuce and in other species (BEWLEY & FOUNTAIN, 1972; THOMAS, PALEVITCH, BIDDINGTON & AUSTIN, 1975; DUNLAP & MORGAN, 1977).

The effects of light and various growth regulators appear to be closely interwoven. In lettuce seeds the induction of secondary dormancy by

prolonged soaking in the dark was prevented by the application of gibberellin GA₄₊₇ or when seeds were irradiated (KHAN, 1980/81; KHAN, KARSSSEN, LEUE & ROE, 1979). Potassium nitrate stimulated germination of *Avena fatua* L. seeds in the light but not in the dark (HILTON, 1984). Red light and GA₃ were synergistic in promoting seed germination of *Senecio madagascariensis* Poir. (GUILLEN, ROMERO & MONTALDI, 1984). Gibberellic acid and potassium nitrate were synergistic in promoting seed germination of *Nicotiana rustica* L., especially in the light (SARMA & PHUKAN, 1981).

From evidence of this kind, the question arose: are endogenous gibberellins involved in light-terminated seed dormancy? A commonly held view is that applied gibberellins replace light in breaking dormancy and therefore light may act by increasing the content of P_{fr} and this in turn acts by inducing the production of gibberellins within the seeds (BLACK, 1980/81).

Several studies have supported this suggestion. KOHLER (1966) found an increase in the extractable gibberellin-like activity in lettuce seeds following a brief red irradiation. The increase in the gibberellin (GA₄) level in partially dormant embryos under various light conditions led SMOLENSKA & LEWAK (1971) to suggest that light activated the production of GA₄. In *Picea sitchensis* Trautv. and *Pinus sylvestris* L. seeds, TAYLOR & WAREING (1979) reported that in response to radiation, there is a rapid increase in gibberellin level followed by a decrease.

BIANCO & BULARD (1981) reported an increase in endogenous levels of GA₉ in lettuce seeds in response to irradiation.

However, there are several case studies which do not support this hypothesis. Light-requiring seeds of several species in the genera *Rumex* and *Amaranthus* are not released from dormancy by gibberellins (TAYLORSON & HENDRICKS, 1976). IKUMA & THIMANN (1960) failed to detect any differences in gibberellin content of red light treated and untreated seeds. Far-red light reverses the promoting effects of red light but had little effect on GA₃-promoted germination (BLACK, 1959; IKUMA & THIMANN, 1960, 1963a).

The contradictory evidence as reviewed by BEWLEY & BLACK (1982) led these authors to suggest that gibberellins do not replace the P_{fr} requirement, but rather complement low levels of P_{fr} present in seeds. However, the relationship between gibberellins and light remains somewhat confusing.

(c) **Temperature and germination**

Temperature has also been closely linked with the phytochrome system. Increased temperatures are known to accelerate inactivation of P_{fr} (EISENSTADT & MANCINELLI, 1974; TAYLORSON & HENDRICKS, 1972; RILEY, 1981). This inactivation may occur either by reversion to P_r or by total destruction of P_{fr} (TAYLORSON & HENDRICKS, 1972). However, under some circumstances, high temperatures may induce the formation of P_{fr}. In *Rumex obtusifolius* L. seeds, high temperature

treatment followed by far-red irradiance resulted in low germination. If, however, high temperature treatments were followed by far-red light and then by further high temperatures, high germination ensued, indicating the conversion of P_r to P_{fr} . (TAKAKI, KENDRICK & DIETRICH, 1981). Effects of light on germination of *Sorghum halepense* (L.) Pers. were related to incubation temperature (HUANG & HSIAO, 1987); it was inhibitory at 22°C, produced no response at 28°C, and was stimulatory at 35°C.

Temperature shifts may induce high germination of light-requiring *Rumex crispus* seeds in the dark (TAYLORSON & HENDRICKS, 1972). This was also seen in *Rumex obtusifolius* seeds (HAND, CRAIG, TAKAKI & KENDRICK, 1982) and in *Sorghum halepense* (HSIAO & HUANG, 1988). It was suggested that the temperature shift increases the sensitivity of seeds to a low level of a pre-existing active form of phytochrome or decreases the threshold of P_{fr} needed to promote germination (HAND, CRAIG, TAKAKI & KENDRICK, 1982; TAKAKI & ZAIA, 1984).

The effect of temperature has also been linked to growth regulators. Thermo-inhibition of seeds is a process inhibiting germination above optimal temperatures which leads to thermodormancy, a type of secondary dormancy (KEYS, SMITH & LYON, 1975; RAO, SANKHLA & KHAN, 1975; BRAUN, RAO & KHAN, 1976). When growth regulators such as cotylenin or fusicoccin were applied to lettuce seeds at 25 or 35°C, thermo-inhibition by these temperatures was reversed and germination occurred rapidly and before the seeds had achieved the state of thermodormancy

(BRAUN, RAO & KHAN, 1976; KHAN, 1980/81) These regulators were ineffective once the thermodormancy/secondary dormancy had been induced by prolonged thermo-inhibition or by other means (KHAN & KARSSSEN, 1980; KHAN, 1980/81).

(d) **Conflicting germination results in the literature**

Conflicting reports of the effect of environmental factors on seed germination in several species have been published. The effect of light on germination of wild mustard was stimulatory according to POVILAITIS, (1956) while other authors reported no marked effect (HOLM & MILLER, 1972; MULLVERSTEDT, 1963). HILTON & BITTERLI (1983) suggested the effect of light in promotion of *Avena fatua* seed germination depends on the level of dormancy. CRESSWELL & GRIME, (1981) attributed the contrasting response of some species to light to the differing light-filtering properties of the tissues which surround the developing seed. Differential rates of chlorophyll loss and/or seed drying within the same inflorescence may also explain the variation in light requirement for germination in certain species (CRESSWELL & GRIME, 1981). These authors further commented that even the harvesting and drying procedures may account for much of the conflicting data concerning the light requirement of seeds.

The response of a seed to a growth regulator may vary markedly depending on the state of dormancy. *Lactuca sativa* L. and *Haplopappus gracilis* (= *Aplopappus gracilis* A. Gray) seeds with a primary dormancy germinate rapidly when treated with the fungal toxin fusicoccin (GALLI, SPARVOLI

& CAROI, 1975; KHAN, 1980/81) or cotylenins (BALLIO, 1977; KHAN, 1980/81). The same growth regulators were ineffective in preventing or breaking the secondary dormancy in lettuce and celery seeds induced by temperatures above optimal or by low water potential solutions (KHAN 1980/81). The requirement of alternating temperatures for germination in *Solanum nigrum* was found to be variable. GIVELBERG & HOROWITZ (1984) suggested this was due to differences in the origin of seed collections. The particular biotype of a species may respond in different ways. For example, atrazine-resistant biotypes of *S. nigrum* subspecies *nigrum* had a prolonged dormancy and did not respond to growth regulators such as GA₃ and fusicoccin (GARCIA-BAUDIN & AGUIRRE, 1983). However, the biotypes susceptible to atrazine control were far less dormant than the atrazine - resistant biotypes and did respond to these growth regulators.

KARSSSEN, ZAGÓRSKI, KEPCZYŃSKI & GROOT (1989) stated that "the feeble position of the hormone theory of dormancy and germination is partially due to technical inadequacies in hormone determination", but it may also be attributed to the wrong experimental approach (WAREING 1982).

BRUINSMA (1980) suggested that the application of exogenous plant growth regulators might interfere with endogenous hormone patterns or supplement components present at suboptimal concentrations. However, the endogenous level of a hormone is not always a valuable indicator of its physiological importance. Turnover rates of these substances may be more

relevant than their absolute amounts. In addition to this, exogenously applied regulators may arrive at sites other than those where the corresponding endogenous hormones reside and accordingly their fate and function may be very different. It was concluded by BRUINSMA (1980) that reliance must be placed not only on experiments with exogenous applications but also on exact determinations of the endogenous compounds involved. However, KARSEN, ZAGÓRSKI, KEPCZYŃSKI & GROOT (1989) suggested that the essential changes in endogenous growth regulators "may only occur in a limited area of the seed and may only involve a small part of the total hormone content". TREWAVAS (1986) advocated that the regulatory principle is changing hormone sensitivity rather than the occurrence of limiting hormone levels.

This study is not intended to investigate the intricate physiological mechanisms that are involved in bringing about seed germination, but rather is related to determining the variable germination responses of bugweed to factors such as light, incubation temperature, temperature transfer and growth regulator (GA_3) interactions. The information required is "what happens" rather than "how" it happens, in order to relate this to possible methods of control for the species. Because a study of the obviously complex seed germination physiology in relation to endogenous and exogenous levels of growth regulators would yield no useful information specific to the control of the species, no attempt has been made to investigate these aspects.

B. MATERIALS AND METHODS

(i) GERMINATION PROCEDURES

Seeds were collected from ripe fruits on trees at Cedara State Forest (Figure 1.6), cleaned by repeated washings in tap water then dried at air temperature in the laboratory. Compartmented plastic repli dishes were used for all germination experiments (Plate 11). Sheets of Whatman No. 1 filter paper were cut into 2x2 cm squares. Six of these squares were placed into each compartment of the trays. The filter paper in each compartment of the trays was moistened with 1.5 ml of the appropriate solution. Sinking seeds which were "apparently viable" (ROBERTS, 1981) were used for all experiments. Seeds were counted into lots of 20 on a Numigral¹ seed counter. There were six replicates of 20 seeds each for every treatment. Treatments were randomized within each replicate. Plastic repli dishes were positioned side by side on moist paper towels and placed inside transparent plastic bags, in order to maintain a high humidity. All experiments were initiated within two weeks of seed collection. Throughout this thesis, seeds were counted as having germinated once radicle protrusion was evident.

(ii) PRESENTATION AND ANALYSIS OF RESULTS

The program Harvard Graphics was used for all graphs in this thesis²

Analysis of variance was done where applicable (Figures 2.8, 2.9 and 2.11)

¹Numigral seed counter. Tripette and renaud [sic]. 20, Avenue Marcelin Berthelot. Zone Industrielle du Val de Seine. 92390 Villeneuve La Garenne. Paris. France.

²Graphs were plotted on a Sekonic X-Y Plotter (SPL-430) kindly loaned by the Resource Section at Cedara Agricultural College.

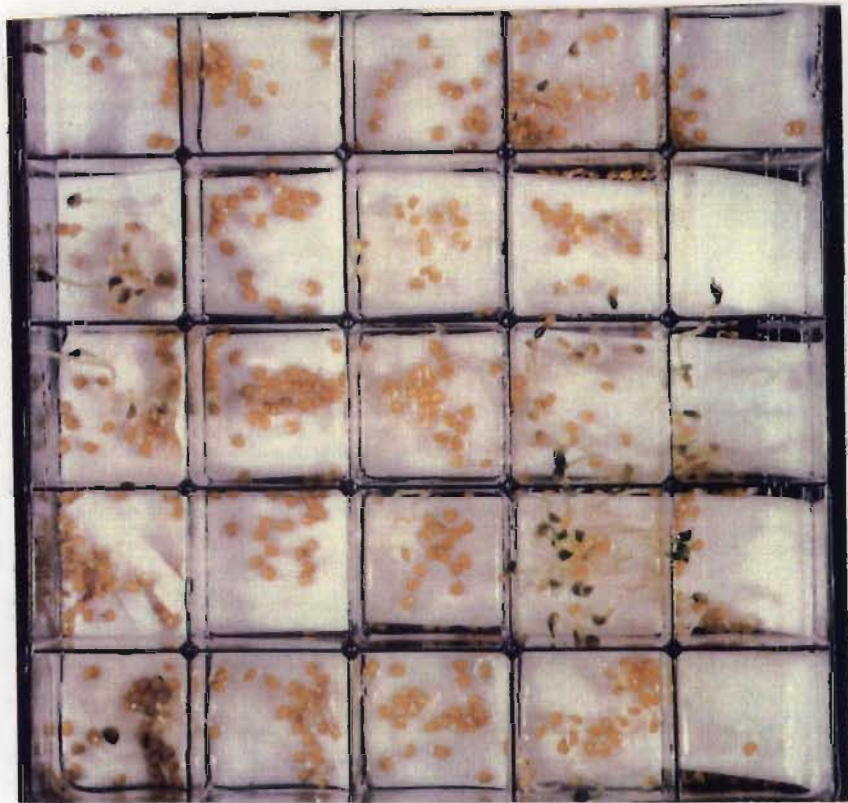


PLATE 11:

Compartmented repli-dish used for germination experiments. Each compartment contained six squares of filter paper moistened with 1.5 ml of the appropriate solution. Seedlings or non-germinated seeds of bugweed are seen in each compartment.

using the Genstat 4 program and then the least significant differences (LSD) between means were calculated (RAYNER, 1967; Chapter 10). LSD's were calculated only between means of the germination responses of seed batches within the same incubator.

(iii) **LIGHT AND TEMPERATURE PROCEDURES**

Plastic repli dishes were wrapped in two layers of aluminium foil for the dark treatments, before being placed in plastic bags. For the light treatments the repli dishes were not wrapped in foil. All these trays were exposed to 14 hours light and 10 hours dark in the incubators. Light was provided by "cool white" fluorescent tubes (spectral irradiance 11Wm^{-2}). Labcon incubators were set to the required temperatures ($\pm 1^{\circ}\text{C}$) and checked regularly for stability with maximum-minimum thermometers. Germinated seedlings were counted and removed each week, and total cumulative percentage germination was determined at the end of the 12 week incubation period. Dark-treated seedlings were counted and removed under a green safe light, which did not affect bugweed seed germination.

Subsequent to incubation at constant temperatures for 12 weeks, seed batches were subjected to a daily cycle of light and dark with alternating temperatures of $15/30^{\circ}\text{C}$, with 15°C in the dark for 10 hours and 30°C in the light for 14 hours for a further 12 weeks. Germination was recorded at weekly intervals and results of the response to this transfer are presented as cumulative percentage germination over the 24 week study period.

(iv) **GROWTH REGULATOR PROCEDURES**

In an initial pilot trial to determine the concentration most effective for germination, several growth regulators were applied to bugweed seeds in the light at 20°C. These growth regulators were: 3, 10 and 30 mM potassium nitrate (KNO_3); 0.3, 1.0 and 3.0 mM sodium azide (NaN_3); 10, 25 and 50 mM chlormequat (CCC); and 0.288, 0.864 and 1.44 mM gibberellic acid (GA_3). KNO_3 , NaN_3 and CCC were dissolved in distilled water, whereas GA_3 required the addition of small aliquots of aqueous sodium hydroxide until solution was complete. The pH of the GA_3 solution was then adjusted to 6.8 using aqueous hydrochloric acid. Solutions were kept refrigerated prior to use and GA_3 solutions were replaced at four-monthly intervals. Each compartment contained an aliquot of 1.5 ml of the appropriate solution. Based on results from the pilot trial the following most effective concentrations of each growth regulator were applied to seeds under different incubation conditions: 3 mM KNO_3 , 0.3 mM NaN_3 , 50 mM CCC and 1.44 mM (equivalent to 500 mg l⁻¹) GA_3 . Later trials in this study were conducted using only the growth regulator GA_3 since this proved the most effective promotor of germination.

(v) **GA_3 AND TEMPERATURE TRANSFER PROCEDURES**

(a) **Germination in the light**

Concentrations of 0, 10, 100, 500 and 1 000 mg l⁻¹ GA_3 were applied in 1.5 ml aliquots to seeds in the light at different incubation temperatures. These were 5, 10, 20, 25, 30 and alternating 15/30°C respectively. Percentage germination was recorded at weekly intervals and results are

presented as cumulative percentage germination over 12 weeks incubation.

(b) Transfer of seeds incubated in the light to alternating temperatures

After 12 weeks, non-germinated seeds from the experiment described above were transferred to alternating 15/30°C in the light. Percentage germination was recorded weekly for a further 12 weeks and cumulative percentage germination is presented for the whole 24 week study period.

(c) Germination in the dark

As for (iv)(a) above, except that solutions were added to seeds in a darkroom under safe green light conditions. Germination was recorded over a period of 12 weeks as for (iv)(a) above but under safe green light conditions.

(d) Transfer of seeds incubated in the dark to alternating temperatures

After 12 weeks, non-germinated seeds from the trial described in Section (iv)(c) above were transferred first to alternating 15/30°C in the dark for 12 weeks, then again to the light for a further 12 week period. Percentage germination was recorded weekly and results of these transfers are presented as cumulative percentage germination after 24 weeks (transfer to the dark) and 36 weeks (transfer to the light).

(vi) **GERMINATION CHARACTERISTICS OF SEEDS OF DIFFERENT ORIGINS**

To test the generality of seed requirements for germination, seed batches collected³ from two localities in South Africa; Cedara (Natal) and Pretoria (Transvaal) (Figure 1.6); from two Indian Island localities (Mauritius and Reunion) and from New South Wales, Australia, were incubated under various conditions. Seeds were incubated at constant 20°C in the light and the dark, and at 15/30°C in the light and dark either in the presence or absence of 500 mg l⁻¹ GA₃.

(vii) **EFFECT OF LIGHT INTENSITY AND QUALITY ON GERMINATION**

(a) **Artificial shading and germination**

Seeds were incubated for 10 hours in the dark at 15°C and for 14 hours in the light at 30°C in shade cloth bags of varying density. These bags screened out 0, 30, 40, 55, 85 and 100 % of the incoming light to the seeds. After 12 weeks incubation, seed batches which showed low overall germination were unwrapped and incubation was continued for another 12 weeks under the same regime.

(b) **Light quality and germination**

Seeds were allowed to imbibe moisture in the dark at 15/30°C for nine days and then subjected to one of four light treatments before further incubation in the dark. The treatments were: one hour red light, one hour far-red light, one hour red/one hour far-red light and one hour red/one hour far-red/one hour red light. After the different light treatments the seeds were incubated at 15/30°C in the dark.

³Seeds were cleaned, dried and stored in a laboratory for approximately 18 months prior to the experiment (NESER, 1986, *pers. comm.*). PPRI, Private Bag X134, Pretoria, 0001.

(viii) **EFFECT OF DIFFERENT TEMPERATURE REGIMES ON GERMINATION**

Seeds were initially incubated for 0, 2, 4, 7, 10 and 14 days at various incubation conditions (#1) and then transferred after each time period to a second set of incubation conditions (#2). These were as follows:

| | #1 | #2 |
|---|-----------------------------------|-----------------------------------|
| A | 20 ⁰ C in the light | 15/30 ⁰ C in the light |
| B | 15/30 ⁰ C in the light | 20 ⁰ C in the light |
| C | 15/30 ⁰ C in the light | 20 ⁰ C in the dark |
| D | 15/30 ⁰ C in the dark | 20 ⁰ C in the dark |
| E | 20 ⁰ C in the dark | 15/30 ⁰ C in the dark |
| F | 20 ⁰ C in the light | 20 ⁰ C in the dark |

(ix) **STORAGE PROCEDURES**

Initially, two storage experiments were planned to run over two years. In the first experiment, seeds were to be stored moist or dry in the cold (5⁰C). In the second, moist or dry storage was to occur under warm conditions (20⁰C). However, repeated power failures increased the temperatures of the refrigerators and consequently the cold storage experiment was abandoned. Twelve months after the initiation of the experiment, two major failures of the 20⁰C storage room occurred. At this point the experiment was terminated.

The procedure for the storage experiment at 20⁰C is therefore described. Seeds were stored on moist or dry paper towels in plastic trays. These trays were wrapped in three layers of aluminium foil. Trays were placed

in a constant 20°C storage room for the duration of the experiment. Seeds were taken out at four monthly intervals and placed in repli dishes under green light conditions prior to incubation in the light or dark at constant or alternating temperatures. Seed batches were incubated for 12 weeks after being moistened with solutions of 0, 100 or 500 mg l⁻¹ GA₃. Germination was recorded at weekly intervals and results are presented as cumulative percentage germination.

C. RESULTS

(i) INFLUENCE OF TEMPERATURE AND LIGHT ON GERMINATION

Germination of bugweed seeds is dependent on the temperature regime during incubation. No germination occurred at constant temperatures of 5, 10, 20, 25 or 30°C (Figure 2.3). However, a high germination response occurred at alternating temperatures of 15/30°C, provided seeds were incubated in the presence of light (Figure 2.3). There is thus a requirement for both light and alternating temperatures in bugweed seeds. These factors act in an additive manner.

To ascertain the optimum magnitude of fluctuation between day and night temperatures for germination, seeds were incubated in the light at 7/30; 15/30; 20/30 or 30/30°C. Thus the magnitude of fluctuations was 23, 15, 10 and 0°C, respectively. Results indicated that the magnitude of temperature fluctuation was not important in promoting a high germination response, since ranges between the low and high temperatures amounting to 10, 15 or 23°C all promoted greater than 90 % germination (Figure 2.4). However, when no fluctuation in temperature occurred, that is, at constant 30°C, the seeds did not germinate (Figure 2.4). Thus at least some measure of fluctuation in temperature was required for germination of bugweed seeds. For the remainder of these studies, alternating temperatures of 15/30°C in the light were used to compare the germination response with that at constant temperatures. The former conditions are considered as "optimum conditions" for germination.

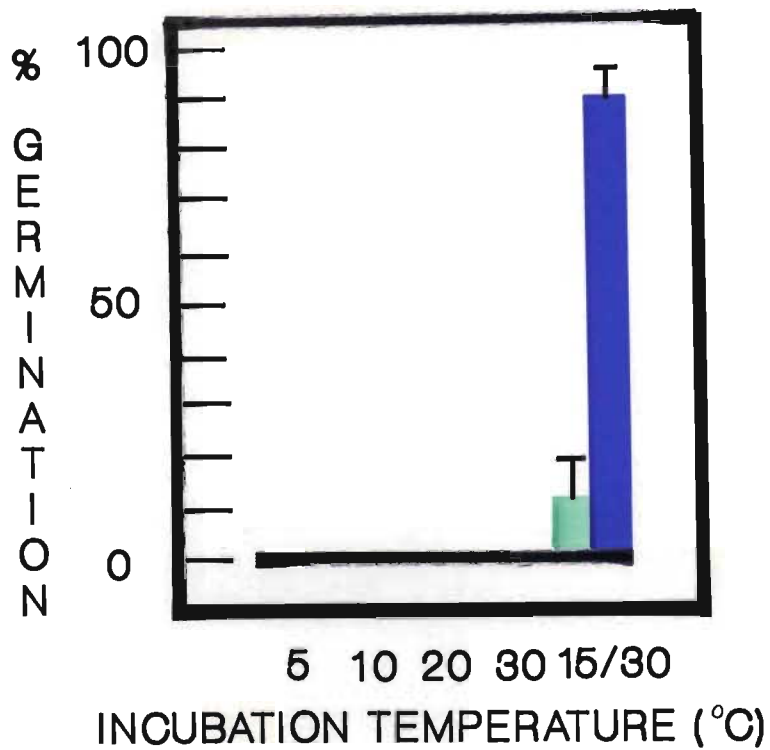


FIGURE 2.3: The effect of constant or alternating temperatures on percentage germination of bugweed in the dark (—) or light (—). Vertical bars represent the standard errors of the means.

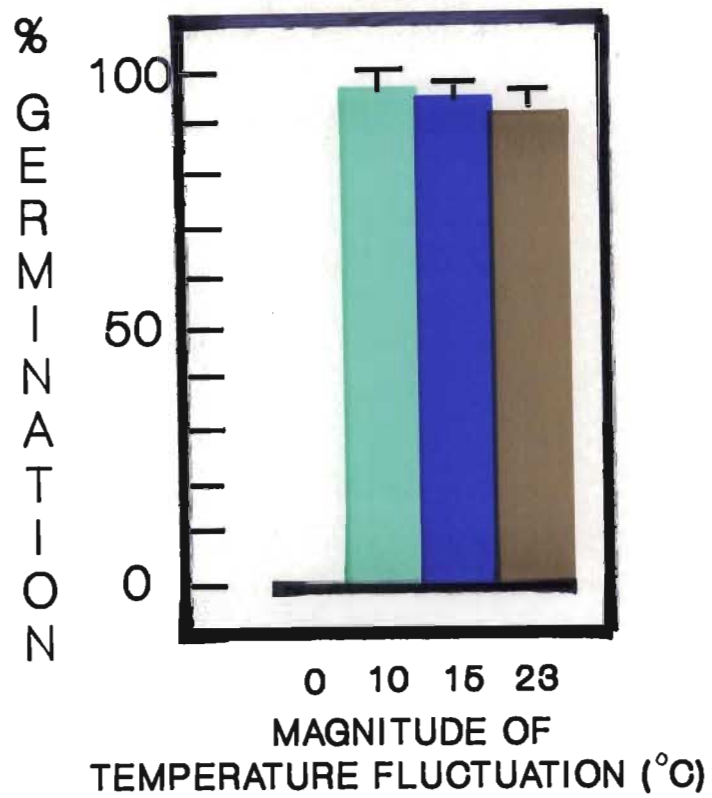


FIGURE 2.4: The effect of magnitude of fluctuation between maximum and minimum temperatures on germination of bugweed in the light. Vertical bars represent the standard errors of the means.

(ii) **INFLUENCE OF TRANSFERRING SEEDS FROM UNFAVOURABLE TO OPTIMUM INCUBATION CONDITIONS**

To investigate further the effects of temperature and light on germination, non-germinated seeds from the first trial (Figure 2.3) were transferred either to the light or dark at alternating 15/30°C.

Seeds incubated at constant temperatures in the light showed an increase in germination response when transferred to optimum conditions, irrespective of initial incubation conditions (Figure 2.5). Transfer of seed batches incubated in the dark at constant temperatures to 15/30°C in the dark stimulated high germination percentages, and this was apparently influenced by the initial incubation conditions (Figure 2.6). A second transfer, to the light, tended to raise the germination response (Figure 2.6).

These results therefore suggest that light, level of temperature and transfer to alternating temperatures may act in an additive manner, since highest germination responses were achieved when all three factors were present during incubation of the seeds. Seeds incubated at constant temperatures in the dark or light were apparently dormant.

This dormancy was readily removed once transfer to favourable environmental conditions occurred, thus bugweed seeds are conditionally dormant, since they germinate only over a narrow range of temperatures (BASKIN & BASKIN, 1984b).

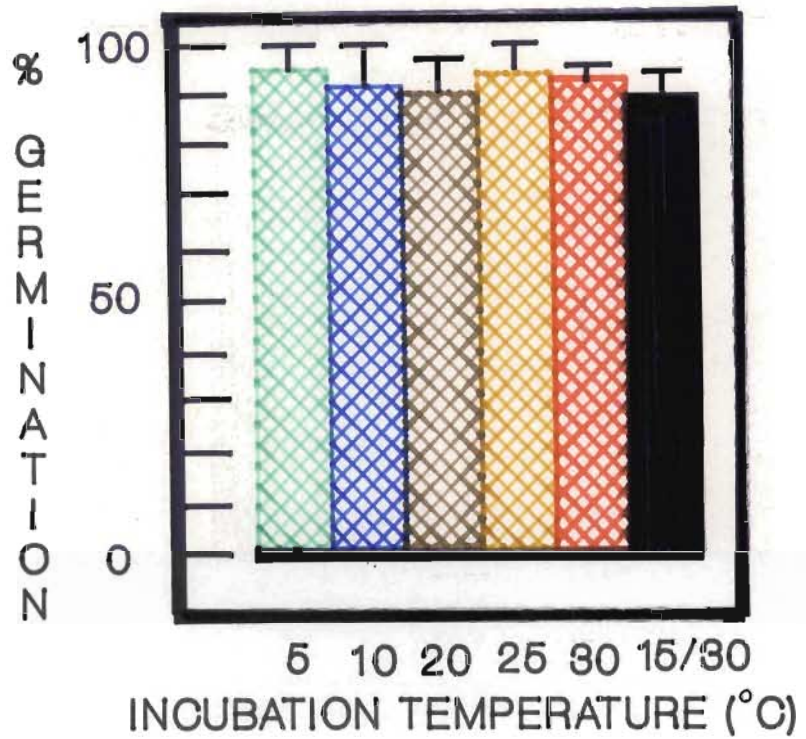


FIGURE 2.5: The effect of temperature transfer on bugweed germination when seeds had initially been incubated in the light at different temperatures. The shaded bar represents germination that occurred at 15/30°C in the light. No germination occurred at constant temperatures in the light. Hatched bars represent germination when seeds were transferred from constant temperatures to 15/30°C in the light. Vertical bars represent the standard errors of the means.

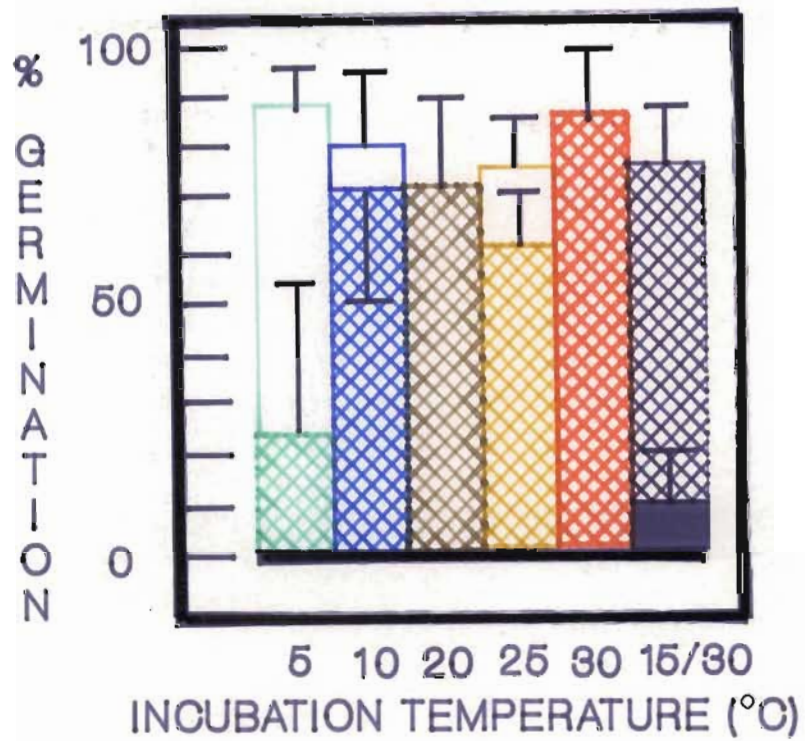


FIGURE 2.6: The effect of temperature transfer on bugweed germination when seeds had initially been incubated in the dark at different temperatures. The shaded portion represents germination that occurred at 15/30°C in the dark. No germination occurred at constant temperatures in the dark. Hatched portions of bars represent germination after 12 weeks when seeds were transferred a first time, to either 15/30°C in the dark (from constant temperatures) or the light (from alternating 15/30°C). The unshaded portions represent germination when seeds were transferred a second time, from 15/30°C in the dark to the light. Vertical bars represent the standard errors of the means.

(iii) **INFLUENCE OF DIFFERENT GROWTH REGULATORS ON GERMINATION**

An initial pilot trial at 20°C in the light involved three concentrations of various growth regulators. These were the germination inhibitor and gibberellin biosynthesis inhibitor, chlormequat (CCC), and the germination promoters KNO₃, NaN₃ and GA₃. Based on the results of this screening trial (which are not presented) the most effective concentration of each chemical was applied to seeds incubated under constant 20°C or alternating 15/30°C temperatures either in the light or dark (Figure 2.7).

When seeds were incubated under optimum temperature conditions, application of the germination promoters KNO₃, NaN₃ and GA₃ had no effect on germination, since the response was similar to that of the control (Figure 2.7A). In the presence of the growth inhibitor CCC, however, germination was significantly inhibited at optimum conditions (Figure 2.7A).

At a constant temperature of 20°C in the light, where the control did not germinate, the application of KNO₃, NaN₃ and GA₃ promoted the germination response, thus these growth regulators substituted for an alternating temperature requirement in bugweed (Figure 2.7B). Once again, application of the inhibitor CCC to seeds resulted in a low germination response, which was similar to that observed in the control seeds (Figure 2.7B). At alternating temperatures in the absence of light, the highest germination response was obtained when the growth regulator GA₃ was applied to the seeds (Figure 2.7C). KNO₃ promoted approximately 70 % germination under these conditions, but NaN₃ had a

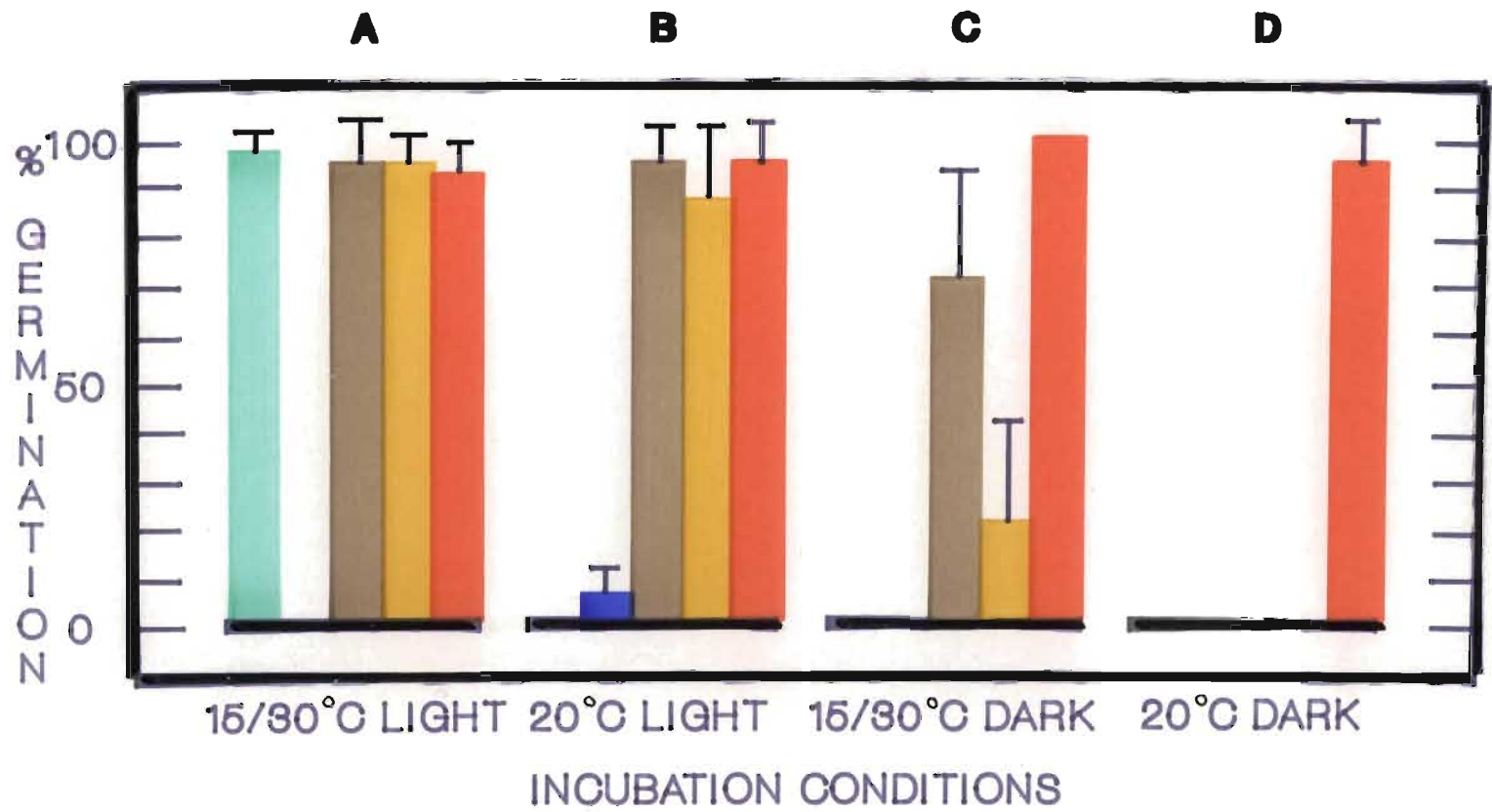


FIGURE 2.7: The effect of distilled water (—), 50 mM CCC (—), 3 mM KNO₃ (—), 0.3 mM NaN₃ (—) and 1.4 mM GA₃ (—) on the germination of bugweed under different incubation conditions. (A) 15/30°C in the light (B) 20°C in the light (C) 15/30°C in the dark (D) 20°C in the dark.

very reduced effect on seed germination (Figure 2.7C). The application of CCC did not apparently affect germination since the response was similar to that of the control (Figure 2.7C).

The most enlightening information from this trial, however, came when seeds were incubated at constant 20⁰C in the dark. The only growth regulator with any promotive effect on seed germination was GA₃ (Figure 2.7D). The other growth regulators did not enhance germination. In a separate screening trial, ethrel was applied to seeds at constant 20⁰C in the dark. This growth regulator also proved totally ineffective under these incubation conditions.

From the growth regulator trial it appears that gibberellins and more specifically GA₃ may be the fundamental growth promotor in the germination of bugweed, since (1) it is the only growth regulator, among those tested, that promoted germination at constant 20⁰C in the absence of light, (2) the growth inhibitor CCC is thought to be specifically antagonistic to the synthesis of endogenous GA₃ (GRAEBE & ROPER, 1978). Thus inhibition of germination at optimum conditions by CCC might be indicative that GA₃ levels in seeds incubated under optimum conditions increase and consequently promote germination. Further germination trials on bugweed therefore included only the growth regulator GA₃.

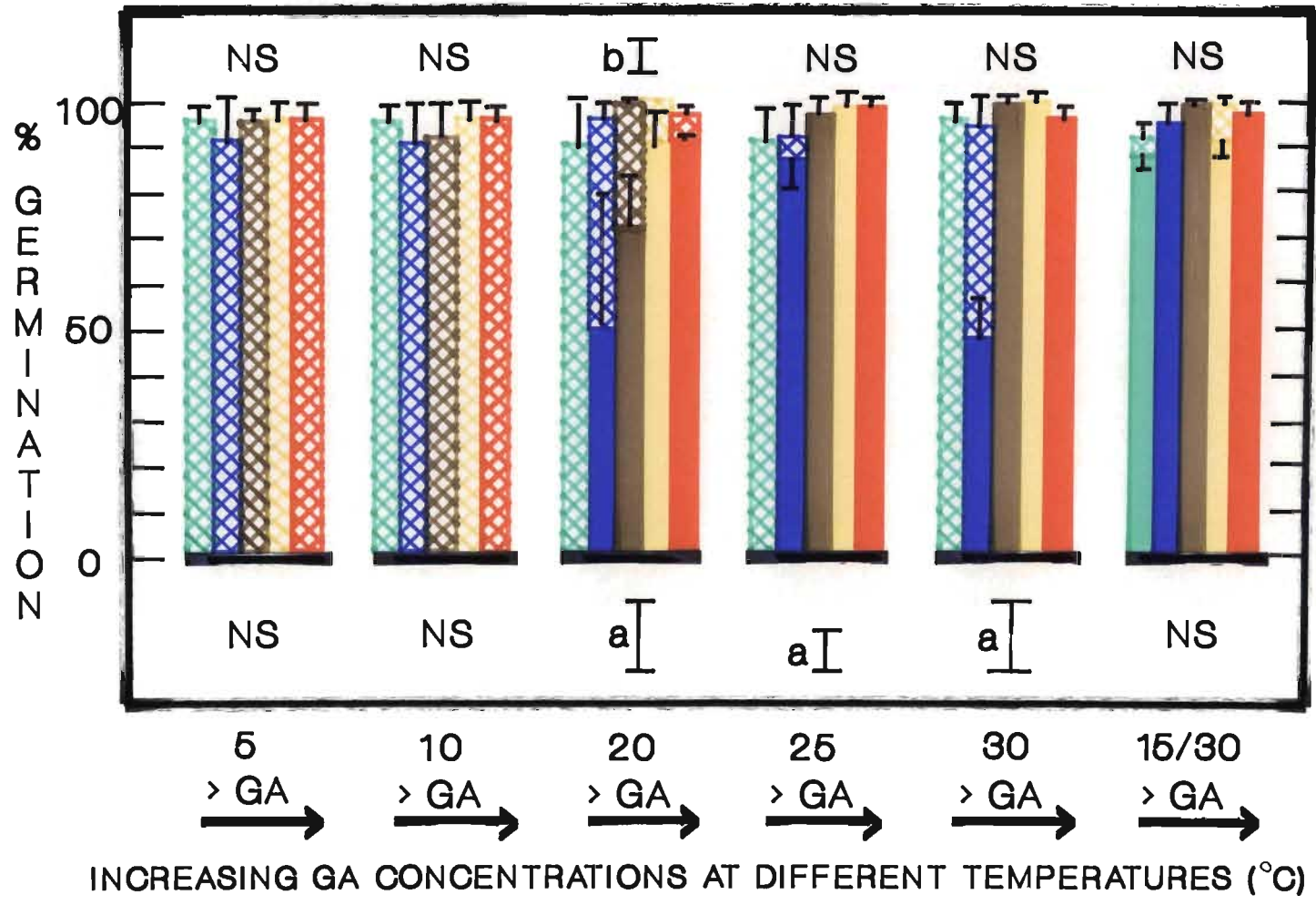
(iv) **INFLUENCE OF GA₃ ON GERMINATION UNDER DIFFERENT INCUBATION CONDITIONS**

(a) **Germination in the light**

The application of GA₃ to seeds incubated at 5 or 10⁰C was totally ineffective in promoting germination, even when high concentrations were used (Figure 2.8). The action of GA₃ on seed germination was thus totally negated at cool temperatures of 5 and 10⁰C. GA₃ did, however, significantly promote germination at constant temperatures of 20, 25 and 30⁰C (Figure 2.8). At optimum conditions of alternating 15/30⁰C, the application of GA₃ did not result in a germination response significantly different from that of the control. Application of GA₃ to seeds therefore substituted for an alternating temperature requirement in the light by improving the response at constant temperatures *i.e.* conditional dormancy was broken.

(b) **Transfer of seeds incubated in the light to alternating 15/30⁰C**

Transfer of non-germinated seeds incubated in the light at constant 5 or 10⁰C to optimum temperature conditions resulted in very high germination responses, irrespective of GA₃ concentration (Figure 2.8). This promotive effect of transfer to optimum conditions was also seen in seeds incubated in the light at constant 20, 25 or 30⁰C, and was most marked when seeds were incubated in distilled water or low concentration of GA₃. This trial therefore suggests high concentrations of GA₃ substitute for a temperature transfer requirement in bugweed seed germination except at cool constant temperatures. Low concentrations of GA₃ were apparently below the threshold level required to break conditional dormancy.



(c) **Germination in the dark**

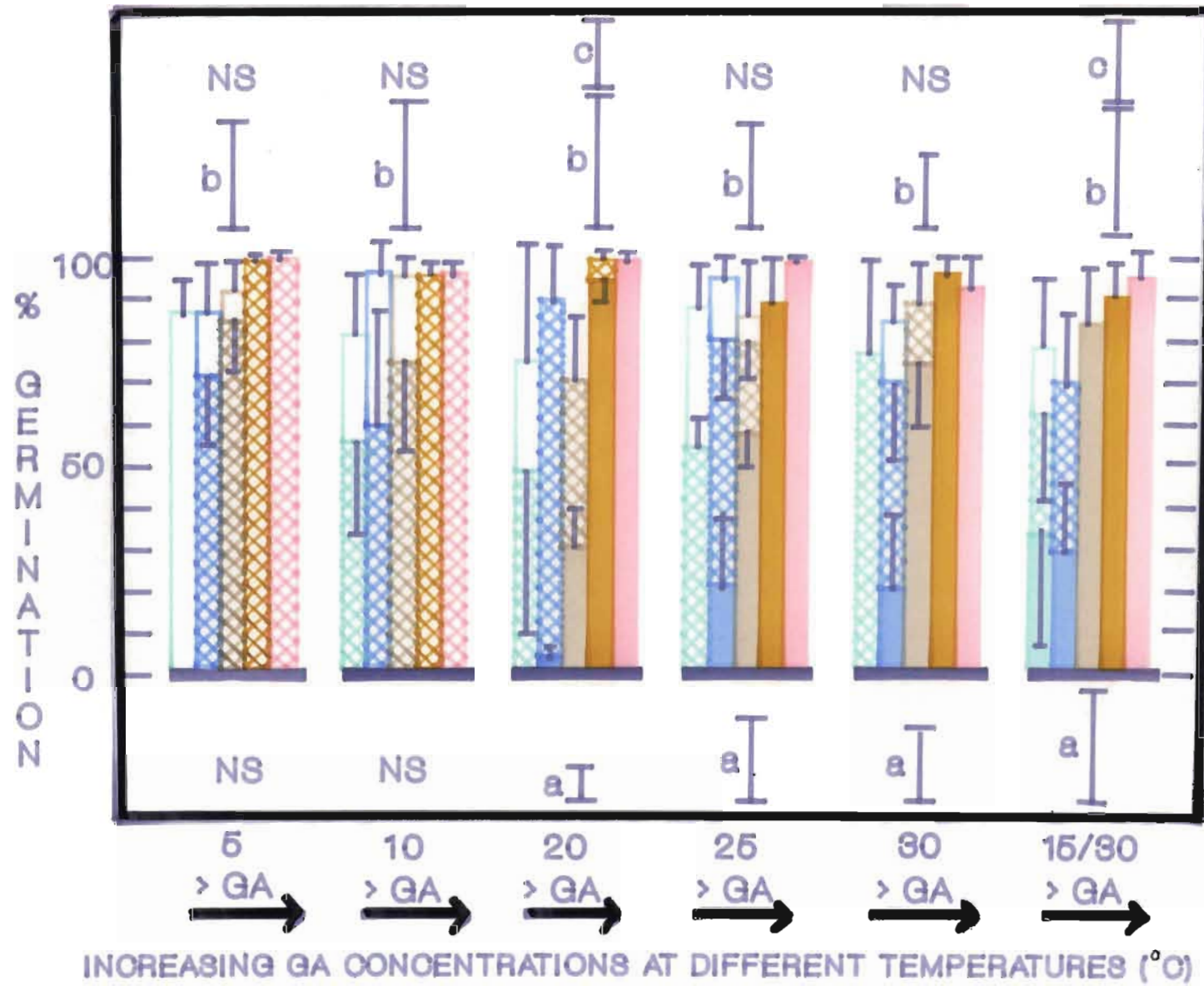
As for incubation in the light, the application of GA₃ to seeds at 5 or 10°C was totally ineffective, regardless of concentration applied (Figure 2.9). A significant ($P < 0.05$) concentration effect of GA₃ was evident in seeds incubated at constant 20, 25 and 30°C and alternating 15/30°C (Figure 2.9). The higher the concentration of GA₃ applied to the seeds, the greater the percentage germination response (Figure 2.9). Application of high levels of GA₃ substituted for both light and alternating temperature requirements by inducing high germination percentages at constant temperatures higher than 10°C, *i.e.* conditional dormancy was broken.

(d) **Transfer of seeds incubated in the dark to alternating 15/30°C**

Transfer of non-germinated seeds incubated in the dark to alternating 15/30°C resulted in high germination percentages, and this was influenced by both initial incubation temperature and the concentration of applied GA₃ (Figure 2.9). A second transfer to the light further promoted germination in seeds which had been initially incubated in low concentrations of GA₃. Thus only high concentrations of GA₃ substituted for a temperature transfer requirement in bugweed germination (except at cool constant temperatures).

(v) **GERMINATION CHARACTERISTICS OF SEEDS FROM DIFFERENT LOCALITIES**

Incubation under optimum conditions of 15/30°C in the light resulted in high germination responses, regardless of origin of collection (Figure 2.10A). Application of 500 mg l⁻¹ GA₃ to seeds under these conditions did not promote a higher germination response over that of the control.



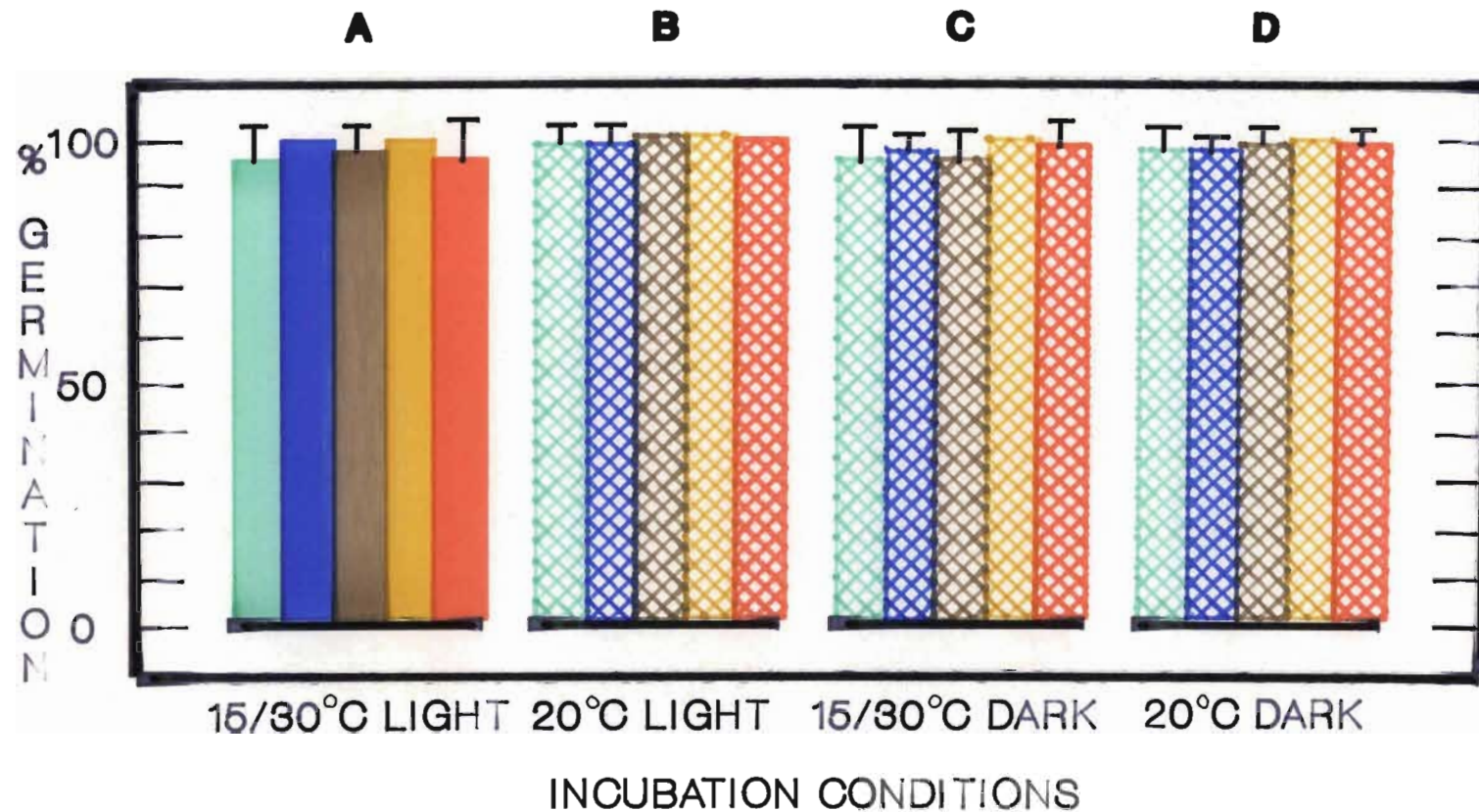


FIGURE 2.10: The germination response of bugweed seeds from different localities to favourable and unfavourable incubation conditions. (A) 15/30°C in the light (B) 20°C in the light (C) 15/30°C in the dark (D) 20°C in the dark. Localities are: Transvaal (—); Natal (—); Mauritius (—); Reunion (—) and New South Wales (—).

Shaded bars represent percentage germination after 12 weeks incubation in distilled water.

Hatched bars represent germination after 12 weeks incubation in 500 mg l⁻¹ GA₃.

Vertical bars represent the standard errors of the means.

Germination was inhibited by incubation at constant temperatures in the light and dark and at alternating temperatures in the absence of light. This held true irrespective of site of seed collection (Figures 2.10B, C and D). Application of 500 mg l⁻¹ GA₃ to seeds incubated under these unfavourable conditions substituted for the requirement of both light and alternating temperatures regardless of seed origin (Figures 2.10B, C and D). From this trial it was clear that bugweed seeds from different localities behaved in a similar manner and required both light and alternating temperatures for germination. These requirements could be substituted for by the application of GA₃.

(vi) **EFFECT OF LIGHT INTENSITY AND QUALITY ON GERMINATION**

(a) **Artificial shading and germination**

Seeds were incubated inside shade cloth bags of different density at alternating temperatures of 15/30°C (Figure 2.11). The range from full light to dense shade did not significantly affect the germination response. However, germination in 100 % shade resulted in a significantly lower germination response (Figure 2.11). Seeds incubated under these latter conditions were in a state of conditional dormancy, since transfer of these completely shaded seeds to the light significantly increased germination to 84 % (Figure 2.11). From this trial it was evident that partial to almost complete reduction of light reaching bugweed seeds did not inhibit germination at alternating 15/30°C. Further implications of this in the field situation are that if seeds are inhibited in germination by complete shade, then a change in light conditions (which would, for example, occur

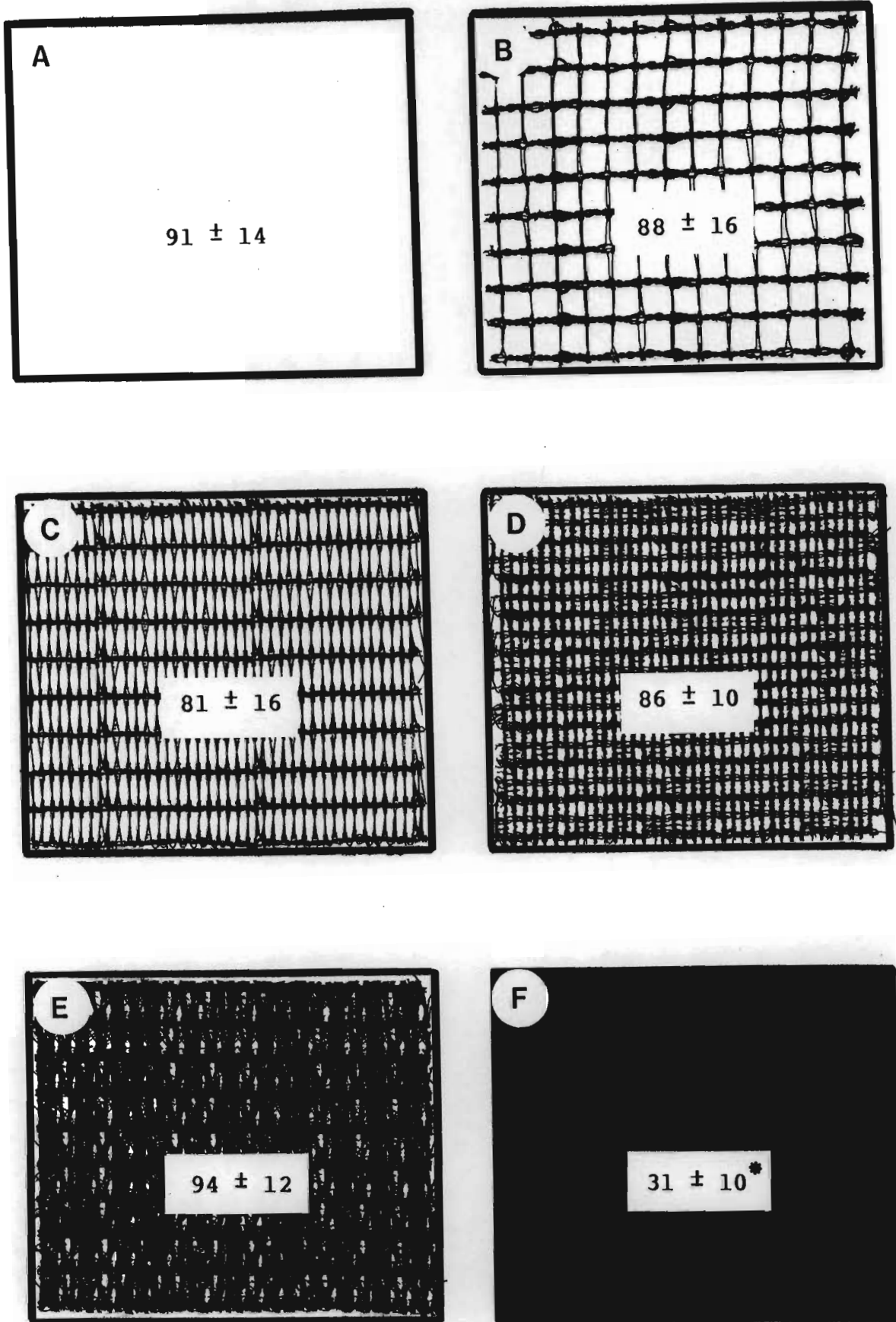


FIGURE 2.11: The effect of increasing shade on the percentage germination of bugweed. Percentage shade is A = 0; B = 30; C = 40; D = 55; E = 85; F = 100. Germination results are given as means \pm SE.
 * Transfer of non-germinated seeds to the light resulted in 84 ± 15 % germination.

after soil disturbance, thinning or clear fell operations) would probably result in a massive germination response.

(b) **Light quality and germination**

The shading effect of a forest canopy not only results in a reduced intensity of light but also a high far-red/red ratio. Those species with germination controlled by the phytochrome system would therefore exhibit inhibition of germination under such conditions. To test for the presence of the phytochrome system in bugweed, seeds were exposed to different light conditions at alternating temperatures of 15/30°C in the dark, a situation where a low germination response occurs (Figure 2.12). Irradiance with far-red light for one hour did not affect the response. However, one hour of exposure to red light significantly increased germination. An exposure to one hour far-red light after one hour red irradiance resulted in a reversal of the promotive effect of red light and therefore a significantly lower germination response. A further exposure to one hour red light (*viz.* red light to far-red light to red light) reversed the effect of far-red light and once again resulted in a significantly higher percentage germination than occurred in the control (Figure 2.12). From this trial it is evident that the phytochrome system is operative in bugweed seeds. Exposure to red light results in a high germination response, whereas exposure to far-red light reverses this effect. This is typical of phytochrome - mediated germination.

Thus bugweed seeds on the forest floor can be expected to vary in their germination response, not due to a reduction in light intensity but rather to the effect of quality of light (the ratio of far-red light to red light)

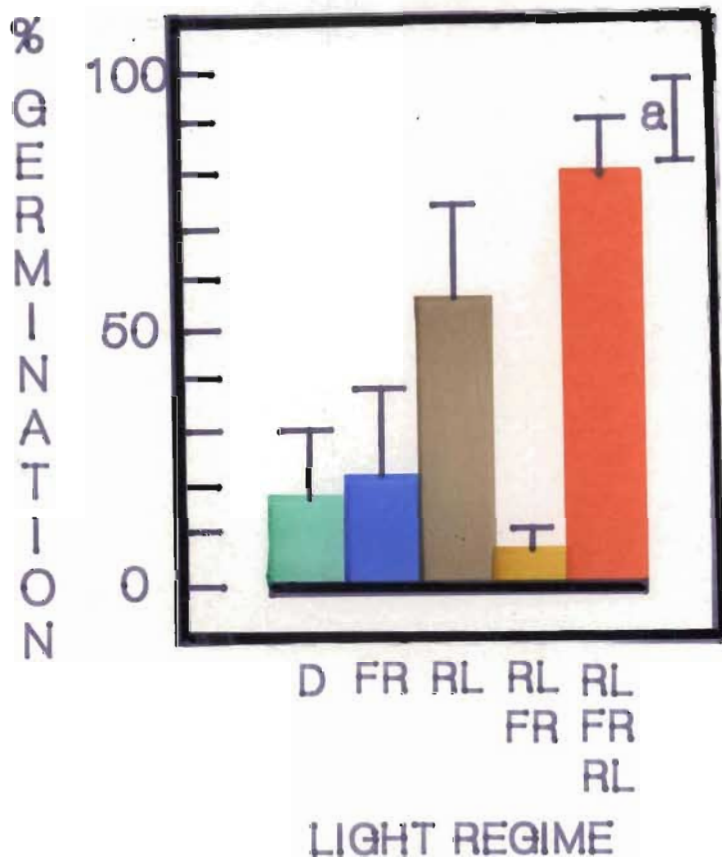


FIGURE 2.12: The effect of red light/far-red light reversibility on seed germination of bugweed. Seeds were imbibed for 9 days in the dark at 15/30°C prior to the light treatment. Seeds were exposed to different light regimes. These were: D = dark, FR = 1 hour far-red illumination, RL = 1 hour red illumination, RLFR = 1 hour red followed by 1 hour far-red illumination, RLFRRL = 1 hour red, 1 hour far-red then 1 hour red illumination. Vertical bars represent the standard errors of the means. a: LSD $p < 0.05$ of bars.

present in the surroundings of the seeds. This ratio changes over time, for example, after thinning pine trees during normal forestry operations. Within a population, variation in endogenous P_{fr} levels would probably also occur. A sporadic seedling emergence could therefore be predicted in the field, with seeds individually germinating when endogenous levels of P_{fr} reach a critical threshold level.

(vii) **EFFECT OF DIFFERENT TEMPERATURE TRANSFERS ON GERMINATION**

Bugweed germination is inhibited by incubation in the dark both at constant and at alternating temperatures. Transfer of seeds between these incubation conditions therefore does not promote high germination responses (Figure 2.13D,E). Incubation of seeds at constant temperatures in the light is also inhibitory to germination, and transfer of these seeds to constant temperatures in the dark also resulted in low germination percentages (Figure 2.13F).

Transfer of seeds from inhibitory conditions in the light, such as constant 20°C, to germination-promoting conditions, such as alternating temperatures of 15/30°C, promoted germination (Figure 2.13A). The reverse transfer gave similar results; incubation in the light at alternating temperatures of 15/30°C for 2, 4, 7, 10 or 14 days resulted in high germination responses when the seeds were moved to constant temperature conditions. Thus, during two days initial incubation at alternating temperatures in the light, physiological changes occurred in the embryo which promoted high germination under conditions that, normally, were inhibitory (Figure 2.13B). This was also evident in seeds transferred from

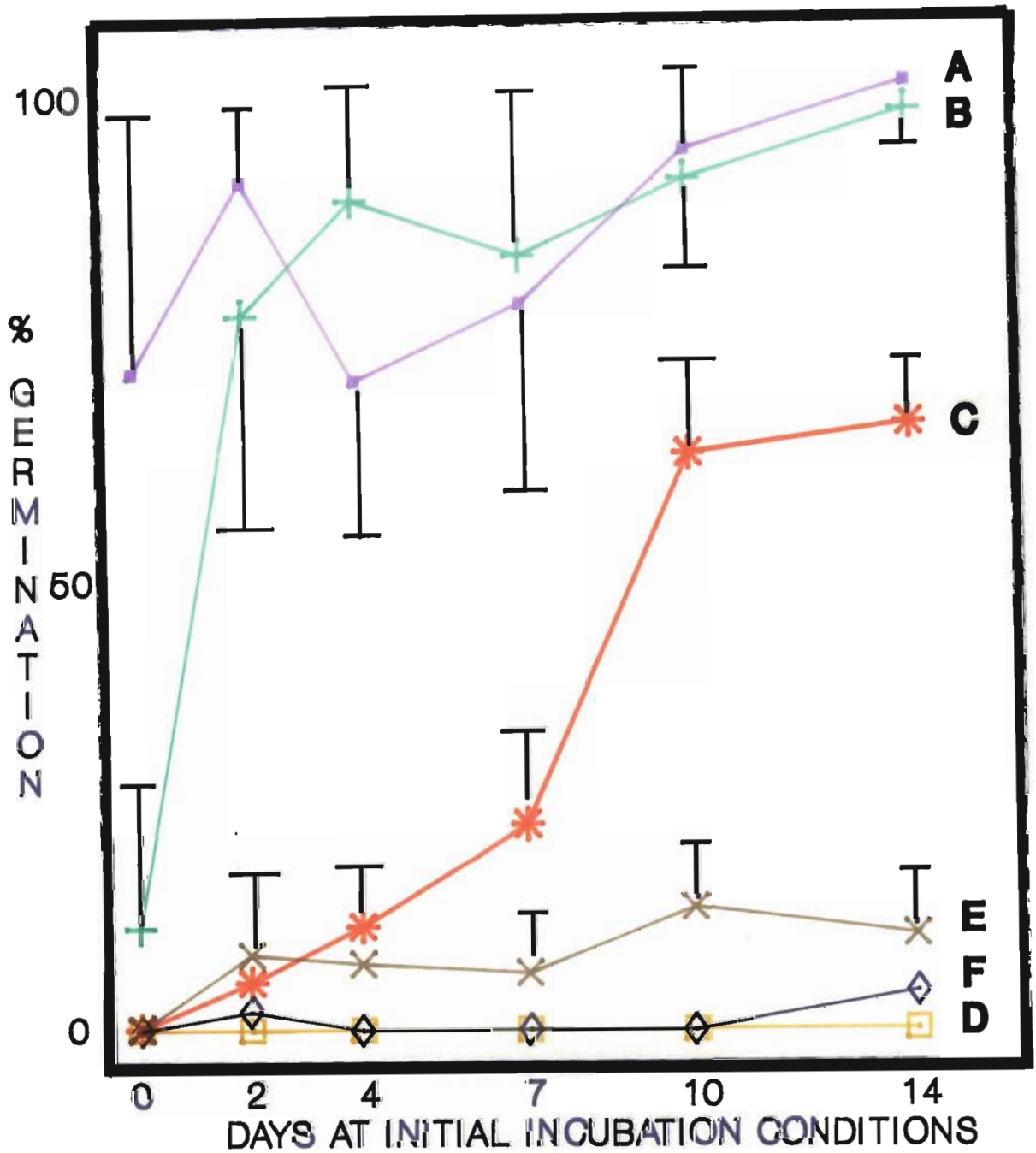
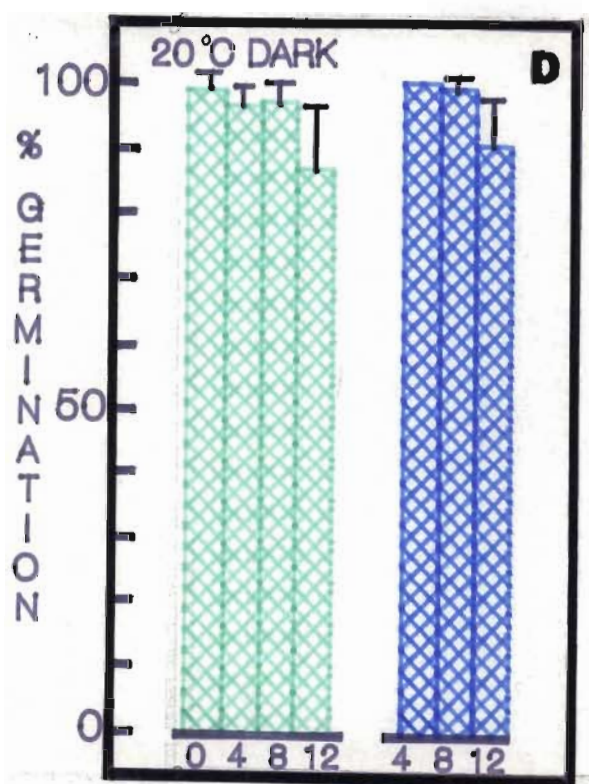
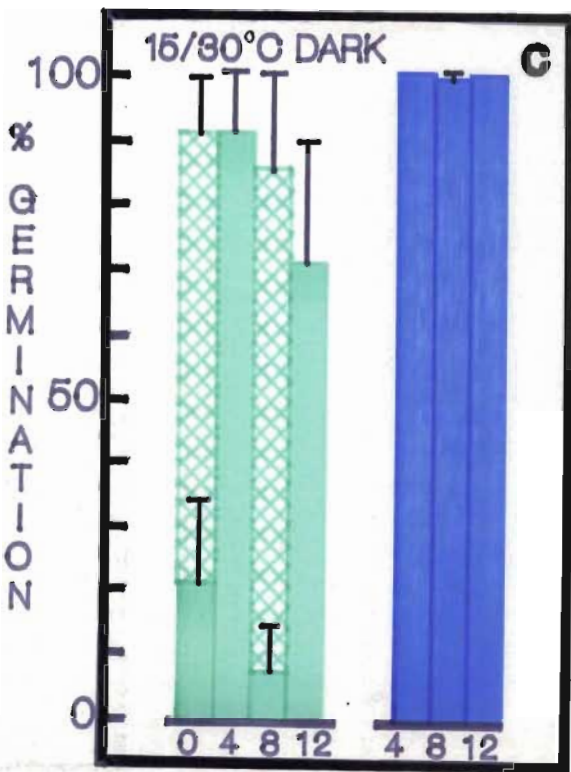
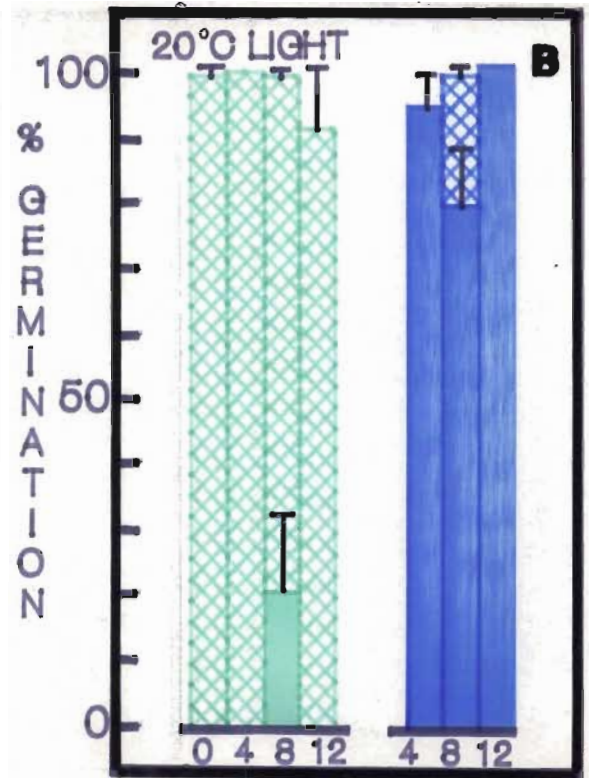
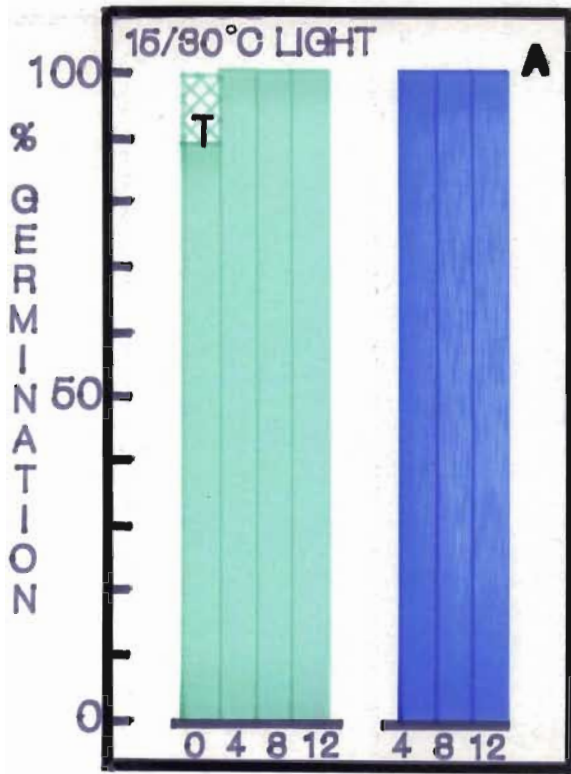


FIGURE 2.13: The effect of transfer to different temperatures on seed germination of bugweed. Seeds were initially incubated (#1) for 0, 2, 4, 7, 10 or 14 days under the temperature regime indicated and then transferred after this time period to a second set of incubation conditions (#2).

- | #1 | #2 |
|-------------------|---------------|
| A — 20°C light | 15/30°C light |
| B — 15/30°C light | 20°C light |
| C — 15/30°C light | 20°C dark |
| D — 15/30°C dark | 20°C dark |
| E — 20°C dark | 15/30°C dark |
| F — 20°C light | 20°C dark |



DURATION OF STORAGE (MONTHS)

FIGURE 2.14: The effect of dry (—) and moist (—) storage at 20°C on germination of bugweed. Seeds were stored for 0, 4, 8 or 12 months prior to incubation in distilled water under different incubation conditions. Shaded portions of bars represent germination after 12 weeks at each incubation temperature. Hatched portions of bars represent germination after 12 weeks when seeds were subsequently transferred to 15/30°C in the light. **A** = 15/30°C in the light; **B** = 20°C in the light; **C** = 15/30°C in the dark; **D** = 20°C in the dark. Vertical bars represent the standard errors of the means.

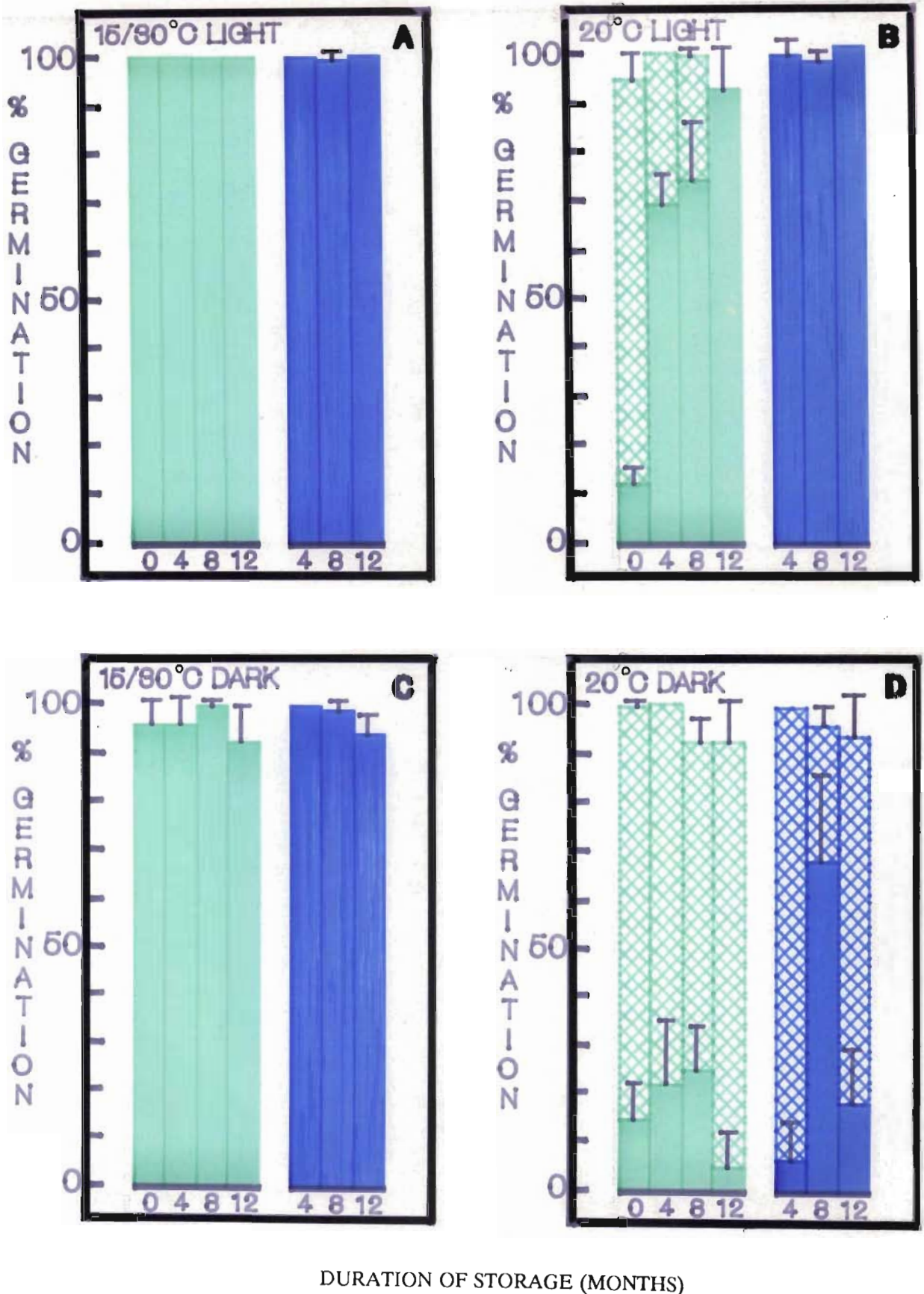
dormancy after moist storage. Transfer of non-germinated seeds to alternating temperatures improved germination of dry-stored seeds (Figure 2.14B).

A requirement for light at alternating temperatures varied with duration of dry storage. Seeds initially lost a requirement for light, then acquired a light sensitivity, then lost it again (Figure 2.14C). Transfer of non-germinated seeds from the eight month dry storage batch to the light resulted in a high percentage germination. This indicated that these seeds were in a state of conditional dormancy during incubation in the dark (Figure 2.14C). Moist stored seeds completely lost their requirement for light, regardless of duration of storage (Figure 2.14C). Both moist and dry storage did not improve germination at constant 20°C in the dark, which indicated that incubation under these conditions was more inhibitory to germination than incubation under other conditions. Transfer to alternating temperatures in the light permitted seeds to germinate (Figure 2.14D), thus seeds were conditionally dormant.

(b) Effect of storage on the requirement of applied GA₃ under unfavourable incubation conditions

When incubated at optimum conditions, application of 100 mg l⁻¹ GA₃ to dry and moist stored seeds did not alter the germination response when compared to that of the control (Figure 2.15A). At constant temperatures in the light, however, both storage conditions promoted higher germination percentages than in the control, with non-germinated dry-stored seeds requiring transfer to alternating temperatures to achieve maximum germination (Figure 2.15B). Incubation in 500 mg l⁻¹ GA₃ substituted for

Legend



DURATION OF STORAGE (MONTHS)

FIGURE 2.15: The germination response of bugweed to the application of $100 \text{ mg l}^{-1} \text{ GA}_3$ after dry (—) or moist (---) storage. Seeds were stored for 0, 4, 8 or 12 months prior to incubation in $100 \text{ mg l}^{-1} \text{ GA}_3$ under different incubation conditions. Shaded portions of bars represent germination after 12 weeks at each incubation temperature. Hatched portions represent germination after 12 weeks when seeds were subsequently transferred to $15/30^\circ\text{C}$ in the light. **A** = $15/30^\circ\text{C}$ in the light; **B** = 20°C in the light; **C** = $15/30^\circ\text{C}$ in the dark; **D** = 20°C in the dark. Vertical bars represent the standard errors of the means.

this transfer requirement in dry stored seeds (results not shown).

Freshly-harvested seeds responded well to alternating temperatures in the dark in the presence of $100 \text{ mg l}^{-1} \text{ GA}_3$. Both types of storage did not change this result (Figure 2.15C).

Freshly-harvested seeds showed a low germination response when incubated in 100 mg l^{-1} solution GA_3 at constant temperatures in the absence of light. This germination response remained low in dry-stored seed batches. This indicated that incubation at 20°C in the dark was more inhibitory to germination than incubation under other conditions. A transfer to optimum conditions was required to elicit a high percentage germination (Figure 2.15D). Moist stored seed batches varied in their germination response, depending on the duration of storage. Once again, transfer to optimum conditions resulted in an increased response (Figure 2.15D). Seeds incubated in $500 \text{ mg l}^{-1} \text{ GA}_3$ at constant temperatures in the dark lost their requirement for transfer to optimum temperature conditions (results not shown). Thus the low responses to $100 \text{ mg l}^{-1} \text{ GA}_3$ of seeds incubated at 20°C in the dark was apparently due to this concentration of GA_3 generally being at a below-threshold level for the promotion of germination.

From these results it is evident that germination is inhibited by incubation at constant temperatures or the absence of light *i.e.* there is a requirement for both light and alternating temperatures for high germination

percentages. Transfer of seeds from unfavourable to optimum conditions enhanced seed germination, thus bugweed seeds are conditionally dormant.

The growth regulator with the greatest effect is GA₃, which could break conditional dormancy if applied in high concentrations. Low concentrations of GA₃ were apparently below the threshold level required to break conditional dormancy. *S. mauritanum* seeds collected from different parts of the world had a similar requirement for both alternating temperatures and the presence of light, which was substituted for by application of high dosages of GA₃.

The phytochrome system is operative in bugweed seeds. The germination response is apparently also influenced both by initial incubation conditions and the conditions to which seeds are transferred. Storage conditions could change germination requirements and also the response of seeds to low dosages of GA₃.

D. DISCUSSION

(i) INFLUENCE OF LIGHT, TEMPERATURE AND TRANSFER OF SEEDS TO OPTIMUM CONDITIONS ON GERMINATION

Bugweed seeds can be described as positively photoblastic since light stimulates germination (EVENARI, 1965). Light-requiring seeds, as defined by KARSSSEN (1980/81a) include those seeds which will only germinate in the light. Bugweed seed does not strictly fall within this definition, since a low germination response was recorded in the absence of light at alternating temperatures of 15/30⁰C. At constant temperatures seeds did not germinate in the light. However, since light plus alternating temperatures greatly enhanced seed germination, describing this species as light-requiring is accurate and this terminology will be used for the remainder of this thesis.

The influence of light on germination is dependent on incubation temperature. The presence of light does not stimulate germination at constant temperatures but is highly effective when seeds are incubated at alternating temperatures. The magnitude of temperature fluctuation is not important for bugweed seeds, since differences in "day" and "night" temperatures of 10⁰C through to 23⁰C, brought about a similar high germination response. This requirement for fluctuating temperatures has also been reported for other *Solanum* species, for example *S. laciniatum*, *S. aviculare* (PORTER & GILMORE, 1976) and *S. dulcamara* (ROBERTS & LOCKETT, 1977). The presence of light also enhanced seed germination of *S. nigrum* (HOROWITZ & GIVELBERG, 1982) and *S. ptycanthum* (LE & ILNICKI, 1983).

Bugweed seeds did not apparently exhibit primary dormancy (KARSSSEN, 1980/81a) since a high germination response was recorded under optimum incubation conditions (15/30°C in the light) immediately after harvest. This requirement for both light and alternating temperatures could be substituted for by the application of GA₃. The effect of gibberellins such as GA₃ have also proved effective in promoting germination of *S. laciniatum*, *S. aviculare* (PORTER & GILMORE, 1976) and *S. dulcamara* (ROBERTS & LOCKETT, 1977).

The types of dormancy operative in bugweed seeds became apparent in the temperature transfer experiments. Incubation at constant temperatures in the light and in the dark or at alternating temperatures in the absence of light was not conducive to germination. However, transfer of seeds to optimum conditions, that is, alternating 15/30°C in the presence of light, brought about a high percentage germination. Thus seeds were in a state of conditional dormancy when incubated under unfavourable environmental conditions (KARSSSEN, 1980/81a; CHANCELLOR, 1982; BASKIN & BASKIN, 1984b). Secondary dormancy was not induced by these unfavourable conditions, since transfer to optimum conditions promoted high germination percentages. The promotive effect of transfer from unfavourable conditions to optimum conditions has also been reported for seeds of *Sorghum halepense* (HSIAO & HUANG, 1988) and *Tagetes minuta* L. (FORSYTH & VAN STADEN, 1983; DRENNAN & VAN STADEN, 1989).

HAND, CRAIG, TAKAKI & KENDRICK (1982); and TAKAKI & ZAIA (1984) hypothesised that the promotive effect of temperature transfer either increases the sensitivity of seeds to a low level of phytochrome pre-existing in the active or P_{fr} form or it decreases the threshold level of phytochrome in this form needed to promote germination. It was also suggested that the temperature shift might influence the formation of P_{fr} in the dark. The mode of action is, however, not, clear.

The phytochrome system is functional in bugweed seeds, since red light and far-red light were antagonistic in their effects on the germination response. PONS (1986) stated that this property is generally interpreted as a means by which the seed can detect the presence of chlorophyll-containing plant tissues in its immediate surroundings and can therefore postpone germination. The risk of high seedling mortality due to competition from established plants is therefore reduced (PONS, 1986).

Seed production of bugweed occurs throughout the year (Figure 1.8), thus a whole range of seasonal environmental conditions could potentially be encountered by the seeds. The seeds are, however, in a state of conditional dormancy, and germination will occur only under favourable environmental conditions. While seeds are surrounded by fruit pulp, they are apparently inhibited from germinating (Figure 1.11). Once this fruit material decomposes and disintegrates, many seeds will still not be able to germinate because of the high far-red/red light ratio under a pine canopy (SMITH, 1982).

The germination potential of seed is also regulated by prevailing temperatures. The magnitude of temperature fluctuation is dependent on depth of burial, and bugweed seeds that become buried would probably be in a state of conditional dormancy, due not only to an absence of light but also because the temperatures would have very low diurnal fluctuations.

Once conditions change, for example, as a result of soil disturbance due to thinning or clear-felling operations, windfall, lightning strikes or fire, conditions of light and temperature change and seeds may germinate rapidly and take advantage of the "new" niche. Thus bugweed seeds exhibit characteristics of a typical pioneer species, which is geared to exploit changes in the environment rapidly and so outcompete other species.

CAVERS (1985) has commented on the advantages of variable germination characteristics in a species. The pattern of germination exhibited by the seeds of a species is usually closely related to the effectiveness of that species as a weed. Seeds that germinate under conditions which make control programmes difficult or ineffective, will have an enhanced chance of continued survival. Greater variability or intermittency in seed germination often increases the success of the weed species, since each time a stand of seedlings or older plants is destroyed, a new stand will soon arise from the soil seed bank to take its place.

In the case of bugweed, germination patterns are apparently variable and this, plus the large numbers of viable seeds produced (7.2 million seeds per hectare during a 20 month period), makes control a very daunting proposition.

(ii) **INFLUENCE OF GROWTH REGULATORS ON GERMINATION**

The growth regulators KNO_3 , NaN_3 and GA_3 did not affect seed germination under optimum germination conditions, since the germination response of bugweed was equally high when seeds were incubated in distilled water under optimum conditions (Figure 2.7A). The effect of CCC, however, was highly inhibitory to seed germination under these optimum conditions. Growth retardants such as CCC are generally regarded as gibberellin antagonists. Part of the action of CCC is thought to lie in inhibition of gibberellin biosynthesis (GRAEBE & ROPER, 1978). CCC is generally considered to inhibit germination, but only in relatively high concentrations and possibly only in combination with other factors, for example, pH (HINTIKKA, 1988). It required a concentration of 6 400 mg l⁻¹ CCC to inhibit germination of *Brassica oleracea* L. seeds (KNYPL, 1967) while 4 000 mg l⁻¹ solution was ineffective in inhibiting *Pinus resinosa* Ait. seed germination (KOZLOWSKI, 1985). In the case of *Barbarea stricta* Andrz. and *Barbarea vulgaris* R.Br., however, much lower concentrations of CCC (from 50 to 150 mg l⁻¹) were effective in reducing seed germination, depending on species and site of collection (HINTIKKA, 1988). These seeds are therefore more sensitive to inhibition than seeds requiring high concentrations of CCC. Inhibition of bugweed seed germination by CCC falls into the former category, since high levels of this growth regulator were required to reduce germination. In the pilot trial 1 580 mg l⁻¹ CCC did not inhibit seed germination at optimum germination conditions.

The results of growth regulator trials suggest that during incubation at optimum conditions, the level of endogenous gibberellin may increase, which results in germination. The application of CCC during incubation under these conditions will inhibit the formation of gibberellin and therefore prevent germination. There may thus be a direct link between temperature and synthesis of gibberellin, but this possibility will require more detailed investigations to clarify the issue. This aspect is of some importance when considering the contradictory evidence of GARDNER (1983) who found that CCC had almost no effect on red-light induced, gibberellin-dependent lettuce seed germination.

Application of growth regulators to seeds incubated under conditions inhibitory to germination, *i.e.* constant temperatures in the light or dark, and alternating temperatures in the absence of light, produced the following results:

1. The growth regulators KNO_3 and NaN_3 stimulated germination at constant temperatures in the light but not in the dark (Figures 2.7B and D). This relationship between light and growth regulators has also been reported for seeds of *Avena fatua* (HILTON, 1984), *Senecio madagascariensis* (GUILLEN, ROMERO & MONTALDI, 1984) and *Nicotiana rustica* (SARMA & PHUKAN, 1981).
2. The most effective regulator applied was 500 mg l^{-1} GA_3 , which stimulated higher germination percentages than the other growth regulators at alternating $15/30^\circ\text{C}$ in the absence of light (Figure 2.7C). GA_3 was the only growth regulator that stimulated

germination at constant 20°C in the dark (Figure 2.7D). The action of GA₃ varied with incubation temperature, and germination percentages were influenced by dosage of this growth regulator. High dosages broke conditional dormancy (except at 5 and 10°C) and substituted for both light and alternating temperature requirements. However, low dosages of GA₃ were only partially effective, and transfer of seeds to optimum conditions was required to promote high germination percentages. This involvement of gibberellin in germination is well documented for other *Solanum* species and it is generally regarded as the main growth regulator functional in this genus.

(iii) **HYPOTHETICAL INVOLVEMENT OF THE PHYTOCHROME SYSTEM, ENDOGENOUS GIBBERELLINS AND TEMPERATURE TRANSFER ON GERMINATION**

This hypothesis is based on results shown in Figure 2.13. Seeds do not germinate at constant 20°C in the dark. Incubation of seeds at alternating 15/30°C in the dark for up to 14 days did not influence the germination response when they were transferred to 20°C in the dark. However, incubation in the light at alternating 15/30°C for 14 days stimulated 50 % of the seeds to germinate on subsequent incubation at 20°C in the dark. Thus the presence of light during incubation at 15/30°C possibly led to an increased level of phytochrome in the P_{fr} form and this level was sufficiently high in 50 % of the seeds to escape inhibition at constant temperatures in the dark. The level of P_{fr} may have continued to increase when seeds were transferred to the light at constant 20°C from 15/30°C,

thus higher germination percentages occurred at 20°C in the light than in the dark.

The increase in P_{fr} level is, however, apparently dependent on temperature. Transfer of seeds to constant 20°C in the dark from 20°C in the light did not promote germination. Thus incubation for up to 14 days at 20°C did not result in increased levels of P_{fr} in the seeds, this only occurred in the light when seeds were incubated at 15/30°C. In addition to this, seeds incubated at constant 20°C in the light required a transfer to 15/30°C for a period of two days or more to enhance germination. Thus light, alone, did not account for the germination responses in these transfer experiments, some other factor changed during incubation at 15/30°C in the light to elicit germination and its action is linked with the phytochrome system.

It is tempting to speculate that endogenous gibberellins are formed during incubation at alternating temperatures in the light. This speculation is based on the following facts, CCC inhibits germination at 15/30°C in the light and this might be by inhibiting the action or formation of gibberellins; and in high concentrations at certain temperatures, GA_3 substitutes for a requirement for transfer to optimum conditions.

If this hypothesis is correct, then enough gibberellins are synthesized during incubation at alternating 15/30°C in the light to allow 50 % of the seeds to germinate at constant 20°C in the dark. Transfer from 15/30°C in the light to constant 20°C in the light would allow endogenous levels of

gibberellin to act together with endogenous P_{fr} to promote a higher level of germination than occurred in seeds transferred to the dark.

(iv) **EFFECT OF SEED STORAGE ON GERMINATION**

Storage of fully imbibed seeds resulted in a decrease in the level of conditional dormancy since high germination percentages occurred at constant temperatures in the light and alternating temperatures in the dark (Figure 2.14). In addition to this, moist storage apparently increased the sensitivity of some seed batches to below-threshold levels (100 mg l^{-1}) of GA_3 (Figure 2.15D).

Thus physiological changes had occurred during moist storage which altered the germination potential of the seeds. These changes were found to a lesser degree in dry-stored seeds. Implications of this are that seeds stored in the field situation at varying stages of imbibition will probably differ in their germination requirements. Thus once again, bugweed seeds exhibit a high degree of variability in their potential germination response. This ultimately ensures survival of the species.

CHANCELLOR (1984) has stated that the next great advance in weed control technology will be to stimulate germination of soil-stored seeds and thus exhaust the soil seed bank. A single application of herbicides could then kill all seedlings.

Substituted phthalimides are growth regulators with action akin to that of gibberellins (LOS, KUST, LAMB & DIEHL, 1980a; DEVLIN, 1981;

SUTTLE & SCHREINER, 1982) and are known to stimulate germination in other gibberellin-responsive species (METZGER, 1983; UPADHYAYA, HSIAO & BONSOR, 1986). However, application of these regulators to the bugweed seed bank in the soil would probably not prove effective against all buried seeds unless high concentrations reached all the seeds, since the presence of light or alternating temperatures is also required for a high germination response to occur (Figures 2.8 and 2.9). Thus not all seeds would germinate simultaneously. Import of these products for use in bugweed control would therefore be inadvisable, because of the variable germination pattern that occurs in this species. Due to unavailability these compounds could however, not be tested.

(v) **STUDY OPTIONS FOR THE REMAINDER OF THIS PROJECT**

At this point, dichotomy of further investigation possibilities arose. The one direction would involve a further elucidation of growth regulator-temperature-light interactions. Here, perhaps endogenous levels of gibberellin may be shown to be influenced by incubation temperature, light or storage conditions, and bugweed germination characteristics may well prove a system which lends itself to this type of research.

However, the outcome of this project for my employer, the Plant Protection Research Institute, should be one of practical application, with potential for control of this weed species, rather than studying highly complex interactions within the system, which would involve extraction of growth regulators from the seed tissue to determine their endogenous levels. Thus further work has involved an overview description of the

system, with the aim of manipulation for control purposes. This will include determining just how variable bugweed seed germination can be, and finally, whether the problem caused by the reproductive potential of bugweed can be overcome by utilization of fruits of the species.

E. SUMMARY AND CONCLUSIONS

1. Bugweed seeds require both light and alternating temperatures for optimum germination. Seeds do not exhibit primary dormancy.
2. The magnitude of temperature fluctuation is not important since differences of 10, 15 and 23°C all promoted a high germination response.
3. Transfer of seeds from unfavourable conditions to optimal conditions promoted a high germination response, indicating seeds were in a state of conditional dormancy during incubation under unfavourable conditions.
4. GA₃ is the only growth regulator tested which could bring about a high germination response under all incubation conditions tested except low constant temperatures.
5. GA₃ breaks conditional dormancy by improving the response at constant temperatures, but this was dependent on dosage.
6. Seeds from different localities had similar germination requirements.
7. The effect of light is more qualitative than quantitative in stimulating germination, and the phytochrome system is functional

in bugweed seeds.

8. Moist storage at 20⁰C for up to 12 months caused seeds to lose their requirement for light at alternating temperatures or to lose their requirement for alternating temperatures in the presence of light. Thus, the level of conditional dormancy was decreased. However, germination did not occur at constant temperatures in the dark. Dry storage at 20⁰C for up to 12 months caused a more variable germination response under unfavourable incubation conditions.
9. Moist storage can apparently increase the sensitivity of some seed batches to below-threshold levels of GA₃.
10. Physiological changes occurring during incubation at 15/30⁰C in the light which promote germination when the seed is transferred to unfavourable conditions may be related to the phytochrome system and to endogenous levels of GA₃.
11. Bugweed seeds exhibit a high degree of variability in their germination response, and this enhances chances of survival of the species. Consequently, a sporadic seedling emergence is ensured which results in increased control costs.

CHAPTER 3

THE EFFECT OF LOCALITY, SEASON AND YEAR OF SEED HARVEST ON GERMINATION

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CHAPTER 3

THE EFFECT OF LOCALITY, SEASON AND YEAR OF SEED HARVEST ON GERMINATION

A. INTRODUCTION

(i) GENETIC AND ENVIRONMENTAL INFLUENCES ON PLANT RESPONSES IN DIFFERENT POPULATIONS

WASER, VICKERY & PRICE, (1982) concluded that species have traditionally been viewed as homogeneous, with their uniformity maintained by gene flow within and among populations (MAYR, 1970; DOBZHANSKY, 1970; GRANT, 1971). Contrary evidence does, however, suggest that gene flow in plant species is localized (EHRlich & RAVEN, 1969; ENDLER, 1977; LEVIN, 1979) and that differentiation within and among populations is extensive (EPLING, LEWIS & BALL, 1960; SOLBRIG & SIMPSON, 1974; LEVIN, 1977; NAYLOR, 1983; NOLAN & UPADHYAYA, 1988). However, EHRlich & RAVEN, (1969) stressed that populations within a species may have similar responses not because of gene flow but because they occupy habitats with similar sets of selective forces.

The analysis of WASER, VICKERY & PRICE (1982) suggested that, in the case of *Mimulus guttatus* Fisch ex. D.C., gene flow played a minor role in maintaining similarities of populations in different localities. Instead, environmental conditions were very important in determining the phenotypic character of a population.

It is now generally accepted that the genotype and the environment in which it develops, interact to give the observed phenotype (CULLIS, 1977). Not only the environment of the progeny but also the maternal environment existing during seed development and maturation may affect germination behaviour. The influence of the maternal environment may be detected not only in the immediate progeny but also in subsequent generations, for example, in *Pisum sativum* L. (HIGHKIN, 1960) and *Plantago lanceolata* Hook., (ALEXANDER & WULFF, 1985).

(ii) **GERMINATION RESPONSES DUE TO IMMEDIATE ENVIRONMENT**

Variation in germination behaviour between populations has been reported for a number of species (CAVERS & HARPER, 1966; NAYLOR & ABDALLA, 1982; FROUD-WILLIAMS, 1987). This variation may be a result of environmental conditions, such as temperature and light, as in the case of *Solanum nigrum* L. (ROGERS & OGG, 1981; VAN DEN VENTER, MEGGITT & PENNER, 1982). There are contradictory data in the literature concerning the germination of *S. nigrum* seeds at constant temperatures. Constant temperatures are effective for *S. nigrum*, according to BURGERT & BURNSIDE, 1972 and GIVELBERG & HOROWITZ, 1984, whereas others claim that alternating temperatures favoured germination (LAUER, 1953; ENGELHART, VICENTE & SILBERSCHMIDT, 1961; ROBERTS & LOCKETT, 1978b; ROGERS & OGG, 1981). GIVELBERG & HOROWITZ, (1984) suggested that variability may have arisen because seeds were of different origins. Place of harvest also greatly influenced the germination potential of *Dactylis glomerata* L., (JUNTILLA 1977; PROBERT, SMITH & BIRCH, 1985),

Cedrus deodara (D. Don) G. Don (THAPLIYAL & GUPTA, 1980), *Pinus taeda* L. (RICHTER & SWITZER, 1982) *Poa annua* L. and *Alopecurus myosuroides* Huds. (NAYLOR & ABDALLA, 1982). The fraction of seeds that are viable may also vary with locality, for example in *Daucus carota* L., (LACEY, 1984).

The microclimate in a habitat may alter over a very small distance, thus variability may be detected in plants harvested within a distance of a few metres on a single hillside as in *Avena barbata* Brot. (ALLARD, MILLER & KAHLER, 1978; HAMRICK & ALLARD, 1972). Variation may be substantial between different trees as seen in *Leptospermum scoparium* J.R. et G. Forst. (MOHAN, MITCHELL & LOVELL, 1984), or *Rhus globra* L. and *Rhus coppalina* L. (FARMER, LOCKLEY & CUNNINGHAM, 1982).

Germination of seed may also vary with season and year of harvest. This was seen in *Spergularia marina* (L.) Grisels. where both the response to the optimum temperature, 5/15⁰C, and the germination percentage at different constant temperatures varied with month of harvest (OKUSANYA & UNGAR, 1983).

The level of dormancy of ten *Rosa* species and their germination pattern varied between years. Climatic conditions before harvest greatly influenced germination. This was particularly true of the average daily temperature for the thirty day period preceding harvest (VON ABRAMS & HAND, 1956). Seasonal differences were also found in *Amaranthus retroflexus* L., where seeds that matured in late autumn were less dormant than those

which matured in late summer (CHADOEUF-HANNEL & BARRALIS, 1983).

Harvest date also influences germination. The later the harvest date after anthesis, the greater the germination response evident in *Solanum melongena* L. (SUZUKI & TAKAHASHI, 1968) and *Vicia faba* L. (EL BAGOURY, 1975). This may be related to increased seed mass for example with *Lactuca sativa* L., (GLOBERSON 1981), *Glycine max* Merr. (SINGH & GUPTU, 1982) and *Sorghum bicolor* Moench, (MAITI, RAJU & BIDINGER, 1985) but not with *Daucus carota*, where, even with seeds of the same size, germination increased with maturity (AUSTIN & LONGDEN, 1967).

(iii) **GERMINATION RESPONSES DUE TO THE MATERNAL ENVIRONMENT DURING DEVELOPMENT**

Climatic conditions under which the seeds develop before harvest may also affect their germination. For example, temperature treatments applied to the mother plant can affect germination. KOLLER (1962) found that high temperatures during seed maturation increased germination in *Lactuca sativa*, while THOMAS & RAPER, (1975) obtained highest germination in tobacco when the parental plant experienced high temperatures prior to flowering and low temperatures during seed maturation. Mother plant temperature influenced germination in *Syringa vulgaris* L. [sic.] (JUNTILLA, 1973) and *Aegilops kotschisi* Boiss. (WURZBURGER & KOLLER, 1976). Photoperiod during maturation may also be important, for example, in *Portulaca oleracea* L. (GUTTERMANN, 1977), *Aegilops kotschisi* (WURZBURGER & KOLLER, 1976) and *Amaranthus retroflexus*

(KIGEL, OFIR & KOLLER, 1977). Light quality was significant for *Portulaca oleracea* (GUTTERMANN, 1977).

Implications of the effects of the external environment on the mother plant are that erroneous interpretation of variability observed between different phenotypes may occur. If seeds from individual plants growing in different localities are planted in a common environment, any phenotypic variation among the plants is generally attributed to genetic differences (CLAUSEN, KECK & HIESEY, 1940). The interpretation of such results is, however, complicated by maternal effects (HUME & CAVERS, 1981). If maternal effects are present, the environment in which the seeds had matured (maternal environment) would influence their growth in the common environment, thus differences among plants could be due to an environment effect, indeed, according to ALEXANDER & WULFF (1985), this effect could be evident for more than one generation. Thus some variation among individuals could result from maternal environmental differences and some from genetic differences (ALEXANDER & WULFF, 1985). One should therefore be wary of sweeping statements made about the germination of a species that are based on seed samples from a single population (NAYLOR & ABDALLA, 1982).

(iv) **IMPLICATIONS OF VARIABILITY FOR WEED CONTROL**

DUKE (1985) describes a "general purpose genotype characteristic" as being a property in certain plants which can permit them to adapt to a wide range of environments, rather than being limited to a narrow range,

as may result from intensive selection in crop breeding, where much flexibility in response to environmental variation may be lost. Thus a less "improved" species may be relatively free from environmental constraints. It follows, then, that some species are inherently more able than others to respond favourably to a wide range of environments. In the case of weeds, there is considerable morphological and physiological plasticity. A very important survival mechanism for plants growing in unpredictable conditions is the ability to develop seed variability or heteroblasty. Thus even under optimum conditions only a part of such a seed population will germinate at any one time. Each time that a stand of seedlings or older plants is destroyed, a new stand will soon arise from the seed bank to take its place (CAVERS, 1974).

Even in the case of species bred for lack of dormancy and for regularity of germination there may still be much variation in germination behaviour within and between seed batches because of differences in factors such as coat thickness, seedling vigour and degree of dormancy. Such seeds could be pretreated or primed to maximise percentage and speed of germination, for example, by means of storage under particular conditions or the addition of chemicals which increase the germination potential of the seed.

A much greater variation in germination response would be expected, and is usually seen, in weed seeds which are geared to survival via natural, not man-made selection. Implications of variable dormancy are, for example, that of high weed control costs to eradicate a soil seed bank. As stated earlier, CHANCELLOR (1984) has pointed out that a new era in weed

control which would greatly improve progress would be the manipulation of dormancy mechanisms to lead to mass germination of the seed, thereby exhausting the soil seed bank. This is, incidently, the same aim as that of breeders of cultivated plants who strive to obtain a uniform and total seedling emergence in crops.

The aim of this section of the study was to ascertain whether seeds of bugweed from different origins exhibited different germination characteristics, or if they followed general *Solanum* trends, in consistently requiring light and alternating temperatures for maximum germination to occur, and whether the response to the application of GA₃ was always the same. An overview approach to these questions has therefore been taken, where seeds from various parts of South Africa were tested under controlled environmental conditions at Cedara to ascertain their germination potential, depending on season and year of harvest. A number of questions were posed; are seeds dormant, if so, does the level of dormancy differ among seed batches? What is the influence of season and year of harvest on subsequent germination patterns? Does the sensitivity of seeds to GA₃ differ among sites at different times of the year? If so, can inferences be drawn regarding the application of GA₃ (or other growth regulators with a similar action) in the field situation to bring about a consequent stimulation of germination in dormant seeds?

B. MATERIALS AND METHODS

(i) HARVESTING AND SEED PREPARATION

Seeds from five localities: Groot Constantia (in the Cape), Pretoria (in the Transvaal), Umndoni Park (along the Natal South Coast) and from Ashburton and Cedara State Forest (two Natal inland sites) were harvested during autumn (mid-April), winter (mid-July), early summer (mid-November) and late summer (mid-February) of two consecutive years (Figure 3.1). Seed batches¹ from ripe fruits were cleaned by repeated washing in tap water, air dried at ambient temperatures and kept in brown paper bags at room temperature until required for use. All experiments were initiated within one week of collection. Sinking seeds which were "apparently viable" (ROBERTS, 1981) were used for all experiments. Seeds from each locality were counted into lots of twenty on a Numigral seedcounter and placed into compartmentalized plastic trays in a random fashion.

(ii) ANALYSIS OF RESULTS

Analysis of variance was done using the Genstat 4 program and then the least significant differences (LSD) between means were calculated (RAYNER, 1967; Chapter 10). LSD's were calculated only between means of the germination responses of seed batches within the same incubator. LSD's are presented for interactions between site x season x year of seed harvest. The correlation coefficient (r) was used in Figures 3.13 and 3.14

¹A seed batch, as described in this study is defined as a sample of seeds harvested from a population growing in one locality during one time period, and subsequently incubated under a particular set of conditions.

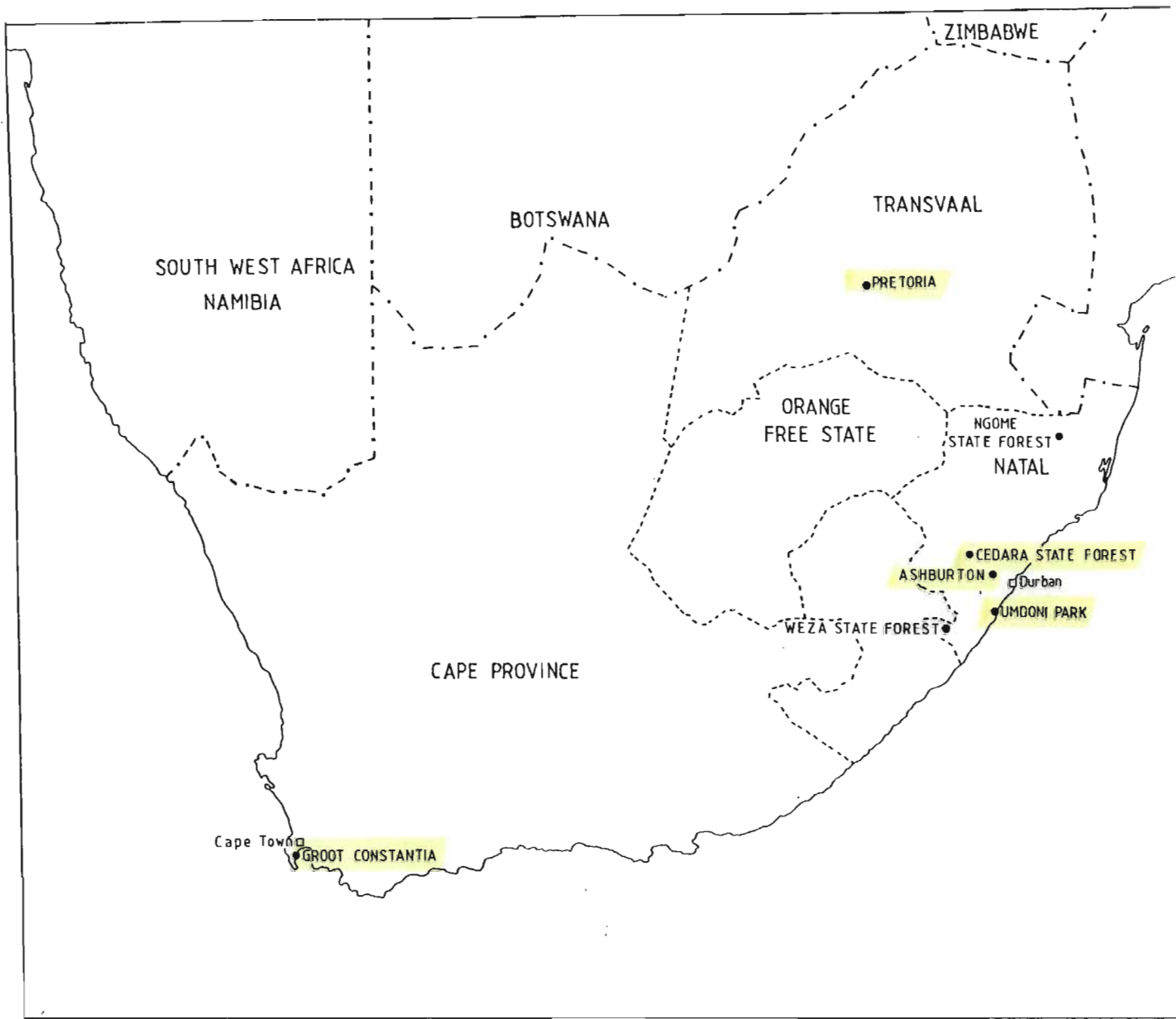


FIGURE 3.1: Map indicating the sites where bugweed fruits were harvested for this study.

to test whether secondary dormancy and annual temperature patterns were correlated (PARKER, 1973; page 57).

(iii) **GROWTH REGULATOR TREATMENTS**

Solutions of 0, 100 or 500 mg l⁻¹ GA₃ were added to the seeds in different compartments of repli dishes and these were kept in the light or dark, according to subsequent incubation conditions. An aliquot of 1.5 ml of each solution was added to each compartment.

(iv) **GERMINATION PROCEDURES**

The plastic repli dishes were placed in incubators at constant 20⁰C or alternating 15/30⁰C in the light or dark. Light was not continuous, either at constant 20⁰C or alternating 15/30⁰C, but was cycled for 14 hours each day, coinciding with the period of 30⁰C in the alternating temperature incubator. For dark germination, dishes were wrapped in two layers of aluminum foil, and the numbers of seeds that germinated were counted under green light conditions in a light proof room. Germination was recorded weekly; but results are expressed as final percentage germination after 12 weeks incubation. Subsequent to 12 weeks incubation, seeds were transferred to a second set of incubation conditions, for a further 12 weeks, as follows:

First incubation²
15/30⁰C Light
15/30⁰C Dark
20⁰C Light
20⁰C Dark

Second incubation²
15/30⁰C Light
15/30⁰C Light
15/30⁰C Light
15/30⁰C Dark³

²for 12 weeks.

³After this incubation for 12 weeks, the non-germinated seeds were transferred to 15/30⁰C in the light for a further 12 weeks incubation period.

C. RESULTS

(i) GERMINATION RESPONSE UNDER OPTIMUM CONDITIONS

(a) Germination in the absence of GA₃

The germination of seeds from the Natal South Coast, Transvaal and the Cape under optimum germination conditions was generally high, regardless of year or season of harvest (Figure 3.2). Seeds from these localities therefore showed no primary dormancy. In contrast, the response of seeds from the Natal inland sites, Cedara and Ashburton, was variable. Either a high germination response was evident, or, in some seed batches, approximately half of the seeds failed to germinate (Figure 3.2). Prolonged incubation of these low-germinating seed batches under optimum conditions did not significantly improve the germination response (Figure 3.2). Non-germinated seeds were "apparently viable" upon dissection⁴, and thus were therefore not dead *but in a state of primary dormancy*.

(b) Germination response with applied GA₃

The primary dormancy exhibited by certain seed batches from Cedara and Ashburton was broken with the application of 100 mg l⁻¹ GA₃ (Figure 3.3). Other seed batches were either unaffected or had a slightly increased germination response when incubated in 100 mg l⁻¹ GA₃ (Figures 3.2 and 3.3). A relatively high concentration of GA₃ (500 mg l⁻¹) was not more effective in promoting germination (results not shown). Implications for the field situation are that not all seeds will germinate when shed from the

⁴ROBERTS (1981) defined "apparently viable seeds" as those which appear to be intact and which resist gentle pressure.

1986/87

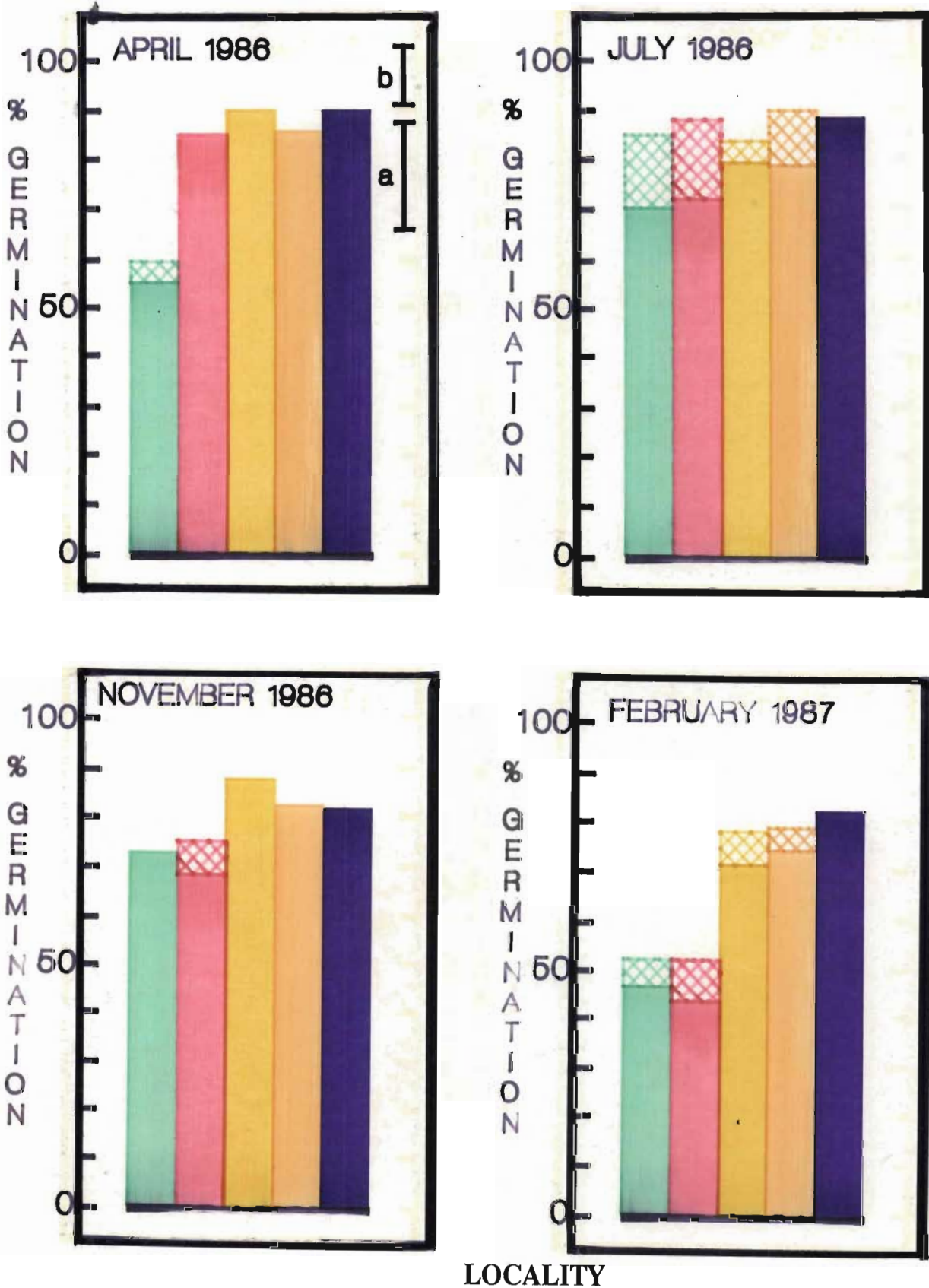
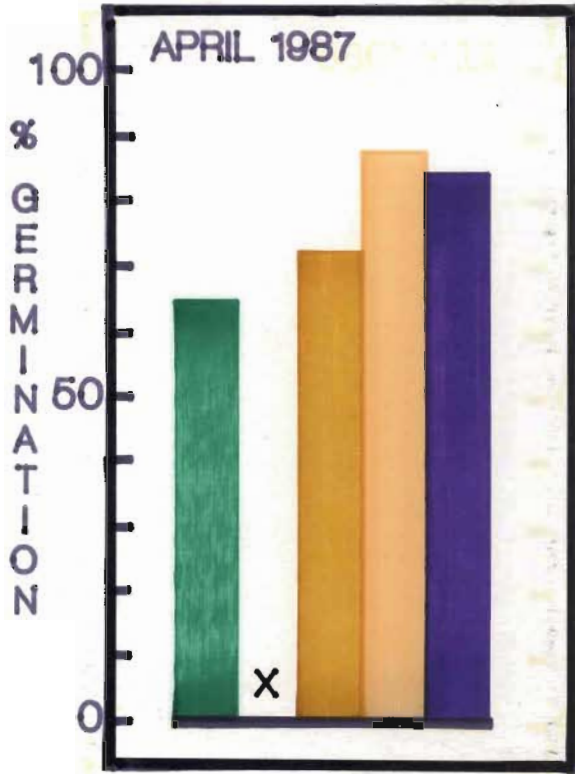


FIGURE 3.2: The germination response of bugweed under optimum conditions. Seeds were harvested at four different times of the year in 1986/87 and 1987/88 from five localities. Seeds were incubated in distilled water. Shaded and hatched portions of each bar represent germination at 15/30°C in the light after 12 and 24 weeks respectively.
a: LSD $p < 0.05$ shaded portions of bars (locality x season x year).
b: LSD $p < 0.05$ hatched portions of bars (locality x season x year).

1987/88



- Cedara - Natal inland
- Ashburton - Natal inland
- Umdoni Park - Natal South Coast
- Pretoria - Transvaal
- Groot Constantia - Cape
- X: No seeds produced during this time

1986/87

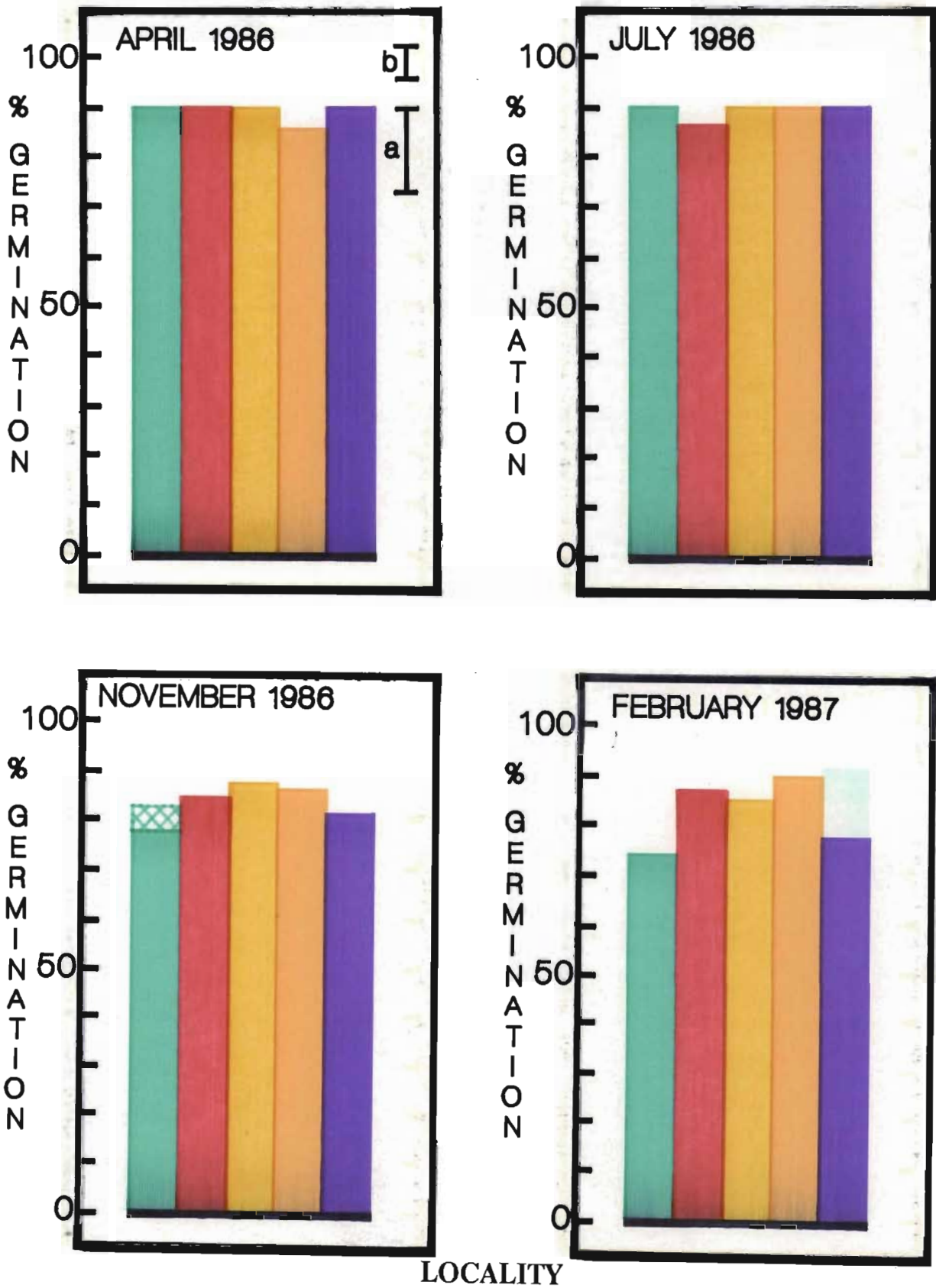


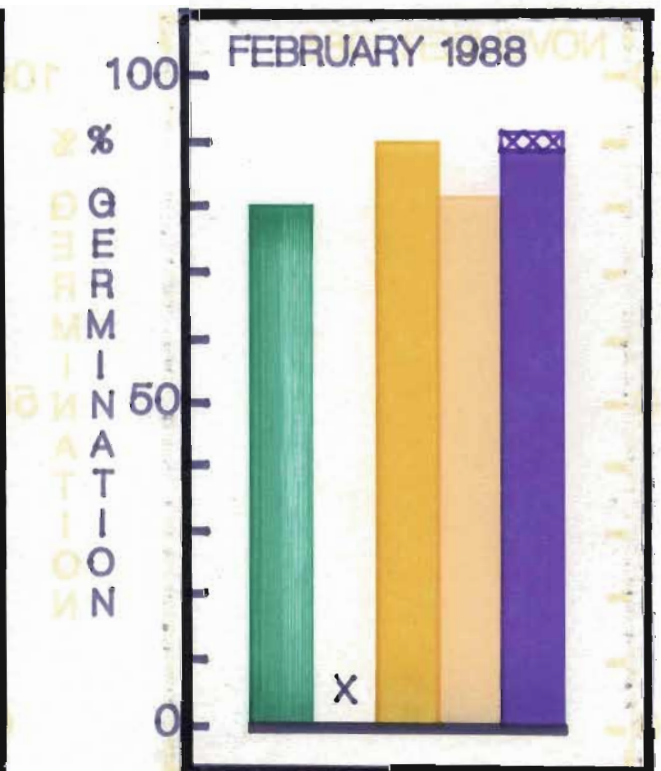
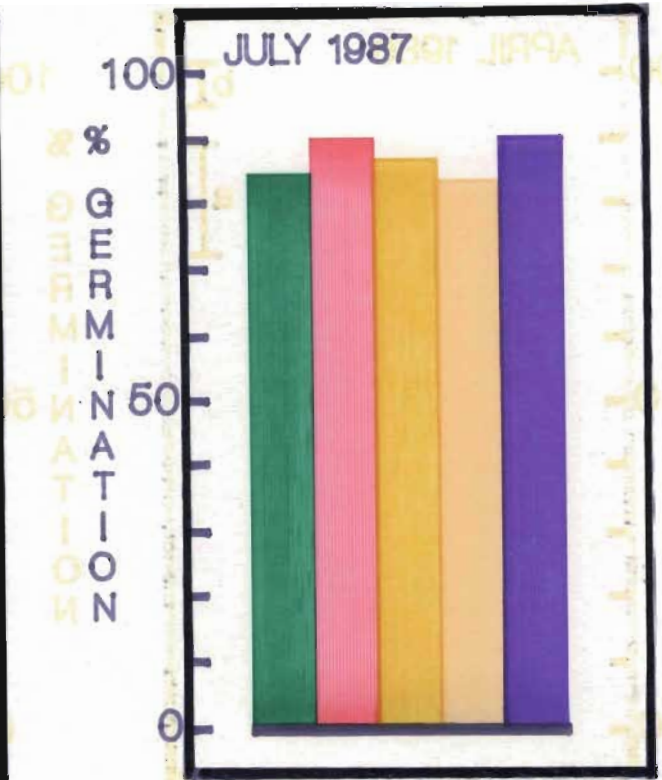
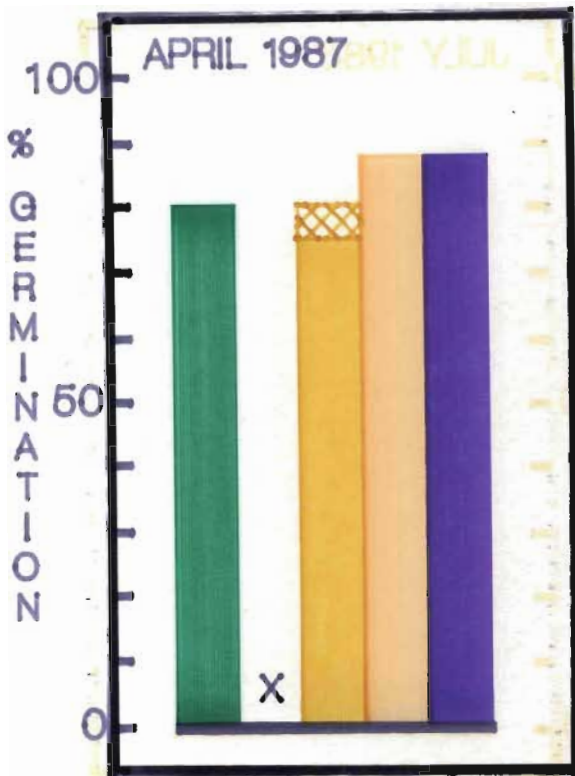
FIGURE 3.3: The germination response of bugweed under optimum conditions. Seeds were harvested at four different times of the year in 1986/87 and 1987/88 from five localities. Seeds were incubated in $100 \text{ mg l}^{-1} \text{ GA}_3$.

Shaded and hatched portions of each bar represent germination at $15/30^\circ\text{C}$ in the light after 12 and 24 weeks respectively.

a: LSD $p < 0.05$ shaded portions of bars (locality x season x year).

b: LSD $p < 0.05$ hatched portions of bars (locality x season x year).

1987/88



- Cedara - Natal inland
 - Ashburton - Natal inland
 - Umdoni Park - Natal South Coast
 - Pretoria - Transvaal
 - Groot Constantia - Cape
- X: No seeds produced during this time

tree; some will remain dormant even when conditions are favourable for germination. However, the application of GA₃ (or other growth regulators with a similar action), even in low concentration, to the soil surface would stimulate germination of most freshly-shed bugweed seeds, provided environmental conditions are optimal for germination.

(ii) **GERMINATION RESPONSE UNDER CONSTANT TEMPERATURES IN THE LIGHT**

(a) **Germination in the absence of GA₃**

The germination response of seeds from the Natal inland sites, Cedara and Ashburton, was inhibited by constant temperatures, irrespective of season or year of harvest. Seeds from the Natal South Coast and the Transvaal rarely showed significant germination responses. In contrast, seeds from the Cape showed highly variable germination responses, with certain seed batches having a partial loss of the alternating temperature requirement. The response was dependent on season and year of harvest (Figure 3.4).

Seeds generally showed greatly enhanced germination when transferred from constant to alternating temperatures, indicating that when the seeds were incubated at constant temperatures, they were *in a state of conditional dormancy* (Figure 3.4). However, this transfer to optimum conditions did not stimulate high germination percentages in every seed batch from the Natal inland sites, and the response varied with season and year of seed harvest (Figure 3.4). The ability of seeds to germinate when transferred to optimum conditions had therefore been lost in some seed batches from the Natal inland sites and seeds had apparently *entered secondary dormancy*.

1986/87

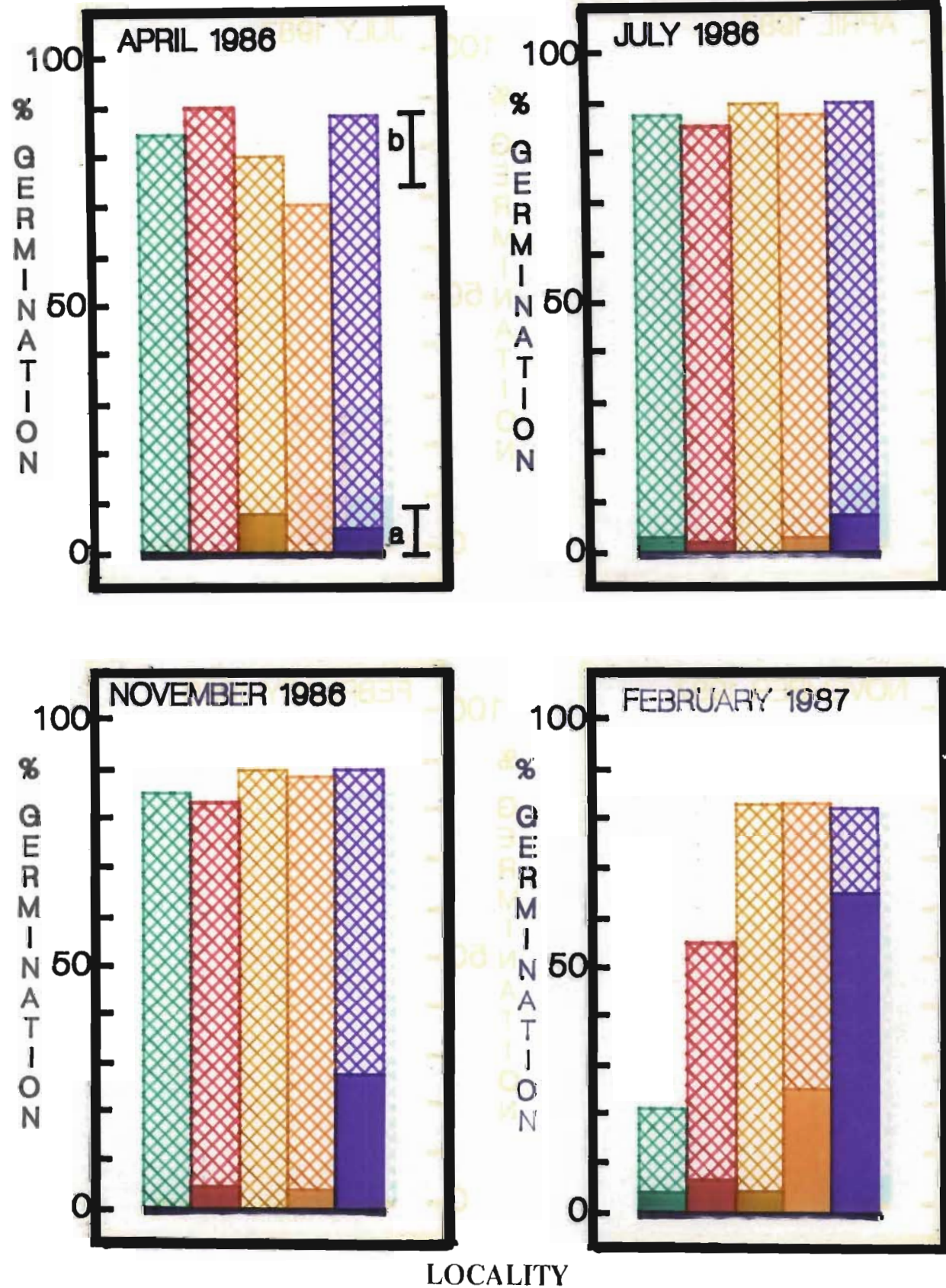


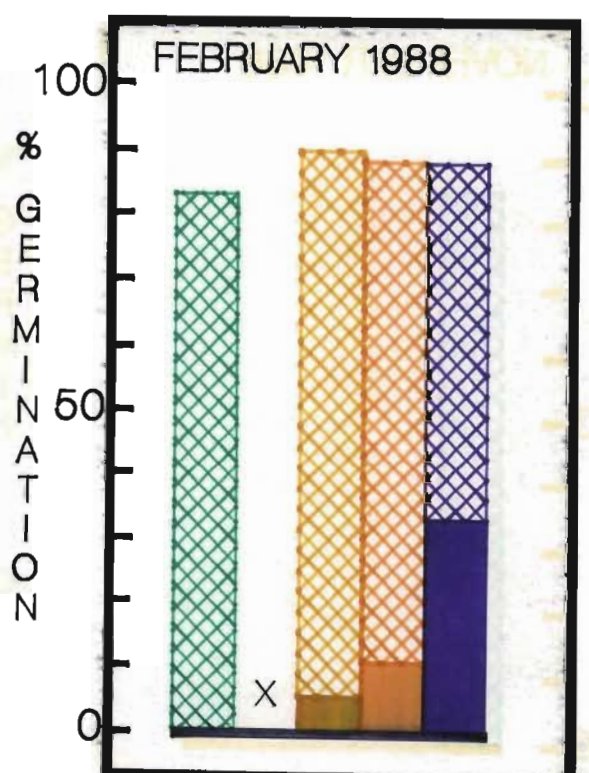
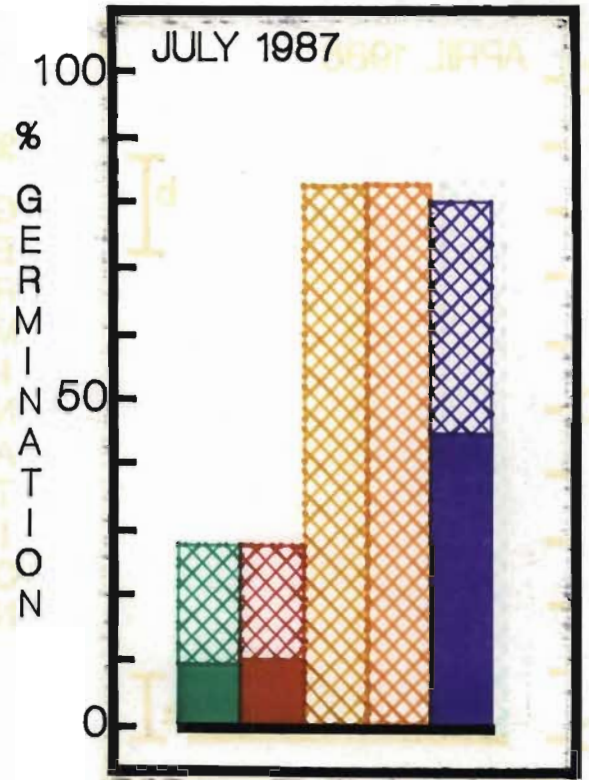
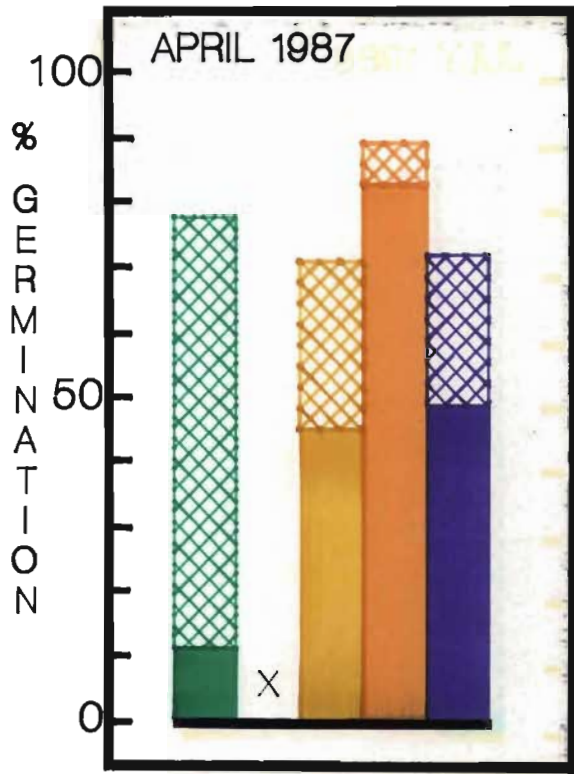
FIGURE 3.4: The germination response of bugweed at 20°C in the light. Seeds were harvested at four different times of the year in 1986/87 and 1987/88 from five localities. Seeds were incubated in distilled water.

Shaded portions of each bar represent germination at 20°C in the light after 12 weeks. Hatched portions of each bar represent germination after 12 weeks when seeds were subsequently transferred to alternating 15/30°C in the light.

a: LSD $p < 0.05$ shaded portions of bars (locality x season x year).

b: LSD $p < 0.05$ hatched portions of bars (locality x season x year).

1987/88



- Cedara - Natal inland
 - Ashburton - Natal inland
 - Umdoni Park - Natal South Coast
 - Pretoria - Transvaal
 - Groot Constantia - Cape
- X: No seeds produced during this time

(b) **Germination response with applied GA₃**

The response of seeds from the Natal South Coast, Transvaal and the Cape to the application of 100 mg l⁻¹ GA₃ in the light at constant temperatures was variable. Germination of around 60 % or above was evident in April, November and February of both 1986/87 and 1987/88. The response was, however, significantly lower in July of both years (Figure 3.5). Thus seeds were apparently more responsive to GA₃ in the warmer months. Seeds from the Natal inland sites either showed a similar response or a significantly lower germination response than seeds from the other three localities (Figure 3.5). Thus the response to GA₃ was influenced by site, season and year of seed harvest.

Transfer of low-germinating seed batches to alternating temperatures resulted in an increased germination response (Figure 3.5).

The application of 500 mg l⁻¹ GA₃ generally promoted a high germination response in all seed batches and was a sufficiently high dosage to substitute for the requirement for transfer to optimum conditions, seen in some of the seed batches (Figures 3.5 and 3.6). This indicated that in some seed batches the application of 100 mg l⁻¹ GA₃ was below the threshold level required for germination at constant temperatures in the light (Figure 3.5). Seed batches that did not respond to 100 mg l⁻¹ GA₃ were therefore in *a deeper state of conditional dormancy*.

1986/87

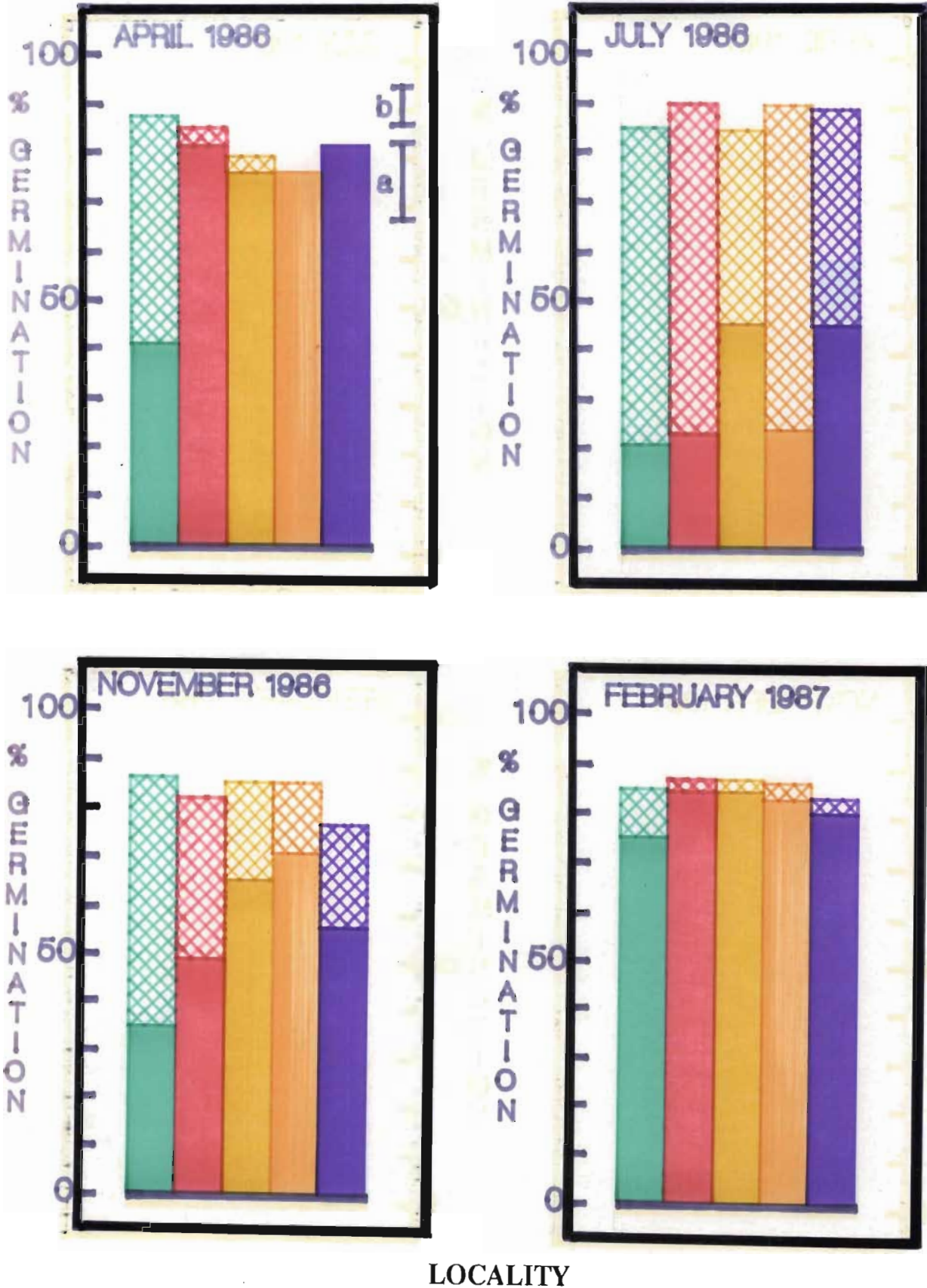


FIGURE 3.5: The germination response of bugweed at 20°C in the light. Seeds were harvested at four different times of the year in 1986/87 and 1987/88 from five localities. Seeds were incubated in 100 mg l⁻¹ GA₃.

Shaded portions of each bar represent germination at 20°C in the light after 12 weeks. Hatched portions of each bar represent germination after 12 weeks when seeds were subsequently transferred to alternating 15/30°C in the light.

a: LSD p<0.05 shaded portions of bars (locality x season x year).

b: LSD p<0.05 hatched portions of bars (locality x season x year).

1987/88



- Cedara - Natal inland
- Ashburton - Natal inland
- Umdoni Park - Natal South Coast
- Pretoria - Transvaal
- Groot Constantia - Cape
- X: No seeds produced during this time

1986/87

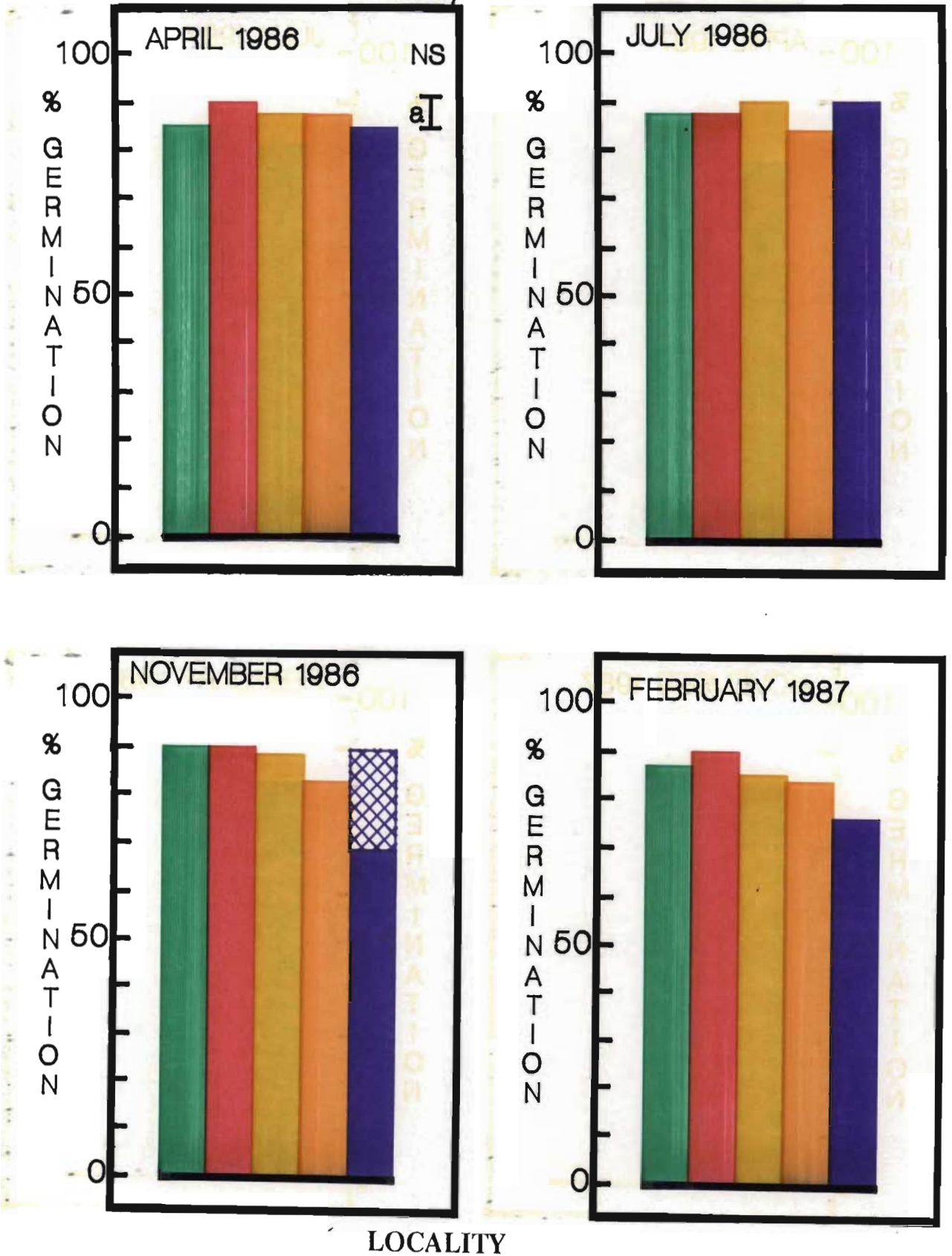
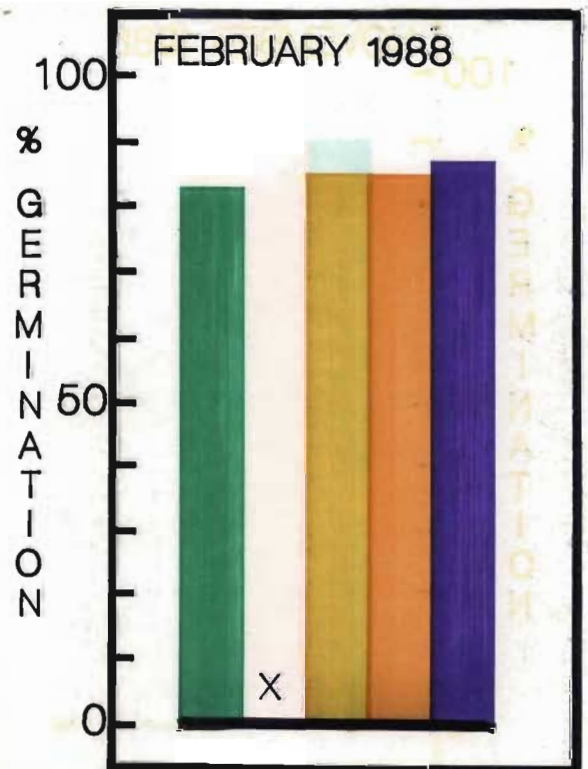
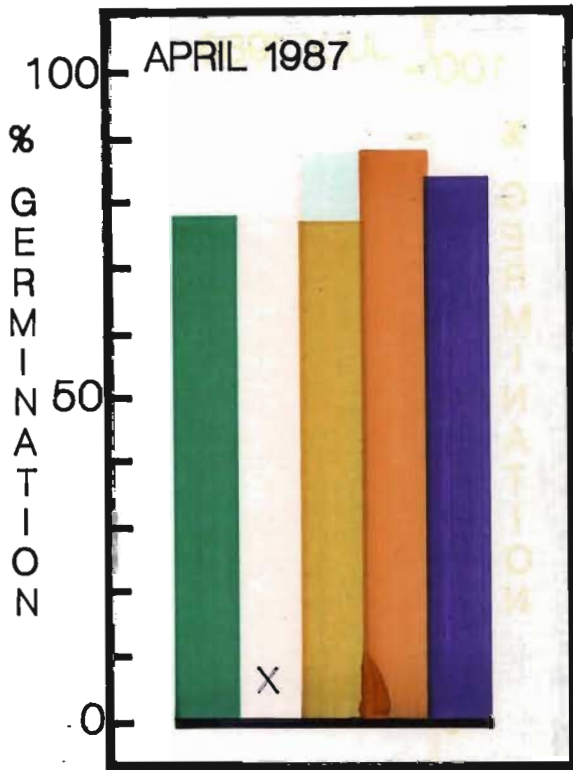


FIGURE 3.6: The germination response of bugweed at 20°C in the light. Seeds were harvested at four different times of the year in 1986/87 and 1987/88 from five localities. Seeds were incubated in 500 mg l⁻¹ GA₃. Shaded portions of each bar represent germination at 20°C in the light after 12 weeks. Hatched portions of each bar represent germination after 12 weeks when seeds were subsequently transferred to alternating 15/30°C in the light.
 a: LSD p<0.05 shaded portions of bars (locality x season x year).
 NS: no significant differences between means of hatched portions of bars (locality x season x year).

1987/88



- Cedar - Natal inland
- Ashburton - Natal inland
- Umdoni Park - Natal South Coast
- Pretoria - Transvaal
- Groot Constantia - Cape
- X: No seeds produced during this time

(iii) **GERMINATION RESPONSE UNDER ALTERNATING TEMPERATURES IN THE DARK**

(a) **Germination in the absence of GA₃**

The germination response of seeds from the Natal South Coast and the Transvaal was generally very low, regardless of season or year of harvest (Figure 3.7). Seeds from the Cape, however, showed a variation in response from 40 to 67 % germination for seed harvested between April 1986 and April 1987. This response declined in the other Cape seed batches (Figure 3.7). A fair proportion of seeds from the Cape therefore did not have a requirement for light at alternating temperatures, depending on year and season of harvest.

During July 1987, 69 % of the seeds from Cedara did not show a requirement for light at alternating temperatures for germination but the response was significantly lower in July 1986 (Figure 3.7). Seed batches harvested from trees at Cedara at other times showed a very low germination response. Seeds from the other Natal inland site, Ashburton, consistently showed a low response to these incubation conditions (Figure 3.7).

Thus it is evident that the germination response of bugweed to alternating temperatures in the absence of light can be highly variable, differences being determined by site, season and year of seed harvest.

Transfer to the light of seeds obtained from trees in the Cape, resulted in a moderate to high germination response, indicating that in the absence of light, *most of the seeds had been in a state of conditional dormancy*. This

1986/87

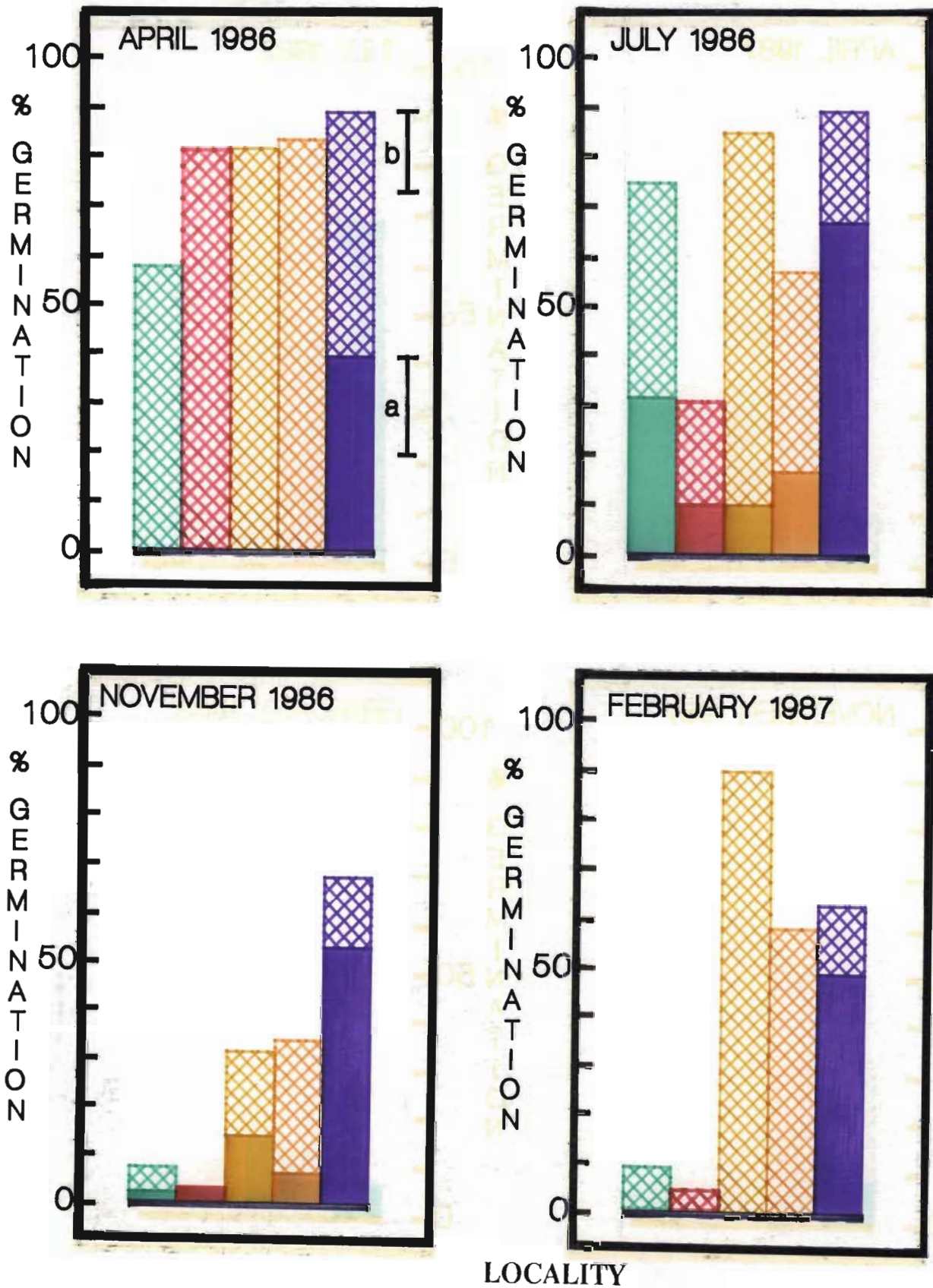


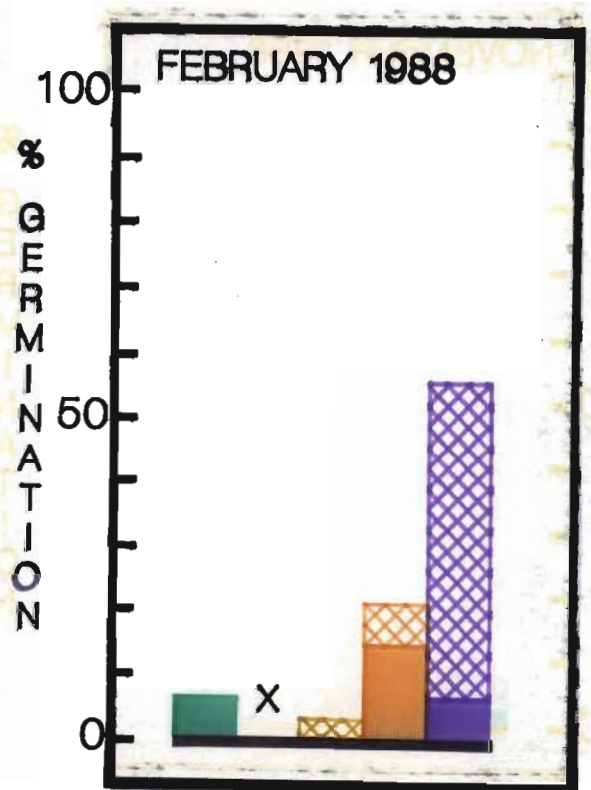
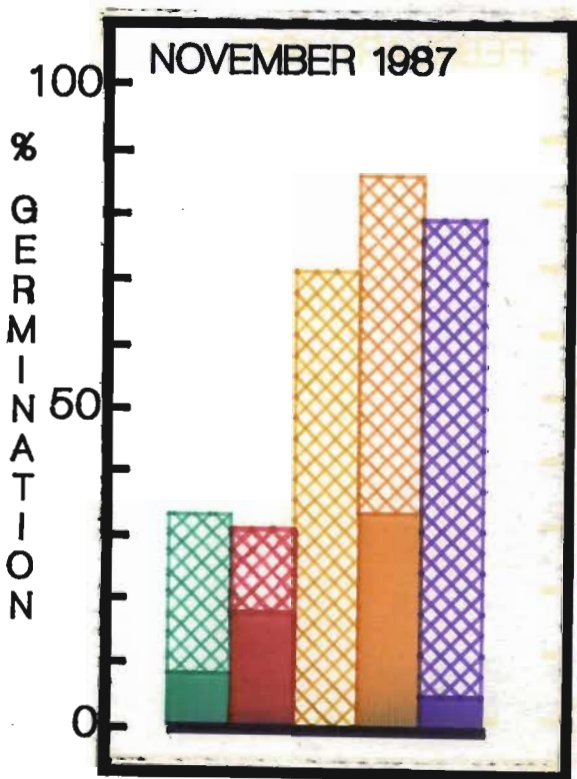
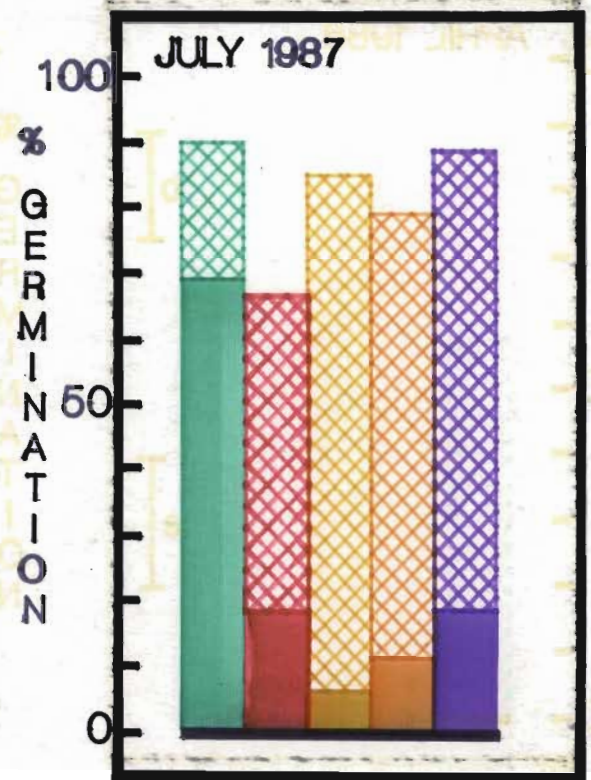
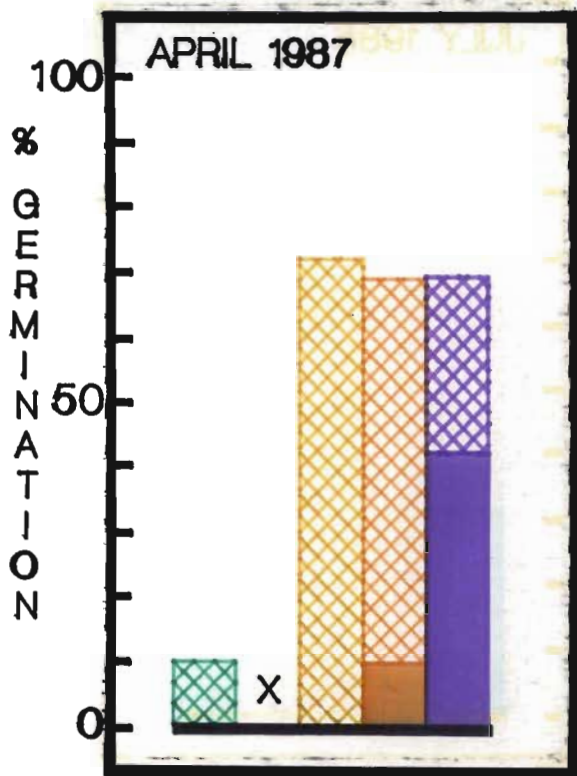
FIGURE 3.7: The germination response of bugweed at 15/30°C in the dark. Seeds were harvested at four different times of the year in 1986/87 and 1987/88 from five localities. Seeds were incubated in distilled water.

Shaded portions of each bar represent germination at 15/30°C in the dark after 12 weeks. Hatched portions of each bar represent germination after 12 weeks when seeds were subsequently transferred to the light.

a: LSD $p < 0.05$ shaded portions of bars (locality x season x year).

b: LSD $p < 0.05$ hatched portions of bars (locality x season x year).

1987/88



- Cedara - Natal inland
- Ashburton - Natal inland
- Umdoni Park - Natal South Coast
- Pretoria - Transvaal
- Groot Constantia - Cape
- X: No seeds produced during this time

situation was also evident in seed batches from the South Coast and the Transvaal, except during November 1986, when less than half of the seeds germinated, and February 1988, when transfer of seeds to the light did not promote further germination (Figure 3.7). Non-germinated seeds were apparently viable upon dissection, and may be described as being in a state of secondary dormancy, induced by incubation in the absence of light. However, *the occurrence of secondary dormancy was most frequent in seed batches from the Natal inland sites* (Figure 3.7). This indicated that seeds from these two sites have a greater ability to avoid unfavourable environmental conditions by entering a period of rest.

(b) **Germination response with applied GA₃**

The germination response of seeds from the Natal South Coast, Transvaal and the Cape to the application of 100 mg l⁻¹ GA₃ was generally higher than 60 %. Sometimes it reached a value higher than 80 % (Figure 3.8). Germination percentages of some seed batches from the two Natal inland sites were significantly lower than those from the other sites, depending on season and year of harvest (Figure 3.8).

Transfer of most seed batches to the light did not markedly improve the germination response (Figure 3.8). However, less than 50 % of the seeds obtained from Cedara and Ashburton in November 1986, germinated when transferred to the light. These seeds had therefore *entered secondary dormancy*. However, the application of 100 mg l⁻¹ GA₃ was generally a sufficiently high dosage to prevent seed batches entering secondary dormancy, except in the latter two cases. A GA₃ concentration effect was

1986/87

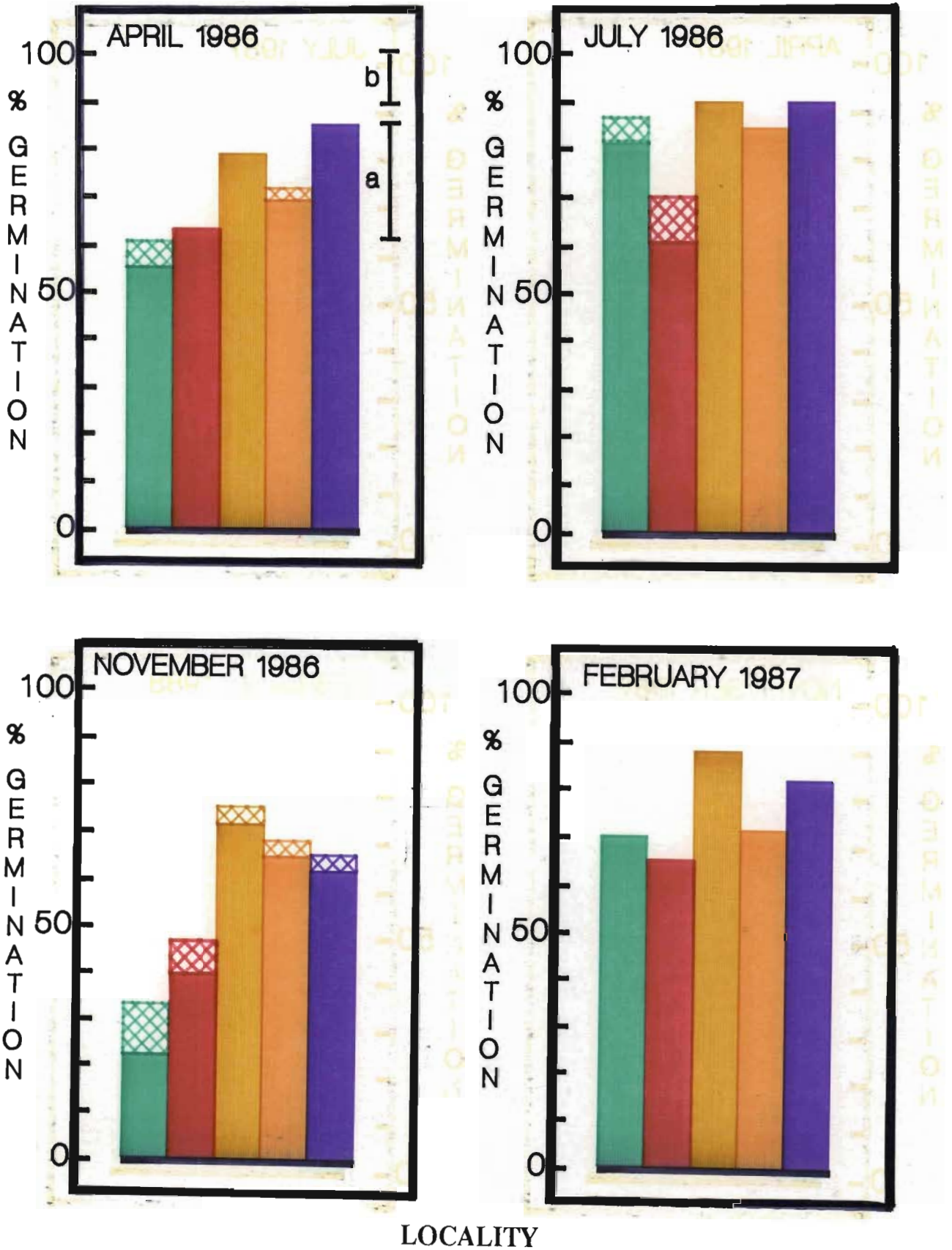
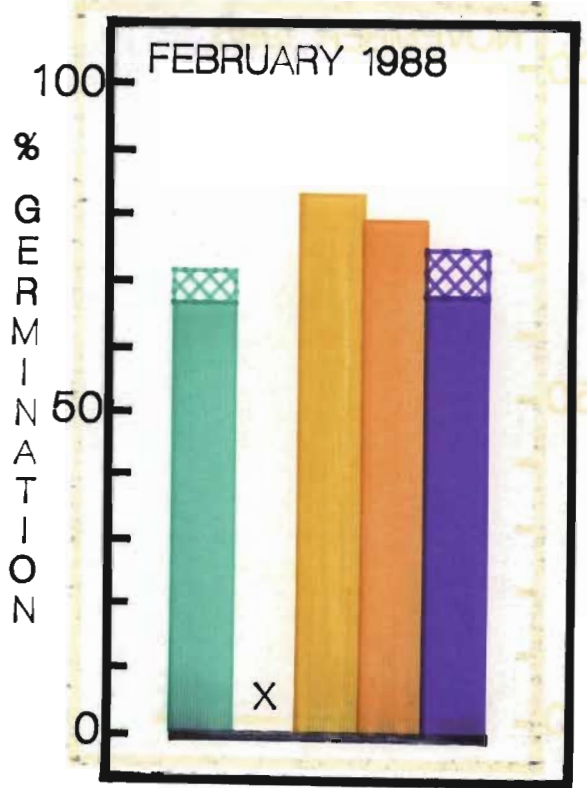
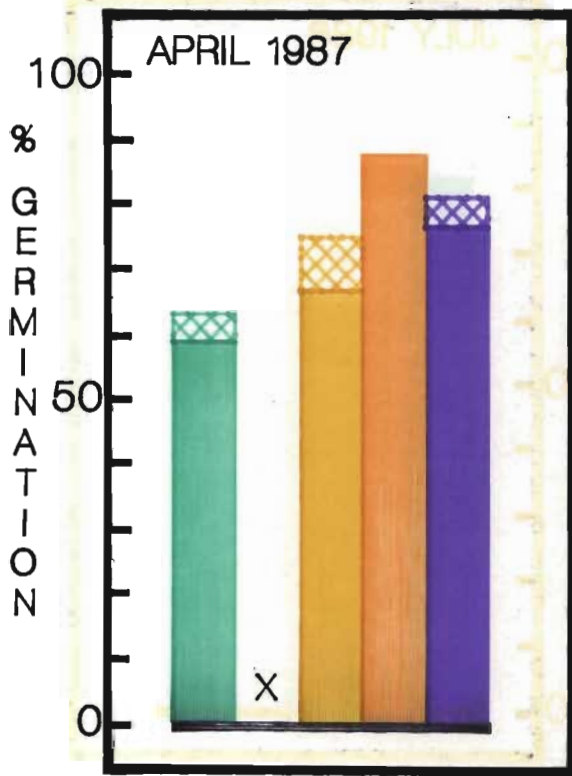


FIGURE 3.8: The germination response of bugweed at 15/30°C in the dark. Seeds were harvested at four different times of the year in 1986/87 and 1987/88 from five localities. Seeds were incubated in 100 mg l⁻¹ GA₃. Shaded portions of each bar represent germination at 15/30°C in the dark after 12 weeks. Hatched portions of each bar represent germination after 12 weeks when seeds were subsequently transferred to the light.

a: LSD p<0.05 shaded portions of bars (locality x season x year).

b: LSD p<0.05 hatched portions of bars (locality x season x year)

1987/88



- Cedar - Natal inland
 - Ashburton - Natal inland
 - Umdoni Park - Natal South Coast
 - Pretoria - Transvaal
 - Groot Constantia - Cape
- X: No seeds produced during this time

1986/87

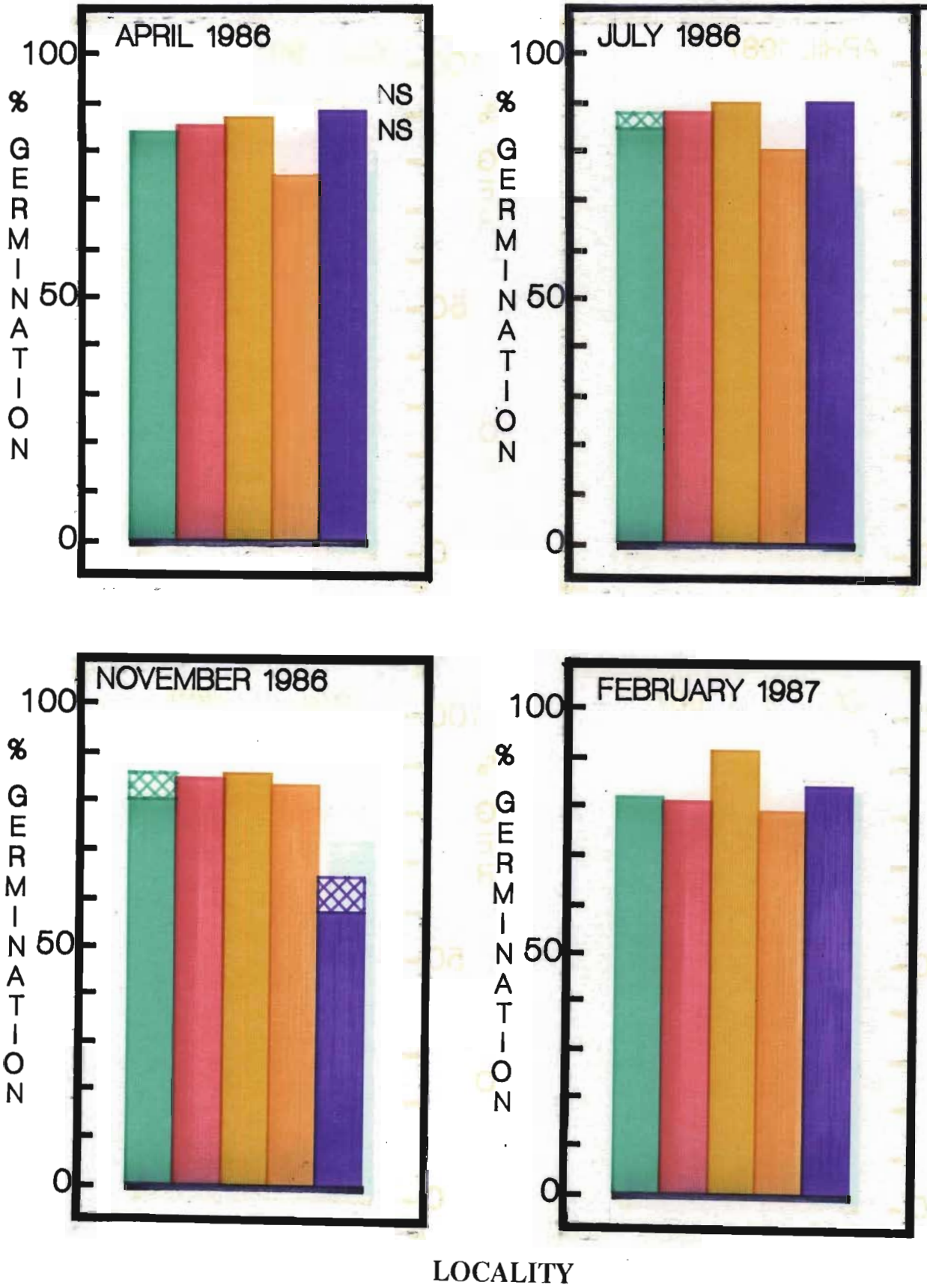
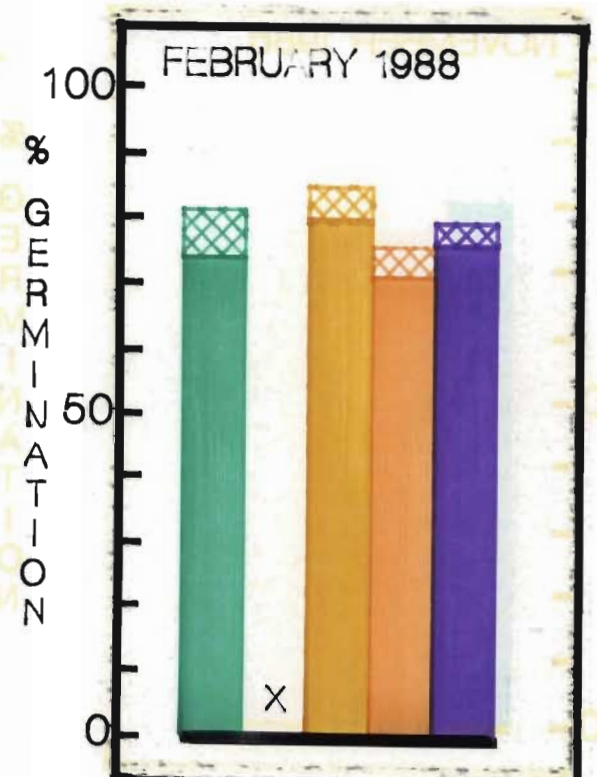
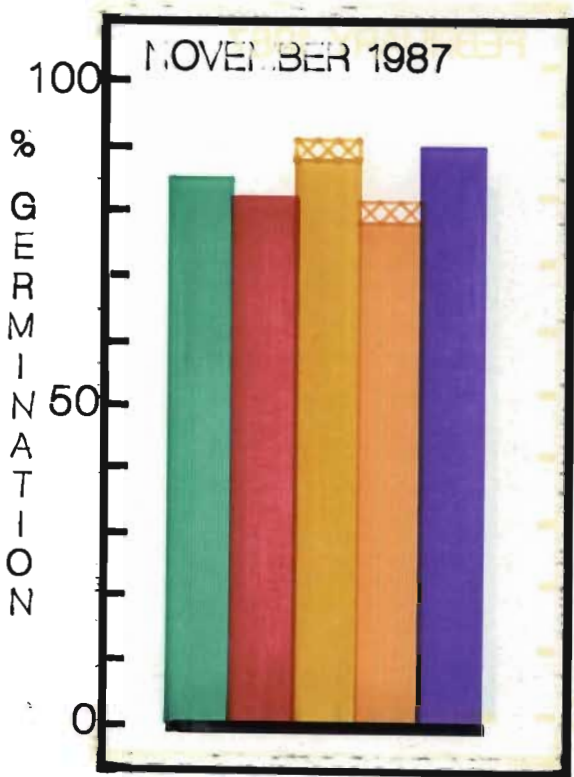
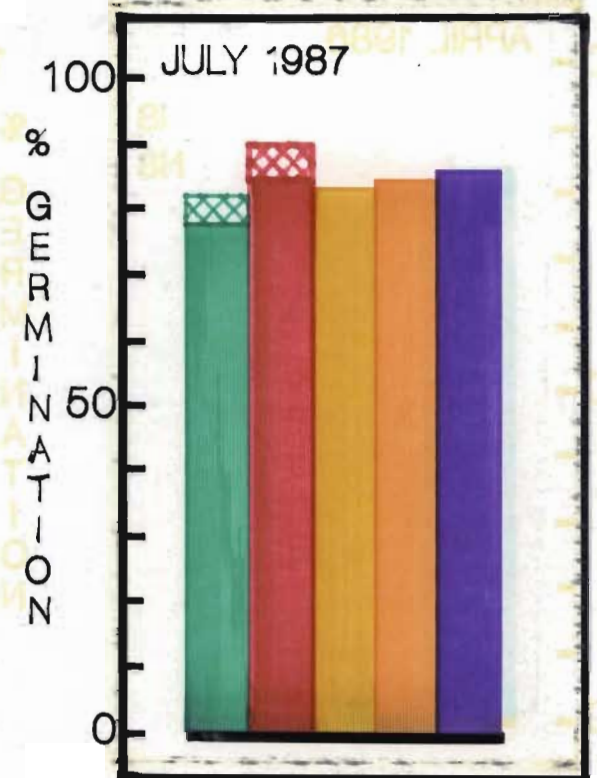
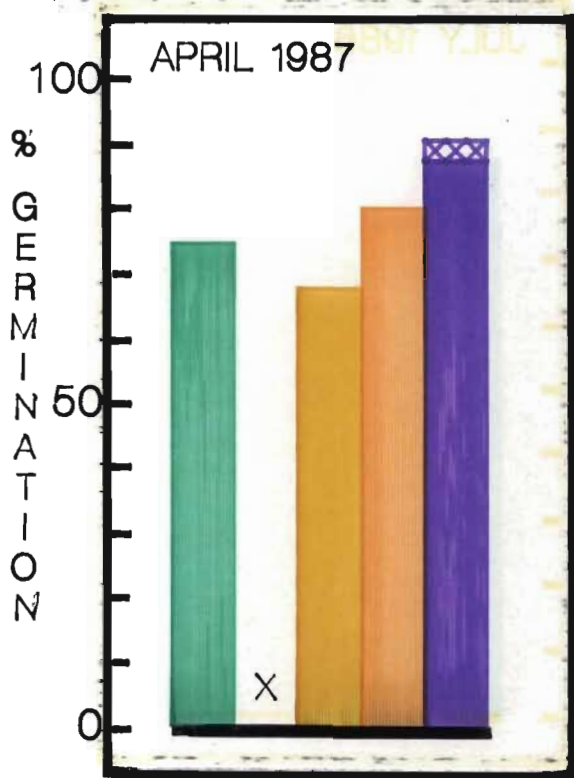


FIGURE 3.9: The germination response of bugweed at 15/30°C in the dark. Seeds were harvested at four different times of the year in 1986/87 and 1987/88 from five localities. Seeds were incubated in 500 mg l⁻¹ GA₃. Shaded portions of each bar represent germination at 15/30°C in the dark after 12 weeks. Hatched portions of each bar represent germination after 12 weeks when seeds were subsequently transferred to the light. NS: no significant differences between (1) shaded portions of bars or (2) hatched portions of bars (locality x season).

1987/88



- Cedar - Natal inland
- Ashburton - Natal inland
- Umdoni Park - Natal South Coast
- Pretoria - Transvaal
- Groot Constantia - Cape
- X: No seeds produced during this time

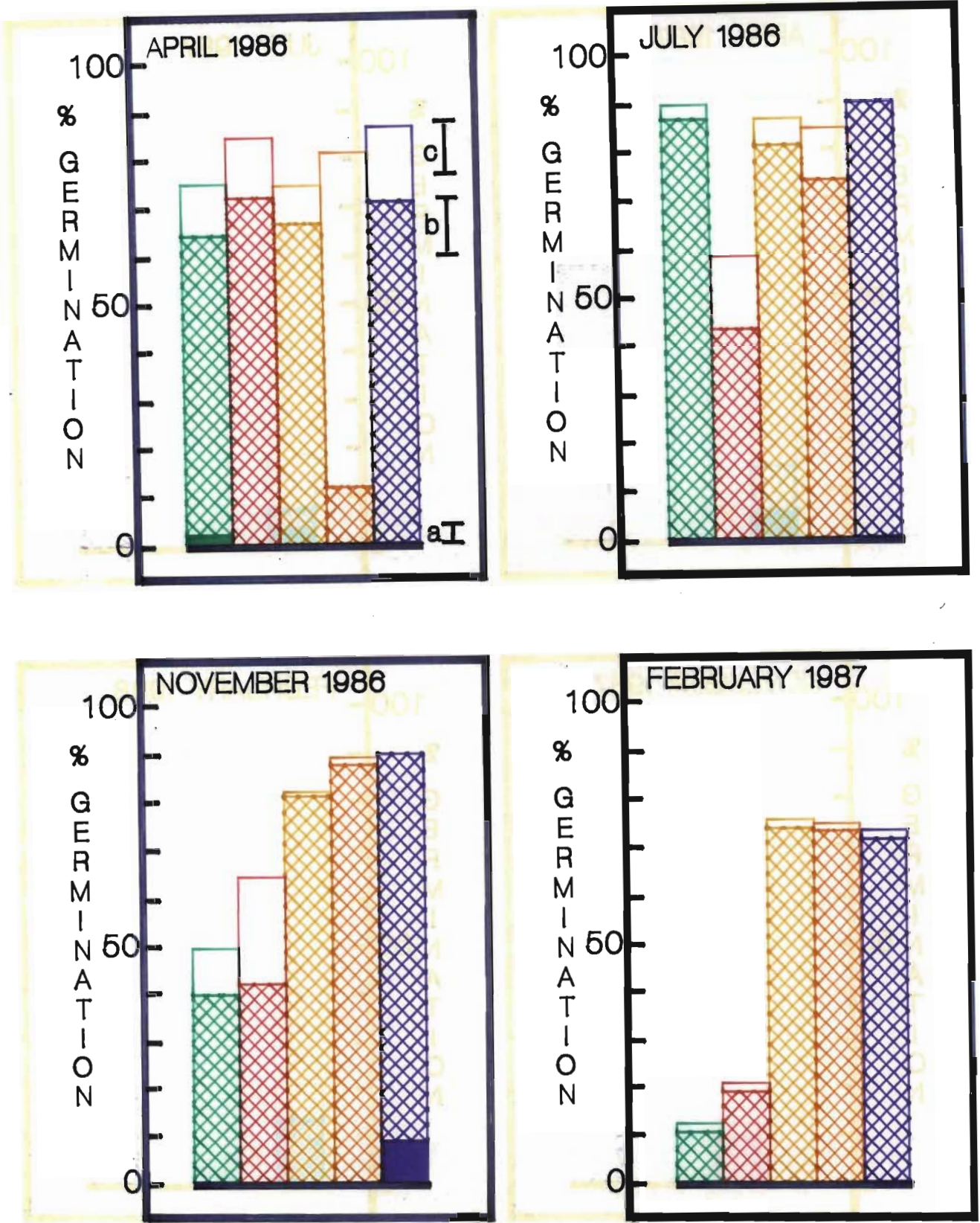
evident, since application of 500 mg l⁻¹ GA₃ promoted higher germination percentages than when seed batches were incubated in 100 mg l⁻¹ GA₃ (Figures 3.8 and 3.9). Implications are that application of low concentrations of GA₃ (or other growth regulators with a similar action) would be below the threshold level required to stimulate high germination percentages of some seed batches dispersed to conditions with fluctuating temperatures in the absence of light (for example, shallow buried seeds). The efficacy of low concentrations of GA₃ in stimulating germination would be influenced by site, season and year of seed dispersal.

(iv) **GERMINATION RESPONSE UNDER CONSTANT TEMPERATURES IN THE DARK**

(a) **Germination in the absence of GA₃**

No significant germination response occurred under these highly unfavourable conditions, except in seed batches harvested from trees at the Cape in November 1986 and April 1987, where a low but significant response was evident (Figure 3.10). Transfer of non-germinated seeds to alternating temperatures in the dark showed an improved germination response in some seed batches but not in others. The improved response was most marked in seeds from April 1986 to February 1987 indicating an end to the conditional dormancy imposed on these seed batches during incubation at constant temperatures (Figure 3.10). A further transfer to the light promoted moderate to high germination responses and was most marked from April 1987 to February 1988 (Figure 3.10). Seeds from the Cape, Transvaal and the Natal South Coast were rarely or never induced to enter secondary dormancy by incubation at constant temperatures in the absence of light. Transfer of seeds to optimum conditions from the two

1986/87



LOCALITY

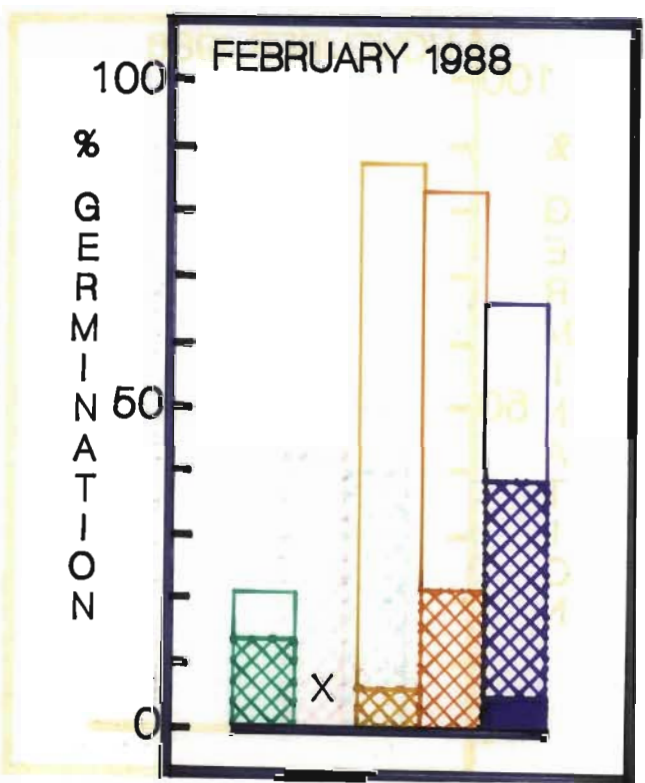
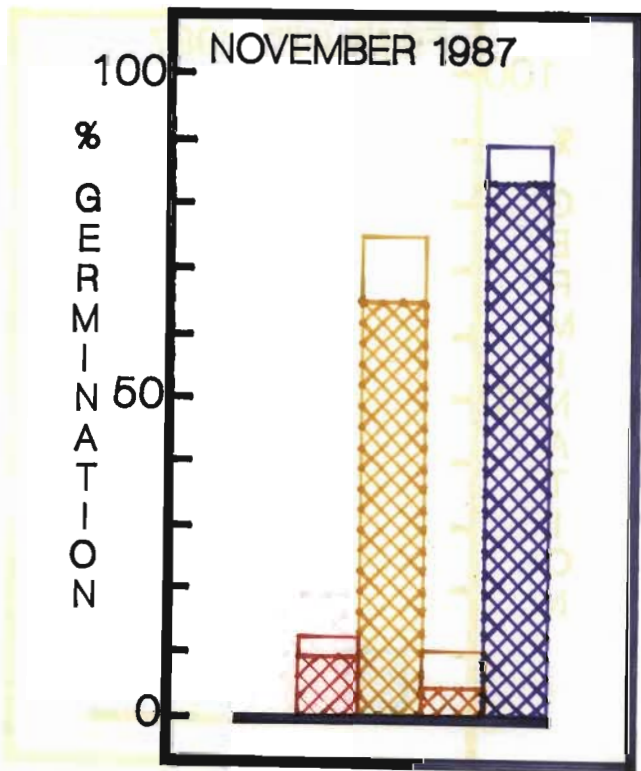
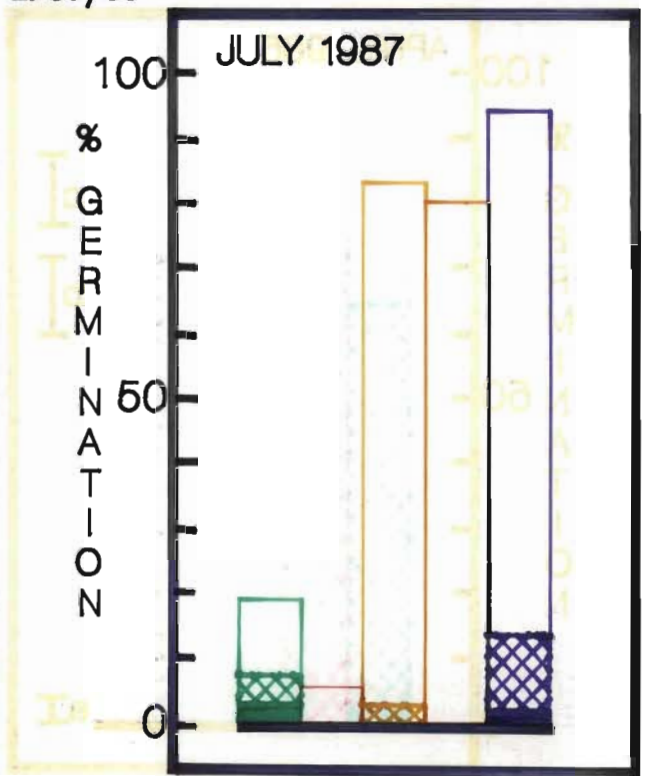
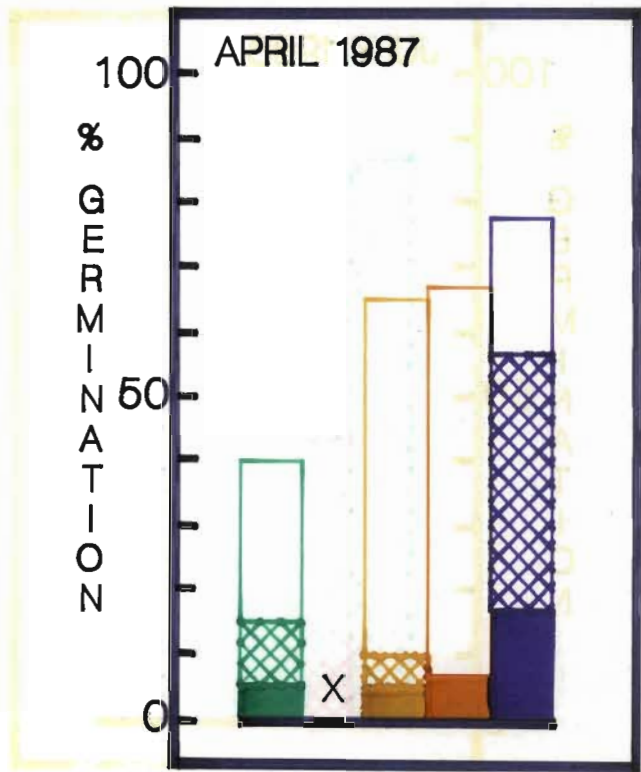
FIGURE 3.10: The germination response of bugweed at 20°C in the dark. Seeds were harvested at four different times of the year in 1986/87 and 1987/88 from five localities. Seeds were incubated in distilled water.

Shaded portions of each bar represent germination at 20°C in the dark after 12 weeks. Hatched portions of each bar represent germination after 12 weeks when seeds were subsequently transferred to alternating 15/30°C in the dark. Unshaded portions represent germination after 12 weeks when seeds were transferred a second time, to the light.

a: LSD $p < 0.05$ shaded portions of bars (locality x season x year).

b: LSD $p < 0.05$ hatched portions of bars (locality x season x year).

1987/88



- Cedara - Natal inland
 - Ashburton - Natal inland
 - Umdoni Park - Natal South Coast
 - Pretoria - Transvaal
 - Groot Constantia - Cape
- X: No seeds produced during this time

Natal inland sites, however, showed a higher frequency in occurrence of secondary dormancy, dependent on season and year of harvest (Figure 3.10).

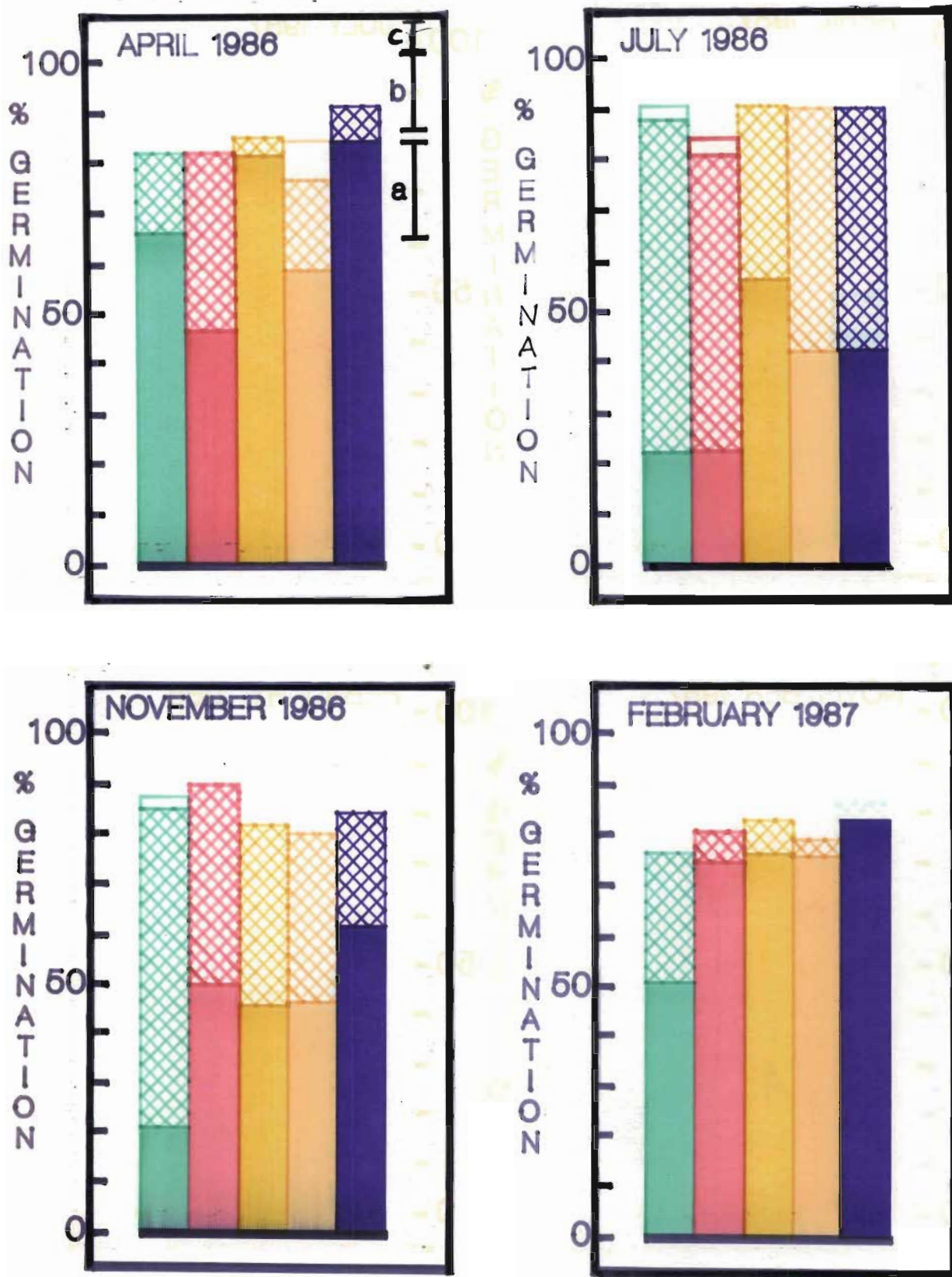
(b) **Germination response with applied GA₃**

Seeds from the South Coast, Transvaal and Cape generally responded moderately well to applications of 100 mg l⁻¹ GA₃ in April and February of both years (Figure 3.11). However, significantly lower responses were seen in July of both years, whereas in November, significantly higher responses were seen in 1987 than in 1986 (Figure 3.11). Seeds harvested from Cedara and Ashburton generally showed similar or significantly lower germination responses than seeds from other sites.

Transfer of most of the seed batches to 15/30°C in the dark promoted germination. Exceptions were seen in seeds from all sites when harvested during July 1987 (Figure 3.11). A further transfer to the light was beneficial to seed harvested from trees at the South Coast, Transvaal and Cape, during this time, promoting approximately two to three times as much seed germination (Figure 3.11). However, there was no further increase in germination of seeds from Cedara and Ashburton during this time (Figure 3.11). *The non-germinated seed from the two Natal inland sites had therefore entered secondary dormancy, which was not prevented by the application of 100 mg l⁻¹ GA₃ during dark incubation.*

A high germination response was seen in the majority of seed batches when 500 mg l⁻¹ GA₃ solutions were applied at 20°C in the dark, although approximately 35 % of seeds harvested at Cedara during February 1988

1986/87



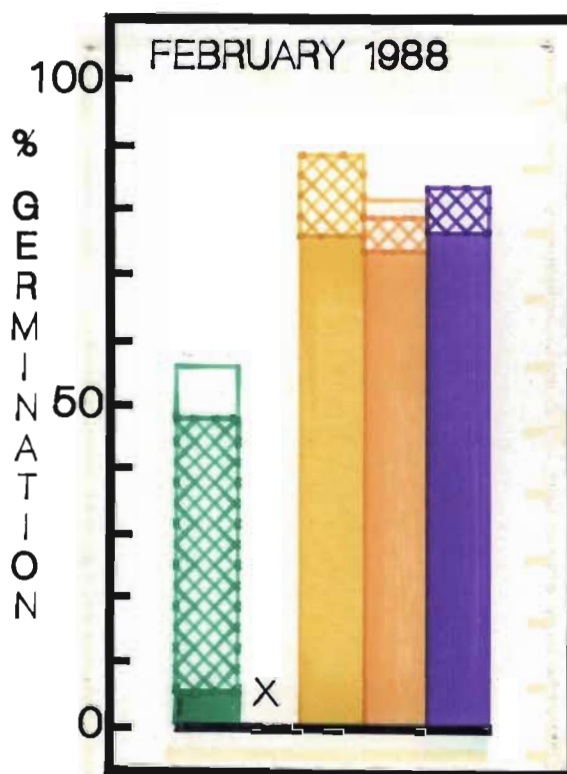
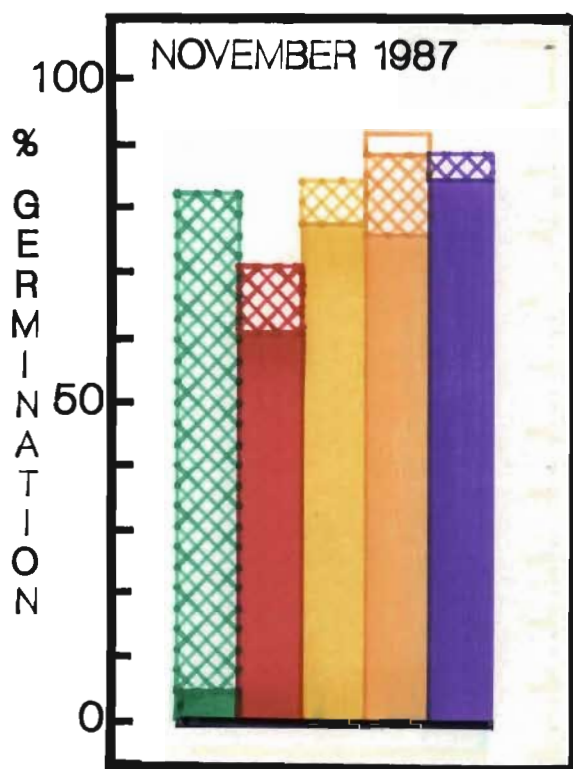
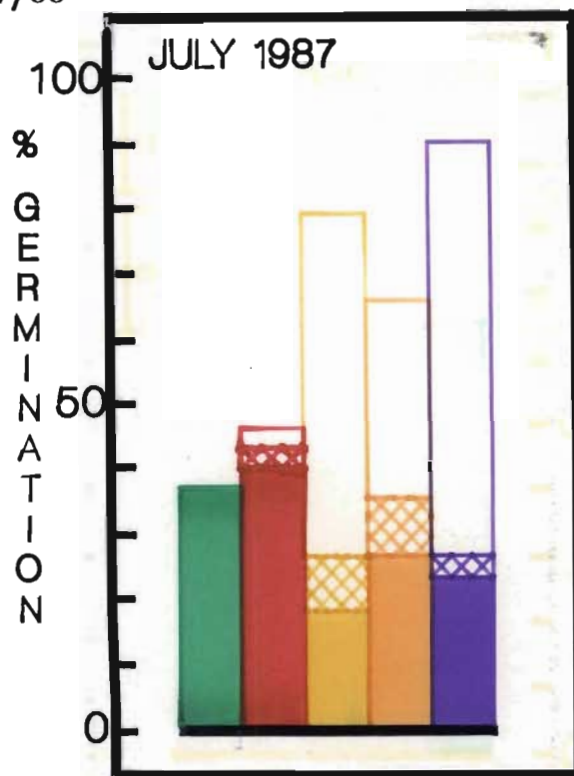
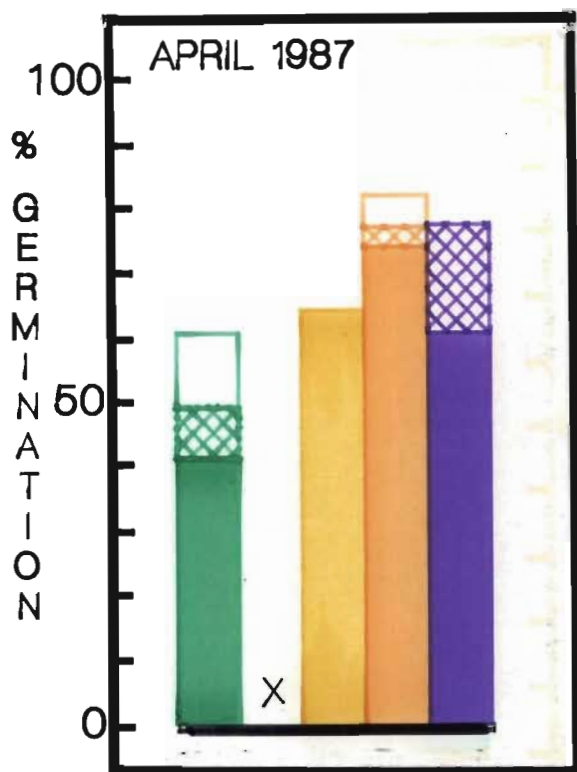
LOCALITY

FIGURE 3.11: The germination response of bugweed at 20°C in the dark. Seeds were harvested at four different times of the year in 1986/87 and 1987/88 from five localities. Seeds were incubated in 100 mg l⁻¹ GA₃ respectively.

Shaded portions of each bar represent germination at 20°C in the dark after 12 weeks. Hatched portions of each bar represent germination after 12 weeks when seeds were subsequently transferred to alternating 15/30°C in the dark. Unshaded portions represent germination after 12 weeks when seeds were transferred a second time, to the light.

a: LSD p < 0.05 shaded portions of bars (locality x season x year).

1987/88



- Cedara - Natal inland
- Ashburton - Natal inland
- Umdoni Park - Natal South Coast
- Pretoria - Transvaal
- Groot Constantia - Cape
- X: No seeds produced during this time

1986/87

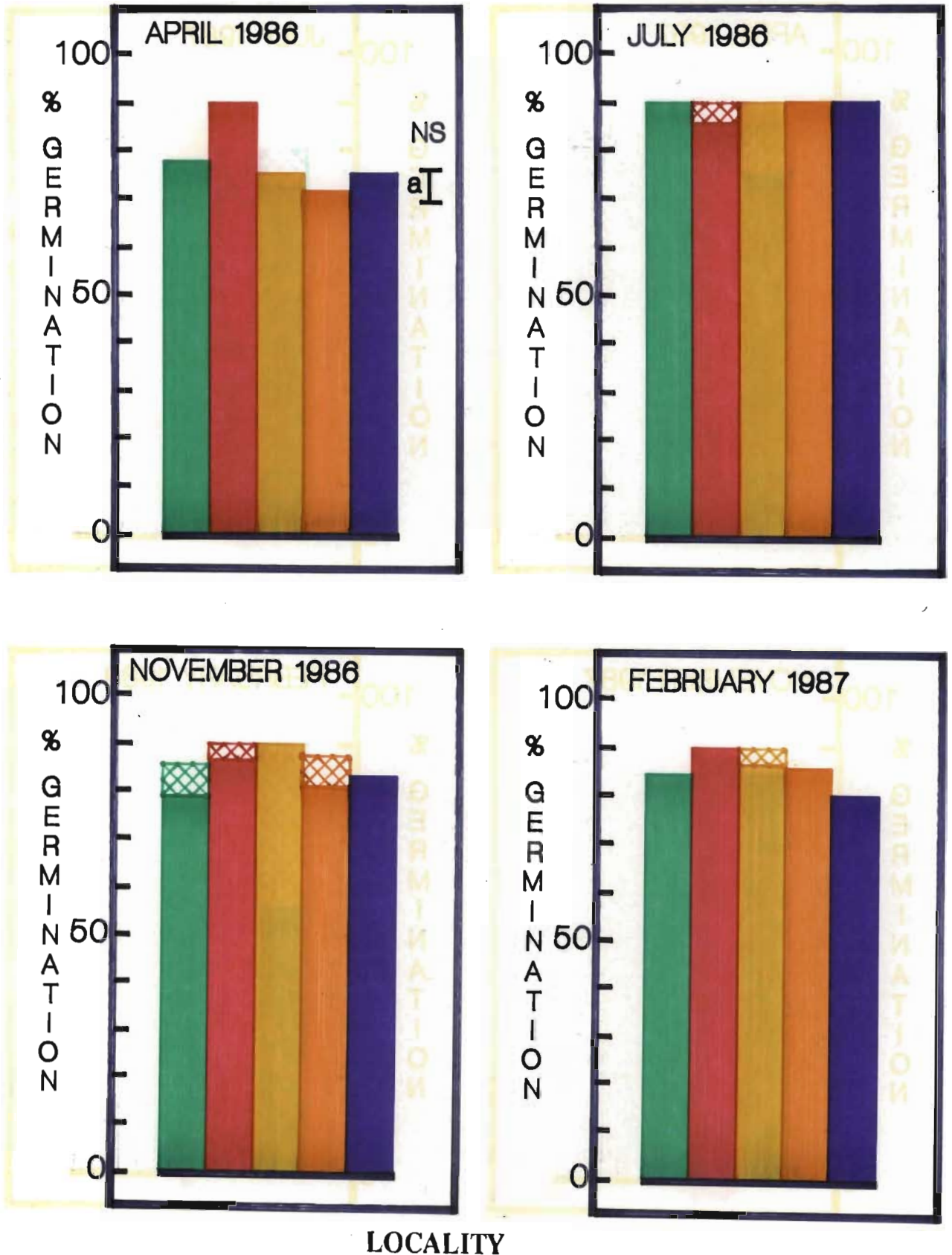


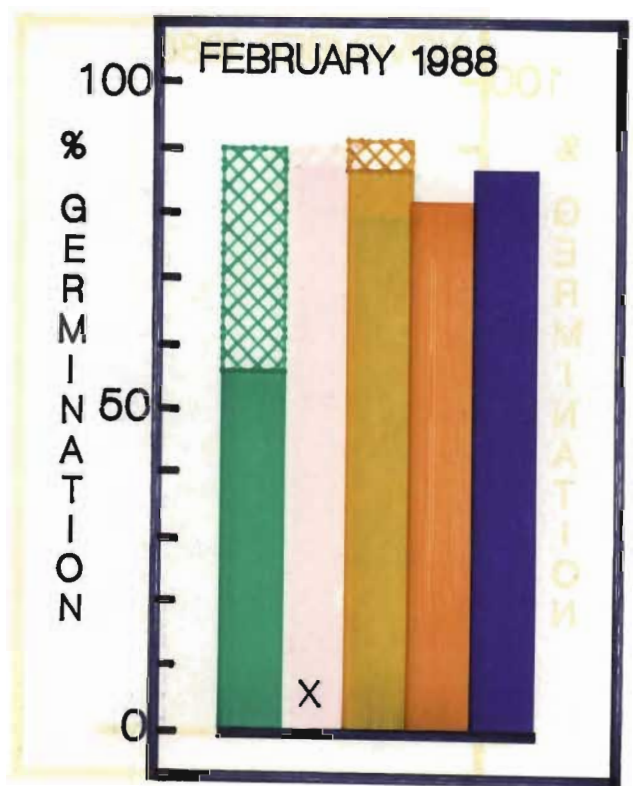
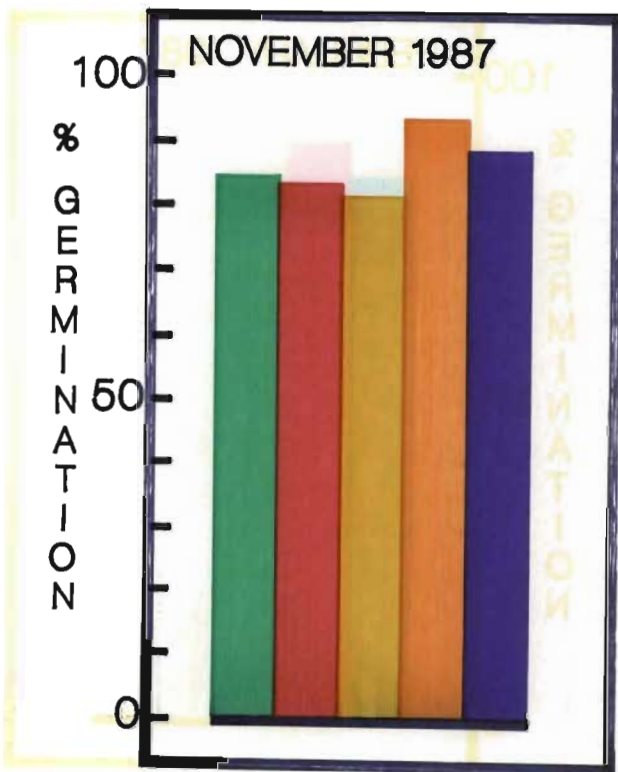
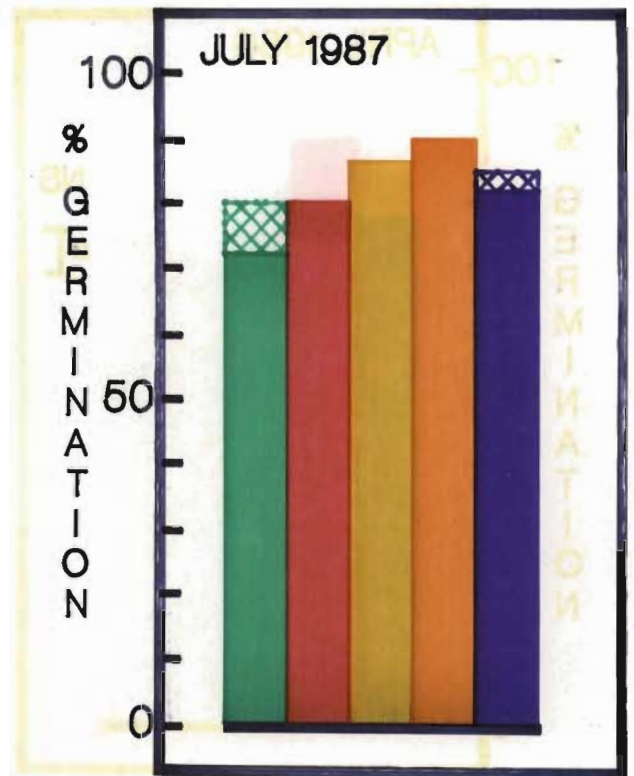
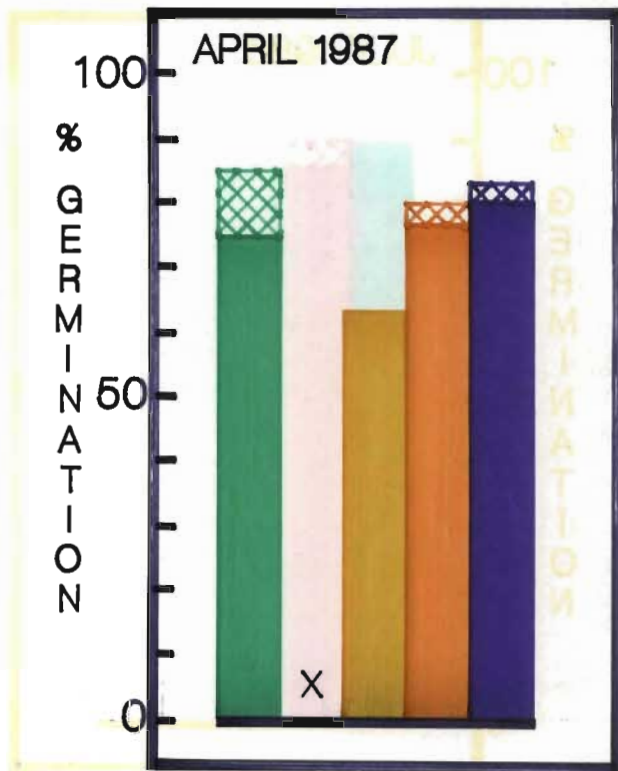
FIGURE 3.12: The germination response of bugweed at 20°C in the dark. Seeds were harvested at four different times of the year in 1986/87 and 1987/88 from five localities. Seeds were incubated in 500 mg l⁻¹ GA₃ respectively.

Shaded portions of each bar represent germination at 20°C in the dark after 12 weeks. Hatched portions of each bar represent germination after 12 weeks when seeds were subsequently transferred to alternating 15/30°C in the dark. No further germination occurred when seeds were transferred a second time, to the light.

a: LSD $p < 0.05$ shaded portions of bars (locality x season x year).

NS: no significant differences between means of hatched portions of bars (locality x season x year).

1987/88



- Cedarra - Natal inland
- Ashburton - Natal inland
- Umdoni Park - Natal South Coast
- Pretoria - Transvaal
- Groot Constantia - Cape
- X: No seeds produced during this time

also required a transfer to 15/30°C in the dark (Figure 3.12). However, this dosage of GA₃ did generally substitute for both light and alternating temperature requirements, and prevented most seed batches from entering secondary dormancy, irrespective of site, season and year of harvest.

From the results it was evident that seed germination is highly variable, and is influenced by the site, season and year of harvest. Seeds from the Cape apparently had the overall highest germination potential (Figures 3.4, 3.7 and 3.9). Seeds from the two Natal inland sites exhibited the lowest overall germination potential. Differences were seen in terms of primary dormancy (low response to optimum conditions); conditional dormancy (low response to unfavourable conditions) and secondary dormancy (low response after transfer to optimum conditions).

GA₃ showed variable efficacy in breaking both primary and conditional dormancy and also in preventing the induction of secondary dormancy by unfavourable incubation conditions. A strong concentration effect was evident and seed batches varied in their response to 100 mg l⁻¹ GA₃. This concentration was sometimes below the threshold level required for high germination percentages at unfavourable conditions. Seed that only responded to the higher concentration (500 mg l⁻¹ GA₃) or required 100 mg l⁻¹ GA₃ plus transfer to optimum conditions for high germination percentages were therefore in a deeper state of conditional dormancy.

D. DISCUSSION

(i) OCCURRENCE OF PRIMARY DORMANCY

The occurrence of primary dormancy was dependent on site, season and year of seed harvest. Seeds from the Natal South Coast, Transvaal and Cape were never encountered in this state, and always showed a high germination response to optimal germination conditions. Seeds from the Natal inland sites, however, had the ability to resist germination under favourable environmental conditions. Approximately half of the seeds harvested during certain times of the year therefore exhibited primary dormancy. Thus should conditions suddenly become unfavourable, for example, an unseasonal drop in temperature, then not all seeds would be destroyed.

Since application of low concentrations (100 mg.l^{-1}) of the growth regulator GA_3 overcame primary dormancy, it is tempting to speculate that endogenous levels of this growth regulator in these seed batches were low and seeds required a supplement of exogenous GA_3 for germination to occur. However, definite statements in this matter could only be made if endogenous growth regulators were extracted and their levels ascertained and compared during these different periods (BRUINSMA, 1980).

(ii) GERMINATION UNDER CONSTANT TEMPERATURES IN THE LIGHT

Seeds from the Natal inland sites always had a very low germination response to these conditions, and seeds from the Natal South Coast and Transvaal rarely showed significant germination responses. Seeds from the

Cape, however, had variable germination responses. Seeds which could germinate under these conditions were not conditionally dormant, and a wider range of environmental conditions would probably result in germination. Seeds from the other sites were generally more specific in their requirements for germination and the presence of alternating temperatures was necessary for germination to occur.

This variation in requirement for alternating temperatures was also reported in seeds of *S. nigrum*. GIVELBERG & HOROWITZ (1984) suggested the conflicting results recorded in the literature for this species were due to differences in localities of harvest. The occurrence of conditional dormancy seen in bugweed seeds was highly variable, depending on site, season and year of harvest. Implications in the field situation are that different populations of dispersed seeds show variation in conditional dormancy which results in *sporadic seedling emergence*. Thus the range of environmental conditions limiting germination is variable. Transfer of most seed batches to alternating temperatures resulted in a high percentage germination, indicating a capacity to germinate once environmental conditions became closer to the optimum.

(iii) **GERMINATION UNDER ALTERNATING TEMPERATURES IN THE DARK**

As occurred with seeds incubated at constant temperatures in the light, the germination response of seeds when incubated at alternating temperatures in the dark was dependent on site, season and year of harvest. Seeds which germinated under these conditions were not conditionally dormant. In the field situation, seeds which have been recently buried and are

positioned near the soil surface, are exposed to diurnal temperature fluctuations but are in the dark. Under these conditions a few seeds would not be conditionally dormant and would germinate immediately. However, most seeds would remain dormant in the soil. Disturbance of the soil exposes seeds to light. This may result in some germination and the percentage is influenced by the site, season and year of seed dispersal. Thus germination would occur sporadically over a long period of time.

(iv) **GERMINATION UNDER CONSTANT TEMPERATURES IN THE DARK**

A very low germination response was observed to the above conditions in all seed batches studied. Transfer of seed batches to alternating temperatures in the dark promoted a high germination response in certain seed batches. This was most marked in seeds harvested in April 1986 to February 1987. Thus these seeds were *in a state of conditional dormancy* during incubation at constant temperatures in the dark and germinated readily once transfer occurred. A further transfer to the light was required before other seed batches could germinate. These seed batches were therefore in a *deeper state of conditional dormancy* than those requiring only a single transfer, to alternating temperatures in the dark; *i.e.* the conditions required for germination in these seed batches were more specific and thus their degree of conditional dormancy was greater than those seed batches capable of responding to a wider range of conditions.

In the field situation deeply-buried seeds, which are subjected to near constant temperatures in the dark, can become disturbed and brought nearer the soil surface, where they are then subjected to alternating

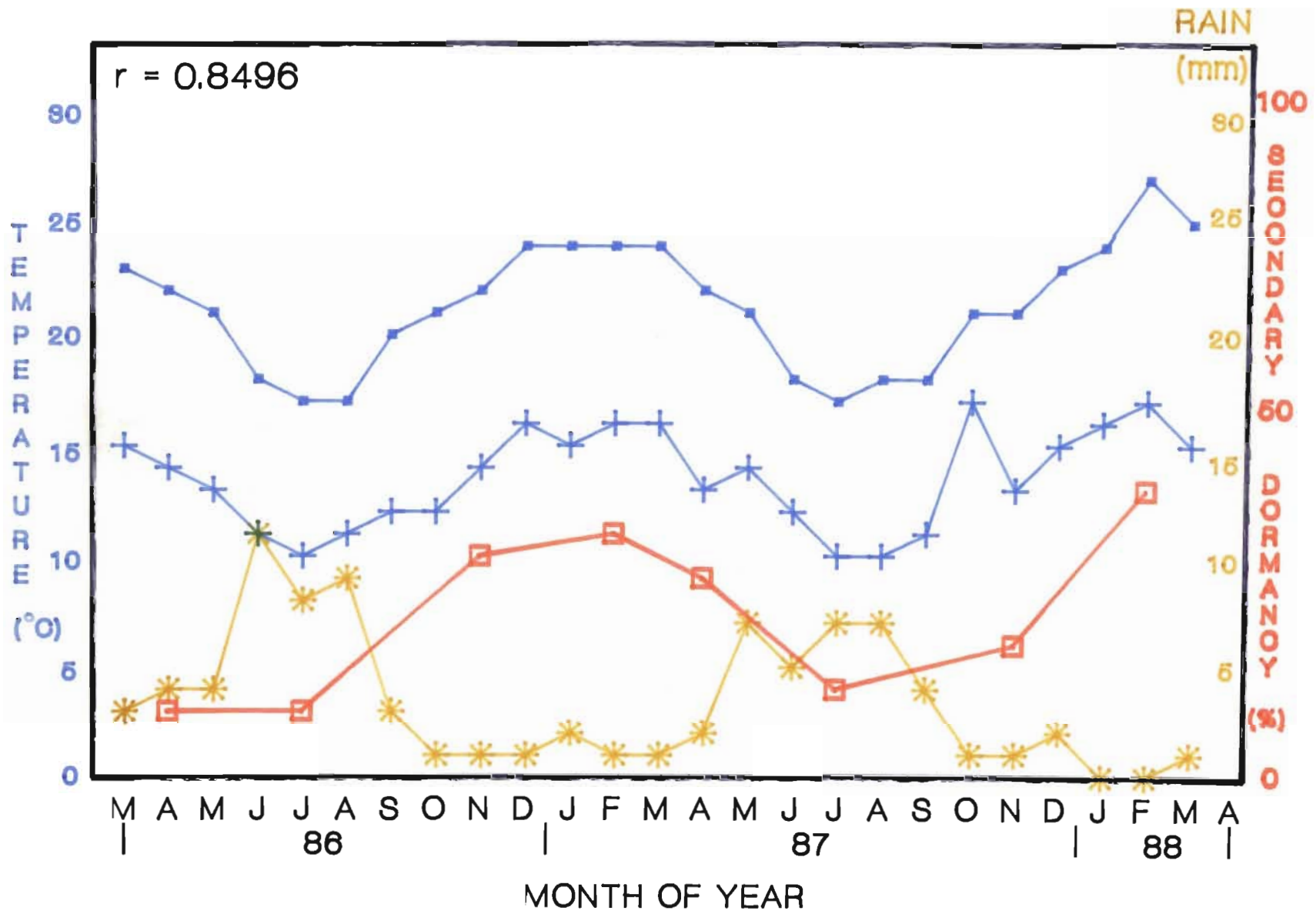
temperatures in the dark, and finally appear above the soil surface, where they will be exposed to the light. However, even under these newly acquired optimum conditions *they will not all germinate*. Their germination potential will be dependent on site, season and year of harvest.

(v) **SECONDARY DORMANCY IN DIFFERENT SEED BATCHES**

The occurrence of secondary dormancy was dependent on site, season and year of harvest as well as the type of initial incubation conditions to which the seeds were subjected. Incubation at constant temperatures in the light only rarely induced secondary dormancy. This occurred only in seed batches harvested from both Natal inland sites during February 1987 and July 1987. Incubation at both constant and alternating temperatures in the dark, however, resulted in a more frequent occurrence of secondary dormancy, and this was especially marked in seed batches from the Natal inland sites. Thus both shallow-buried seeds (subject to alternating temperatures in the dark) and deeply-buried seeds (subject to constant temperatures in the dark) at the Natal inland sites will enter secondary dormancy, and this is influenced by season and year of harvest. *Seeds from these Natal inland regions will thus be far more difficult to control than those from the other regions, since a more sporadic seedling emergence will occur.*

An attempt was then made to see whether the occurrence of secondary dormancy was influenced by prevailing weather conditions. When the climatic pattern at Groot Constantia is considered, it is evident that seasonal temperature fluctuations do not deviate markedly from the mean (Figure 3.13). Thus maximum temperatures have a variation of around 3°C

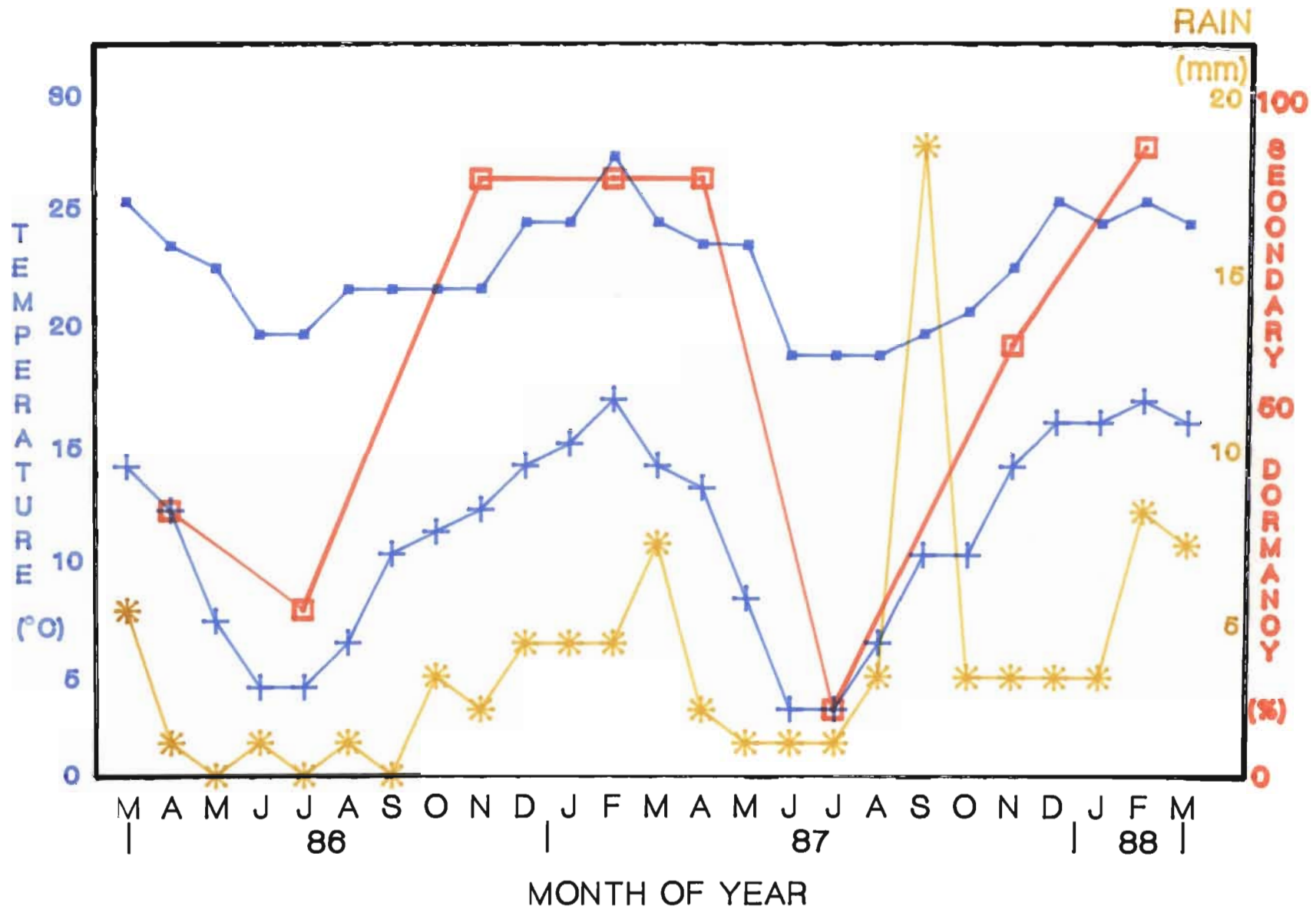
FIGURE 3.13: The relationship between monthly maximum (■) and minimum (+) temperatures and rainfall (☁) at Groot Constantia and percentage secondary dormancy (●) of bugweed. Seeds were initially incubated for 12 weeks at 15/30°C in the dark then subsequently transferred to the light. Non-germinated seeds had entered secondary dormancy.



above or below a mean of 20°C, and minimum temperatures have a variation of around 4°C above or below a mean of 13°C. A winter rainfall pattern occurs in this region (Figure 3.13).

Of all the seed batches, seeds harvested from the Cape (Groot Constantia) overall showed the highest potential for germination under unfavourable conditions, and also the lowest ability to enter secondary dormancy. Freshly-harvested seed batches will lie on the soil surface and may be completely or partially covered by soil, leaf detritus or animal faecal material. These seeds would therefore be subject to alternating temperatures in the dark. Secondary dormancy in Cape seed batches induced by an alternating temperature (15/30°C) in the dark, is therefore considered. The percentage of seeds that exhibited secondary dormancy (following transfer to the light after incubation in the dark) correlated with the annual temperature cycle at the Cape (with a significance of $p < 0.01$) and was apparently inversely related to the annual rainfall cycle (Figure 3.13). During the warmer months, from November to April, when temperatures of 15/30°C are most likely to periodically occur, approximately 35 % of the seeds entered secondary dormancy (Figure 3.13).

Seeds harvested from Cedara overall showed the lowest ability to germinate under unfavourable conditions and the highest ability to enter secondary dormancy. Climatic conditions here are very different from those at Groot Constantia in the Cape, having a summer rainfall (Figure 3.14). The mean maximum temperatures are higher than those at the



Cape, with a variation of 4°C above or below a mean of 24°C, and minimum temperatures are lower than those at the Cape, with a variation of around 6°C above or below a mean of 9°C (Figure 3.14). In spite of these climatic differences, a similar correlation occurred in secondary dormancy to those of seed harvested from the Cape, in terms of relationship to temperature, with a significance of $P < 0.1$. However, a much higher percentage of seeds became secondarily dormant during the summer months (approximately 90 %) than those at the Cape (35 %) and this perhaps reflects a necessity for seeds to escape the onset of the harsher winters of this region (Figure 3.14).

The trigger for these differences in dormancy response seen in seeds harvested from these two sites may be the magnitude of minimum seasonal temperature fluctuation. Large minimum temperature fluctuations at Cedara may be the cause of large fluctuations in secondary dormancy seen in seed batches from this area. In contrast, less sizeable fluctuations around the mean minimum temperature at the Cape are reflected in less sizeable fluctuations in secondary dormancy.

It is therefore evident that temperature but not rainfall pattern may be critical in determining the occurrence of secondary dormancy, since neither a summer nor a winter rainfall pattern apparently influenced the secondary dormancy responses of bugweed seeds.

However, the relationship between temperature and secondary dormancy in seeds from the other three sites was not significant. Here, peaks and

troughs were displaced with respect to temperature fluctuations (results not shown). Nonetheless, peaks of secondary dormancy (induced by initial incubation at 15/30°C in the dark) still *tended* to occur in warmer months of the year. Other climatic factors at these sites probably interact to influence the occurrence of secondary dormancy.

A certain proportion of seeds shed at all sites during the warmer months of the year can therefore be expected to enter secondary dormancy. Implications of this are that germination will be sporadic and that *late emerging seedlings will escape herbicide treatments used for the control of bugweed during its active growth period.*

(vi) **GERMINATION RESPONSE WITH APPLIED GA₃**

The effect of a low concentration of GA₃ on the germination response depended on site, season and year of seed harvest as well as on incubation conditions. Under optimum conditions, the application of 100 mg l⁻¹ GA₃ resulted in a high germination response, which overcame primary dormancy.

At constant temperatures in the light, however, this level of GA₃ was not sufficient to promote a high percentage germination in all seed batches. Either the application of 500 mg l⁻¹ GA₃ was required for maximum germination or a transfer to alternating temperatures was required at low GA₃ concentrations.

The application of 100 mg l⁻¹ GA₃ to seeds incubated at alternating

temperatures in the dark promoted high germination percentages in most seed batches, thereby substituting for a requirement for transfer to the light. However, a concentration effect was evident, since the application of 500 mg l⁻¹ GA₃ improved the germination percentages of certain seed batches. Secondary dormancy was prevented in most seed batches by application of 100 mg l⁻¹ GA₃. However, during November 1986, seeds from the Natal inland sites required the application of 500 mg l⁻¹ GA₃ during incubation in the dark to prevent the induction of secondary dormancy.

Incubation at 20°C in the absence of light inhibited germination. Application of 100 mg l⁻¹ GA₃ during dark incubation at 20°C resulted in variable germination, depending on site, season and year of seed harvest. Generally, this concentration of GA₃ was below the threshold level to substitute for a transfer requirement. A single transfer, to alternating temperatures in the dark, greatly enhanced germination of most low-germinating seed batches, and this was especially marked during April 1986 to February 1987. A further transfer to light enhanced the germination response of other seed batches. However, the occurrence of secondary dormancy was evident in approximately 40 to 60 % of seeds of some batches from the Natal inland sites. The application of 500 mg l⁻¹ GA₃ during incubation in the dark at 20°C was required to prevent these batches entering secondary dormancy.

Implications in the field situation are that the *application of GA₃ (or other growth regulators with a similar action) will always be effective in promoting*

bugweed seed germination even under unfavourable environmental conditions, but with one proviso, the concentration of applied growth regulator must be sufficiently high and must reach all of the seeds in a concentrated form. In practice, this would be highly unlikely to occur, especially when considering control of bugweed seeds lying under a dense mat of pine needles. The highly variable nature of germination responses prevalent in bugweed seeds therefore ensures success of the species, by rendering control options via stimulation of the soil seed bank both impractical and uneconomic.

Seed batches shed from trees in different localities in this study had been subjected during development to very different maternal environmental conditions. In addition to this, it is highly probable that seed batches from different localities were genetically different, although this has not been established. Such genetic and maternal environmental factors will interact in their influence on the germination responses of different seed batches when subsequently incubated in a common environment.

It is very difficult to design experiments to separate the influences of these different factors. For example, PEGTEL (1985) posed a similar question for germination of *Solanum dulcamara* L. seed. Whether "the sort of variability discovered in my experiment has an inherent physiological basis resulting from (long-term) natural selection and/or is due to (short-term) phenotypic acclimatization during development, state of maturity, physiological condition and age of seeds at the moment of sampling"? From his results, PEGTEL (1985) concluded that there was no evidence of major differences in behaviour between two populations of *S. dulcamara*,

but that differences might be masked by environmental influences which acted upon the seed.

It is certainly not within the scope of this study to try and unravel the complexities of these relationships in bugweed seed germination. Rather, this study has served to illustrate that great variability exists, for whatever reason(s), and that this must be considered in explaining why this species has proved so difficult to control.

E. SUMMARY AND CONCLUSIONS

1. The site of plant growth as well as season and year of seed collection influence:
 - (a) The occurrence of primary dormancy.
 - (b) The occurrence of secondary dormancy.
 - (c) The ability to germinate at alternating temperatures in the dark (conditional dormancy).
 - (d) The ability to germinate at constant temperatures in the light (conditional dormancy).
 - (e) The depth of conditional dormancy, since some seed batches require both transfer to alternating temperatures and a further transfer to the light before germination will ensue. Other batches respond to a single transfer to alternating temperatures and do not require a further transfer to the light to elicit germination.
 - (f) The combined effects of a low concentration of GA_3 and of incubation conditions on germination.
 - (g) The combined effects of a low concentration of GA_3 and of incubation conditions in preventing seeds from entering secondary dormancy.
2. Germination ability is highly variable therefore seedling emergence will occur sporadically over a long period of time.
3. Seeds from the two Natal inland sites germinate less readily and

enter secondary dormancy more easily than seed from the other localities. Therefore these seeds will be harder to control, due to delayed emergence and some seeds escaping control measures.

4. The percentage secondary dormancy in seeds from the Cape or Cedara was apparently influenced by seasonal temperature fluctuations. This relationship was not seen clearly at the other sites.
5. Application of GA₃ (or related compounds) at the soil seed bank will prove inadequate as a control measure for bugweed since not all the seed present will be stimulated to germinate.

CHAPTER 4

SEED BURIAL AND GERMINATION

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CHAPTER 4

SEED BURIAL AND GERMINATION

A. INTRODUCTION

(i) VIABILITY

It has been known at least since the nineteenth century, that seeds of some species can remain viable for many years in the soil. FROUD-WILLIAMS (1987) stated that essentially two approaches to the study of seed longevity had been adopted. The first involves long-term burial of seeds in containers, which are subsequently exhumed at various intervals to determine percentage viability (EGLEY & CHANDLER, 1983). The second approach involves exhumation of seeds from soils that have remained undisturbed for long periods (CHANCELLOR, 1986). Indications of considerable longevity of weed seed populations has been obtained from examination of seedbanks beneath pastures of known age. For example, viable weed seeds have been detected beneath soils not disturbed for 58 years (BRENCHLEY, 1918).

FROUD-WILLIAMS (1987) comments, however, that a major limitation of both approaches adopted is that they involve determination of seed lifespan under atypical conditions. Seeds obtained as a result of these techniques are not subjected to normal cultivation practices which may result in exposure to light, increased amplitude of temperature fluctuation on a daily basis, and modification of the gaseous environment. These are

factors which are known to influence dormancy loss and seed germination. These problems have been overcome to some extent by mixing seed at various depths with soil contained in open-ended cylinders and monitoring subsequent seedling emergence (FROUD-WILLIAMSON, 1987). Periodically the soil was disturbed to simulate cultivation, and at the end of a specified period, survival of remaining viable ungerminated seeds was determined. The number of viable seeds was found to decrease exponentially with time, the rates of depletion differing with species (ROBERTS, 1962). Furthermore, depletion rates were higher with an increase in disturbance (ROBERTS & DAWKINS, 1967) and also higher in superficial soil layers than in deeper layers (ROBERTS & FEAST, 1972; KANNANGARA & FIELD, 1985). Even for the most long-lived species the annual depletion rate was usually several per cent.

The trend of increasing seed survival with depth has been observed for a wide range of crop and weed species. Burial of *Kochia scoparia* Schrad. seeds in the top 10 cm of soil (ZORNER, ZIMDAHL & SCHWEIZER, 1984) or *Achillea millefolium* L. seeds in the top 8 cm of soil (KANNANGARA & FIELD, 1985) caused a significantly higher decline in viability than those buried at around 30 cm. Similarly, seeds nearer the surface lost viability more quickly in *Alopecurus geniculatus* L. (SYNNES, 1984), *Eleusine indica* Gaertn. and *Avena fatua* L. (SCHWERZEL, THOMAS & OOSTERMAN, 1978/9). HORNG & LEU (1978) reported that nine out of ten weed species investigated had higher mortality on the soil surface. THOMAS, BANTING & BOWES (1986) found that whereas only 1 % of *Setaria viridis* Beauv. seeds sown on the soil surface were

viable after six years, buried seeds remained viable for up to 17 years.

In contrast, BOUHACHE & TANJI (1985) found little difference in seed viability of *Solanum elaeagnifolium* Cav. seeds with an increase in burial depth of up to 60 cm below the soil surface. CHEAM (1987) found a locality effect; *Bromus diandrus* Roth. seed buried at a cool site with high rainfall showed increased viability with burial depth, whereas at warmer sites with lower rainfall there was an inverse relationship between viability and burial depth. Certain populations of *Panicum miliaceum* L. showed greatest seed survival at the soil surface (COLOSI, CAVERS & BOUGH, 1988) as did seeds of *Bromus diandrus* which had entered enforced dormancy on the soil surface. The latter survived for six months whereas seeds buried at 5 or 15 cm were not dormant but germinated or lost viability within one month (HARRADINE, 1986).

Measurement of viability has been variable in studies of this type. ROBERTS (1981) stated "for many purposes, assessment of the apparently viable seeds may be adequate". He defined "apparently viable seeds" as those "which appear to be intact and which resist gentle pressure ...". However, COLOSI, CAVERS & BOUGH (1988) found wide discrepancies between the percentage of apparently viable seeds and the percentage viable seeds determined by germination and tetrazolium tests for *Panicum miliaceum*. For this species, it was concluded that while the two measures of seed mortality are correlated, the estimate of mortality based on apparent viability would give misleadingly low values for seed survival. Thus many apparently sound seeds were dead. It is therefore clear that

the best assessment of viability for bugweed seeds will be achieved by combining the flotation method¹ with germination tests. This has been done in the present study.

Although the size of the seedbank may be considerable, it has been found that comparatively few seeds contribute to annual seedling recruitment (ROBERTS & FEAST, 1973a,b). This may be attributed to post-germination mortality of seedlings in the soil. A similar conclusion was reached by SCHAFER & CHILCOTE (1970), who found 85% pre-emergence mortality of germinated *Lolium perenne* L. seeds.

In summary, FROUD-WILLIAMS (1987) has noted that the size of a seed population on a soil surface for a given area at a specific time depends on four factors: the rate of recruitment from seed rain; the rate of seed emigration from, and immigration into the soil; the rate of germination; and the rate of loss through seed mortality and predation. Consequently any discussion of the dynamics of seed populations must take cognisance of these parameters.

(ii) GERMINATION AND CYCLES OF DORMANCY

Retention of seed viability is not the only effect that soil storage has on seeds. Germination requirements may also change following burial. For example, winter burial of *Aethusa cynapium* L. mericarps increased both percentage germination and the temperature range over which subsequent

¹This method separates floating and sinking fractions of seed in water. "Apparently viable" bugweed seeds sink in water, whereas "apparently dead" seeds, which do not resist gentle pressure, float.

germination took place (ROBERTS & BODDRELL, 1985). Germination after exhumation may increase with duration of burial, as is the case for *Matricaria perforata* Mérat (LONCHAMP, CHADOEUF-HANNEL & BARRALIS, 1984); or in some species, with increased burial depth, as was seen in *Panicum dichotomiflorum* Michx. var. *geniculatum* (ALEX, 1980), *Chenopodium album* L. and *Echinochloa crus-galli* Beauv. var. *praticola* (WATANABE 1981), but not for *Thlaspi arvense* L., where storage both on the soil surface or in the soil led to an equal decrease in seed dormancy (HUME, 1984).

CHADOEUF-HANNEL, MAGRIE, LONCHAMP & BARRALIS (1980) reported that for 15 weed species, burial resulted in an increase in germination, with a broader optimum temperature range, and loss of photosensitivity as reflected by an ability to germinate in the dark. The effect of locality on subsequent germination was reported by BURNSIDE, FENSTER, EVETTS & MUMM (1981), for seeds of 12 economic weed species buried at two sites. For *Amaranthus retroflexus* L., the germination response of seed buried in east Nebraska was higher than that of seed buried in west Nebraska. However, for *Abutilon theophrasti* Medic., the reverse was true. Germination of most of the other species in this study was not affected by locality. Thus site of burial affects the germination potential of some species.

At this point, the dormancy terminology used in Chapter 2 will be re-stated to prevent any possible confusion. Seeds that are dormant at the time of release from the parent plant are in a state of *primary dormancy*

(KARSSSEN, 1980/81a; BEWLEY & BLACK, 1982). In this state, they have little or no germination even when conditions normally favourable for germination occur (BASKIN & BASKIN 1984b). *Non-dormant* seeds germinate to high percentages over a wide range of temperatures (BASKIN & BASKIN 1984b) *i.e.* they do not exhibit primary dormancy.

Seeds in the soil may cycle between dormancy and non-dormancy; germination or continued dormancy is dependent on soil environmental conditions (EGLEY, 1986). Primary dormancy may be alleviated when conditions in the soil environment bring about changes in seed physiology which result in germination over a wide range of conditions.

Alternatively, seeds may lose their primary dormancy, and because of unfavourable environmental conditions, fail to germinate. Germination is thus inhibited. These seeds may germinate after the unfavourable conditions are removed. These seeds will only germinate over a narrow range of temperatures and are therefore considered to be different from non-dormant seeds. This is described as *conditional dormancy* (BASKIN & BASKIN, 1984b). If the inhibiting conditions are not removed by a critical time (EGLEY, 1986), the seeds may pass from this state of conditional dormancy into *secondary dormancy*. Alternatively, seeds may pass straight from primary dormancy into secondary dormancy. Seeds with secondary dormancy have a requirement for another set of dormancy-breaking conditions before germination can occur (EGLEY, 1986). Seeds may break off from the cycle and germinate rapidly, or else continue through the cycle several times and thus exhibit delayed emergence.

Growth regulators may substitute for these environmental conditions and can manipulate or modify this cycle. Thus application of GA₃, for example, may break primary or conditional dormancy or prevent primary or conditionally dormant seeds entering secondary dormancy.

Cyclic changes in dormancy, have been shown for seeds of *Lamium purpureum* L. (BASKIN & BASKIN 1984a) and for *Aethusa cynapium* (ROBERTS & BODDRELL, 1985). Burial of *Aethusa cynapium* during winter increased germination and the temperature range over which it took place. In late spring the range narrowed, widening again in autumn (ROBERTS & BODDRELL, 1985). The cyclic annual pattern of germination may be related to soil temperature, moisture and burial time, as in the case of seeds of *Aethusa cynapium*, *Alopecurus myosuroides* Huds. and *Euphorbia exigua* L. (LONCHAMP, CHADOEUF-HANNEL & BARRALIS, 1984).

Other species which exhibited cyclic dormancy patterns in relation to burial include *Panicum capillare* L., (BASKIN & BASKIN, 1986) and *Solanum sarrachoides* Sendt. (ROBERTS & BODDRELL, 1983). Annual cycles of dormancy as a result of burial may also be related to light, where seeds may pass from full dormancy (light-irresponsiveness) to having a requirement for light, and back again to full dormancy (TAYLORSON 1970, 1972). Cyclic changes may also occur in secondary dormancy under field conditions. This has been observed in seeds of many annual wild species, and follows a seasonal pattern (COURTNEY, 1968;

TAYLORSON 1970; STOLLER & WAX 1974; WILLEMSEN, 1975; ROBERTS & LOCKETT, 1978a; KARSSSEN, 1980/81b).

(iii) **THE INFLUENCE OF THE SOIL ENVIRONMENT ON GERMINATION**

Burial of seeds is a natural way of inhibiting germination (KARSSSEN 1980/1981b). Burial may enforce dormancy, for example in *Poa annua* L. and *P. trivialis* L. (HOWE & CHANCELLOR, 1983) and *Veronica hederifolia* St. Lag. (LAUER 1953) but not in *Festuca rubra* L. and *Lolium perenne* L. (HOWE & CHANCELLOR, 1983). A factor involved in the inhibition of germination may be an unfavourable temperature. For instance, seeds of *Veronica hederifolia*, which have a low temperature requirement, will not germinate in summer because ambient soil temperatures are above the temperature limits for germination (LAUER 1953).

Another factor may be the absence of light (GRANSTRÖM, 1987). Less than 1 % of incident light penetrates further than 2.2 mm in clay, loam or sand (WOOLLEY & STOLLER, 1978). However, light-independent seeds, capable of germination in the dark, may be inhibited during burial (HOLM 1972; FRANKLAND 1977) perhaps because of decreased oxygen or increased carbon dioxide tensions (CROCKER 1948; HARPER 1957). Increased carbon dioxide levels of up to 2 or 4 % inhibited germination of *Capsella bursa-pastoris* (L.) Medic. and *Senecio vulgaris* L. (POPAY & ROBERTS 1970). Although carbon dioxide levels in soil are rarely greater than 1 %, and oxygen levels usually around 19 % (ROBERTS 1972), the soil water content may markedly influence oxygen availability to seeds.

Increased soil moisture stimulated development of secondary dormancy in *Sisymbrium officinale* Scop., an effect attributed to interference with the availability of oxygen (KARSSSEN, 1980/81a). Under anaerobic conditions seeds are known to produce metabolites such as ethanol, acetaldehyde and acetone, which inhibit germination (HOLM, 1972). Allelopathic substances such as phenolic acids, secreted by roots of early invaders and second year perennials may also inhibit germination of first year annuals (JACKSON & WILLIAMSON 1976).

The most commonly reported factor influencing germination in soil appears to be that of alternating temperatures. From an ecological viewpoint the response to alternating temperatures is generally regarded as an adaptation for gap-detection and depth-sensing (THOMPSON & GRIME, 1983). Germination may be delayed due to nearly constant temperatures if the seeds are deeply buried in the soil or if the soil is covered by vegetation. Germination may be enhanced when the seeds are brought near the surface or when the plant cover is removed. A stimulation of seed germination in darkness by diurnal temperature fluctuations generally occurs in species with relatively large seeds. Other species which form a persistent seed bank require light in addition to fluctuating temperatures. Species with very small seeds and/or from wet habitats belong in this group (THOMPSON & GRIME, 1983).

The response to alternating temperatures also functions as a season-sensing mechanism in *Rumex obtusifolius* L. In the spring the soil is rapidly heated and the seeds near the surface are subjected to large temperature

fluctuations and germinate. Temperatures gradually decline in autumn and diurnal fluctuations decrease, which inhibits germination (VAN ASSCHE & VANLERBERGHE, 1989).

In seeds of *Sisymbrium officinale*, the effect of temperature depended on nitrate levels in the soil. Buried seeds did not lose dormancy during low winter temperatures when the nitrate content of the soil was very low but this dormancy was overcome when nitrate was added resulting in a rapid increase in germination (KARSSSEN, 1980/81b).

The influence of temperature on germination of many *Solanum* species is marked. Thus, it is reasonable to assume that this is true in the case of buried *S. mauritianum* seeds. Extensive variations have been noted in soil temperature patterns. At shallow depths temperature variation is largely due to annual differences in solar radiation, air temperature and soil water content (QUELLET & DES JARDINS, 1975). Soil and air temperature follow similar trends and are both dependent on solar radiation (QUELLET, 1972).

Because the amount of radiation reaching the soil surface fluctuates during a 24 hour period, so does the soil surface temperature. Typical soil temperature variations at different depths on a cloudless day have been reported by OKE (1978) (Figure 4.1). The near surface temperature variation is wave-like and similar to that at the soil surface. At greater depths the amplitude decreases and there is a lag between the times of maximum and minimum temperature. Because of this time lag, the soil

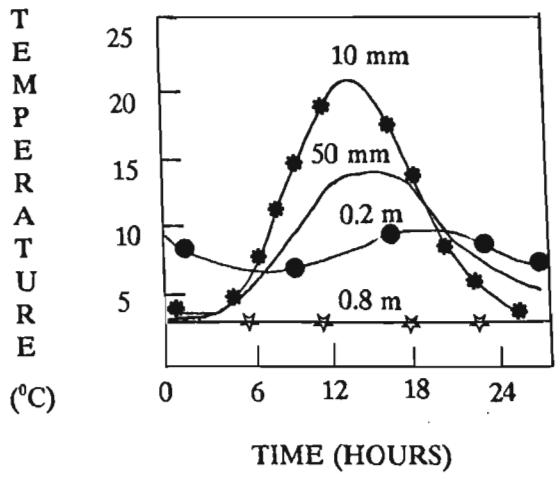


FIGURE 4.1: Generalized pattern of soil temperatures at different depths over a daily interval (OKE, 1978).

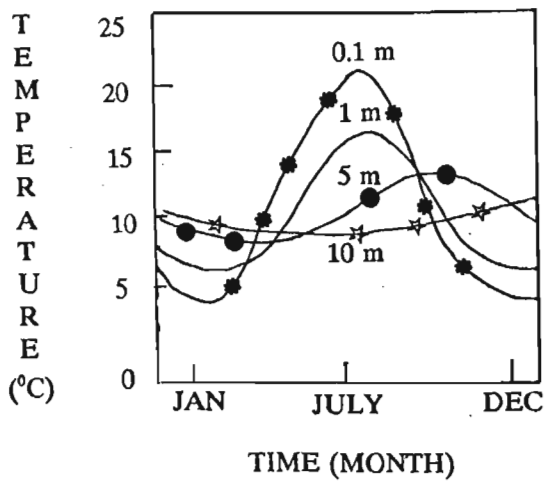


FIGURE 4.2: Generalized pattern of soil temperatures in the Northern Hemisphere at different depths over a year (OKE, 1978).

may at any given time, be cooling in its upper layers but warming at only a short distance beneath, and *vice versa* (OKE, 1978). Clouds affect the diurnal soil temperature pattern. With overcast skies, absolute temperatures are lower by day but warmer at night and the wave amplitude is smaller at shallow depths.

The annual soil temperature regime also follows a wave-like pattern (Figure 4.2). During the warm season, soil temperatures decrease with depth and the associated downward heat flux builds up the heat store in the soil. In the cold season the gradient is reversed and the heat store is gradually depleted.

Freshly-produced seeds are conditionally dormant, and this is influenced by time of year and place of harvest (Chapter 3). In order for any control programme to be successful for bugweed, knowledge of the effect of burial on conditional dormancy should also be established. In this Chapter, the results of experiments to determine these requirements are reported.

B. MATERIALS AND METHODS

(i) SITE DESCRIPTION

The three sites used for seed burial are shown in Figure 3.1. Umdoni Park is a conservation trust situated on the Natal South Coast. Vegetation is of a typical coastal type (ACOCKS, 1988). Common invader species are *Lantana camara* L., and *Chromolena odorata* (L.) R.M. KING & H. ROBINSON. The presence of *Solanum mauritianum* is, however, not common. Seeds were buried under grass at Umdoni Park, on level ground with sandy soil.

Ukulinga is a research station situated on the outskirts of Pietermaritzburg, near Ashburton. The surrounding vegetation type is thornveld (ACOCKS, 1988). Bugweed seldom occurs as an invader species in this type of vegetation, since conditions are considered too dry. A typical invader species is *Acacia karroo* Hayne. A shallow layer of soil rests on shale, which retains water, resulting in poor drainage. Seeds were buried under grass at this site.

Cedara is a research station concerned with both Agriculture and Forestry. Bugweed is considered a prime invader species in the pine plantations of this region. Seeds were buried under grass at this site.

(ii) **BURIAL TECHNIQUES**

(a) **Experiment 1**

Seeds were collected from Cedara, Ukulinga and Umdoni Park in July 1986, cleaned and sorted. Sinking seeds which were "apparently viable" (ROBERTS, 1981) were used for all experiments. Seeds were contained in terylene bags in batches of 400. Bags were buried at 0, 4 and 15 cm depths at the site of collection. To avoid any possibility of the seeds being subjected to a light stimulus seeds were exhumed during cloudy, moonless nights at 4, 8, 12, 16, 20 and 24 months after burial, and transported to the laboratory in light-tight containers. All further manipulations of seed occurred under green safelight conditions.

(b) **Experiment 2**

Seeds from Cedara were harvested in February 1987, cleaned then placed into terylene bags and buried at 0, 4 and 15 cm at Cedara, Ukulinga or Umdoni Park. At 4, 8, 12 and 16 months after burial seeds were exhumed and manipulated under the conditions described above.

(c) **Experiment 3**

Seeds harvested during July 1987 were buried at Cedara, at depths of 0, 4 and 15 cm. At 4, 8 and 12 months after burial these seeds were manipulated as described above. Results were compared with those of seeds harvested from Cedara during July 1986 (Experiment 1) and February 1987 (Experiment 2).

(iii) **INCUBATION CONDITIONS**

As indicated in Chapter 2, incubation under an alternating temperature of 15/30°C in the dark, or under a constant temperature of 20°C in either the light or the dark, inhibited seed germination. Germination was stimulated by the application of 500 mg l⁻¹ GA₃ or alternating temperatures of 15/30°C in the light. Transfer of seeds from unfavourable to optimum conditions frequently increased the germination of seeds.

Using this information, the changed germination potential of seeds following burial was ascertained by incubation under laboratory conditions of constant 20°C or alternating 15/30°C, either in the light or in the dark. Light was not continuous, either at constant 20°C or alternating 15/30°C, but was cycled for 14 hours each day, coinciding with the period of 30°C in the alternating temperature incubator. Seeds that sank in water were either incubated in the presence of distilled water or 500 mg l⁻¹ GA₃.

Since the majority of seed batches gave a high percentage germination following the application of 500 mg l⁻¹ GA₃, only the results for buried seeds incubated in distilled water are presented. Reference is made to the GA₃ results where necessary. After 12 weeks incubation under unfavourable conditions, all non-germinated seeds were transferred to optimum conditions (15/30°C in the light) to test for the development of secondary dormancy. Floating seeds were tested for viability by incubation at alternating 15/30°C in the light in the presence of 500 mg l⁻¹ GA₃.

(iv) **MEASUREMENT OF SOIL TEMPERATURE**

To record the temperature fluctuations for the burial experiments, a continuous recorder with mercury probes² was used at each site. The mercury probes were placed horizontally on the soil surface or at either 4 or 15 cm below the soil surface.

²These recorders were obtained on loan from Mr J Morrison, Private Bag X9059, Pietermaritzburg, 3200. Specifications for these recorders were obtained from Adolf Thies and Company. Local distributors were Labotec (Natal Pty. Ltd.) 291 Smith street, Durban, 4001.

C. RESULTS AND DISCUSSION

(i) BURIAL OF SEEDS AT THE SITE OF HARVEST

The aim of the first burial experiment was to ascertain the effects of seed burial on germination. In spite of some predation by mammals and birds, it is likely that most seeds will not be dispersed far from the maternal environment (Chapter 1). Thus, seeds harvested from three sites; Cedara and Ukulinga (Natal interior) and from Umdoni Park (Natal South Coast) during July 1986 were buried at the site of harvest. Differences in germination results between sites could therefore be due to a combination of both environmental and genetic factors.

(a) Predation

Of the seeds stored on the soil surface at Cedara, $34 \pm 20\%$ were lost due to predation (Table 4.1). Bite marks in the terylene bags suggested that rodents were responsible for this predation. Predation was also evident at Ukulinga, with $58 \pm 38\%$ of the seeds on the soil surface lost (Table 4.1). The variation around the mean was large at both sites since some bags were almost completely empty while others were untouched. Buried seeds were not subject to predation (Table 4.1). No predation occurred in seeds stored on the soil surface at Umdoni Park (Table 4.1). Thus, the occurrence of predation was influenced by site and depth of burial, and the presence or absence of predators.

TABLE 4.1: Predation of bugweed seeds over 24 months as affected by site and depth of burial.

| Burial locality | % Predation (\pm SE) of buried seed | | |
|-----------------|--|------|-------|
| | 0 cm | 4 cm | 15 cm |
| Cedara | 34 \pm 20 | 0 | 0 |
| Ukulinga | 58 \pm 38 | 0 | 0 |
| Umdoni Park | 0 | 0 | 0 |

(b) **Viability**

Seeds from packets which showed no sign of rodent predation were used for viability studies. After removal from exhumed packets, seeds were separated into two fractions in water (Materials and Methods, Chapter 1). The fraction that sank had high germination percentages under optimum conditions. Floating seeds showed very low germination percentages in the presence of 500 mg l⁻¹ GA₃ at alternating 15/30°C in the light. Careful examination of floating seeds indicated that the embryos had rotted or become shrivelled. These seeds were counted as non-viable. There was no indication in any of the three burial trials that seeds had germinated prior to exhumation and then died.

Seeds progressively lost viability when stored on the soil surface at Cedara. Twelve months storage resulted in 83 % seed death, and by the end of two years, viable seed numbers had declined to 10 % (Figure 4.3A). Floating non-viable seeds appeared intact but with brown, shrivelled embryos. In contrast, seeds buried at 4 or 15 cm depths retained their viability during the study, with only 1 % seed death (Figure 4.3A). Seed death at Ukulinga was consistently low regardless of depth of burial (Figure 4.3B).

Seeds stored on the soil surface at Umdoni Park showed a significant decrease in viability with time (Figure 4.3C). After two years storage 94 % of the seeds had lost viability. This pattern of seed mortality was similar to that of seeds stored on the soil surface at Cedara (Figure 4.3A). As seen for seeds buried at the other two sites, low seed mortality was evident for up to 24 months when burial occurred at 4 or 15 cm (Figure 4.3C).

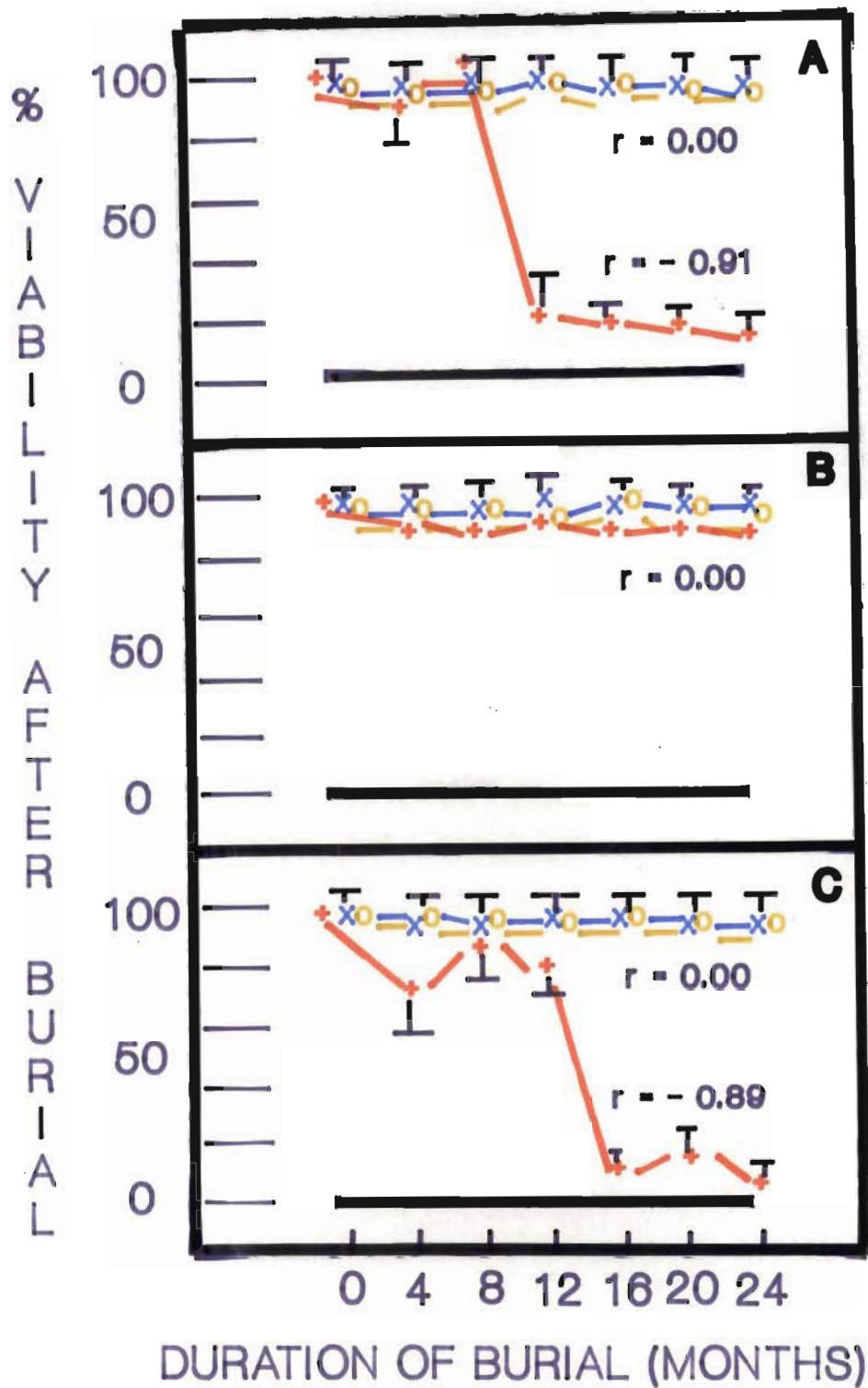


FIGURE 43: The effect of locality, depth and duration of burial of bugweed seeds on their viability.

Experiment 1: Seed burial at the site of collection. The correlation coefficient (r) for each relationship is given.

Seeds were buried at (A) Cedara; (B) Ukulinga and (C) Umdoni Park. Seeds were buried for up to 24 months at 0 cm (+), 4 cm (x) or 15 cm (o).

The occurrence of seed death (loss of viability) was therefore influenced by site, depth and duration of burial and was not apparently the result of seedling mortality.

Both loss of viability and predation seriously affected numbers of viable seed recovered from the soil surface. Seeds buried at 4 or 15 cm were not affected. Viable seed numbers recovered from the surface at Cedara were low due both to predation and high loss of viability. Seeds on the soil surface at Ukulinga retained their viability but were subject to predation. Surface-stored seeds at Umdoni Park were not eaten but did lose viability (Figure 4.3 and Table 4.1). As a result there was not always sufficient numbers of viable seed from the soil surface for experimentation, and consequently, this incomplete data has been omitted.

(c) *In situ* germination

Preliminary germination trials showed that the terylene bags used in the trials did not inhibit seed germination (Plate 12A). It is thus clear that passage of water and air through the bags was not impeded. Throughout the study seeds held in the terylene bags did not germinate regardless of whether they were buried or stored on the soil surface.

To test whether the inhibition of seed germination was caused by high CO₂ tensions, seeds were incubated under atmospheres of up to 5 % CO₂ at optimum germination conditions; 15/30°C in the light. Germination was not inhibited by CO₂ (Plate 12B) suggesting that the inhibition of seed germination was controlled by some other factor

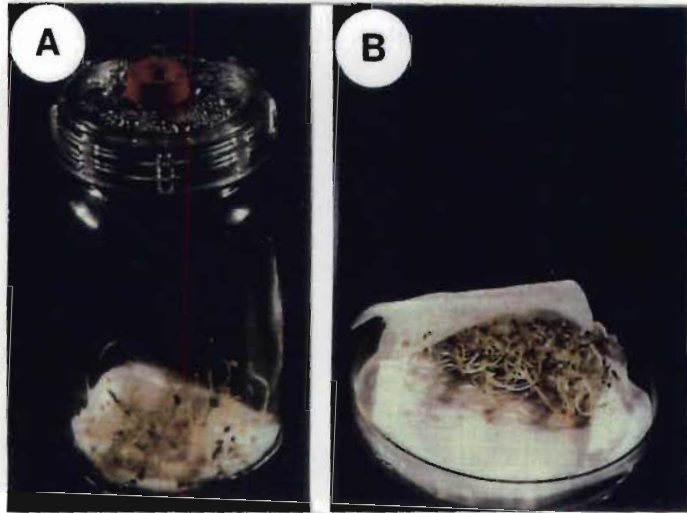


PLATE 12: The effect of (A) 5 per cent CO_2 and (B) terylene bags on germination of bugweed when incubated under optimum conditions.

(d) **Burial and dormancy**

Freshly-harvested seeds from all three sites at the beginning of this study (July, 1986) showed high germination percentages under optimum conditions (Figure 4.4). This indicated an absence of primary dormancy in bugweed seeds collected at this time. Freshly-harvested seeds did not germinate under unfavourable conditions such as alternating 15/30°C in the absence of light, or constant 20°C, either in the light or the dark, and can therefore be described as conditionally dormant. Transfer of seeds to optimum incubation conditions brought about high germination percentages (Figure 4.4). It would therefore appear as if seeds harvested during July 1986 did not exhibit secondary dormancy, irrespective of site of harvest.

These dormancy characteristics may not be permanent and are likely to change with time. To investigate this, seeds were buried at different depths at the site of harvest, exhumed at four monthly intervals, and then divided into two sub-samples. The first sub-sample of seeds was tested for germination either at (1) optimum conditions (15/30°C in the light) or else at conditions normally unfavourable for germination; these being (2) alternating 15/30°C in the dark; (3) constant 20°C in the light; or (4) constant 20°C in the dark. Seeds incubated under these conditions are described as batches³. After 12 weeks of such treatment non-germinated seeds from either (2), (3) or (4) were transferred to optimum conditions. Seeds from (4) were first transferred to alternating 15/30°C in the dark for

³A seed batch as described in this study is defined as a sample of seeds harvested from a population growing in one locality during one time period, buried at one site at a particular depth for a specified duration, and subsequently subjected to a specified set of incubation conditions in the laboratory.

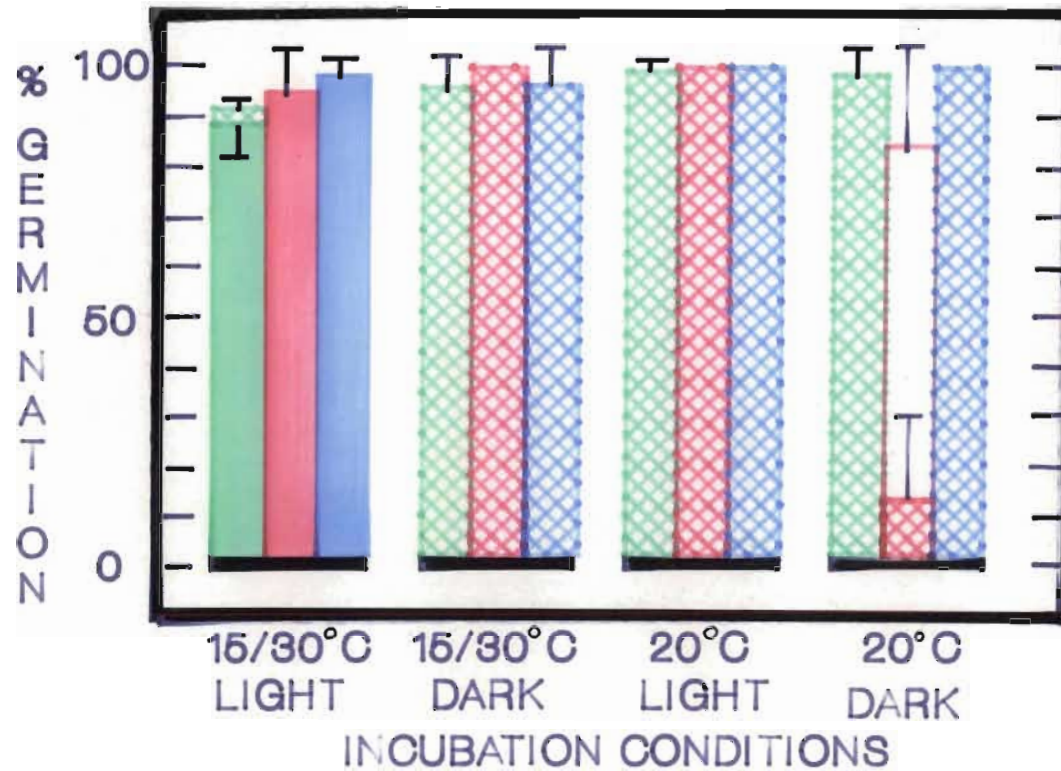


FIGURE 4.4: Germination response of bugweed seeds harvested from Cedar (—), Ukulinga (—) or Umdoni Park (—) during July 1986 (Experiment 1). Shaded bars represent germination after 12 weeks at initial incubation conditions. Hatched bars represent germination after 12 weeks when seeds were transferred a first time, either to alternating 15/30°C in the light (from 15/30°C dark or 20°C light) or the dark (from 20°C dark). Unshaded bars represent germination after 12 weeks when seeds from 20°C dark were transferred a second time, to the light. Vertical bars represent the standard errors of the means.

12 weeks, followed by a further transfer for 12 weeks in the light. The second sub-sample of seeds was incubated under these conditions (1-4 above) in the presence of $500 \text{ mg l}^{-1} \text{ GA}_3$, in order to determine whether seeds which had become secondarily dormant subsequent to burial could be induced to germinate. These results are not given, for the sake of brevity, but where relevant, are discussed.

As only few seeds survived exposure on the soil surface (due to predation or other causes) reliable data could not be obtained, thus only germination of seed batches buried at 4 or 15 cm depth are presented.

Because of the large number of separate experiments conducted, short conclusions will be presented at the end of each section for the sake of clarity and perspective.

(aa) *Germination under optimum conditions*⁴

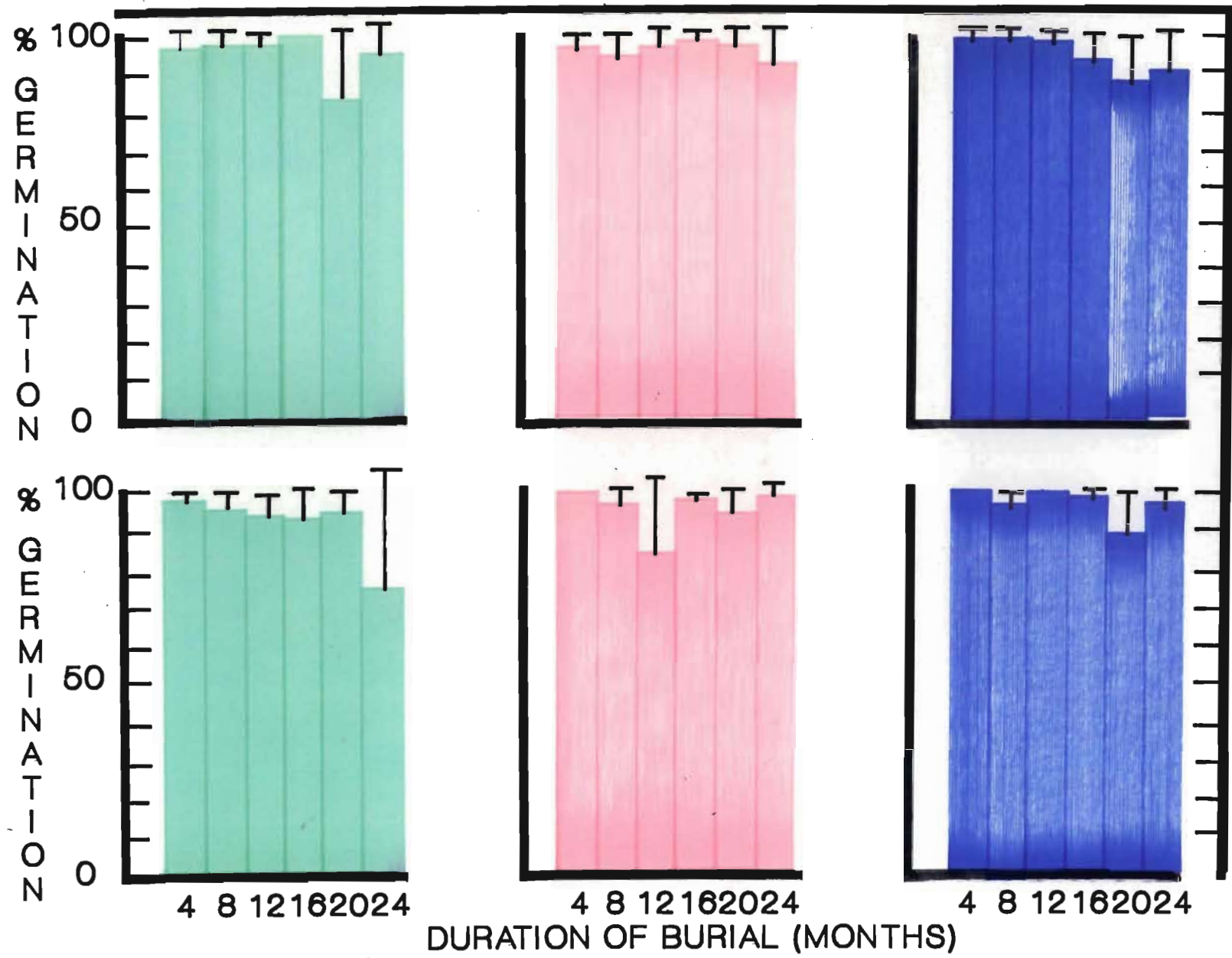
Burial at either 4 or 15 cm depths resulted in high germination percentages following exhumation and incubation at alternating 15/30°C in the light. Neither depth, nor duration, nor site of seed burial directly resulted in secondary dormancy (Figure 4.5). Thus bugweed seeds were apparently always able to germinate when exposed to optimum conditions.

(bb) *Germination at alternating 15/30°C in the dark*

Burial at a depth of 4 cm broke conditional dormancy of some seed

⁴ By definition, a low germination response under optimum conditions after burial is attributed to secondary dormancy and not to primary dormancy. Primary dormancy occurs at the time of release from the parent plant.

DEPTH



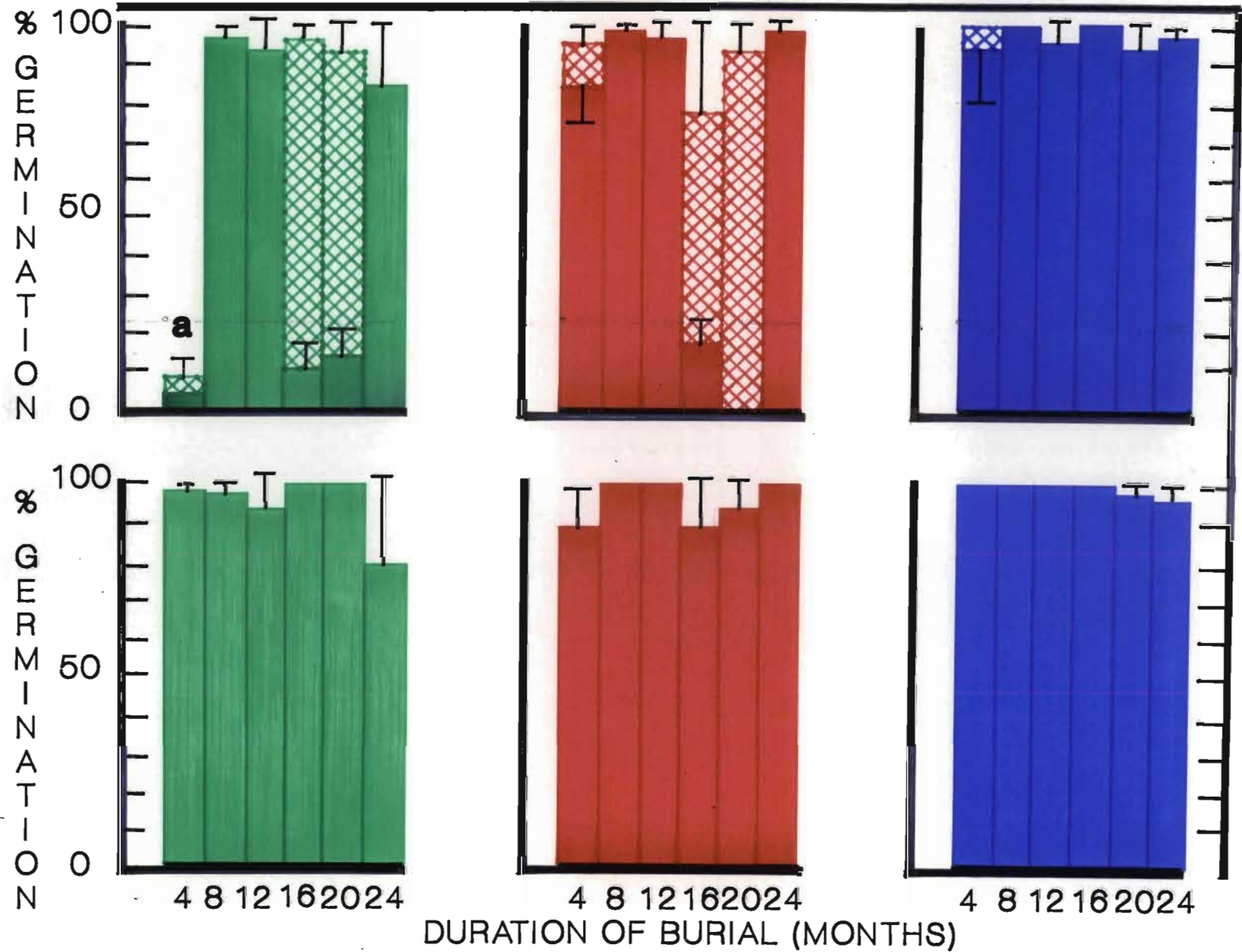
batches, depending on site and duration of burial. At both Cedara and Ukulinga, dormancy-breaking was variable whereas at Umdoni Park, uniform high germination percentages were recorded for seed incubated under these normally inhibitory conditions (Figure 4.6). Burial at 15 cm broke dormancy of seed harvested from all localities, irrespective of duration of burial (Figure 4.6). Transfer to the light of low-germinating seed batches generally promoted germination. One exception was the seed batch exhumed from Cedara after 4 months of burial at a depth of 4 cm. These seeds had apparently become secondarily dormant since they no longer responded to optimum conditions of 15/30°C in light (Figure 4.6). However, seeds from this batch did not exhibit secondary dormancy when 500 ml⁻¹ GA₃ was applied during the initial incubation period in the dark. *Thus the ability of seeds to enter secondary dormancy, induced by incubation at alternating temperatures in the dark subsequent to burial, was dependent on site, depth and duration of burial, and was prevented by the addition of GA₃.*

(cc) *Germination at constant 20°C in the light*

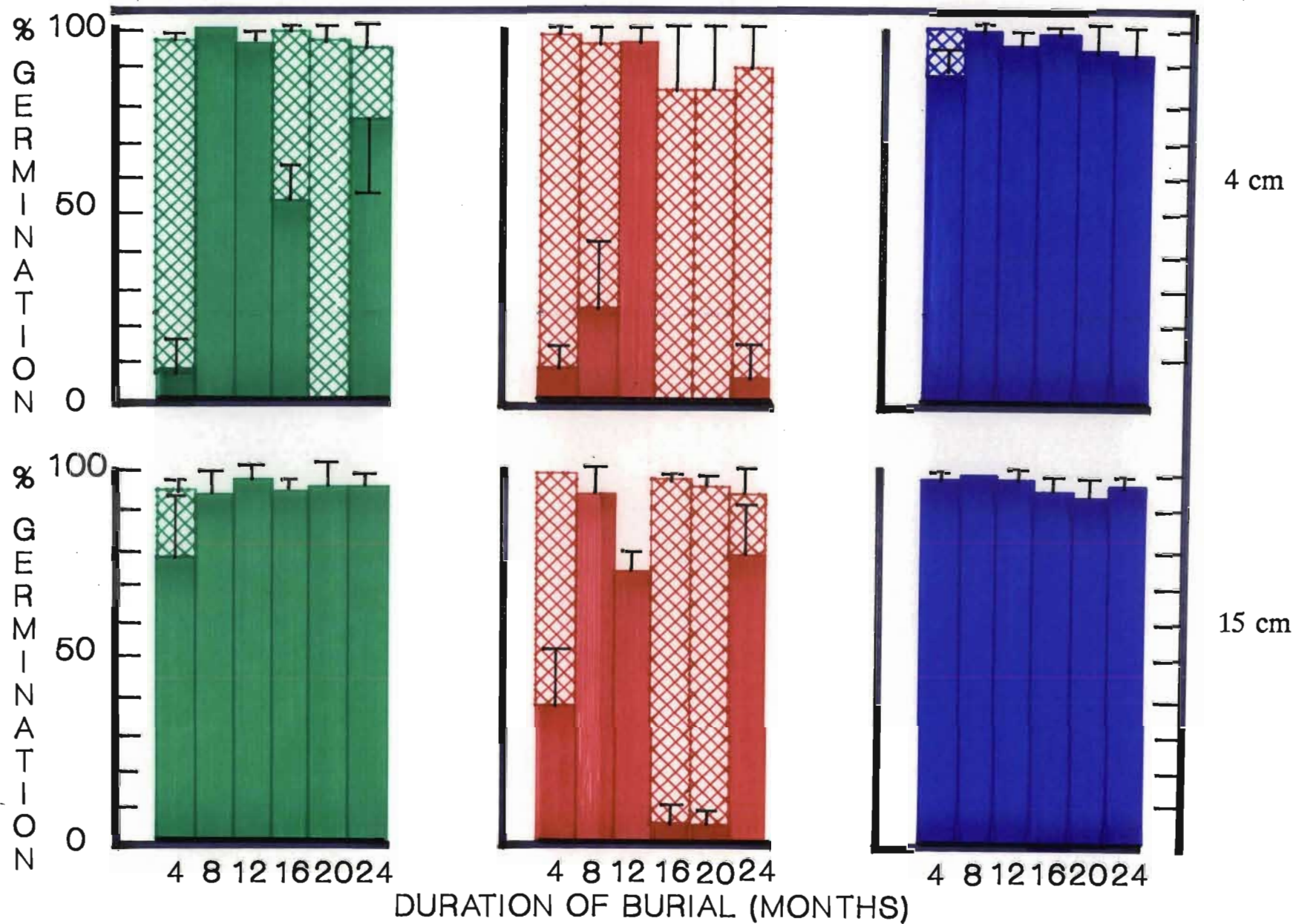
Seeds from both Natal inland sites showed variable germination following burial at 4 cm, whereas seeds from Umdoni Park consistently showed high germination percentages. Storage at 15 cm at the two Natal inland sites was generally more effective in promoting high germination percentages than storage at 4 cm (Figure 4.7). Thus a strong depth effect was evident at two of the sites, but not at Umdoni Park.

Transfer of all seed batches to alternating 15/30°C in the light resulted in

DEPTH



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high germination percentages, from which it may be inferred that secondary dormancy was not induced by incubation at constant 20°C in the light subsequent to burial (Figure 4.7).

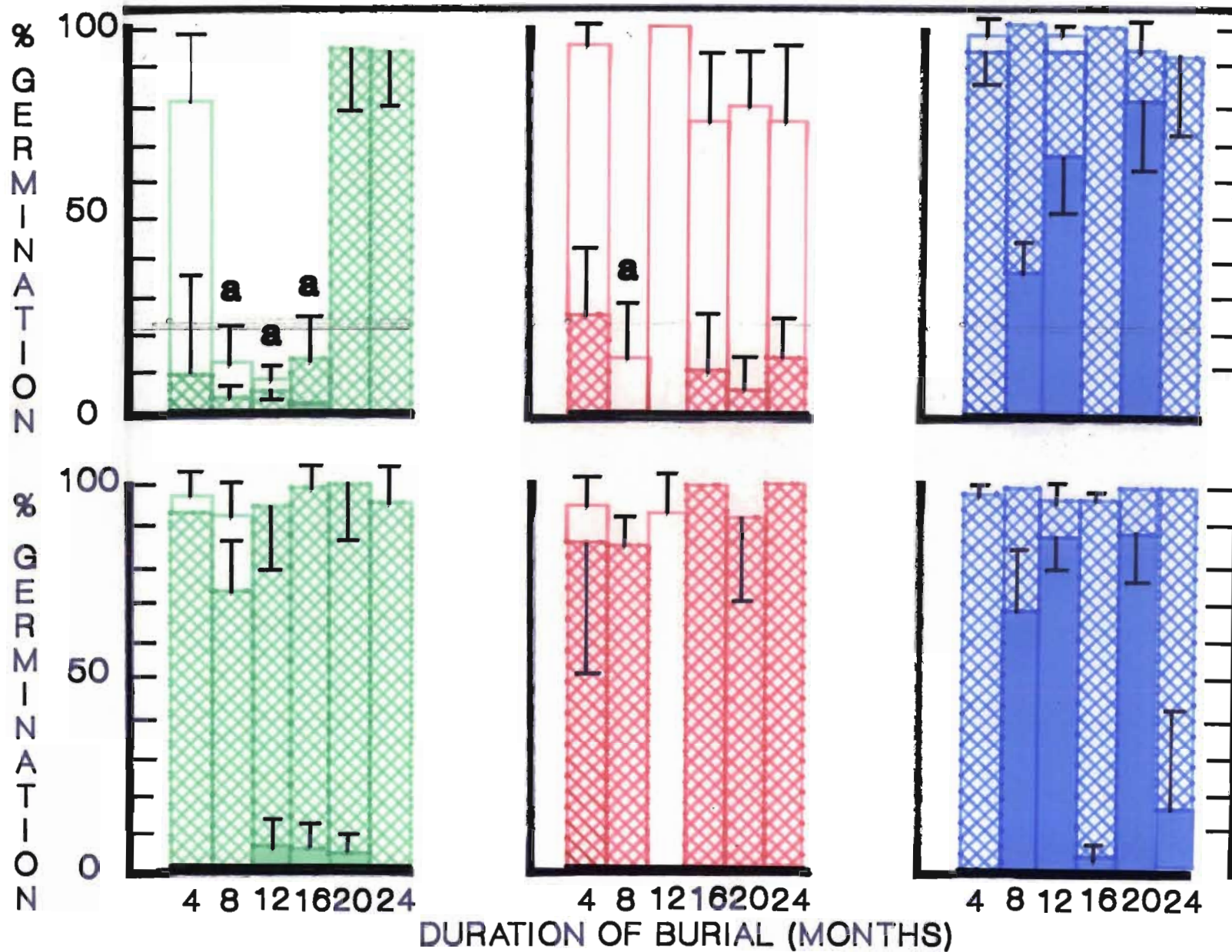
(dd) *Germination at constant 20°C in the dark*

Burial at the two Natal inland sites did not alleviate conditional dormancy, whereas burial at Umdoni Park allowed high germination percentages of some seed batches under these conditions. *Thus bugweed seeds lost the requirements for both light and alternating temperatures, and this was influenced by the site of burial.*

Transfer of seeds to alternating 15/30°C in the dark resulted in variable germination percentages, depending on site, depth, and duration of burial. Thus all seed batches from Umdoni Park showed high germination, irrespective of duration or depth of burial; whereas germination of seeds from the two Natal inland sites was influenced by both depth and duration of burial (Figure 4.8).

A second transfer, to the light, was sometimes effective in promoting germination, depending on site, depth, and duration of burial. Seeds which were buried at Umdoni Park apparently had no requirement for light, since germination responses were not greatly different from those recorded after 15/30°C dark treatments. Seeds from Umdoni Park did not exhibit secondary dormancy. Seeds which had been buried at 4 cm at Cedara showed variable germination, either not requiring the presence of light or else not responding to these optimum conditions, thereby exhibiting

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secondary dormancy. A strong depth effect was evident in seeds from this site, since burial at 15 cm resulted in seeds with a requirement for alternating 15/30°C, but not the presence of light. *Seeds that were buried at 15 cm thus showed a lower level of conditional dormancy than those buried at 4 cm.*

Seeds buried at a depth of 4 cm at Ukulinga generally showed high germination percentages after this second transfer to the light and only one observation of secondary dormancy was recorded. Most batches buried at a depth of 15 cm did not require this second transfer, indicating a lower level of conditional dormancy than those buried at 4 cm (Figure 4.8). Application of 500 ml⁻¹ GA₃ during incubation at constant 20°C in the dark prevented the induction of secondary dormancy.

(e) **Measurement of soil temperature**

The above findings indicated that the breaking of conditional dormancy and induction of secondary dormancy appeared to be largely dependent on initial incubation conditions following burial and also site, depth, and duration of burial. In order to explore this aspect further, and to relate the findings in an ecological context, an attempt was made to examine soil temperatures, since these have been known to play a crucial role in germination and dormancy of many other weed species.

Temperatures at the soil surface at Umdoni Park showed marked differences between maxima and minima throughout the year and these differences declined with increasing depth (Figure 4.9). Thus seeds stored

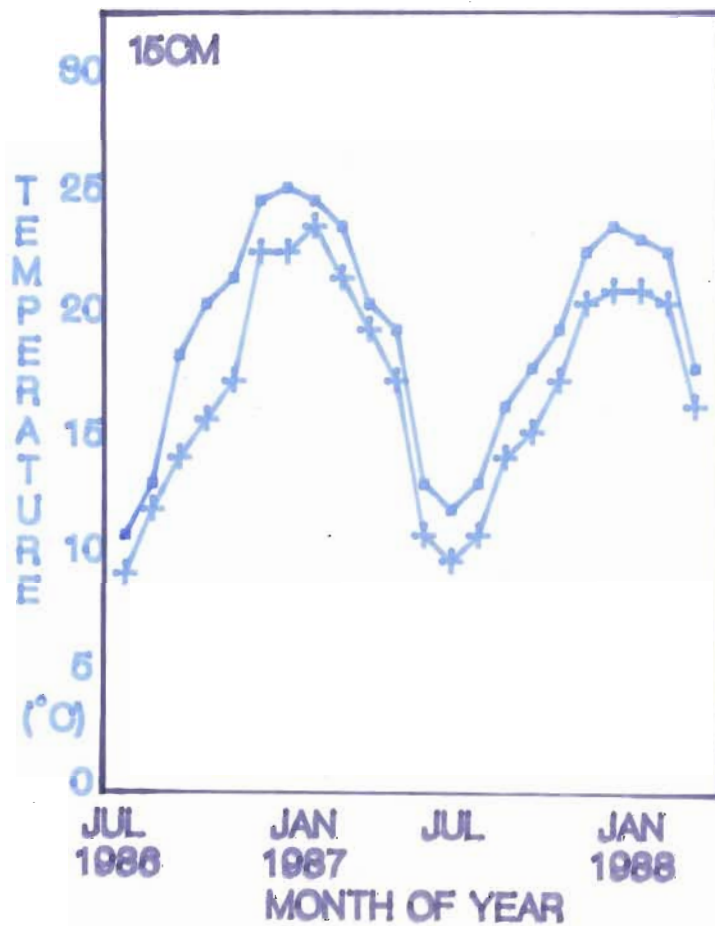
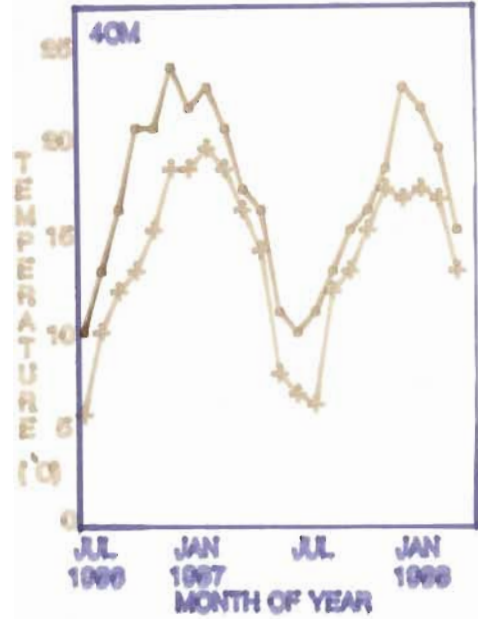
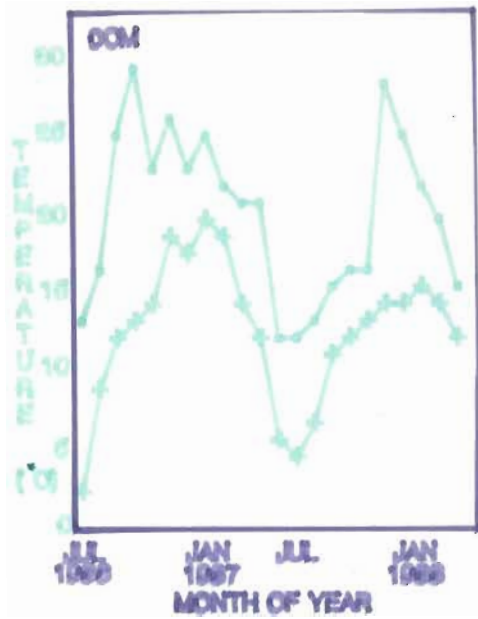


FIGURE 4.9: Seasonal variation in mean monthly temperature measured at three depths at Umdoni Park; 0, 4 and 15 cm.
 (■) maximum temperatures.
 (+) minimum temperatures.

at the soil surface would be subjected to more widely fluctuating diurnal temperatures while those buried at 15 cm would be subjected to narrower diurnal temperature fluctuations. These reduced fluctuations at 15 cm appeared to be more effective in breaking conditional dormancy, and also to prevent seeds entering secondary dormancy. Similar temperature patterns were found at the two Natal inland sites (results not presented).

When germination of different seed batches under unfavourable incubation conditions was plotted together with temperature data at the three sites, no significant correlations were evident (Figure 4.10)⁵. Temperature conditions at Umdoni Park showed milder winters than the two Natal inland sites, and differences between maximum and minimum temperatures were less pronounced. Burial of seeds at Umdoni Park broke conditional dormancy more frequently than burial at either of the Natal inland sites (Figure 4.10). However, no significant correlations were evident between germination responses (at any of the tested incubation conditions) after burial and soil temperature measured during burial of seeds. It is thus apparent that temperature alone did not account for differences in germination responses seen after burial of seeds at the different sites.

From the above set of experiments, it was evident that germination of bugweed seeds was highly variable after burial, and was influenced by site, depth and duration of burial. Conditional dormancy was broken most effectively by burial at Umdoni Park rather than the cooler inland sites. Depth of burial

⁵ Data is only given for a depth of 4 cm since differences in germination of seed batches from different sites were more clearly evident than those at 15 cm, and seed batches from the soil surface were not sufficiently numerous for the patterns to be ascertained.

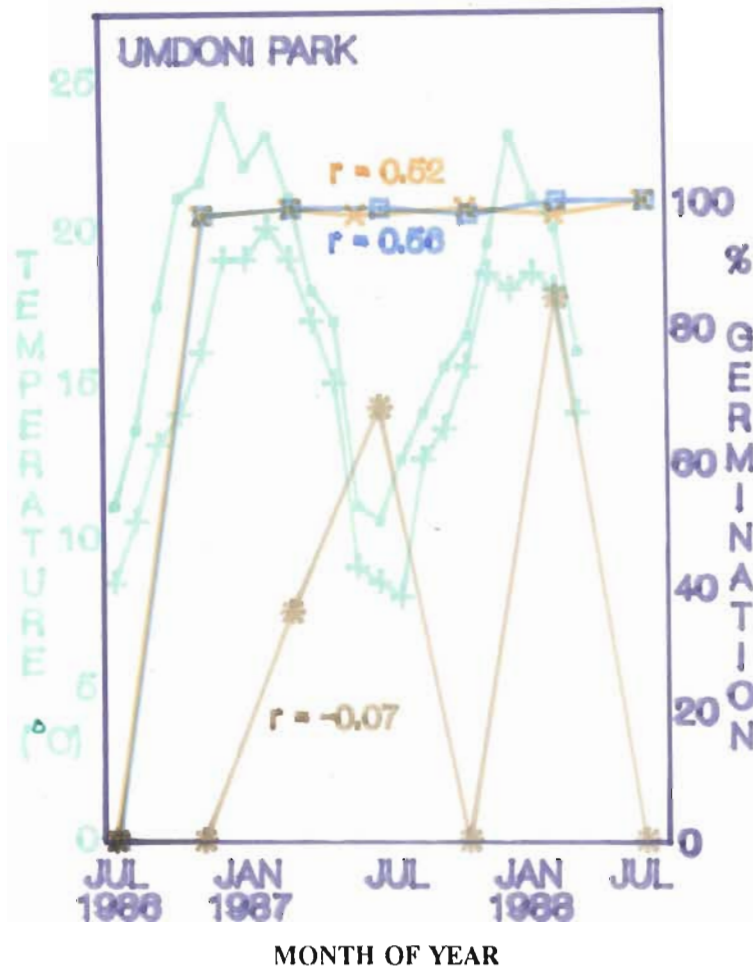
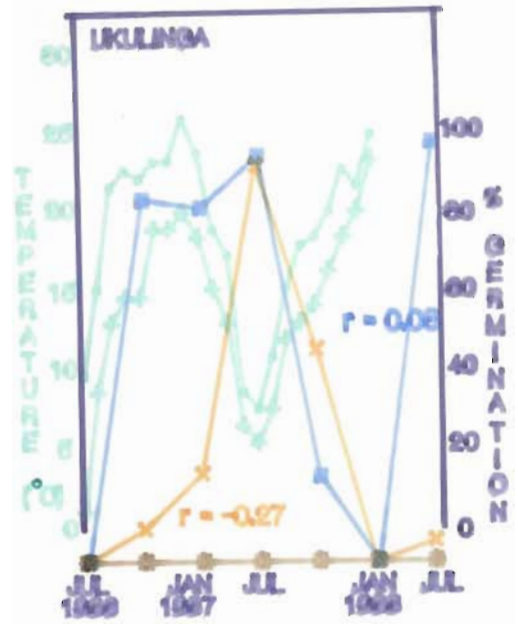
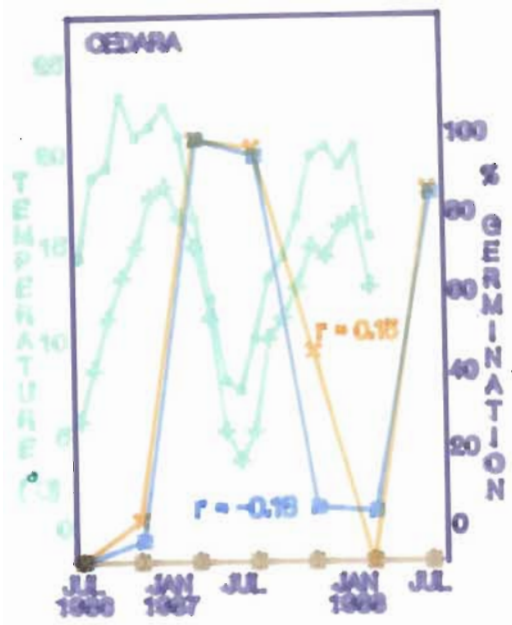


FIGURE 4.10: The relationship between germination of bugweed and seasonal variation in mean monthly temperature at a depth of 4 cm at three localities in Natal. Localities were Cedara, Ukulinga and Umdoni Park.

The correlation coefficient (r) between temperature and percentage germination under different conditions are given.

(□) maximum temperatures.

(+) minimum temperatures.

(x) Germination percentage at 15/30°C in the dark.

influenced germination requirements at all three sites, since burial at 15 cm was more effective in breaking conditional dormancy than burial at 4 cm. Secondary dormancy was more frequently evident at the cooler inland sites than at Umdoni Park and declined with increased depth of burial, irrespective of site. The application of a growth regulator (GA₃) to seeds during incubation at unfavourable conditions did not always prevent seeds becoming secondarily dormant, since germination of some seed batches remained low after transfer to optimum conditions.

(ii) **SEED TRANSPLANTATION EXPERIMENTS**

A second burial experiment was undertaken to ascertain the influence of different environments on seeds *from the same population* and thus to see whether viability or germination was influenced by altered environmental conditions following dispersal. Seeds from Cedara were harvested in February 1987, separated into floating and sinking batches, whereafter sinking viable seeds from different trees were thoroughly mixed and then buried at Cedara, Ukulinga and Umdoni Park. Differences in germination results between sites could therefore to a large degree be attributed to the dispersal environment of the seeds.

(a) **Predation**

When terylene seed bags were stored on the soil surface at Cedara and at Ukulinga inside rodent-proof enclosures, no seeds were lost due to predation.

(b) **Viability**

A loss of viability was seen in seeds stored on the soil surface at Cedara and Umdoni Park but not at Ukulinga (Figure 4.11). *Thus the environment into which seeds were dispersed, rather than the maternal environment or seed genotype, could markedly influence seed mortality* (compare Figures 4.3 and 4.11). Seeds buried for up to 16 months at 4 or 15 cm retained their viability regardless of site or duration of burial (Figure 4.11).

Thus percentage mortality of seeds is determined both by dispersal site and depth of burial. If conditions for germination remain unfavourable for a long time, then it is likely that seeds lying on the soil surface will lose viability.

(c) ***In situ* germination**

Germination did not occur inside the terylene bags except after 16 months storage on the soil surface at Umdoni Park. In this seed batch, only three seedlings were observed, each approximately 5 cm tall, with roots growing from one (out of three) of the bags. Thus in general, no *in situ* germination occurred, irrespective of site or depth of burial.

(d) **Burial and dormancy**

At the beginning of this study seeds freshly-harvested from Cedara during February 1987 showed a fairly high germination under optimum conditions, indicating that these seeds had a low level of primary dormancy. Conditional dormancy nevertheless became evident under all unfavourable incubation conditions tested. All incubation conditions induced secondary

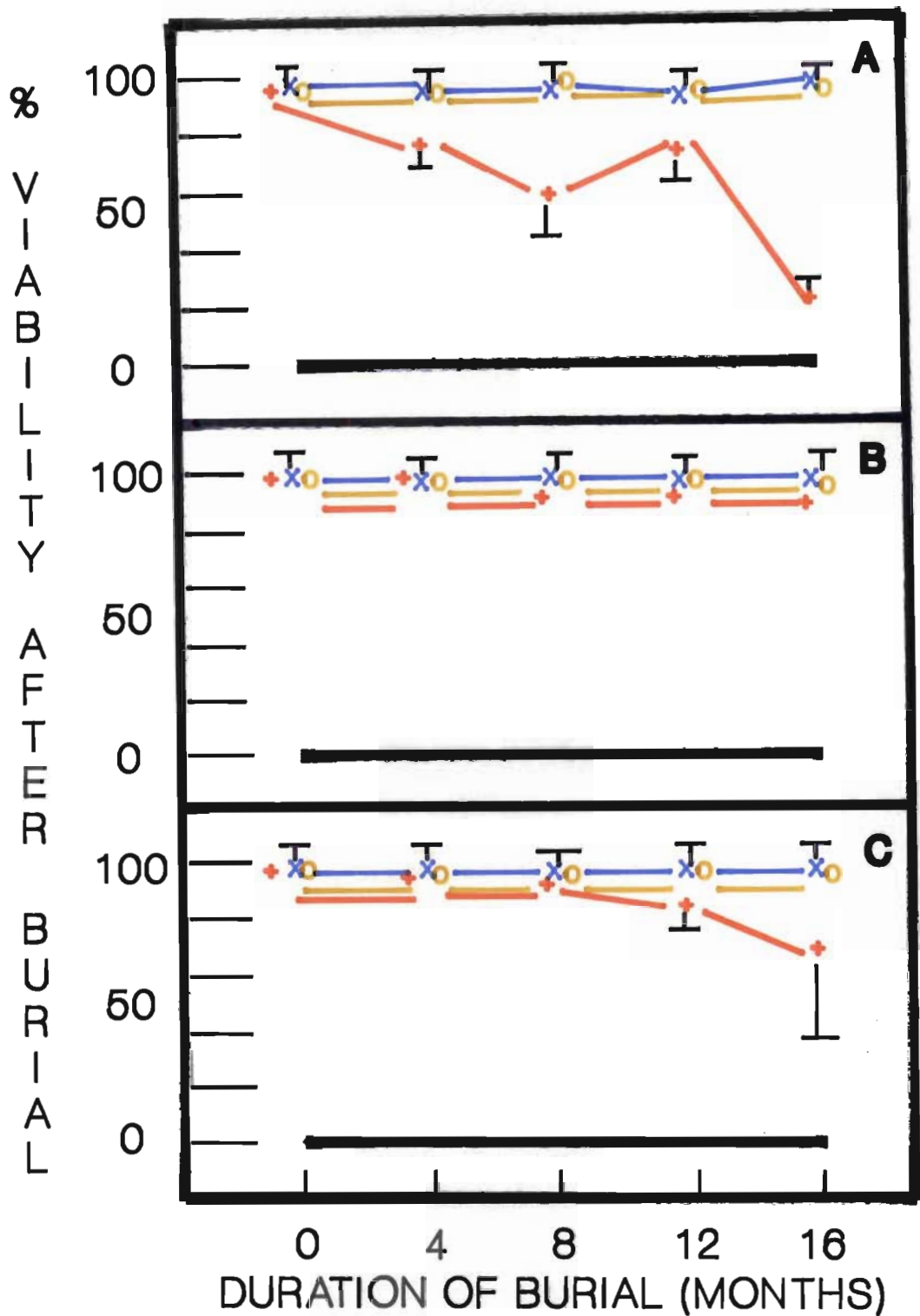


FIGURE 4.11: The effect of locality, depth and duration of burial of bugweed seeds on their viability.

Experiment 2: Seeds collected from Cedara were buried at three sites. Seeds were buried at (A) Cedara (B) Ukulinga and (C) Umdoni Park.

Seeds were buried for up to 16 months at 0 cm (+), 4 cm (x) or 15 cm (o).

dormancy since high germination percentages did not occur after transfer to optimum conditions (Figure 4.12). Application of 500 mg l⁻¹ GA₃ to seed batches during incubation at unfavourable conditions prevented the induction of secondary dormancy (Figure 4.12). The effect of burial at three different sites on the germination characteristics of seeds was determined, in order to establish whether conditional dormancy could be broken, and, if not, whether the ability to enter secondary dormancy was retained after burial.

(aa) *Germination under optimum conditions*

Depth of seed burial strongly influenced subsequent germination under optimum conditions. All seed batches showed high germination percentages following burial at depths of 4 or 15 cm, irrespective of site of burial. In contrast, some of the seed batches which had been stored on the soil surface entered secondary dormancy, and showed low to medium germination percentages at alternating 15/30°C in the light. The response was influenced by both site and duration of seed storage (Figure 4.13). A prolonged incubation at 15/30°C in the light for a further 12 weeks did not show a marked improvement in germination.

Application of 500 mg l⁻¹ GA₃ immediately after burial generally prevented the induction of secondary dormancy. One exception was the seed batch stored on the soil surface at Cedara for 16 months prior to incubation. This seed batch did not respond to application of GA₃ (Figure 4.13).

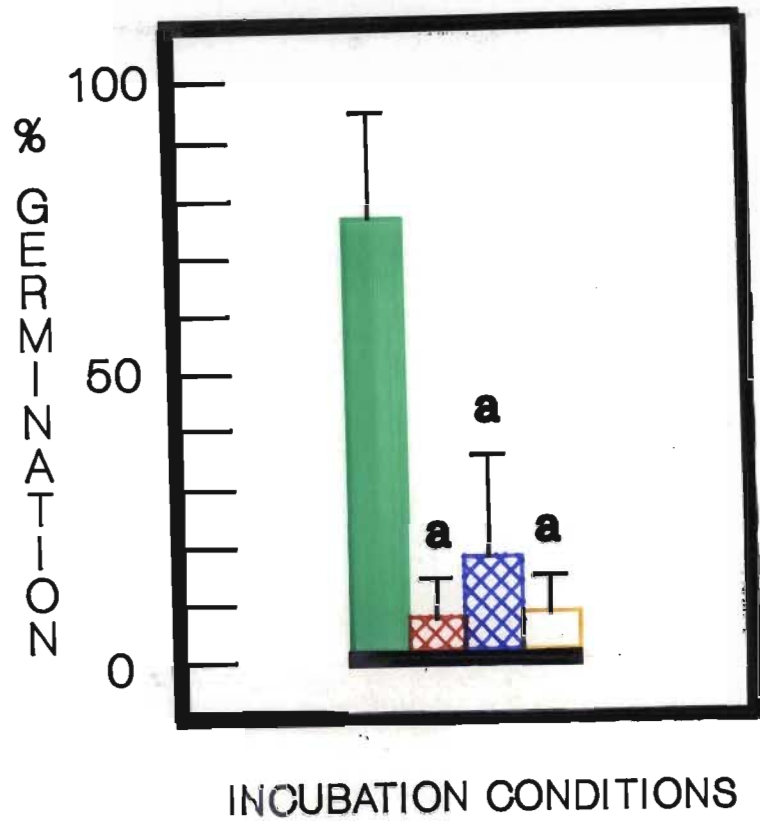


FIGURE 4.12: Germination response of bugweed seeds harvested from Cedara during February 1987 (Experiment 2). Germination was recorded after 12 weeks incubation at 15/30°C in the light (—) or dark (—) or at constant 20°C in the light (—) or dark (—). Vertical bars represent the standard errors of the means.
a: Represents the seed batches which germinated poorly when transferred from unfavourable conditions to optimum conditions (15/30°C in the light). Application of 500 mg l⁻¹ GA₃ to these seed batches during incubation under unfavourable conditions resulted in high germination percentages after transfer to 15/30°C in the light.

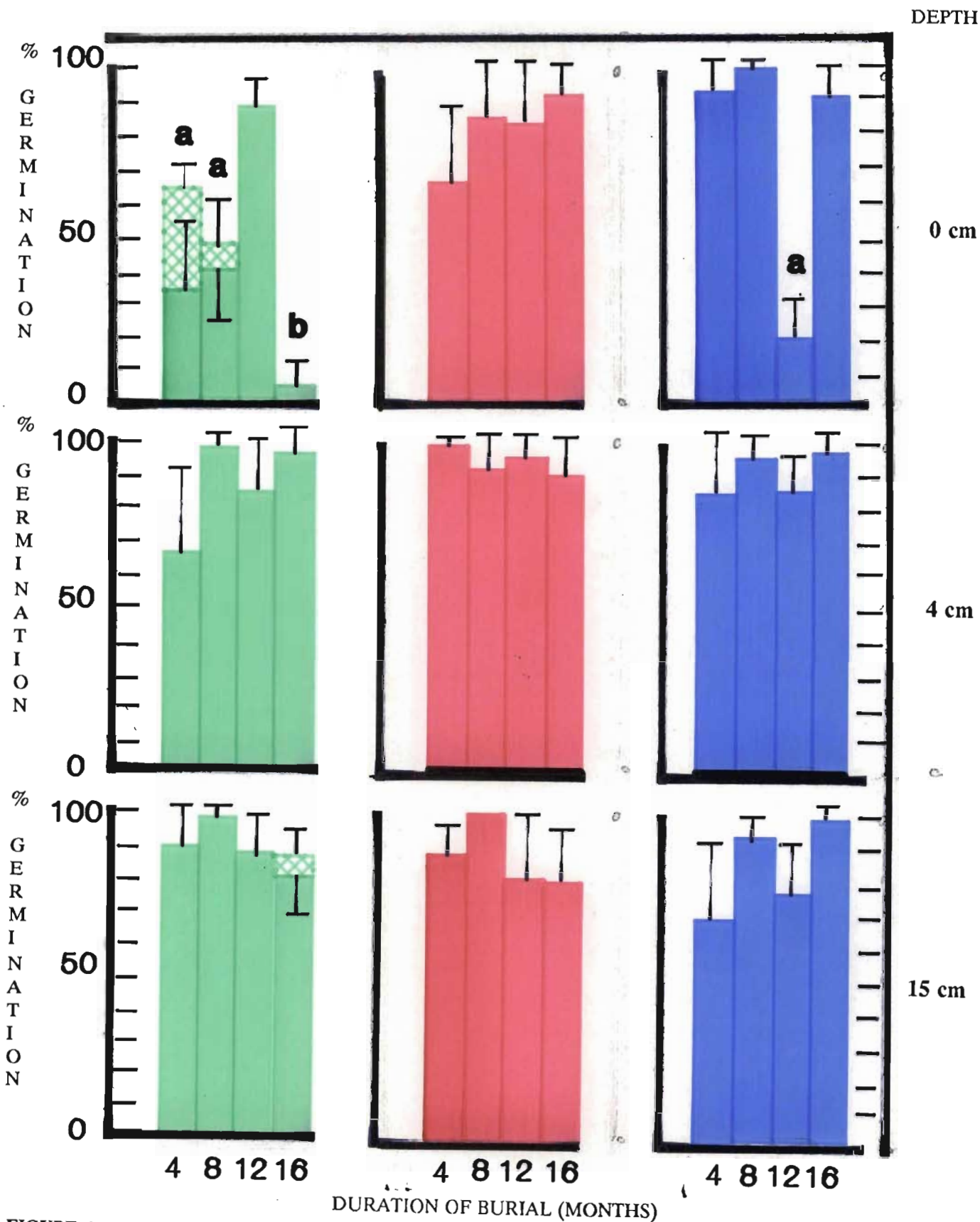


FIGURE 4.13: The effect of seed burial on germination of bugweed under optimum conditions (15/30°C in the light). **Experiment 2:** Seeds collected from Cedara were buried at three sites, Cedara (—), Ukulinga (—) or Umdoni Park (—). Seeds were buried at 0, 4 or 15 cm and exhumed at four-monthly intervals. Shaded and hatched portions of each bar represent germination at 15/30°C in the light after 12 and 24 weeks respectively.

Vertical bars represent the standard errors of the means.

a: Represents the seed batches which after burial germinated poorly under optimum conditions. Application of 500 mg l⁻¹ GA₃ to these seed batches after burial resulted in high germination percentages under optimum conditions.

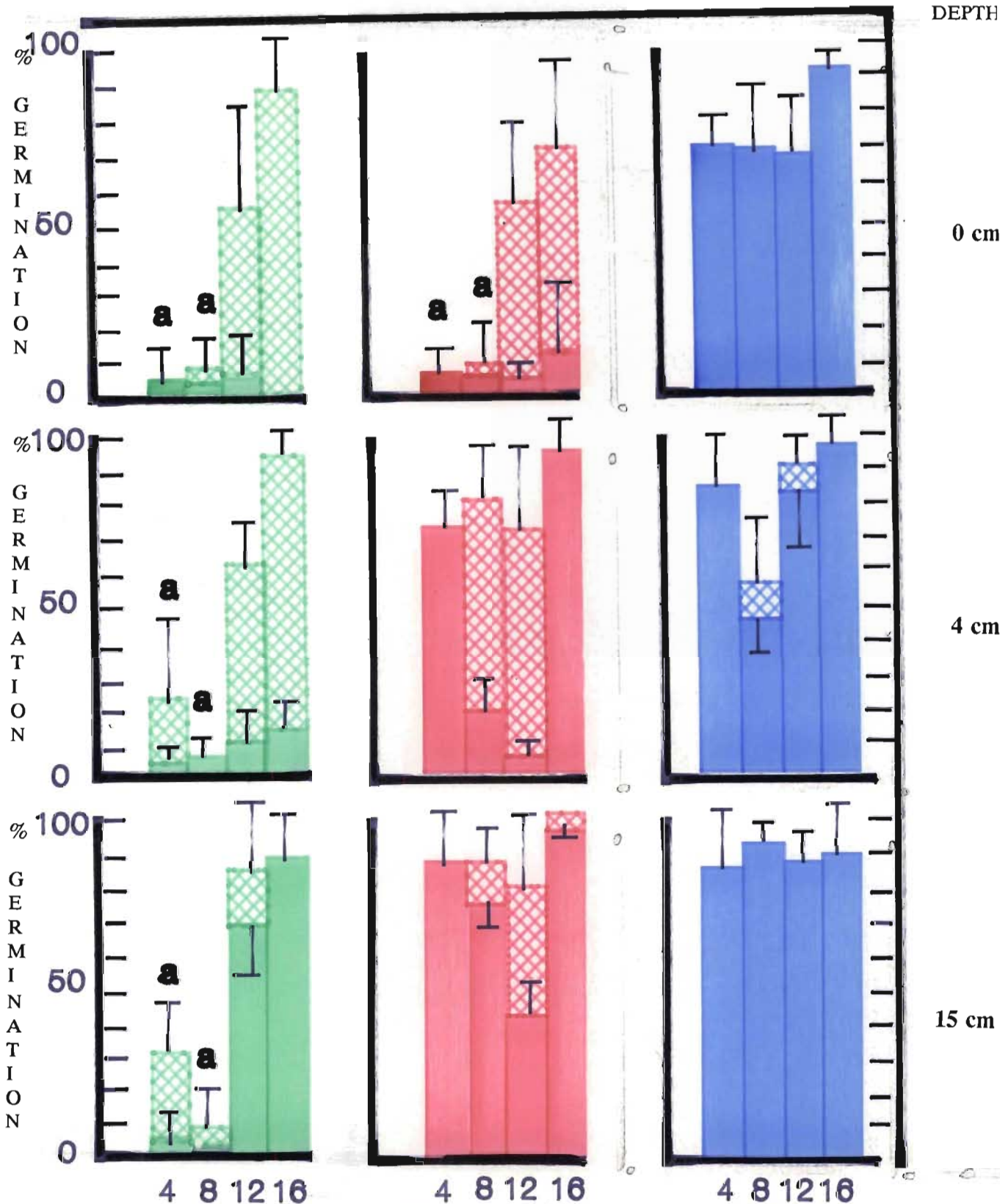
b: Represents the seed batch which after burial did not respond to 500 mg l⁻¹ GA₃.

(bb) *Germination at alternating 15/30°C in the dark*

Site, depth and duration of seed burial strongly influenced subsequent germination under these conditions. Seeds stored on the soil surface at both Natal inland sites did not show high germination percentages at alternating temperatures when incubated in the dark. In contrast, when the seeds were stored at Umdoni Park, conditional dormancy was subsequently lost (Figure 4.14).

Burial at a depth of 4 cm did not overcome the conditional dormancy of seeds buried at Cedara, whereas two of the seed batches which had been buried at Ukulinga lost their requirement for light. Prolonged burial at 15 cm broke the dormancy of seeds buried at Cedara, whereas this was not seen at 4 cm or on the soil surface. Higher germination percentages were recorded for seeds buried at 15 cm than at 4 cm or on the soil surface at Ukulinga. However, this depth effect was less marked when burial occurred at Umdoni Park (Figure 4.14).

Transfer of non-germinated seeds from the above conditions to the light yielded variable germination. This transfer effect was influenced by both depth and site of burial. Greater promotion of germination was obtained after prolonged storage at 0 or 4 cm at the two Natal inland sites. Secondary dormancy did not occur in seeds which had been buried at Umdoni Park, but was seen in two seed batches previously buried at Ukulinga and in six batches which had been buried at Cedara (Figure 4.14). Application of 500 mg l⁻¹ GA₃ during incubation in the dark prevented the induction of secondary dormancy, since a subsequent transfer



DEPTH OF BURIAL (MONTHS)

FIGURE 4.14: The effect of seed burial on germination of bugweed at 15/30°C in the dark. **Experiment 2:** Seeds collected from Cedara were buried at three sites, Cedara (→), Ukulinga (→) or Umdoni Park (→). Seeds were buried at 0, 4 or 15 cm and exhumed at four-monthly intervals. Shaded portions of each bar represent germination at 15/30°C in the dark after 12 weeks. Hatched portions of each bar represent germination after 12 weeks when seeds were subsequently transferred to the light.

Vertical bars represent the standard errors of the means.

a: Represents the seed batches which after burial germinated poorly when transferred from 15/30°C in the dark to optimum conditions (15/30°C in the light). Application of 500 mg l⁻¹ GA₃ to these seed batches after burial and during incubation at 15/30°C in the dark resulted in high germination percentages after transfer to

to the light resulted in high germination percentages (Figure 4.14).

(cc) *Germination at constant 20°C in the light*

Seeds stored on the soil surface at the two Natal inland sites did not germinate, while storage of seed at Umdoni Park resulted in a loss of conditional dormancy, since high germination percentages occurred at constant 20°C (Figure 4.15).

Seed batches buried at a depth of 4 cm at the two Natal inland sites showed variable germination under these conditions. This contrasted with seeds buried at Umdoni Park which consistently gave high germination percentages and therefore broke conditional dormancy (Figure 4.9). Burial at a depth of 15 cm effectively broke conditional dormancy in most of the seed batches, irrespective of site or duration of burial (Figure 4.15).

Storage on the soil surface resulted in variable germination after transfer from constant to alternating temperatures. This was influenced by site of storage. Seeds stored at Umdoni Park showed only marginal increases in germination whereas one seed batch stored at Ukulinga and two batches stored at Cedara entered secondary dormancy (Figure 4.15). Application of 500 mg l⁻¹ GA₃ during incubation at constant 20°C prevented the induction of secondary dormancy after subsequent transfer to alternating 15/30°C (Figure 4.15).

(dd) *Germination at constant 20°C in the dark*

Depth and site of burial strongly influenced subsequent germination under

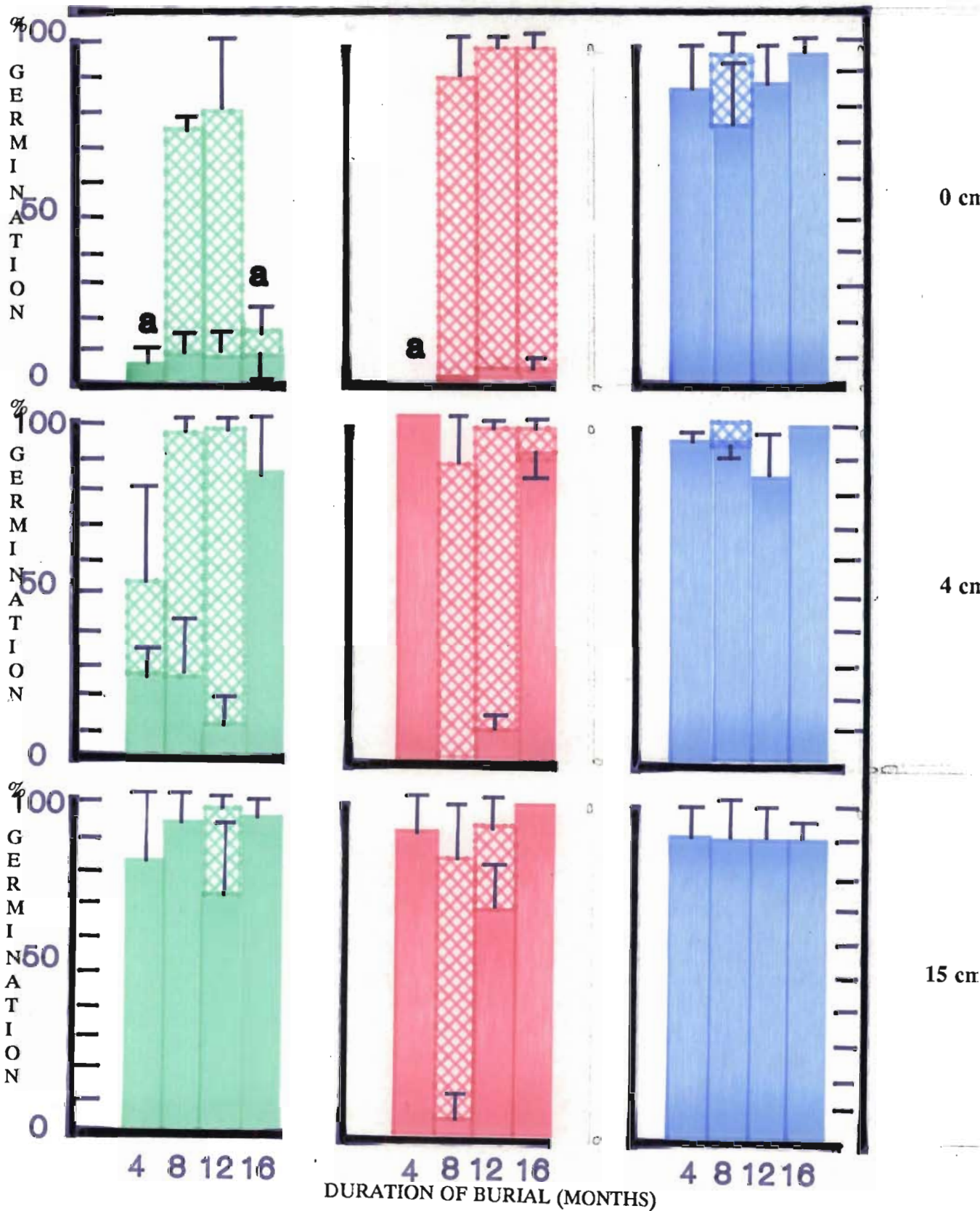


FIGURE 4.15: The effect of seed burial on germination of bugweed at constant 20°C in the light. **Experiment 2:** Seeds collected from Cedara were buried at three sites, Cedara (→), Ukulinga (←) or Umdoni (←). Seeds were buried at 0, 4 or 15 cm and exhumed at four-monthly intervals. Shaded portions of each bar represent germination at 20°C in the light after 12 weeks. Hatched portions of each bar represent germination after 12 weeks when seeds were subsequently transferred to alternating 15/30°C. Vertical bars represent the standard errors of the means. **a:** Represents the seed batches which after burial germinated poorly when transferred from 20°C in the light to optimum conditions (15/30°C in the light). Application of 500 mg l⁻¹ GA₃ to these seed batches after burial and during incubation at 20°C in the light resulted in high germination percentages after transfer to optimum conditions.

these conditions. Storage at the soil surface or at 4 cm was ineffective in breaking conditional dormancy, irrespective of site of burial. Storage at a depth of 15 cm at the two Natal inland sites was ineffective in breaking conditional dormancy. However, seeds buried for 12 months at Umdoni Park lost their requirement for both light and alternating temperatures (Figure 4.16). It was therefore clear that burial at this site could be highly effective in breaking conditional dormancy. *It was also evident that the germination characteristics of seeds had altered and this was due to the dispersal environment of the seeds.*

A single transfer to alternating temperatures was sufficient to permit high germination in some seed batches, whereas others required a second transfer, to the light, before this could occur. Those seed batches which only required one transfer were interpreted as having a lower level of conditional dormancy than those requiring a further transfer to the light (Figure 4.16). This varied with site, depth, and duration of burial.

Secondary dormancy was not seen in seed batches which had been stored at Umdoni Park, but occurred in two seed batches from each of the two Natal inland sites (Figure 4.16).

Application of 500 mg l⁻¹ GA₃ during incubation at 20°C in the dark prevented the induction of secondary dormancy in seed batches which had been buried at 15 cm (Figure 4.16). However, two of the seed batches stored at 0 cm did not fully respond to GA₃, and approximately half of the seeds showed secondary dormancy (Figure 4.16).

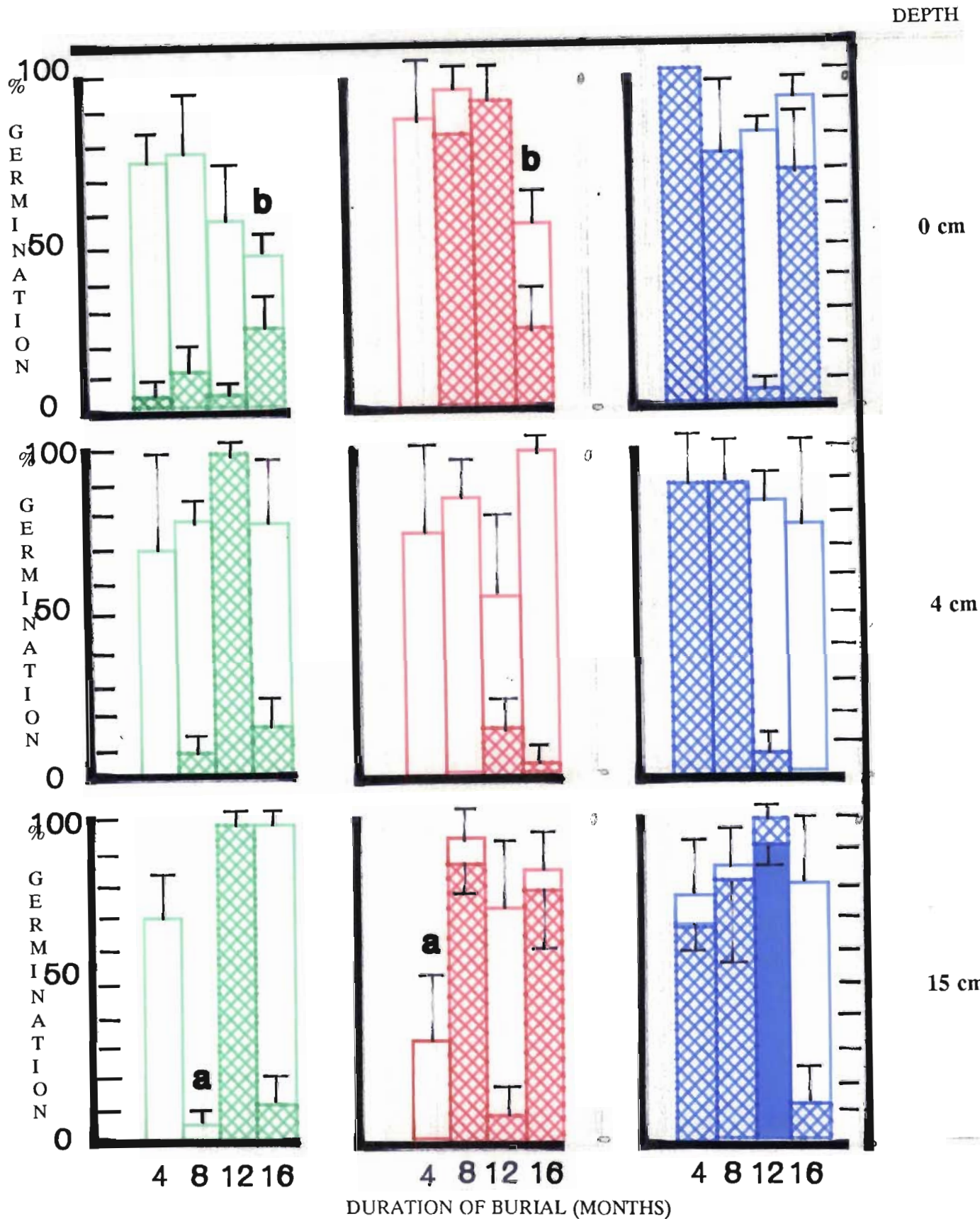


FIGURE 4.16: The effect of seed burial on germination of bugweed at constant 20°C in the dark. **Experiment 2:** Seeds collected from Cedara were buried at three sites, Cedara (→), Ukulinga (→) or Umdoni Park (→). Seeds were buried at 0, 4 or 15 cm and exhumed at four-monthly intervals. Shaded portions of each bar represent germination at 20°C in the dark after 12 weeks. Hatched portions of each bar represent germination after 12 weeks when seeds were subsequently transferred to alternating 15/30°C in the dark. Unshaded portions represent germination after 12 weeks when seeds were transferred a second time, to the light. Vertical bars represent the standard errors of the means.

a: Represents the seed batches which after burial germinated poorly when transferred from unfavourable conditions to optimum conditions (15/30°C in the light). Application of 500 mg l⁻¹ GA₃ to these seed batches after burial and during incubation at 20°C in the dark resulted in high germination percentages after transfer

From the above set of experiments, it was evident that germination of bugweed seeds is highly variable after burial, and is to a large degree influenced by site of dispersal. Thus given a similar genotype, dispersal away from the maternal environment will result in dormancy characteristics very different from those shown by seeds remaining in the maternal environment. Seeds will show changes in the patterns of conditional dormancy, where germination percentages become increased under normally unfavourable conditions; and also in terms of secondary dormancy, where germination percentages may become decreased under normally favourable conditions. Implications of this variability are that seedling emergence will be altered according to site of dispersal and this will markedly complicate procedures aimed at control of this weed.

(iii) **SEASONAL EFFECTS OF BURIAL**

The aim of this burial experiment was to ascertain whether seasonal factors influenced the dormancy characteristics of buried seeds. Thus, seeds were harvested from Cedara during either July 1986 (Experiment 1), February 1987 (Experiment 2) and July 1987. These seed batches were buried *at different times at the site of harvest, viz.* Cedara, and exhumed at 4, 8 and 12 months after burial. Incubation of seeds was either under optimum conditions, or else under conditions not normally conducive to germination. It was argued that differences in germination between seed batches could be accounted for to a large degree by the differing maternal environment both during seed development and burial.

(a) **Predation**

Predation of seeds in the February 1987 and July 1987 batches was prevented by placing surface-stored terylene seed bags in rodent-proof cages. This proved effective in excluding predators, and no seeds were lost due to predation during this study.

(b) **Viability**

A comparison of seed batches harvested during two consecutive winters and an intervening summer showed similar exponential decreases in seed viability with time of storage on the soil surface at Cedara. Viability was retained, however, when seeds were buried at depths of 4 and 15 cm. Thus time of harvest did not influence viability of seeds, but duration and depth of burial did influence seed mortality (Figure 4.17).

(c) ***In situ* germination**

No germination occurred within the terylene bags during this trial, regardless of whether they were buried or stored on the soil surface.

(d) **Burial and dormancy**

Freshly-harvested seeds gave high germination percentages under optimum conditions, thus seeds exhibited low primary dormancy irrespective of season of harvest (Figure 4.18). Seeds harvested during July 1986 or February 1987 exhibited conditional dormancy and did not germinate under unfavourable conditions (Figure 4.18). However, seeds harvested during July 1987 lost their requirement for light when incubated at alternating temperatures (Figure 4.18). The induction of secondary

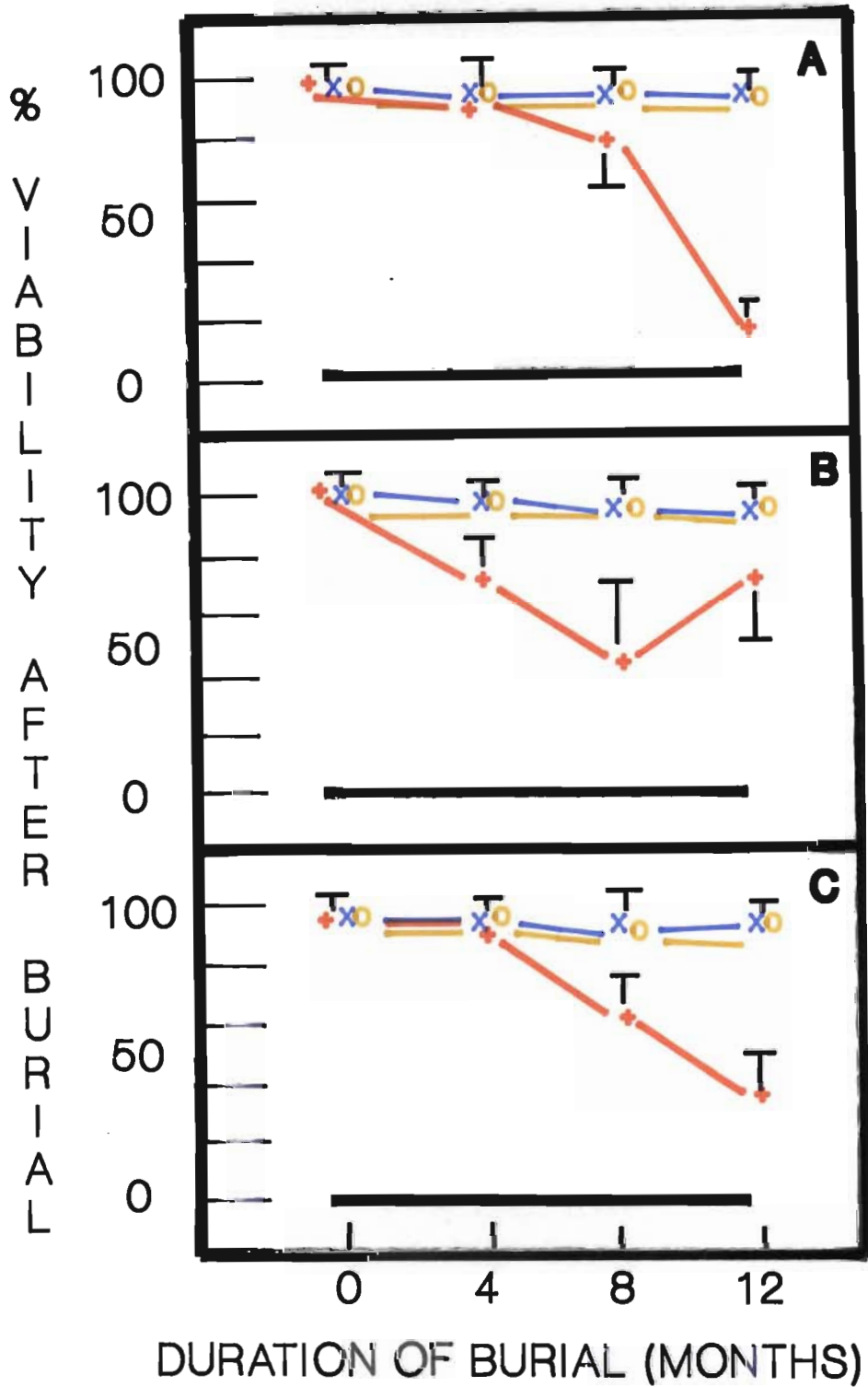


FIGURE 4.17: The effect of time of harvest, depth and duration of burial of bugweed seeds on their viability.

Experiment 3: Seeds were collected and buried at Cedara during July 1968 (A), February 1987 (B) and July 1987 (C).

Seeds were buried for up to 12 months at 0 cm (+), 4 cm (x) or 15 (o).

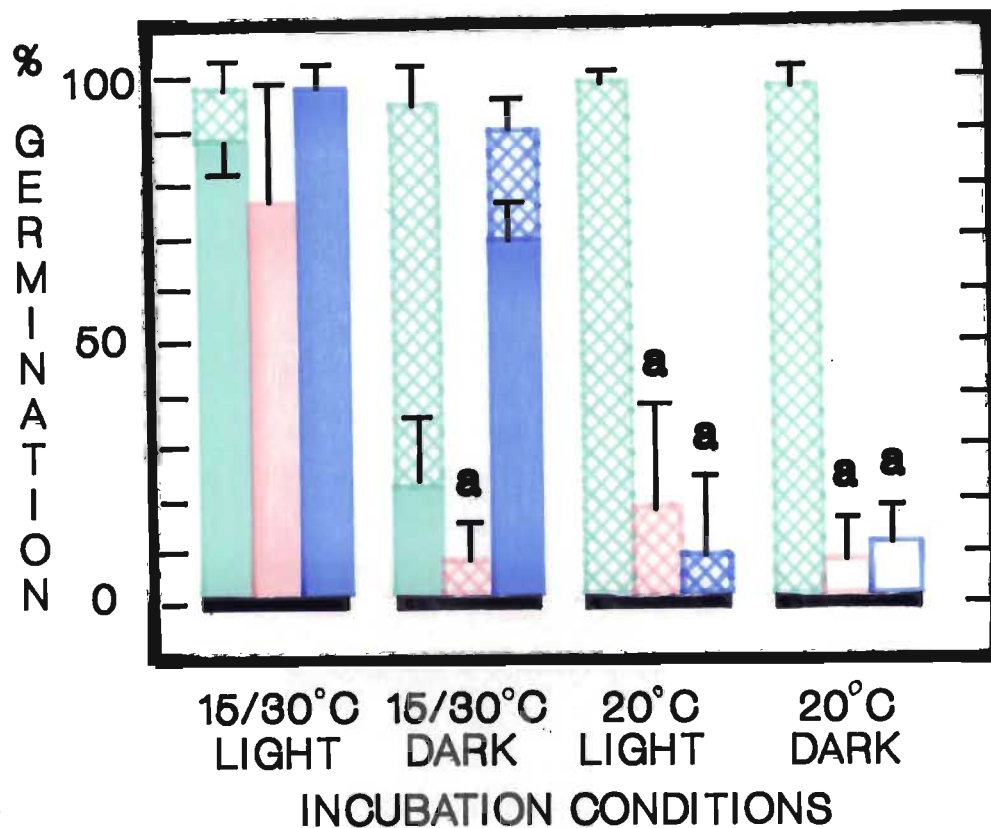


FIGURE 4.18: Germination response of bugweed seeds harvested from Cedara during July 1986 (—), February 1987 (—) and July 1987 (—) (Experiment 3). Shaded portions of each bar represent germination after 12 weeks at initial incubation conditions. Hatched portions of each bar represent germination after 12 weeks when seeds were transferred a first time, either to alternating 15/30°C in the light (from 15/30°C dark or 20°C light) or the dark (from 20°C dark). Unshaded bars represent germination after 12 weeks when seeds from 20°C dark were transferred a second time, to the light.

Vertical bars represent the standard errors of the means.

a: Represents the seed batches which germinated poorly when transferred from unfavourable conditions to optimum conditions (15/30°C in the light). Application of 500 mg l⁻¹ GA₃ to these seed batches during incubation under unfavourable conditions resulted in high germination percentages after transfer to 15/30°C in the light.

dormancy by unfavourable incubation conditions was influenced by season of harvest and prevented by the application of 500 mg l⁻¹ GA₃ (Figure 4.18). The effect of seed burial on germination characteristics was then determined, to see whether conditional dormancy could be broken, and if the ability to enter secondary dormancy was altered.

(aa) *Germination under optimum conditions*

Seeds harvested and buried in July 1986 retained their ability to germinate under optimum conditions, and this was not influenced by depth or duration of burial (Figure 4.19). In contrast, seeds harvested and buried during July of the following year did not germinate under optimum conditions after storage on the soil surface for 8 or 12 months. Thus these seeds had entered secondary dormancy, induced by storage on the soil surface for a time period longer than 4 months (Figure 4.19). Seeds harvested during the summer of 1987 also showed a reduced response to optimum conditions after storage on the soil surface. Here, storage for 4 or 8 months resulted in approximately half of the seeds entering secondary dormancy (Figure 4.19). Again, a depth effect was evident, since burial of summer batches promoted high germination.

Prolonged incubation for a further 12 weeks at 15/30°C did not markedly increase germination percentages (Figure 4.19). Application of 500 mg l⁻¹ GA₃ prevented the induction of secondary dormancy in two of the seed batches but was ineffective in the 1987 winter seed batch which had been stored on the soil surface for 12 months (Figure 4.19). Thus this latter seed batch apparently had a deeper level of secondary dormancy.

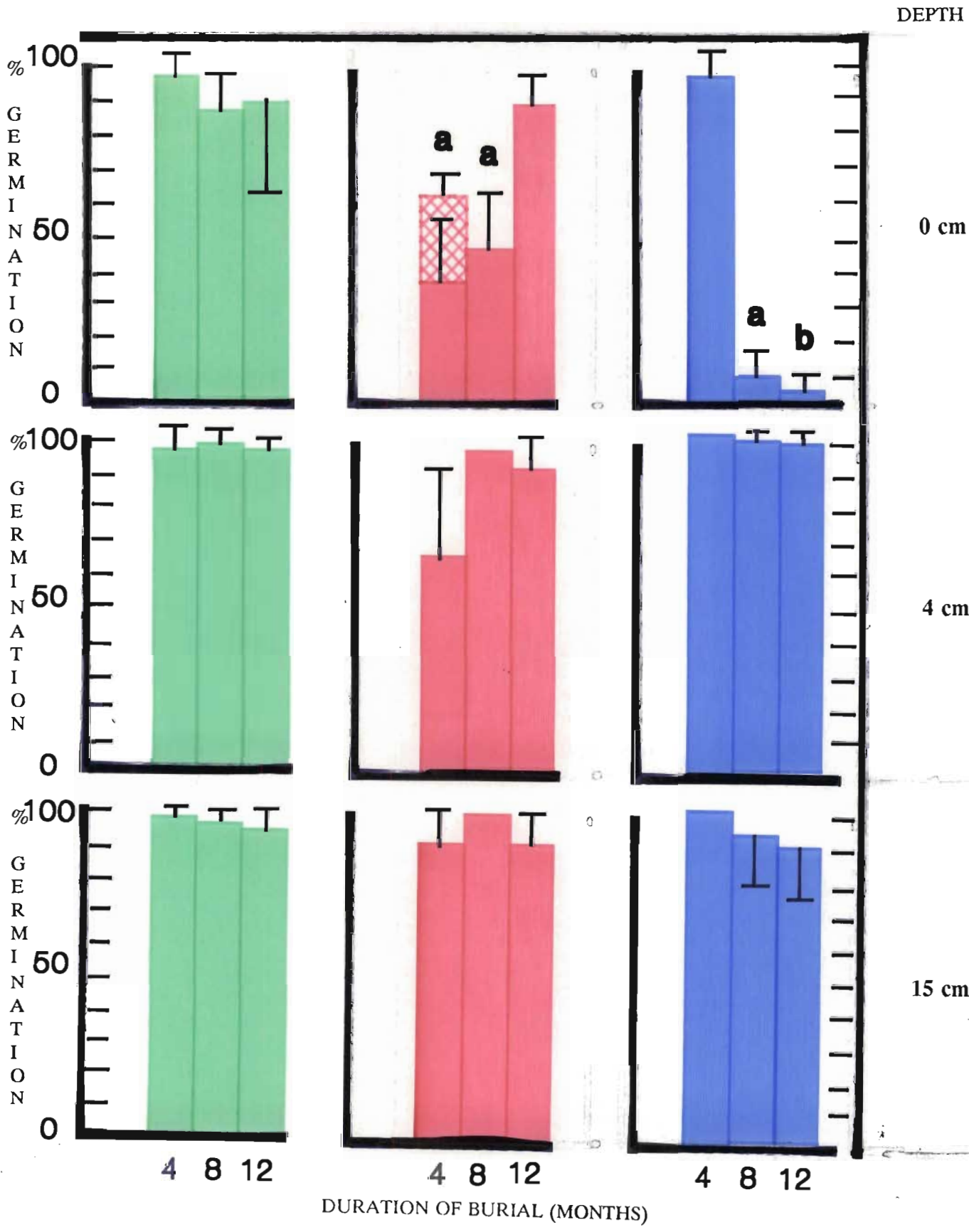


FIGURE 4.19: The effect of seed burial on germination of bugweed under optimum conditions (15/30°C in the light). **Experiment 3:** Seeds were collected and buried at Cedara during July 1986 (→), February 1987 (→) and July 1987 (→). Seeds were buried at 0, 4 or 15 cm and exhumed at four-monthly intervals. Shaded and hatched portions of each bar represent germination at 15/30°C in the light after 12 and 24 weeks respectively.

Vertical bars represent the standard errors of the means.
a: Represents the seed batches which after burial germinated poorly when incubated under optimum conditions. Application of 500 mg l⁻¹ GA₃ to these seed batches after burial resulted in high germination percentages under optimum conditions.
b: Represents the seed batches which germinated poorly after burial.

(bb) *Germination at alternating 15/30°C in the dark*

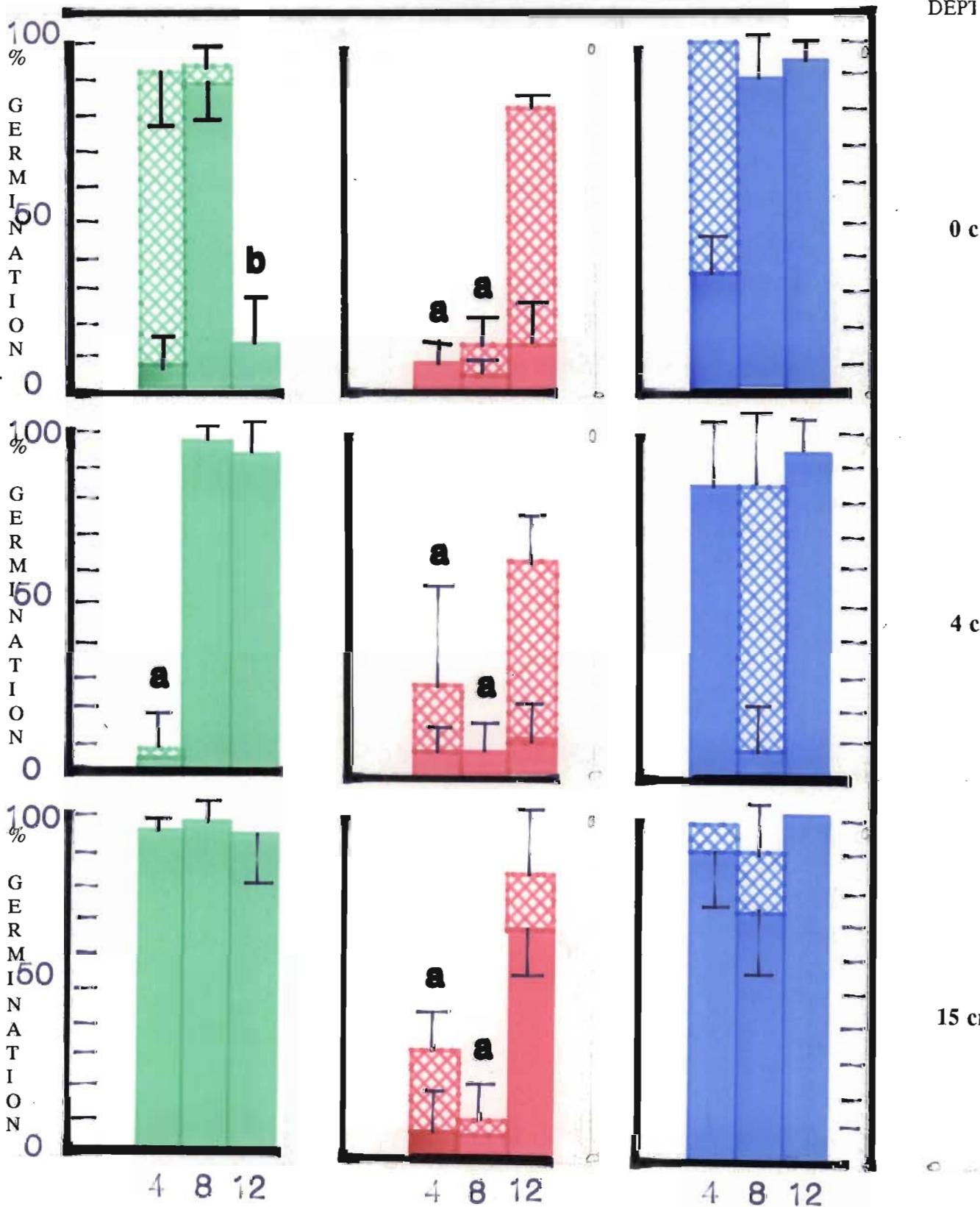
Burial of seeds resulted in highly variable germination. A depth effect was evident, since conditional dormancy was more frequently lost as burial depth increased from 0 to 15 cm (Figure 4.20). A strong seasonal effect was evident, where seeds harvested during February lost conditional dormancy only after prolonged burial at 15 cm compared with the winter harvests where conditional dormancy was consistently broken (Figure 4.20).

Transfer of non-germinated seeds to the light, promoted germination in some batches but not in others. Secondary dormancy occurred in two July 1986 seed batches and six summer seed batches. *This implied that summer-shed seeds will be more difficult to control than winter-shed seeds.*

Application of 500 mg l⁻¹ GA₃ prevented the induction of secondary dormancy in seven out of the eight seed batches mentioned above. The only exception was the seed batch harvested during July 1986, and which had been stored on the soil surface for 12 months. These seeds had lost the ability to respond to GA₃ when incubated in the dark at alternating temperatures following burial (Figure 4.20).

(cc) *Germination at constant 20°C in the light*

Season of harvest and both depth and duration of burial resulted in highly variable germination under these incubation conditions subsequent to burial. A strong seasonal effect was evident, since storage on the soil surface only promoted germination at constant 20°C when seeds had been harvested in July 1986 (Figure 4.21). Burial at a depth of 4 cm broke



DEPTH OF BURIAL (MONTHS)

FIGURE 4.20: The effect of seed burial on germination of bugweed at 15/30°C in the dark. **Experiment 3:** Seeds were collected and buried at Cedara during July 1986 (—), February 1987 (—) and July 1987 (—). Seeds were buried at 0, 4 or 15 cm and exhumed at four-monthly intervals. Shaded portions of each bar represent germination at 15/30°C in the dark after 12 weeks. Hatched portions of each bar represent germination after 12 weeks when seeds were subsequently transferred to the light. Vertical bars represent the standard errors of the means.

a: Represents the seed batches which after burial germinated poorly when transferred from 15/30°C dark to optimum conditions (15/30°C in the light). Application of 500 mg l⁻¹ GA₃ to these seed batches after burial and during incubation at 15/30°C in the dark resulted in high germination percentages after transfer to optimum conditions.

conditional dormancy in July 1987 seeds after 12 months burial, but was effective after only 8 months in seeds harvested during July 1986. Burial at this depth was only slightly effective in breaking dormancy of seeds that were harvested in February (Figure 4.21). Burial at a depth of 15 cm, however, consistently broke conditional dormancy of seed batches collected in July 1986 and February 1987, but was no more effective than burial at 4 cm of seeds harvested in July 1987 (Figure 4.21).

Transfer to the light brought about high germination in seed batches which had been buried at 4 or 15 cm. However, some of the seed batches which had been stored on the soil surface were induced to enter secondary dormancy, depending on season of harvest and duration of storage (Figure 4.21). Application of 500 mg l⁻¹ GA₃ during incubation at 20°C was effective in preventing the induction of secondary dormancy in two out of the three low-germinating seed batches. Seeds harvested in July 1987 did not respond to GA₃ following storage on the soil surface for 12 months (Figure 4.21).

(dd) *Germination at constant 20°C in the dark*

Burial of seeds did not promote germination at constant 20°C in the dark, regardless of season of harvest, depth, or duration of burial. The effect of transferring seeds to alternating 15/30°C in the dark was highly variable. Seeds harvested in July 1986 only responded to this transfer after burial at a depth of 15 cm. Prolonged burial for 12 months at 4 or 15 cm depths caused seeds harvested in February to respond to this transfer while the other seed batches did not. Seeds harvested in July 1987 were initially

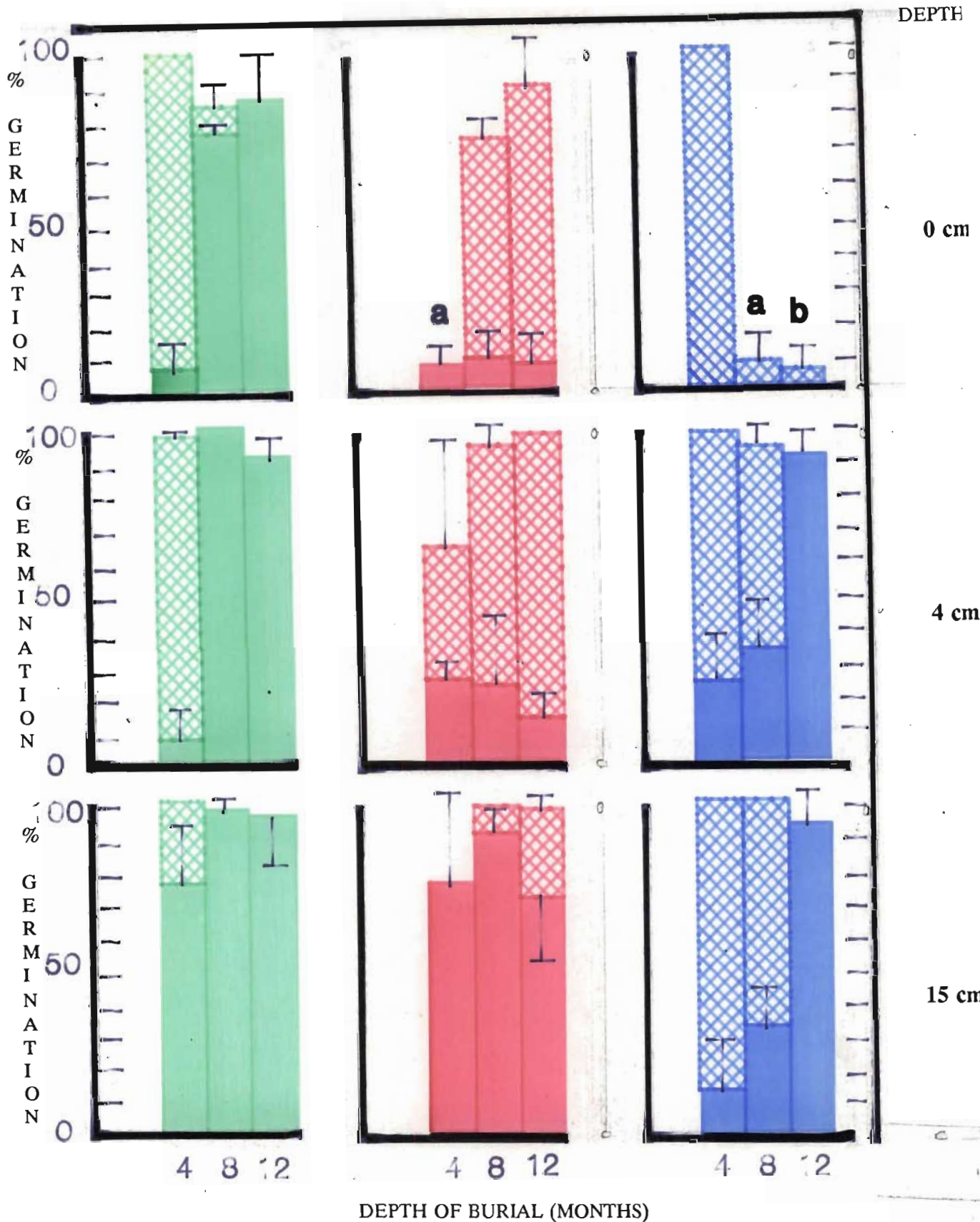


FIGURE 4.21: The effect of seed burial on germination of bugweed at constant 20°C in the light. **Experiment 3:** Seeds were collected and buried at Cedara during July 1986 (→), February 1987 (→) and July 1987 (→). Seeds were buried at 0, 4 or 15 cm and exhumed at four-monthly intervals. Shaded portions of each bar represent germination at 20°C in the light after 12 weeks. Hatched portions of each bar represent germination after 12 weeks when seeds were subsequently transferred to alternating 15/30°C. Vertical bars represent the standard errors of the means.

a: Represents the seed batches which after burial germinated poorly when transferred from 20°C in the light to optimum conditions (15/30°C in the light). Application of 500 mg l⁻¹ GA₃ to these seed batches after burial and during incubation at 20°C in the light resulted in high germination percentages after transfer to optimum conditions.

responsive to this transfer, but after storage for 8 or 12 months a depth effect was evident (Figure 4.22).

A second transfer to the light also resulted in variable seed germination. Secondary dormancy was induced in five seed batches, and this was not always prevented by the application of $500 \text{ mg l}^{-1} \text{ GA}_3$ to seeds during their initial incubation period at 20°C in the dark. The two seed batches which had been stored on the soil surface failed to respond to this treatment (Figure 4.22).

From the above set of experiments, it was evident that germination of bugweed seeds is highly variable after burial, and is influenced by season of harvest and burial, and also both depth and duration of burial. Seeds harvested in summer apparently had a higher level of conditional dormancy than those harvested in winter. However, germination characteristics also varied with year of harvest, thus seeds shed in two consecutive winters showed different dormancy characteristics.

Secondary dormancy was evident in some seed batches from all harvest periods, thus seeds shed at Cedara throughout the year will be difficult to control due to sporadic emergence. Secondary dormancy apparently declined with increased depth of burial. The application of a growth regulator (GA_3) to seeds during incubation at unfavourable conditions did not always prevent seeds entering secondary dormancy, since germination no longer occurred when seeds were transferred to optimum conditions.

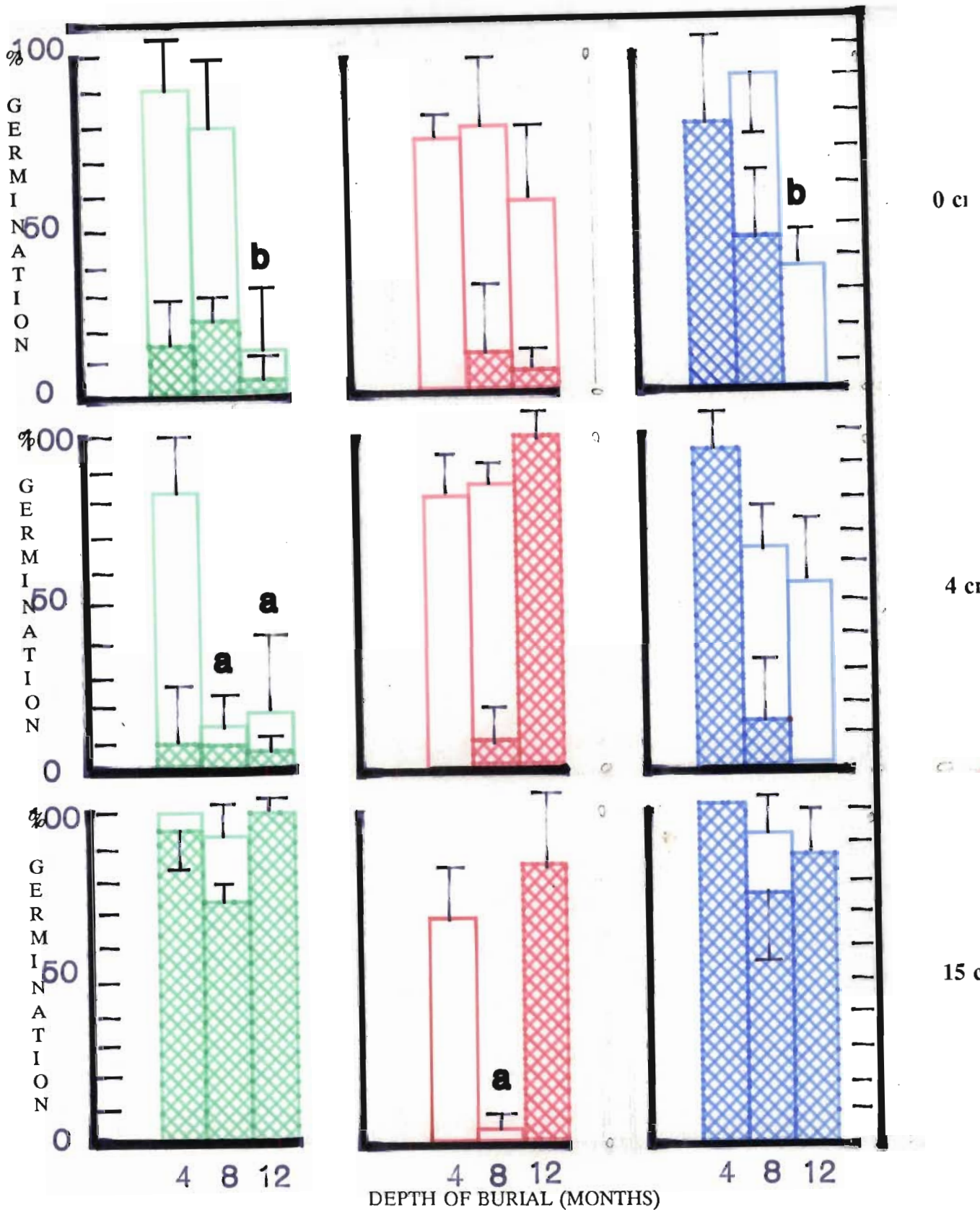


FIGURE 4.22: The effect of seed burial on germination of bugweed at constant 20°C in the dark. **Experiment 3:** Seeds were collected and buried at Cedara during July 1986 (→), February 1987 (←) and July 1987 (⇐). Seeds were buried at 0, 4 or 15 cm and exhumed at four-monthly intervals. Shaded portions of each bar represent germination at 20°C in the dark after 12 weeks. Hatched portions of each bar represent germination after 12 weeks when seeds were subsequently transferred to alternating 15/30°C in the dark. Unshaded portions represent germination after 12 weeks when seeds were transferred a second time, to the light. Vertical bars represent the standard errors of the means.

a: Represents the seed batches which after burial germinated poorly when transferred from unfavourable conditions to optimum conditions (15/30°C in the light). Application of 500 mg l⁻¹ GA₃ to these seed batches after burial and during incubation at 20°C in the dark resulted in high germination percentages after transfer to optimum conditions.

D. CONCLUDING DISCUSSION

(i) PREDATION

From the size and spacing of the bite marks on the terylene bags, it is believed that rodents were responsible for loss of seeds stored on the soil surface at Cedara and Ukulinga but not at Umdoni Park. The fate of these seeds is not known (*i.e.* whether predation or dispersal occurred).

(ii) VIABILITY

The results obtained showed clearly that viability of bugweed seeds is determined by both the site and depth of burial. The depth effect on viability is probably one of insulation. Seeds are protected from the fluctuating environmental conditions which occur at the surface of the soil.

The site effect is surprising, since seeds stored on the soil surface at Ukulinga were subjected to much drier conditions than those at Cedara or Umdoni Park, yet this was the only site where surface-stored seeds retained viability. This could be the result of the halting or slowing of the activity of micro-organisms at the drier locality.

(iii) *IN SITU* GERMINATION

There is an apparent anomaly between loss of conditional dormancy in soil-stored seeds, and germination within bags in the field. The removal of seeds from bags allowed a high percentage germination under incubation conditions similar to those observed in the field, thus the bags themselves must in some way have inhibited germination in the field.

There are at least two authors who have reported the same phenomenon. VAN ASSCHE & VANLERBERGHE (1989) found that in their experiments with bagged seeds of *Rumex obtusifolius*, the germination of buried seeds in the field was lower than germination obtained in petri dishes. Seeds exhumed in summer and autumn germinated under temperature conditions similar to those occurring in the field, while germination in the soil was absent or negligible. They cited the theory of WESSON & WAREING (1969) that some inhibitor is accumulated during burial and the work of HOLM (1972) who found certain inhibitory volatile metabolites are produced by buried seeds. BASKIN & BASKIN (1989) reported that germinated *Capsella bursa-pastoris* seeds "were found in the bags of exhumed seeds on only one occasion ... less than 1 % of the seeds had germinated. Also, the maximum germination of seeds buried in nylon bags in the glasshouse ... was only 4 %... ". " Thus it appears that very few seeds germinate during burial and that some factor(s) of the burial environment, in addition to darkness, may be preventing germination of buried seeds".

In the case of bugweed seeds, a preliminary trial indicated high germination percentages when seeds were contained in bags and incubated under optimum conditions (Plate 12A). The terylene bags used in the present study were completely permeable to water and air, and it is therefore unlikely that gaseous or water-soluble inhibitors accumulated in the vicinity of the seeds. When seeds were stored on the soil surface in bags they did not germinate *in situ*, thus it was also not an interaction between the bags and soil which caused inhibition of germination. Since

terylene is fully polymerized in its final form and may therefore be considered inert when in contact with most aqueous media, it must be an artificial physical restraint due to the bag, which is "sensed" by the seeds in the field situation and which prevents their germination. Thus during periods when conditional dormancy is broken in the field, a "restriction dormancy" which is probably physical and not chemical in nature, and which was not seen under optimum conditions in the laboratory, might be responsible for inhibiting germination. Further investigations of this phenomenon could reinterpret conclusions drawn from many burial trials reported in the literature, which used nylon or terylene sachets (sometimes two layers) for ease of exhumation of seeds.

(iv) **BURIAL AND DORMANCY**

As described by BASKIN & BASKIN (1987), a *fully dormant* seed may come out of dormancy and exhibit a continuum of germination responses, gradually acquiring the capacity to germinate over a wider and wider range of conditions until it becomes *fully non-dormant*, in which state it can germinate over the widest range of conditions possible for that seed. This transitional state between dormancy and non-dormancy is known as *conditional dormancy*, where germination may occur only over a narrow range of conditions.

(a) **Depth of seed burial and conditional dormancy**

When considering storage in the soil, conditions of burial differ with depth in terms of moisture, carbon dioxide, oxygen tensions, and temperature. In a recent review, TAYLORSON (1987) stated that since alternating

rather than constant temperatures are more favourable for germination in many species, the more frequent and larger oscillations near the surface play a significant role in both changes in relative (conditional) dormancy and initiation of germination. TAYLORSON (1987) concluded that the extremes of temperature and water stress encountered near the surface must interact strongly with levels of relative dormancy. Thus, seeds stored near the soil surface are more frequently changing their status of dormancy than are those buried deeper in the soil (TAYLORSON, 1987).

In the first experiment, it was clearly seen that depth of seed burial influenced germination under conditions not normally conducive to germination. In general, burial at a depth of 15 cm was consistently more effective in reducing the level of conditional dormancy in seeds than burial at a depth of 4 cm. A similar depth effect was reported for another solanaceous weed, *Datura stramonium* L. (REISMAN-BERMAN, KIGEL & RUBIN, in press).

Burial site was also seen to have an effect, since burial at Umdoni Park generally resulted in higher germination percentages following burial than burial at either of the two Natal inland sites. Furthermore, germination was promoted at constant temperatures in the dark, a situation normally highly inhibitory to germination of *Solanum* species.

Additional confirmation of the above results was seen in the second experiment. Thus burial of seeds collected during summer in 1987 at three sites widely different in climate altered their dormancy characteristics

following burial. Increased depth of burial resulted in a more effective and consistent reduction in level of conditional dormancy in seeds. This effect was more pronounced for seeds buried at Umdoni Park than after seed burial at the two inland sites. It was evident, therefore, that the dispersal environment of seeds exerts a marked effect on seedling emergence.

At Umdoni Park, burial of seeds at 4 cm was seen to be more effective in breaking conditional dormancy than at the other two sites. The effect here probably relates to the mildly fluctuating temperatures and near-constant water status of the coastal environment. A far more pronounced depth effect was evident at the two inland sites, since storage at 4 cm was much less effective than storage at 15 cm in breaking conditional dormancy. Here, fluctuating climatic temperatures will be "sensed" by seeds buried at 4 cm as being potentially harsh, thus a larger percentage of seeds are protected by remaining conditionally dormant.

Seeds stored at 15 cm are more insulated from climatic fluctuations and therefore do not receive the same "strength of signal" as that of seeds stored at 4 cm, thus a higher percentage lose conditional dormancy but are still inhibited (by some or other factor) from germinating in the field.

Storage on the soil surface results in full exposure of seeds to all elemental changes and was found to be least conducive to breaking conditional dormancy. This is interpreted as being a strong protective mechanism for seeds to survive potentially harsh environmental conditions.

Attempts to relate the breaking of conditional dormancy with soil temperature data indicated no apparent direct relationship between monthly temperature maxima or minima and percentage seed germination at any of the sites (Figure 4.10), since peaks of germination were displaced with respect to either seasonal peaks or drops in temperature. From this it was concluded that factors other than seasonal changes in temperature were also involved in determining the dormancy characteristics of seed.

REISMAN-BERMAN, KIGEL & RUBIN (in press) reported that seed burial had a dual antagonistic effect on the seeds of *Datura ferox* and *D. stramonium*. While increasing depth of burial inhibited germination and emergence, at the same time it also alleviated dormancy, apparently by accelerating after-ripening processes. A similar inhibition of germination by burial has been reported in other species (KARSSSEN, 1982). This inhibition has been attributed to the lack of promotive factor(s) such as light and/or temperature fluctuations at increasing depths in the soil (BEWLEY & BLACK, 1982; KARSSSEN, 1982). Other factors which may cause inhibition of germination in deeply-buried seeds are the presence of volatile metabolites such as acetaldehyde, ethanol or acetone (HOLM, 1972), ethylene or non-volatile allelopathic compounds, or high levels of CO₂ resulting from biological activity (KARSSSEN, 1982).

Bugweed seeds which had been buried at Umdoni Park lost conditional dormancy and subsequently showed high germination at constant 20°C in the dark. However, they did not germinate in the field. Reasons for this are obscure. The build-up of volatile or non-volatile compounds is not

thought to be a likely occurrence in the very sandy soils of this region, and, under laboratory conditions, high levels of CO₂ did not inhibit germination. Thus some other factor(s) inhibited germination of seeds buried at 15 cm at Umdoni Park. One factor may even be artificial, due to the physical restrictive presence of the terylene bags containing the seeds (discussed earlier).

The interaction of several factors which influence germination is well recognized in the literature, and workers frequently investigate relationships between germination and temperature under conditions where variation in other factors is eliminated. For example, BASKIN & BASKIN (1989) found good correlation between mean monthly temperature and germination of *Capsella bursa-pastoris* buried in clay pots which were watered to field capacity each week. Once the variation in moisture had been eliminated, significant correlations between temperature and dormancy were achieved.

However, conditions in the field situation are the sum of many interacting factors such as those described above, and these cause variation in dormancy characteristics. This variation in dormancy which occurs especially at the Natal inland sites, results in a sporadic seedling emergence which greatly adds to the difficulty of controlling this species.

(b) Depth of seed burial and secondary dormancy

When seed was buried at the site of harvest, storage at 15 cm did not induce secondary dormancy at any of the sites, and a low germination

response to unfavourable incubation conditions was attributed to conditional dormancy. This could be broken when seeds were transferred to optimum conditions. The ecological significance of this observation would be that once conditions improve, such as may occur after soil disturbance, then non-germinated seeds at this depth which have retained their conditional dormancy, are fully able to germinate.

On the other hand a strong site effect was shown by storage at 4 cm. The occurrence of secondary dormancy, induced by unfavourable conditions after burial, was more frequent at Cedara than at Ukulinga. This contrasted with the absence of secondary dormancy seen after seed burial at Umdoni Park. Thus *seeds stored at Cedara enter secondary dormancy most readily and will therefore be more resistant to control by the application of herbicides by virtue of a delayed emergence*. Seeds buried at Umdoni Park did not enter secondary dormancy, again reflecting an environment where harmful climatic changes seldom occur. Conditionally dormant seeds at Umdoni Park will therefore readily germinate when conditions are more conducive to germination.

A similar site effect was evident for seeds stored on the soil surface, with a high occurrence of secondary dormancy at Cedara, and very little at Umdoni Park. Thus depth of burial affects frequency of secondary dormancy, an effect which was especially evident at Cedara. Seeds on the soil surface are likely to be exposed to very strong climatic signals and show a strong tendency to enter secondary dormancy. Shallow burial insulates seed from climatic variation to a certain degree, leading to

weaker signals and a smaller incidence of secondary dormancy. Finally, the greater insulation of seeds at a 15 cm depth leads to a failure of seeds to enter secondary dormancy. *Thus, as for conditional dormancy, amplitude of diurnal fluctuation is probably a strong triggering mechanism for entry into secondary dormancy.*

Where seeds harvested from one site were buried at three sites, a strong depth effect was generally evident at each of the sites, with secondary dormancy occurring more often after seeds had been stored on the soil surface. This was much reduced with increasing depth of burial. A site effect was also evident, with a much rarer occurrence of secondary dormancy at Umdoni Park than at Cedara. Thus site differences seen in the first burial experiment were due to the dispersal environment, and not to genetic differences between populations.

(c) Season of harvest and dormancy potential

Depth effects were evident, regardless of the time of year the seeds were harvested and buried, thus loss of conditional dormancy increased with depth of burial. Seeds harvested in summer were subjected to declining temperatures during burial, and this resulted in a lower germination potential (or a higher dormancy potential) than in those harvested during winter months. However, seeds harvested during two consecutive winters did not show similar dormancy characteristics. This suggests there are other factors which differ sufficiently between years, apart from increasing temperatures, to cause differences in germination. Factors such as tree size, position of seeds within the fruit, position of fruits on the

inflorescence and on the trees, and the effect of neighbouring species may be some of the biotic factors which account for these differences. In addition to this, a highly complex light, temperature and soil water regime exists under pine trees, and this can be expected to change on a yearly basis. No doubt these changes also account partially for the results obtained in this study.

It is interesting to note that the incidence of conditional dormancy was much higher than that of secondary dormancy, irrespective of site or time of burial. This indicates that bugweed is a very successful pioneer species, rapidly colonizing areas where conditions have improved, such as after clear-felling or soil disturbance along roadsides. The species is also variable in its ability to enter secondary dormancy, thus not all seeds will germinate once conditions have improved and a sudden reversion to unfavourable conditions would not eliminate the whole population.

(d) **GA₃ and dormancy**

The effect of GA₃ on breaking conditional dormancy or preventing induction of secondary dormancy after seed burial was variable. Implications of this in the field situation are that the application of GA₃ (or products causing similar responses to those induced by GA₃) will not stimulate germination of all seeds. Seeds stored under certain conditions will retain their conditional dormancy or their ability to enter secondary dormancy with a consequent delayed germination. Sporadic emergence will allow the reinfestation of a "chemically controlled" area.

(e) **Implications for control of bugweed**

QUINN & COLOSI (1977) posed the question "are ecologically significant results obtained from comparing germination responses of seeds produced under artificial conditions?" These authors believe that comparisons between germination responses of seed populations produced only in uniform environments ignore the "strategic genetic-environmental interactions which occur in a species-population's natural habitat, and, in reality, determine any adaptive germination responses." Furthermore, "it will be difficult (sometimes impossible) within time- and space-limited experimental designs to unravel all the complexities of the genotype-environmental interactions determining germination responses within and among populations."

In these studies on bugweed, the influence of the maternal environment during seed development, the genotype of the seed, and the local environment after shedding have all been implicated in determining dormancy characteristics of seeds. Dormancy is highly variable in bugweed seeds, being dependent on depth and time of burial, and this can also change completely when seeds are dispersed to a different site. This interaction of the genome with the environment, resulting in variable germination, is of utmost importance when considering control of the species. For example, most pre-emergent herbicides are not persistent enough to be effective, since a significant proportion of late-emerging seedlings would escape treatment. In addition to this, high concentrations of such herbicides, or more persistent herbicides, may be phytotoxic to pine trees, the situation in which bugweed is economically most costly.

Furthermore, use of chemicals similar to GA₃ to stimulate germination would not only be uneconomic, but would also be ineffective against a small but significant proportion of seeds, which would retain their dormancy. Thus, other alternative options for long-term control of species via manipulation of seed must be considered in the case of bugweed.

E. SUMMARY & CONCLUSIONS

1. Seed viability declines with time of storage on the soil surface. This is influenced by site of storage.
2. Seed viability is retained during two years burial at 4 or 15 cm depths, regardless of site of burial.
3. Predation of seeds during winter occurred at Natal inland sites but not at Umdoni Park. Predation only occurred on the soil surface.
4. For seeds harvested and buried in the same vicinity as the parent trees, depth of burial greatly influenced both loss of conditional dormancy and induction of secondary dormancy.
5. Seeds harvested at one site may change both their conditional and secondary dormancy characteristics, depending on dispersal site.
6. Seeds harvested at one site during different times of the year show different dormancy characteristics, depending on duration and depth of burial.
7. While temperature was shown to play a crucial role in determining dormancy characteristics of seeds, other factors were also important.

8. Application of GA₃ to seeds does not always break conditional dormancy after burial and also does not always prevent seeds from entering secondary dormancy. Use of GA₃ or other plant growth regulators is contra-indicated in view of the varied response of seeds and short soil lifespans of such chemicals.

CHAPTER 5

CONTROL OPTIONS FOR BUGWEED

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CHAPTER 5

CONTROL OPTIONS FOR BUGWEED

A. INTRODUCTION

(i) INADEQUACY OF CURRENT CONTROL MEASURES

(a) Importance of the soil seedbank

Current control measures for bugweed generally involve treatment of mature trees. Mechanical ringbarking, where bark is stripped away from the lower portion of stems, or the application of chemicals to cut stumps, coppice or basal stems of trees have all been recommended for controlling this species (LITTLE, 1980; KVITZAU & DARROW, 1983). Despite these measures, bugweed is still a serious invader weed in Natal forestry, veld, roadside and streambank habitats (HENDERSON, 1989) and is continuing to expand at a rate of approximately 16 % per year (LE ROUX, 1984). This is principally due to the attributes of the seed component:

1. Seeds of *Solanum* species may be long-lived, for example *S. nigrum* L. seeds buried at a depth of 8" (approximately 20 cm) showed 82 % germination after 30 years (TOOLE & BROWN, 1946). This may also be true of bugweed seeds, under favourable storage conditions.
2. High numbers of viable seeds are produced throughout the year (7.2 million per hectare over 20 months) (Chapter 1).

3. Seeds survive ingestion by birds and animals and are dispersed by these agents (Chapter 1).
4. Germination requirements are highly variable and factors such as locality, season and year of collection (Chapter 3) and depth, locality and duration of burial (Chapter 4) influence dormancy and the germination response. All these factors contribute to sporadic seedling emergence from the soil seed bank. Assuming then that chemical or mechanical control of the vegetative phase is effective, the soil seed bank and fruit production of the weed species must be considered for any long-term control programme to be successful.

(b) **Reduction of the soil seed bank**

(aa) *Destruction of buried seeds*

To eradicate a soil seed bank, two approaches have generally been considered, either to kill the seeds or, alternatively, to stimulate all seeds in the soil to germinate. In the crop situation, incorporation of pre-emergent herbicides into the soil have been used to control several *Solanum* species. Although these may not affect the seeds in the soil *per se* they do apparently reduce emergence and survival of weed seedlings.

Many pre-emergent herbicides have been found effective in the control of *Solanum* species. A major weed, *Solanum nigrum*, has been successfully controlled in various crops with presowing applications of 5-ethyl dipropylthiocarbamate (EPTC), dinitramine, pendimethalin, fluorochloridon, chloridazan, metribuzin, alachlor, acetochlor, metachlor, and linuron (CARRINGER & BRYANT, 1980; REGEV, KLEIFELD, BARGUTTI & JUNIS, 1982; POTTER & SMITH, 1982/83; EVSIKOV,

1983; HINDERSMANN, PEREIRO & NOHYNEK, 1984; ORSI, VECCHIO, CASINI & ROCCA, 1986). As seedlings emerge, these herbicides are taken up through the roots and then kill the young plants.

There are however, a number of drawbacks to such treatments. The efficacy of soil herbicides varied considerably between years (VANPARYS, HIMME & VAN HIMME, 1986) and with locality (HOPKINS, STROUBE, KROETZ & FURRER, 1980). Late emerging plants of *S. nigrum* escape herbicide treatment, thereby necessitating sequential treatments (ROUAS, 1981; HIMME, BULCKE, STRYCKERS & VAN HIMME, 1986). Too high a concentration of herbicide can harm the crop, for example, tomato yields were reduced (WELLER, 1984), pine seedlings were stunted (METCALFE, 1985), growth of wheat and radish was reduced (DEVLIN & KOSGANSKI, 1986), and maize was adversely affected by a build-up in the soil of residues of atrazine (RAPPARINI, 1986). Combinations of herbicides are frequently required to overcome hard-to-kill/herbicide-resistant *Solanum* species. Weeds previously sensitive may develop a resistance to herbicides, for example *S. nigrum* was eventually not controlled by triazine herbicides alone (ROUAS, 1981; GARCIA-BAUDIN & AGUIRRE, 1983; HIMME, STRYCKERS & BULCKE, 1983; BARLOW & HICKS, 1985; RAPPARINI, 1986; JACOBS, DUESING, ANTONOVICS & PATTERSON, 1988). The use of selective herbicides to kill some weeds can allow encroachment by other weeds, for example, *Senecio vulgaris* L. and *Polygonum persicaria* L. spread rapidly after control of *Solanum nigrum* in the vineyards of Champagne (ROUAS, 1981) and *Solanum* species have become major weeds in cotton,

beans, onions, tomatoes, garlic, potatoes, peppers and melons in California after summer grasses and other weeds had been controlled by selective herbicides (KEMPEN & BELLUOMINI, 1985).

Alternatives to synthetic herbicides have been suggested by various researchers. For example, PUTNAM (1986) suggested that research on allelochemicals which impose allelopathic influences¹, could lead to a new generation of pesticides and plant growth regulatory chemicals. Allelochemicals may be released as volatiles, as exudations from roots, or leached by rainwater from leaves or stems. Dead or decaying plant material may provide a major source of allelochemicals (PUTNAM, 1984). These allelochemicals may be inhibitory to growth of seedlings of the same species, for example, aqueous extracts of fresh fir needles (*Abies alba* Mill.) inhibited seed germination and growth of young seedlings. Such a mechanism may be important in natural regeneration of the species (BECKER & DRAPIER, 1984). However, this is not always the case, for example, sprouting leaves of *Leucaena leucocephala* (Lam.) De Wit (Syn. *L. glauca* (L.) Benth.) contained products which inhibited radicle growth in certain crops but not in *Leucaena* seedlings (KUO, CHOU & HU, 1983/84). In addition to this, soil beneath the canopy of the parent plant may be allelopathic to other species. This was seen in *Eucalyptus tereticornis* Sm. (BHASKAR, 1983), *Pinus ponderosa* Dougl. (LODHI & KILLINGBECK, 1982), *Azadirachta indica* A. Juss (HUSSAIN, HAQ & ANJUM, 1985), *Acacia dealbata* Link. (REIGOSA, CASAL &

¹ Allelopathy is characterized by a reduction in plant emergence or growth, reducing performance of at least some individuals in the association (PUTNAM, 1988).

CARBALLEIRA, 1984) and *Datura stramonium* L. (LOVETT, LEVITT, DUFFIELD & SMITH, 1981).

Compounds extracted from plant material which are responsible for this allelopathy are frequently phenolics, for example, from leaves of *Leucaena leucocephala* (KUO, CHOU & HU, 1983/84), or *Lantana camara* L. (JAIN, SINGH & DEZMAN, 1989) or are tropane alkaloids from seeds, foliage and soil under *Datura stramonium* (PUTNAM, 1988).

Usually, authors have attributed the reason for the release of these allelochemicals to increasing the competitive advantage of the species. These agents reduce the vigour or prevent germination of other species. Although allelopathy may be undesirable, DIONELLO-BASTA & BASTA (1984) suggested that natural inhibitors of seed germination, present in weeds such as *Solanum paniculatum* L., might be used as alternatives to synthetic herbicides. However, PUTNAM (1988) concluded in his review that "it seems doubtful that higher plant products will be active enough to be packaged and sold as herbicides unless some unique compounds are discovered that are more active than those now known." Rather, natural products may provide clues to new herbicide chemistry. "By modifying these natural products, the end products could be more active, selective, or persistent." The author proposed that future research could involve the genetic manipulation of plants to increase their yields of useful secondary metabolites.

An entirely different approach to weed control has been exposure of weed seed infested soil to either solar or microwave radiation. Solar heating of soil is a new method of disinfestation, developed in Israel and tried or adapted in at least 13 countries since 1976 (KATAN, 1983). This can be very successful in some cases, for example, shallow-buried seeds of *Digitaria sanguinalis* Scop., *Malva parviflora* Huds., *Echinochloa crus-galli* Beauv., *Chenopodium album* L., *Amaranthus retroflexus* L. and *Solanum nigrum* were controlled by increased temperatures due to solarization (ELMORE, 1983). KATAN (1981) suggested that the possible mechanisms of weed control due to solarization were direct killing of weed seeds by heat; indirect microbial killing of non-dormant seeds weakened by sublethal heating; killing of non-dormant seeds stimulated to germinate in the moistened soil; and killing of germinating seeds whose dormancy was broken in the heated soil.

Microwave energy has been used to kill both dormant and non-dormant seeds present in the soil. This can be accomplished at a single pass (PATAY & LONNE, 1981). However, there are a number of disadvantages; efficacy is affected by exposure time, microwave energy level and moisture content of the seed (LAL & REED, 1980), soil temperature and humidity (PATAY & LONNE, 1981), soil type (MOOSMAN, KOCH, WELKER & HABERMEHL, 1986), and depth of burial (ELMORE, 1983). LAL & REED (1980) concluded that field application of microwave energy was not practical because of the very high energy cost.

Similarly, use of fumigants for total sterilization would not be practical in many situations. Problems associated with fumigation of the soil include reaching the inoculum and eradicating it effectively at the desired depth, reinfestation, detrimental effects on non-target and beneficial organisms, residual effects on the plant, application hazards and costs. These factors are all prohibitive and make soil fumigation on a large scale both impractical and uneconomic.

(bb) *Stimulating germination of buried seeds*

Rather than attempting to elicit seed death, one of the most promising approaches to decreasing seed numbers is to find a chemical which will break dormancy in buried seeds (CHANCELLOR, 1984). SAINI, BASSI, GOUDEY & SPENCER (1986) considered that the use of such germination stimulants may lead to a reduction in herbicide usage.

The ability of many annual weeds to persist in a cultivated field, despite intensive efforts to eradicate them, is due in part to the ability of seeds to remain dormant in the soil for many years (HARPER, 1957). This reserve of dormant seeds in the soil has hampered weed control efforts because germination of seeds produced in one season can be spread over several subsequent years. The soil's reservoir of weed seeds could be depleted if dormant seeds in the soil could be forced to germinate by some mechanical or chemical treatment, and the resultant seedlings destroyed by unfavourable environmental conditions, herbicides or tillage.

WESSON & WAREING (1969) maintained that cultivation of the soil is an excellent means of encouraging weed seed germination; it aerates the soil, it brings seeds which have lost their dormancy near the surface and it gives light to many seeds which during burial have acquired a need for light to germinate. This method is, however, not practical in many situations.

Emergence in the field can be stimulated by nitrogen fertilizers (SEXSMITH & PITTMAN, 1963; RADEMACHER & KIEWNICH, 1964; SINYAGIN & TEPER, 1967; HILTON, 1984, 1985b; SAINI, BASSI, GOUDEY & SPENCER, 1986). Another stimulant of great potential is ethylene, which can be injected directly into the soil or applied as an ethylene-generating chemical such as ethephon (2-chloro-ethylphosphoric acid). This has been found effective for a range of arable weeds (EGLEY & DALE, 1970; CHANCELLOR, PARKER & TEFEREDEGN, 1971; HALL & WAREING, 1972; EPLEE & LANGSTON, 1976). Sodium azide was considered the most consistent stimulant of indigenous weed emergence (HURTT & TAYLORSON, 1986), and has also broken dormancy of wild oat seeds (FAY & GORECKI, 1978). Other chemicals are also known to stimulate germination of dormant wild oats seeds under laboratory conditions. These include the respiratory inhibitors azide and cyanide, ethanol, nitrates and nitrites, ethylene, cytokinins and gibberellins.

However, while these chemicals have proven useful in seed physiological studies, their use under field conditions has been limited due to their cost, toxicity and/or rapid inactivation in the soil (UPADHYAYA, HSIAO &

BONSOR, 1986). In addition to this, there may be great variability in the effects of growth stimulants, depending on rate of application, season of application and the post-treatment environment (HURTT & TAYLORSON, 1986).

In Solanaceous species the most effective growth stimulant is generally gibberellin. This is one of the most effective phytohormones in terms of the range of species that is affected (TAYLORSON & HENDRICKS, 1972, 1977). However the use of gibberellins in the field has serious economic limitations. It is interesting to note that several plant growth regulators, not occurring in higher plants, mimic gibberellin effects in inducing dark germination of "Grand Rapids" lettuce seeds. Fusicoccin, a diterpene glucoside from *Fusicoccin amygdali* acts like gibberellins in inducing dark germination in lettuce (LADO, RASI-CAIDOGNO & COLOMBO, 1974), but fails to do so in several other species (ZENG & KHAN, 1984). Unlike gibberellins, it fails to remove secondary dormancy in lettuce and other seeds (KHAN, 1980/81; KHAN & SAMIMY, 1982). The effect of cotylenins, isolated from *Cladosporium* species (SASSA, TOGASHI & KITAGUCHI, 1975) closely resembles that of fusicoccin (BALLIO, 1977; KHAN & SAMIMY, 1982). However, these products are presently too costly to be applied to a soil seed bank².

Substituted phthalimides are a new class of plant growth regulator that mimic the growth regulating activity of gibberellin (LOS, KUST, LAMB & DIEHL, 1980a,b; DEVLIN, 1981) and apparently have "intrinsic

²1mg Fusicoccin = \$47.20 (SIGMA, 1990)

gibberellin-like activity" (SUTTLE & SCHREINER, 1982). Such effects include stimulation of shoot growth, peduncle elongation, bolting, change in sex expression, increase in leaf number and stimulation of seed germination in some weeds (UPADHYAYA, HSIAO & BONSOR, 1986). These authors found that these products were also similar to GA₃ in that they were effective at inducing α - amylase production and the release of reducing sugars by de-embryonated endosperm segments of wild oats. However, the germination-stimulating effect was not inhibited by up to 500 mM chlormequat (CCC), an inhibitor of gibberellin biosynthesis, indicating that GA₃ biosynthesis is not required for this effect (UPADHYAYA, HSIAO & BONSOR, 1986).

METZGER (1983) suggested that substituted phthalimides promoted germination of dormant seeds only in species that also respond to treatment with gibberellin. The coded product, AC-94377, was the most effective germination promoter on a weight-to-weight basis. METZGER (1983) therefore concluded that AC-94377 might be useful in the field to promote germination of dormant weed seeds in the soil. However, there is some doubt about this in studies with *Solanum nigrum* L. and *Avena fatua* L. Although *S. nigrum* seeds may be highly responsive to applications of AC-94377, the activity of this product in stimulation of germination was dependent on soil type (BOND & BURCH, 1990). Performance of this class of product also varied with seed biotype. Field populations of *Avena fatua* consist of genetically different biotypes which differ in seed germination behaviour (NAYLOR & JANA, 1976). UPADHYAYA, HSIAO & BONSOR (1986) found that genetically pure

lines of *A. fatua* differed significantly in their germination response to substituted phthalimides, with one line having 100 % stimulation whereas another only had a 50 % germination response. The authors argued that the effectiveness of any germination stimulant in facilitating control of this weed by depleting its seed bank is expected to depend on its ability to stimulate germination of all biotypes comprising a particular population. If biotypes comprising a population differ in their response to germination stimulants, their continued use could increase the proportion of less responsive biotypes, a situation analogous to herbicide resistance development (UPADHYAYA, HSIAO & BONSOR, 1986). Thus use of such chemicals may eventually cause more harm than good by causing the evolution of a "super-weed".

(ii) **UTILIZATION OF FRUITS TO DEplete SEED RESERVES**

One totally different approach to weed control would be the successful use of the plant (including the seeds) for extraction of economically important natural products. This idea is feasible for members of the *Solanaceae* and is inherent in the name, coming from latin "*solacium*" meaning sedative, referring to the glycoalkaloids of the genus. Solasodine, an alkaloid found in over 100 *Solanum* species, is a precursor for corticosteroid hormones (MANN, 1978).

Contraceptive hormones and anti-inflammatory agents derived from corticosteroids are the two major groups of steroids presently manufactured on an industrial scale, with the sex hormones filling a minor role (WESTON, 1976). Total synthesis of steroids is a complex and expensive

process, although there is an ever-increasing degree of industrial research in this field. The continued supply of steroid drugs therefore relies heavily on the availability of naturally occurring raw materials (WESTON, 1976).

In 1967, total world consumption of steroid precursors was 1 000 tons, mainly from diosgenin, which was available in sufficient quantities at low prices (MANN, 1978). Diosgenin is a sapogenin which occurs as a glycoside in the yam *Dioscorea mexicana* Scheiew., a plant indigenous to Central America (MANN, 1978). The aglycone can be converted to pregnadienolone acetate in 70-75 % overall yield, and this product is then used for the manufacture of a wide range of sex hormones, contraceptive drugs and corticosteroids (FIESER & FIESER, 1959). However, supplies of diosgenin are ultimately limited although recent fluctuations in supply have had social and political rather than agricultural causes (MANN, 1978). A search for alternative raw materials has been motivated by various reasons, for example, an unwillingness to rely solely on one supplier, and in many cases, a desire to use internal production to conserve foreign exchange. The supply of diosgenin was not keeping up with the demand and other sources of steroid raw material were sought. Other major naturally-occurring products used for the industrial preparation of steroids are stigmasterol, which is isolated from soya beans and hecogenin from the sisal plant and cattle bile acids, both of which are used solely for the preparation of corticosteroids (MANN, 1978).

Two other steroidal alkaloids that might become available in large quantities are tomatidine and solanidine, from tomato and potato waste

respectively. However, although an enzymatic conversion of tomatidine to allopregnoenolone has been described by HEFTMANN & SCHWIMMER (1972), neither of these materials are well suited to chemical conversion to steroid drugs. Hecogenin is a byproduct from sisal hemp production, and Brazilian production of paper from sisal might make large quantities available (MANN, 1978). Cholesterol from wool grease and sitosterol from soy oil have been used to make diuretic and contraceptive drugs by microbial fermentation (MANN, 1978).

Solasodine, a nitrogen isomer of diosgenin is in a strong competitive position for steroid conversion (MANN, 1978). This alkaloid can be degraded to pregnadienolone acetate in *ca* 64 % overall yield by a process almost identical to that degrading diosgenin (WESTON, 1976). Solasodine is therefore a valuable raw material for steroid synthesis and much research has been directed towards evaluating its potential as a substitute for diosgenin in synthesis of steroid hormones (SCHREIBER, 1968).

An intensive phytochemical survey of the genus *Solanum* was therefore undertaken to determine candidate species for extraction of alkaloids (SCHREIBER, 1968; SETH & CHATTERJEE, 1969; BRADLEY, COLLINS, CRABBE, EASTWOOD, IRVINE, SWAN & SYMON, 1978). Nearly 100 different *Solanum* species were reported to contain solasodine. Only a handful of these species, however, have been considered for commercial production of solasodine. Workers in Hungary, Russia, Bulgaria, Czechoslovakia, Yugoslavia, Poland, Rumania, China and India studied the cultivation and extraction of the solasodine-rich species of *S.*

aviculare Forst. and *S. laciniatum* Ait., which are natives of Australia and New Zealand. Commercial production of solasodine was achieved in Russia and in Hungary in the 1960s. Other species of *Solanum* which have been investigated as commercial sources of solasodine include *S. khasianum* C.B. Clarke and the North African *S. marginatum* L.F. The European species *S. dulcamara* L. has a variable solasodine content. Potentially useful amounts of solasodine have also been reported in *S. incanum* L., *S. indicum* L., *S. trilobatum* L., *S. elaeagnifolium* Cav. and *S. platanifolium* Sendt. (BRADLEY, COLLINS, CRABBE, EASTWOOD, IRVINE, SWAN & SYMON, 1978).

The solasodine content of the plants is generally highest in green fruits, for example in *S. aviculare* and *S. laciniatum* (MOURSİ & AHMED, 1973a; LANCASTER & MANN, 1975) and *S. khasianum* (MILLER & DAVIES, 1979). In most *Solanum* species, ripe fruits contain very little alkaloid. Exceptions are *S. khasianum*, *S. aculeatissimum* Jacq., *S. elaeagnifolium*, *S. trilobatum*, *S. giganteum* Jacq., *S. torvum* Schlecht., *S. xanthocarpum* Schrad., *S. plantanifolium*, *S. marginatum* and *S. mammosum* Herb. (MANN, 1978). Some fruits, for example, those of *S. aculeatissimum*, may have a high (3-4.6 %) solasodine content but sparse fruiting is associated with this high concentration (MANN, 1978). The location of the glycoalkaloid in the extracellular mucilage may protect against the loss of solasodine that commonly occurs in other species during fruit ripening, since in most other species the alkaloid is intracellular.

Solasodine may also be present in vegetative parts of the plant at moderately high levels, for example, in both *S. aviculare* and *S. laciniatum* (MANN, 1978) but not in *S. khasianum* (MILLER & DAVIES, 1979) and can be higher in immature vegetative parts, for example young leaves, than in mature leaves (MOURSI & AHMED, 1973a; LANCASTER & MANN, 1975).

Solasodine yields vary with climate and soil conditions (MOURSI & AHMED, 1973b; LANCASTER & MANN, 1975). For example, long-day photoperiods favoured accumulation and short day photoperiods inhibited the formation of solasodine. Reduced light intensity and longer wavelength (700 nm) decreased solasodine content whereas both short wavelengths (400-490 nm) and water stress enhanced solasodine content. Planting distance also affected total yield of solasodine (BHARATI & CHATTERJEE, 1986). The alkaloid composition of *S. dulcamara* varied with geographical origin (MÁTHÉ & MÁTHÉ, 1979). THEOPHRASTOS mentioned that herb gatherers of his time, in the 4th Century B.C., prescribed that "some roots should be gathered at night, others by day and some before the sun strikes on them" (cited in ROBINSON, 1974). This folk lore has been confirmed by recent work, as diurnal fluctuations of various alkaloids have been found. For example, atropine in *Atropa belladonna* L., total alkaloids in *Conium maculatum* L., and morphine, codeine and thebaine in *Papaver somniferum* L. (ROBINSON, 1974).

In most parts of the world, solasodine-producing plants are cultivated (*i.e.* they are not present as invader weed species) and much research involves

optimising conditions for maximum solasodine production. Much effort to increase solasodine yields by chemical means; for example, several combinations of gibberellins and chlormequat or nicotinic acid (MANN, 1978), or 2,4-D (KADKADE, 1984) have been tested. Cultivation of a solasodine-producing species is fraught with pitfalls. For example, use of insecticides is necessary to protect against pests such as the Colorado beetle and the eggplant caterpillar (MANN, 1978). Much research has been devoted to the use of fertilizers to increase alkaloid content, for example in *Rauwolfia serpentina* (Linn.) Benth. ex Kurz (MAHESHWARI, YADAV, GANGRADE & TRIVEDI, 1988). However results may not be consistent, for example, a correlation between the nitrogen level and alkaloid content of *Datura innoxia* Mill. and other Solanaceae was found (NOWACKI, JURZYSTA & GÓRSKI, 1975; AFRIDI, WASIUDDIUN & KHALIQUE, 1977) while in other experiments, the nitrogen level only effected the crop yield of *Datura innoxia* or *Datura stramonium* (STECKA, MRUK-LUCZKIEWICZ & WILL, 1975; RUMINSKA & EL GAMAL, 1978).

S. khasianum is a native of India. This species is spiny and therefore there is a significant limitation for its extensive cultivation. Spineless mutants have been obtained but with poor fruiting and reduced solasodine content (BHARATI & CHATTERJEE, 1986). Serious attempts have been made to reduce spininess, for example, by application of high doses of gibberellins, colchicine or gamma irradiation (MANN, 1978). Because of these problems, *S. laciniatum*, a native of New Zealand and Australia, was introduced into India as an alternative source of solasodine. This species

was more desirable for cultivation than *S. khasianum* because of its non-spiny character and because all the plant parts contain solasodine (BHARATI & CHATTERJEE, 1986).

In his review article, MANN (1978) comments that yield estimates of solasodine are generally unreliable because they are based on small-plot tests or else are unpublished because they are proprietary and confidential. *S. laciniatum* yielded 10 - 15 kg ha⁻¹ in Hungary but in Russia it was three times more. Other trials reported yields of 65 kg ha⁻¹. Small-plot trials of *S. aviculare* in New Zealand gave 89 - 197 kg ha⁻¹ the first year and 190 - 356 kg ha⁻¹ subsequently. *S. khasianum* yields were estimated at 431 kg ha⁻¹. MANN (1978) considers most of these estimations are overhigh, and are based on results from small-plot trials.

The aim of this chapter was to explore the available options for reducing the seed population of bugweed. This included reduction of the soil seedbank and fruit production on trees. This latter approach involves utilization of fruits and seeds for alkaloid production. A system of harvesting fruits for utilization is proposed which is geared to obtaining revenue for the control of this species.

B. MATERIALS AND METHODS

Seeds and fruit used in these studies were collected from bugweed trees growing under a pine canopy at Cedara State Forest (Figure 1.6). Sinking seeds which were "apparently viable" (ROBERTS, 1981) were used for all germination experiments.

(i) ANALYSIS OF RESULTS

Where applicable, analysis of variance was done using the Genstat 4 program and then the least significant differences (LSD) between means was calculated (RAYNER, 1967; Chapter 10).

(ii) REDUCTION OF THE SOIL SEED BANK

(a) Moist heat germination

Viable seeds were immersed in water at 60, 70, 80, 90 or 100°C for varying periods of time. These were 0, 0.5, 1, 2, 5, 10 or 15 minutes. After each time interval, the heated water was discarded, and the seeds allowed to cool to ambient laboratory temperatures. Seeds were incubated under optimum conditions (15/30°C in the light) for up to 12 weeks, with seedlings being removed at weekly intervals. Subsequently, non-germinated seeds were soaked in 500 mg l⁻¹ GA₃ and incubated under the same conditions for a further 12 weeks.

(b) **Leachates and germination**

Fresh leaf material (new and mature leaves) was harvested from *S. mauritianum* (bugweed), *Acacia dealbata* (silver wattle), *Melia azedarach* (syringa), *Pinus patula* (pine) and *Eucalyptus* spp. (gum) trees. Other samples include leaf litter and soil beneath leaf litter. This material was ground into small fragments. Different concentrations were applied to seeds. These were either:

1. fragments moistened with 2 ml water; or
2. 10 g of ground material was soaked in 200 ml water overnight and the leachate added to the seeds in 2 ml aliquots; or
3. the leachate from the above was diluted tenfold and added to the seeds in 2 ml aliquots.

Seeds were incubated at optimum germination conditions (alternating 15/30⁰C in the light) for 12 weeks. Subsequently, non-germinated seeds were rinsed for one hour in running tap water and incubated for a further 12 weeks at optimum germination conditions.

(c) **Herbicides and germination**

(aa) *Pilot trial*

A range of synthetic herbicides, which are active in soil, were applied in a pilot trial to dry or fully-imbibed bugweed seeds, which were subsequently incubated at optimum conditions (15/30⁰C in the light) to promote germination. Details of the herbicide characteristics are presented in Table 5.1.

TABLE 5.1: Characteristics of the herbicides applied to bugweed seeds. The names of active ingredients, concentration of active ingredient in the formulated product, the chemical formula of each active ingredient and soil activity of the compounds is listed (BESTE, 1983)

| Product name | Active ingredient | Active ingredient rate | Formula | Adsorption + soil activity |
|--------------|-------------------|------------------------|--|--|
| Basagran | bendioxide | 480 g l ⁻¹ | C ₁₀ H ₁₂ N ₂ O ₃ S | Not adsorbed to soil. Adsorbed to organic matter. |
| Arsenal | imazapyr | 250 g l ⁻¹ | C ₁₆ H ₂₄ N ₄ O ₃ | Leached in sandy soils. |
| Banvel | dicamba | 480 g l ⁻¹ | C ₉ H ₆ ClN ₃ O ₃ S | Rapidly leached in soil. |
| 2,4-D | amine formula | 480 g l ⁻¹ | C ₈ H ₆ Cl ₂ O ₃ | Leached in sandy soils. |
| Gesapax | ametryne | 490 g l ⁻¹ | C ₉ H ₁₉ N ₅ S | Adsorbed in clay soils. |
| Hyvar | bromacil wp | 80 % wp | C ₉ H ₁₃ BrN ₂ O ₂ | Relatively low adsorption on soil colloids. |
| Karmex | diuron | 80 % wp | C ₉ H ₁₀ Cl ₂ N ₂ O ₂ | Adsorption > with clay and om. |
| Gesaprim | atrazine | 47 % m/v | C ₈ H ₁₄ ClN ₅ | Adsorption > with clay and om. |
| Fortrol | cyanazine | 500 g l ⁻¹ | C ₉ H ₁₃ ClN ₆ | Adsorption varies with soil texture, water content and om content. |
| Garlon amine | triclopyr | 360 g l ⁻¹ | C ₇ H ₄ Cl ₃ N ₃ O ₃ | Not strong adsorption unless high om. |

om = organic matter content

wp = wettable powder

m/v = mass/volume

(bb) *Effect of herbicides in different soils*

In the second trial, seeds were imbibed for 22 days in the dark at 20⁰C prior to the application of herbicides. The soils used either had a low clay content (9 % clay, 3 % organic matter and 88 % sand) or else a high clay content with either low organic matter (39 % clay, 2.3 % organic matter) or high organic matter (39 % clay, 7 % organic matter) content³. Subsequent to imbibition, seeds were transferred to petri dishes containing the different types of soil described above, moistened with different solutions of herbicide. Herbicides were applied at the concentrations listed in Table 5.2. Petri dishes were incubated at optimal conditions (15/30⁰C in the light) to promote germination. Herbicides applied to seeds were thus tested for efficacy in inhibiting germination or causing seedling death in various soil types.

(d) **Growth regulators and germination**

The growth regulators atractyloside, carboxyatractyloside and GA₃ were applied at rates of 100 and 500 mg l⁻¹ to seeds under safelight conditions. Seeds were subsequently incubated in the dark at constant 20⁰C for 12 weeks. Non-germinated seeds were transferred to optimum conditions (15/30⁰C in the light) for a further 12 weeks.

(iii) **UTILIZATION OF FRUITS AND SEEDS**

(a) **Utilization of alkaloids extracted from fruits**

Green fruits were collected from trees growing under a mature pine canopy

³Soil was obtained from Mr J Lawrence, Department of Soil Science, Department of Agriculture and Water Supply, Private Bag X9059, Pietermaritzburg, 3200.

TABLE 5.2: Concentrations of various herbicides used to investigate their effect on the germination of bugweed. Concentrations were chosen (under the advice of Dr P E L THOMAS (Private Bag X9059, Pietermaritzburg, 3200, Natal) and BESTE (1983)) to give a direct comparison of active ingredients.

| Herbicide | Active ingredient (mg l ⁻¹) | Formulated product (lha ⁻¹) | | | Kg active ingredient (l ha ⁻¹) | | |
|-------------------|---|---|--------|------|--|--------|------|
| | | Low | Medium | High | Low | Medium | High |
| Dicamba | 480 | 0.6 | 1.2 | 2.4 | 0.3 | 0.6 | 1.2 |
| Triclopyr (amine) | 360 | 3.3 | 6.7 | 13.3 | 1.2 | 2.4 | 4.8 |
| 2,4-D | 480 | 2.5 | 5.0 | 10.0 | 1.2 | 2.4 | 4.8 |
| Bendioxide | 480 | 1.2 | 2.4 | 4.8 | 1.2 | 2.4 | 4.8 |
| Imazapyr | 250 | 2.4 | 4.8 | 9.6 | 0.6 | 1.2 | 2.4 |

at Cedara State Forest. These were oven-dried at 55°C and ground to a fine powder. The method developed for the extraction and purification of solasodine is described in the results section of this chapter.

(b) Alkaloid yields

Crystalline solasodine was weighed, and yield was expressed as a percentage of original dry weight of the fruit material. This procedure was repeated for the low R_f alkaloid. Total alkaloid yield was the sum of these two values. Based on fruit production yields from Table 1.22 alkaloid yield per hectare was calculated.

(c) Potential uses of fruit material after extraction of alkaloids

(aa) Feed for crops

The dried wastes after extraction of alkaloids were weighed into 0.01 g; 0.1 g or 1 g samples. These were applied to newly emerged radish or wheat seedlings, which were grown in vermiculate in pots in a glasshouse in which natural lighting was supplemented by fluorescent tubes and ordinary incandescent light bulbs on a 16 hour day. The mean minimum and maximum daily temperatures were 8.0°C and 26.2°C. The bugweed wastes were applied four times during the experiment, at two-weekly intervals. As a control, 5 %, 10 % or 20 % Hoagland's nutrient solutions (HEWITT, 1966) were applied to wheat seedlings, and 10 %, 20 % and 50 % Hoagland's solutions were applied to radish seedlings at the same time intervals as above.

Each treatment comprised four pots arranged in a randomized block

design. Each pot contained four radish plants or ten wheat seedlings. Pots were watered with distilled water at two day intervals during the trial. After eight weeks, plants were destructively harvested to ascertain the effect of different treatments on their mass. Roots, shoots and tubers were massed separately.

(bb) *Feed for livestock*

After extraction of alkaloids from bugweed fruit material, a sample was sent to the Cedara Feed Analysis Laboratory, where test data is presented on a 100 % dry matter basis. Results were compared with those reported for *S. elaeagnifolium* (WASSERMANN, ZIMMERMANN & NESER, 1988) and *S. laciniatum* (GONZÁLEZ, FERNÁNDEZ & COSÍN, 1990).

C. RESULTS

(i) NUMBER OF SEEDS IN THE SOIL

The fruits of bugweed are either eaten and dispersed by birds and animals or dropped nearby the parent plant. Seed numbers present in the soil vary with site and depth of burial (DENNY & GOODALL *pers. comm.*). Although approximately 90 % of seeds in soil at two Cedara sites were dead, the proportion of viable seeds was numerically sufficiently high to reinfest a cleared area if they were to germinate (Chapter 1).

In a separate study, DENNY & GOODALL (*pers. comm.*) found that a massive seedling mortality occurs in bugweed. The problems caused by prolific seed production in bugweed are compounded by a sporadic seedling emergence, since germination requirements change over time (Chapter 4). This supports the work of DENNY & GOODALL (*pers. comm.*) who found a prolonged seedling emergence (5 years) in a transect under pine where bugweed parent trees had been removed (Chapter 1). Initially massive germination occurred after removal of the parent trees, but seedlings were still emerging five years later, with the vast majority being produced after the summer months (Chapter 1). Thus it is clear that although most seeds which fall from bugweed trees die, and seedling mortality is very high, the problem lies with the much reduced but still significant numbers of viable seed, whose changing levels of dormancy lead to sporadic emergence. This plus the input of new seed from other trees has rendered current control operations aimed at parent trees ineffective in the long-term.

(ii) **REDUCTION OF THE SOIL SEED BANK**

The target for control of bugweed therefore seems to be either to eradicate the small but significant viable portion of the soil seed bank and/or to halt fruit production. Eradication of the soil seed bank involves either treatments that render seeds non-viable, or which stimulate germination of most viable seeds simultaneously.

(a) **Moist heat and germination**

The first method investigated to render seeds non-viable was the application of heat. There was a significant interaction between time of immersion and temperature. Seeds immersed in water at 60°C showed a decline in germination after 10 or 15 minutes immersion (Table 5.3). At 70°C the immersion time required for a significant reduction in seed germination was only one minute. Higher temperatures of 80, 90 or 100°C for only 0.5 minutes inhibited germination of all or most of the seeds. Non-germinated seeds were found to be non-viable (Table 5.3). Conclusions drawn from these results are that bugweed seeds are highly sensitive to heat, with temperatures of 80°C and above being most effective in inducing seed death.

(b) **Leachates and germination**

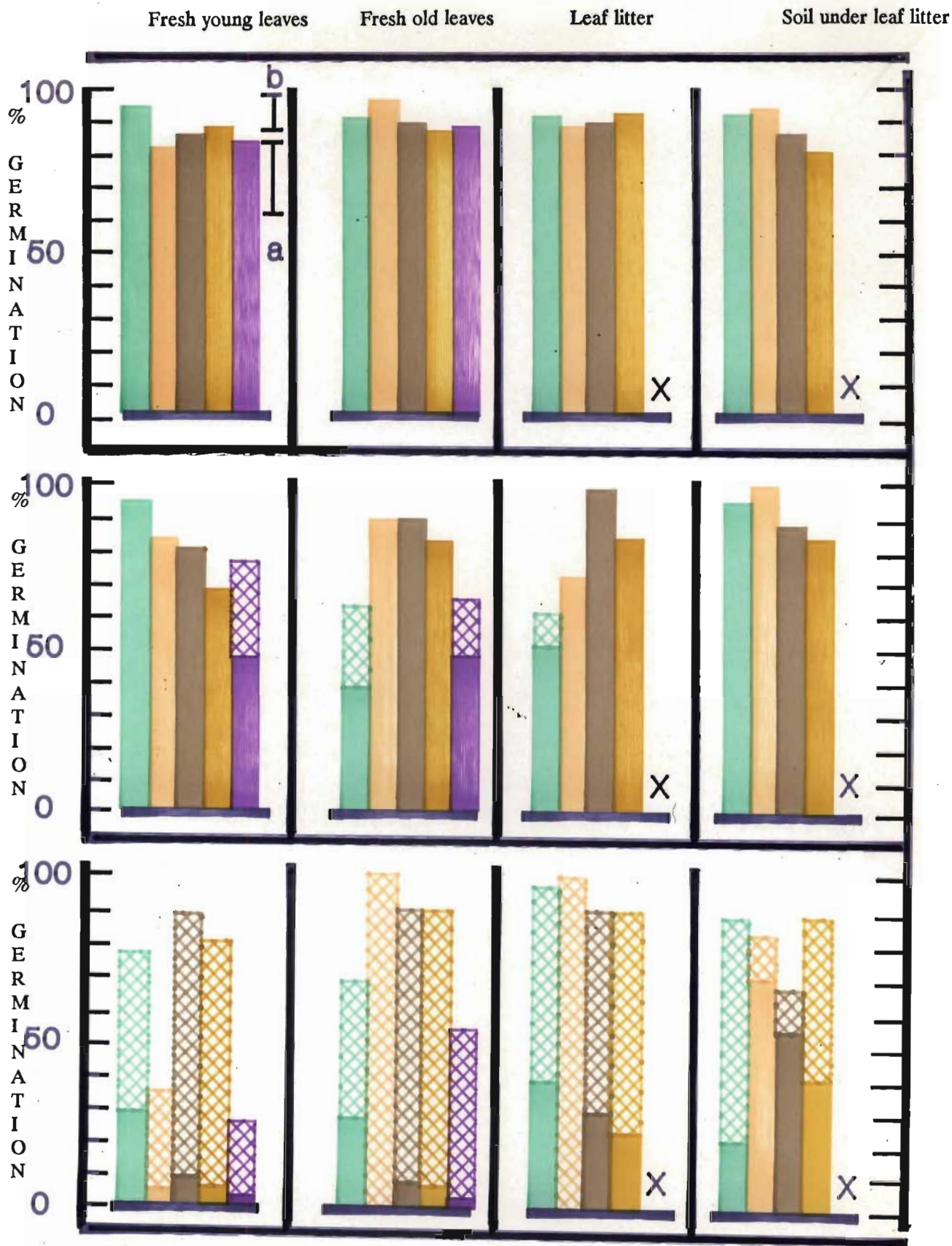
One phenomenon that may inhibit germination of bugweed seeds is that of allelopathy. Leachates from fresh leaves, leaf litter and soil beneath litter of bugweed, gum, silver wattle, pine and syringa trees were applied to bugweed seeds (Figure 5.1). Effective inhibition was seen only at the highest concentrations. However, washing these seeds free of the presence

TABLE 5.3: The effect of temperature and time of immersion on percentage germination of bugweed.

| Temperature (°C) | Time of immersion (minutes) | | | | | | |
|------------------|-----------------------------|-----|----|----|----|----|----|
| | 0 | 0.5 | 1 | 2 | 5 | 10 | 15 |
| 60 | 92 | 62 | 61 | 93 | 87 | 31 | 40 |
| 70 | 63 | 54 | 7 | 5 | 4 | 0 | 2 |
| 80 | 89 | 0 | 0 | 0 | 0 | 7 | 0 |
| 90 | 72 | 0 | 0 | 1 | 0 | 0 | 0 |
| 100 | 68 | 0 | 0 | 0 | 0 | 0 | 0 |

LSD $P < 0.05 = 18$ for interaction between temperature and time of immersion.

Non-germinated seeds did not respond to $500 \text{ mg l}^{-1} \text{ GA}_3$; were soft and spongy and therefore were regarded as non-viable due to the heat treatment.



SPECIES

FIGURE 5.1: The effect of leachates from different species on germination of bugweed. Seeds were incubated under ideal conditions (15/30°C in the light) in the presence of low (A), medium (B), or high (C) concentrations of either fresh young leaves, fresh old leaves, leaf litter or soil under leaf litter, respectively. Shaded portions of each bar represent germination in the presence of leachates. Unshaded portions of each bar represent germination after washing seeds free of leachates, 12 weeks after the initial incubation treatment. Leachates tested were from:

(→) bugweed; (→) gum; (→) silver wattle; (→) pine; (→) syringa.

a: LSD $p < 0.05$ shaded portions of bars (species x concentration x sample extract).

b: LSD $p < 0.05$ hatched portions of bars (species x concentration x sample extract).

x: leaf litter was not found in those samples.

of these leachates resulted in moderate to high germination percentages (Figure 5.1). Thus, in the field situation, any allelopathic effect of these substances would not be permanent. It is interesting that in gum, even the leaf litter inhibited germination. This could explain why heavy bugweed infestations are not generally seen in mature gum plantations. Extraction of any of these water-soluble inhibitors from species such as syringa and gum for treatment of bugweed seeds in a pine plantation, where this weed is most prevalent, would therefore only suppress germination temporarily. Repeated application of such inhibitors would therefore be required for control which would obviously not be a viable proposition.

(c) **Herbicides and germination**

(aa) *Pilot trial*

A range of synthetic herbicides was applied to either dry or fully imbibed seeds incubated under optimum conditions. Results of this preliminary screening trial showed that five of the herbicides; ametryne, bromacil, diuron, atrazine and cyanazine allowed some germination irrespective of whether seeds were dry or fully imbibed prior to application of the herbicides. At least some of the seedlings were healthy (Table 5.4). A concentration effect was evident for these five herbicides, with fewer seeds germinating with application of the stronger solution (Table 5.4). These herbicide candidates were therefore not expected to perform well in the field situation since the amount of active ingredient reaching buried seeds would be reduced due to adsorption to soil colloids (Table 5.1).

TABLE 5.4: The effect of herbicides on germination of dry or fully imbibed bugweed seeds under optimum conditions (15/30°C in the light). Five of the herbicides allowed germination (A). Four of the herbicides inhibited germination (B).

| Herbicide | Dry seeds | | Imbibed seeds | | Seeds ^c or seedlings dead 4 weeks after application |
|------------|----------------|----------------|---------------|----|--|
| | L ^a | H ^b | L | H | |
| A | | | | | |
| Ametryne | 5 | 0 | 25 | 25 | No |
| Bromacil | 50 | 5 | 95 | 7 | No |
| Diuron | 52 | 0 | 50 | 20 | No |
| Atrazine | 98 | 40 | 99 | 30 | No |
| Cyanazine | 60 | 14 | 0 | 12 | No |
| Control | 90 | - | 100 | - | No |
| B | | | | | |
| Dicamba | 0 | 0 | 0 | 0 | Yes |
| 2,4-D | 0 | 0 | 0 | 0 | No |
| Bendioxide | 0 | 0 | 0 | 0 | Yes |
| Imazapyr | 0 | 0 | 0 | 0 | No |

^a L = low concentration = 0.1 %

^b H = high concentration = 1.0 %

except for bromacil which is 0.3 or 3 kg ha⁻¹ for low and high concentrations respectively.

^c Seeds were sectioned and embryos examined. Healthy embryos were white and firm.

Four herbicides retarded seed germination under optimum conditions: dicamba, 2,4-D, bendioxide and imazapyr. Of these, bendioxide and dicamba killed seedlings (Table 5.4). These four candidates were considered for further trials, with application to seeds buried in different soil types.

Imbibed seeds generally germinated better than, or equally as well as dry seeds (Table 5.4). This suggested that in the dry state, seeds absorbed more of the herbicide than when fully imbibed. Consequently, the herbicides were more effective in suppressing germination. In the field situation, seeds will be fully or only partially imbibed and will therefore probably absorb herbicides to a lesser extent. Fully imbibed seeds will probably be the most difficult to kill, and further trials should therefore involve such seeds.

(bb) *Effect of herbicides on germination in different soils*

In the second trial, the herbicides from group B (Table 5.4) were applied to sandy soil or else to soil with a high clay content and either low or high % organic matter. In the sandy soil, two of the herbicides, bendioxide and imazapyr, did not perform well, allowing from 84 to 100 % germination of bugweeds seeds (Table 5.5A). This situation was also evident in soils with a high clay content, regardless of organic matter content (Tables 5.5B and 5.5C). Seedlings were healthy. Thus the performance of these two herbicides in soils of various type did not prove adequate and could therefore not be considered in field applications.

TABLE 5.5: The effect of herbicides on percentage germination of fully imbibed bugweed seeds in different soils. Results are expressed as the mean \pm S E.

(A): Seeds were imbibed in soil containing 9 % clay, 3 % organic matter and 88 % sand.

| Herbicide | Low dose* | Medium dose | High dose |
|-------------------|-------------|-------------|-------------|
| Dicamba | 0 | 0 | 0 |
| Triclopyr (amine) | 0 | 0 | 0 |
| 2,4-D | 0 | 0 | 0 |
| Bendioxide | 100 \pm 0 | 100 \pm 0 | 100 \pm 0 |
| Imazapyr | 84 \pm 7 | 91 \pm 12 | 96 \pm 2 |
| Control | 99 \pm 2 | - | - |

(B): Seeds were imbibed in soil containing 39 % clay and 2.3 % organic matter.

| Herbicide | Low dose | Medium dose | High dose |
|-------------------|-------------|-------------|-------------|
| Dicamba | 81 \pm 4 | 92 \pm 6 | 61 \pm 11 |
| Triclopyr (amine) | 1 \pm 3 | 9 \pm 7 | 0 |
| 2,4-D | 62 \pm 7 | 0 | 0 |
| Bendioxide | 82 \pm 14 | 94 \pm 9 | 98 \pm 3 |
| Imazapyr | 96 \pm 5 | 86 \pm 10 | 90 \pm 11 |
| Control | 99 \pm 2 | - | - |

(C): Seeds were imbibed in soil containing 39 % clay and 7 % organic matter.

| Herbicide | Low dose | Medium dose | High dose |
|-------------------|-------------|-------------|-------------|
| Dicamba | 77 \pm 9 | 77 \pm 8 | 65 \pm 10 |
| Triclopyr (amine) | 0 | 4 \pm 5 | 0 |
| 2,4-D | 21 \pm 6 | 16 \pm 6 | 0 |
| Bendioxide | 98 \pm 5 | 100 \pm 0 | 100 \pm 0 |
| Imazapyr | 86 \pm 10 | 89 \pm 10 | 87 \pm 13 |
| Control | 100 \pm 0 | - | - |

* Dosage varies according to active ingredient (refer to Materials and Methods)

Dicamba looked promising in sandy soils but allowed 60 to 90 % germination in high clay soils irrespective of organic matter content (Tables 5.5A, B and C). Healthy seedlings were produced. Thus herbicide efficacy was variable and was influenced by soil type.

Both triclopyr in the amine formulation (not included in the preliminary trial) and 2,4-D showed a far greater inhibitory effect on germination, regardless of soil type (Tables 5.5A, B and C). Although these may both be considered for further trials, resulting economic and phytotoxicity considerations are likely to exclude them. In addition to this, some germination did occur (Tables 5.5B and C). Thus a complete suppression of germination would not be achieved by using these products.

(d) Growth regulators and germination

An alternative line of investigation concerned with the soil seed bank did not involve suppression of germination or achieving seedling death but rather stimulation of germination. Germination of bugweed seeds is affected under unfavourable conditions only by the application of gibberellins, a growth regulator that cannot be applied economically in the field situation. Thus an alternative more economic product with an action similar to gibberellic acid would have to be found and applied to the soil, and tested for stimulation of germination.

Growth regulators with similar characteristics to gibberellins, atractyloside and carboxyatractyloside (FOWLDS, SMITH & VAN STADEN, 1990), failed to elicit any response. Only GA₃ stimulated germination under

adverse conditions, *viz.* constant 20°C in the dark (Table 5.6). It was also evident that these chemicals did not inhibit germination (and were therefore not similar to CCC), since transfer of non-germinated seeds to optimum conditions, alternating 15/30°C in the light, promoted more than 90 % germination (Table 5.6). Thus these chemicals would be totally ineffective in the field situation.

Substituted phthalimides, which are in the same family as the herbicide imazapyr, reportedly bring about a very similar response to gibberellins in germination of lettuce seeds. However, these products are not available for experimental use in South Africa (S A CYANAMID (PTY) LTD.⁴ *pers. comm.*). Thus at this stage, these products could not be tested for their effect on germination of bugweed seeds.

(iii) **UTILIZATION OF FRUITS TO DEplete SEED RESERVES**

An alternative approach to weed control via manipulation of the seed would be the reduction of fruit yield. If this could be achieved, then the seed population would decline and the rapid spread of bugweed would no longer occur. In addition to this, the soil seed bank would not be enhanced by falling seeds, and would gradually be depleted via germination and seed death. Two options for control are considered here; the first is that of biocontrol and the second, the utilization of seeds or fruits.

Prospects for biocontrol of *Solanum* species in South Africa have been reviewed by NESER, ZIMMERMANN, ERB & HOFFMANN, (1988). It

⁴S A CYANAMID (PTY) LTD., P O Box 58, Isando, 1600.

TABLE 5.6: The effect of atractyloside and carboxyatractyloside on percentage germination of bugweed. Seeds were initially incubated for 12 weeks at 20°C in the dark. Non-germinated seeds were subsequently transferred to 15/30°C in the light, and the germination recorded after 12 weeks. Results are expressed as mean \pm SE.

| Incubation conditions | Control | Atractyloside (mg l ⁻¹) | | Carboxyatractyloside (mg l ⁻¹) | | GA ₃ | |
|---------------------------|-------------|-------------------------------------|------------|--|------------|-----------------|-------------|
| | 0 | 100 | 500 | 100 | 500 | 100 | 500 |
| 20°C dark | 0 | 0 | 0 | 0 | 0 | 81 \pm 3 | 100 \pm 0 |
| Transfer to 15/30°C light | 100 \pm 0 | 100 \pm 0 | 99 \pm 2 | 99 \pm 2 | 95 \pm 3 | 100 \pm 0 | 97 \pm 2 |

was concluded that non-host specificity of all candidate insects tested was prohibitive to future insect release. Eggplants, potatoes and tomatoes were among the most important crops attacked by these insects (NESER, ZIMMERMANN, ERB & HOFFMANN, 1988). At a recent biocontrol working group held in the Cape during April 1989, it was decided that further work in this sphere would have to involve tests in the country of origin of these weeds.

(a) **Utilization of alkaloids extracted from fruits**

A second option considered was that of utilization of fruit and seeds. The natural product solasodine is present in green fruits of over 100 *Solanum* species. Extraction and purification of this on a commercial basis might be an economically viable proposition for control of bugweed. Subsequent conversion of solasodine to corticosteroid hormones is a relatively simple chemical procedure (SATO & NAGAI, 1972). In order to establish the feasibility of preparing purified solasodine from bugweed fruits, the following procedure was evolved, based on a combination of methods suggested by VÁGÚJFALVI, MARÓTI & TÉTÉNYI, 1971; LANCASTER & MANN, 1975; WESTON, 1976; LANCASTER, MANN & BLYTH, 1977; MANN, 1978; BRADLEY, COLLINS, CRABBE, EASTWOOD, IRVINE, SWAN & SYMON, 1978; CRABBE & FRYER, 1980.

A sample of unripe bugweed fruits was harvested, crushed and dried in an oven at approximately 55°C for 2 days (until no further reduction in weight occurred). The crude alkaloids were extracted from finely ground fruits in 80 % ethanol according to the procedure in Figure 5.2. The solution was

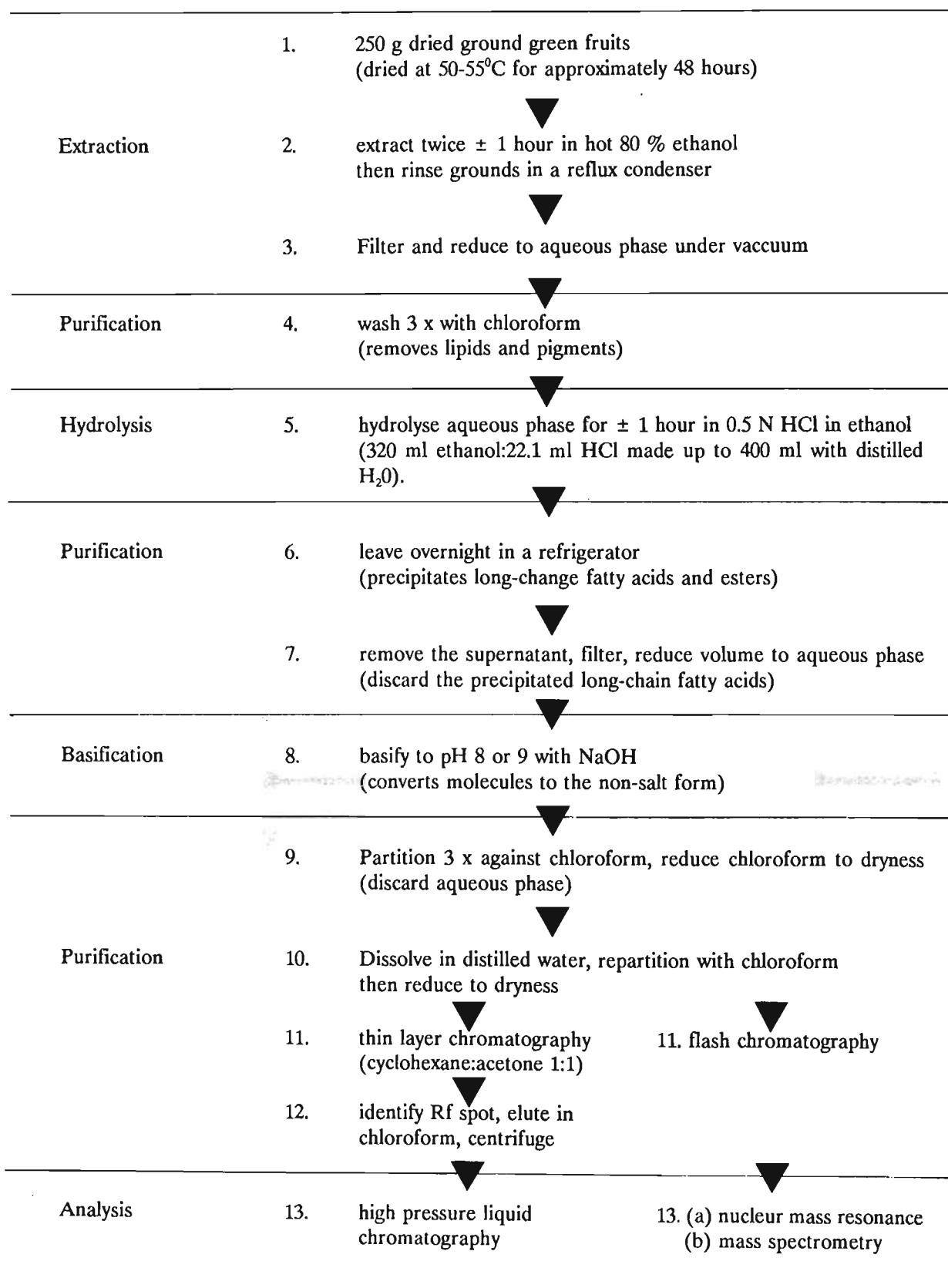


FIGURE 5.2: Flow diagram of procedure of extraction, purification and identification of alkaloids from green bugweed fruits.

then filtered through mutton cloth and concentrated to the aqueous phase under vacuum. Three washings in chloroform extracted most of the chlorophyll pigments and lipids. The aqueous phase was then hydrolysed for approximately one hour in 0.5 N HCL with an ethanol-water mixture. Following this, the sample was filtered and then reduced to the aqueous phase and the pH was raised to 8 or 9 using sodium hydroxide. The alkaloid was obtained by repeated extraction with chloroform. After evaporation of the chloroform fraction to dryness, the residue was examined by TLC (one direction) using cyclohexane:acetone (1:1) as solvent (BENNETT & HEFTMANN, 1962). With Dragendorff reagent (STAHL, 1969) one orange spot was found at R_f 0.5 which corresponded to authentic solasodine. A corresponding spot was subsequently eluted in chloroform, centrifuged and the supernatant dried.

Injection of authentic solasodine into a High Pressure Liquid Chromatogram (HPLC) showed a uv peak at 205 nm, the value commonly quoted in the literature (CRABBE & FRYER, 1980). However, a second more pronounced peak was evident at 282 nm (Figure 5.3A). Injection of the TLC sample detected at R_f 0.5 showed absorption peaks corresponding to those of authentic solasodine (Figure 5.3B). Chromatograms of the TLC sample and authentic solasodine were then compared. A flow rate of 1 ml per minute over a 30 minute run, using a hyperbolic gradient (not linear) up to 50:50 methanol/water gave the best results, *i.e.* the methanol fraction was first increased rapidly then gradually in a hyperbolic fashion. This resulted in the separation of of solasodine. The retention time for authentic solasodine was 15.1 minutes (Figure 5.4A). The TLC sample

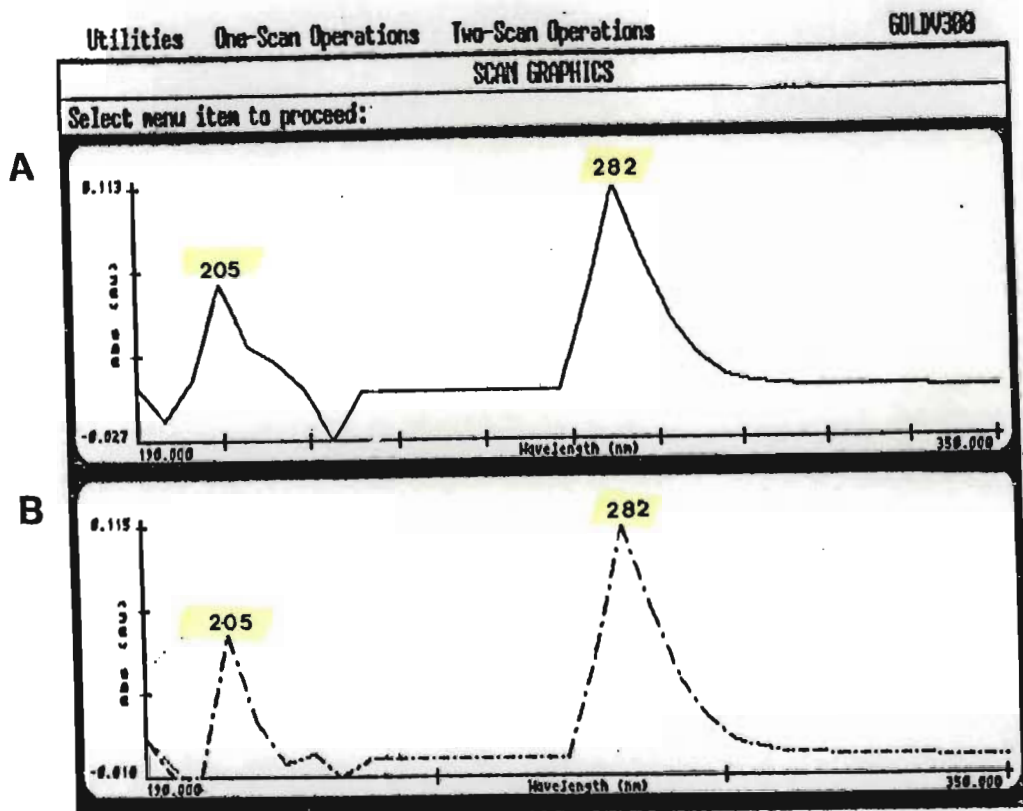


FIGURE 5.3: U-V absorption spectrum of solasodine (A) and a purified sample from green fruits of bugweed (B).

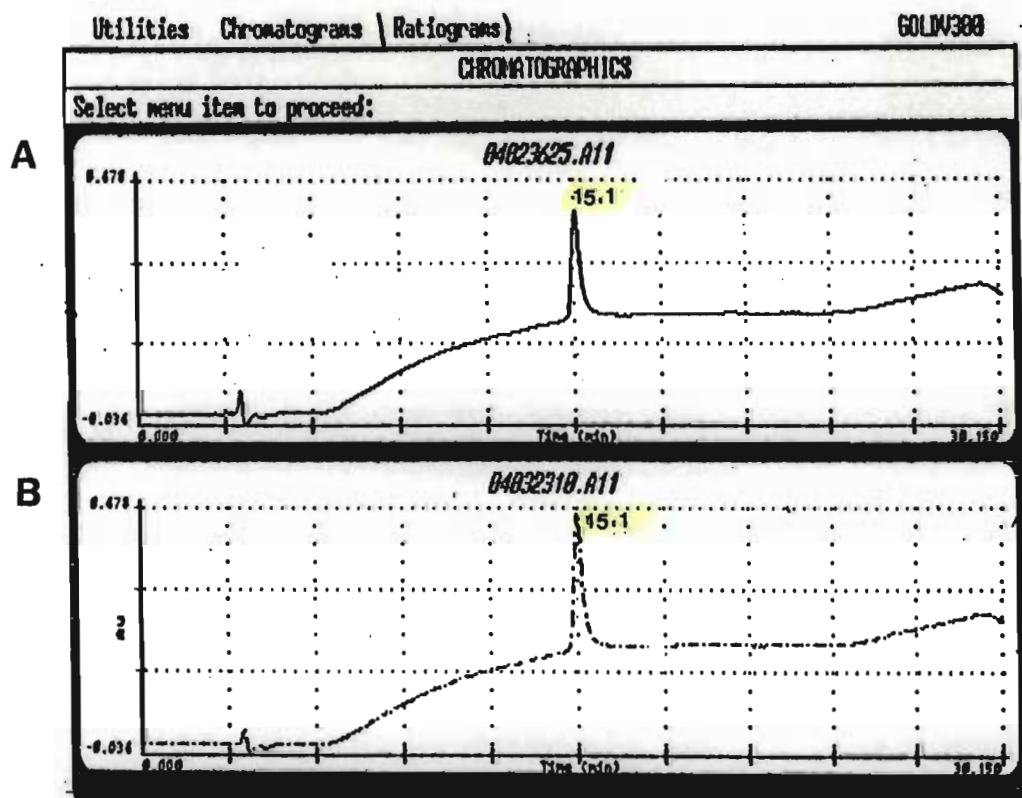


FIGURE 5.4: Retention times of solasodine (A) and a purified sample from green fruits of bugweed (B).

corresponded with this (Figure 5.4B). From this it was concluded that the extraction and purification method was effective, and that the product obtained was in pure form and had identical properties to that of solasodine.

The next task was to extract sufficient bugweed fruits to obtain enough product for chemical analysis. In February 1989, 20 kg of bugweed fruits were collected from Cedara State Forest, broken open then dried in ovens at 55°C for 2-3 days. The dried fruit material was then finely ground to increase the surface area for extraction. The powdered fruit material was boiled three times for approximately one hour each time in 80 % ethanol, filtered and the filtrates then flash-evaporated to remove the ethanol. The previously described extraction procedure was followed, except that after hydrolysis and before raising the pH, the extract was cooled overnight at 10°C which precipitated long-chain fatty acids and esters. The filtered extract was then reduced to the aqueous phase and the rest of the procedure was followed (Figure 5.2).

The crude alkaloid extract was thick, black and syrupy and could not be separated on a TLC plate. The sample was therefore redissolved in water, washed three times in chloroform and the combined chloroform extracts concentrated to dryness. The dipping stains, anisaldehyde and cobalt chloride (MERCK, 1970) were then used to indicate alkaloid presence, since using these dyes in thin layer chromatography was more convenient than spraying with Dragendorff reagent.

The bulk of the crude alkaloid extract was then redissolved in 100 ml of chloroform and flash-chromatographed (STILL, KAHN & MITRA, 1978). No alkaloids were eluted from the column in the chloroform. However, a more polar mixture of 20 % acetone in hexane eluted an alkaloid and this crystallised subsequently. These crystals migrated to R_f 0.5 when subjected to TLC, as described above. The eluate was evaporated to dryness, weighed to obtain yield then further tested to verify its identity.

Pure acetone subsequently eluted a second alkaloid from the column. Examination by TLC showed that *the alkaloid did not migrate from the baseline, and was therefore not solasodine*. The eluate was evaporated to dryness, weighed to obtain yields then further tested to verify its identity.

Crystals extracted by acetone/hexane from column separation were then examined by nuclear magnetic resonance (NMR) spectrometry. The chemical shifts of the various proton resonances are shown in Figure 5.5A. A comparison with authentic solasodine showed that the extracted crystals were solasodine. This was further verified by mass spectrometry. Mass spectra showed that authentic solasodine and the acetone/hexane crystals had similar fragmentation patterns, with prominent peaks at m/z 234, 253, 267, 385 and 413 (Figure 5.6A). These crystals were therefore positively identified as solasodine (DREWES, *pers. comm.*)⁵.

Proton NMR showed that the crystals extracted by acetone from column

⁵Professor S E Drewes, Department of Chemistry, University of Natal, P O Box 375, Pietermaritzburg, 3200.

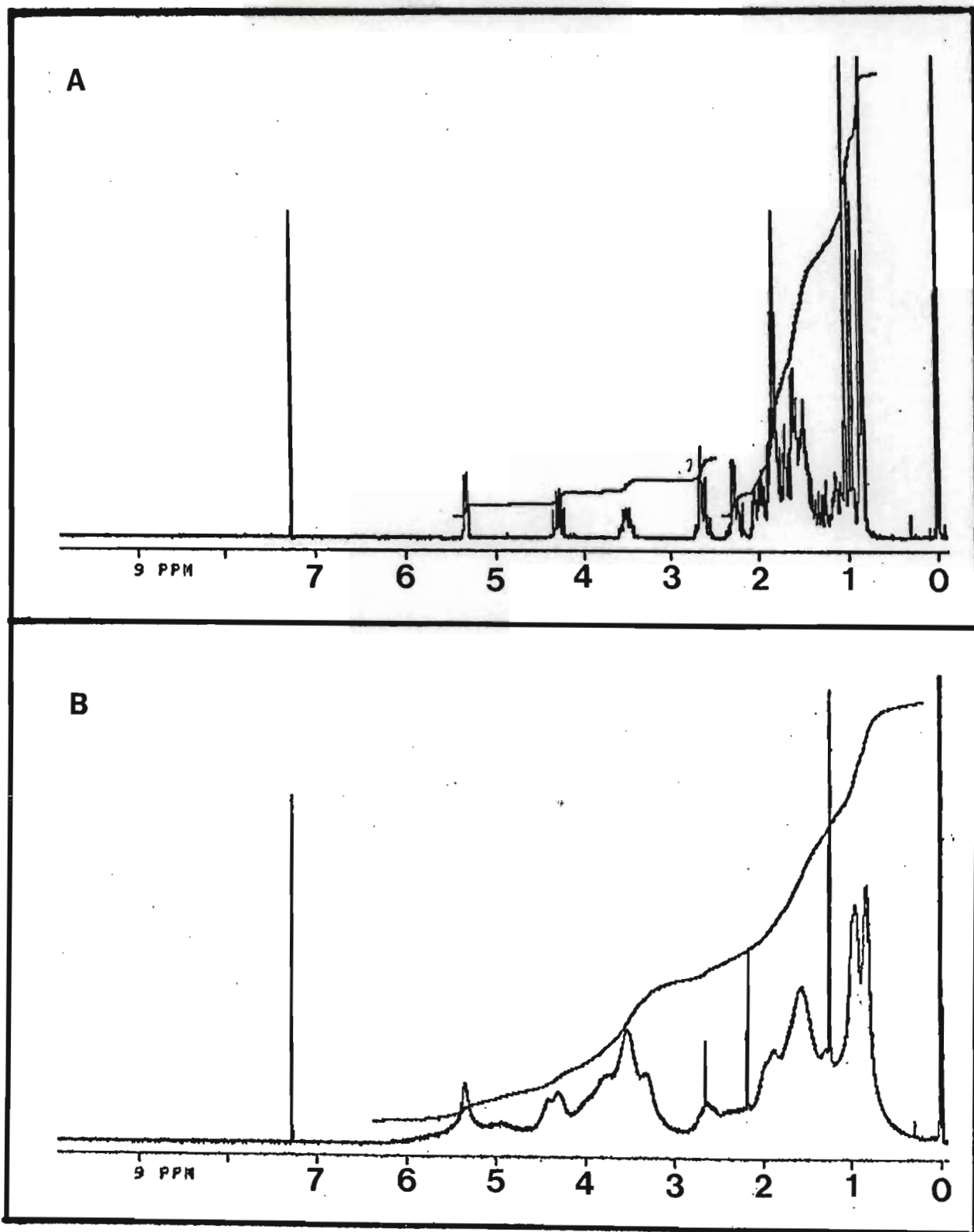
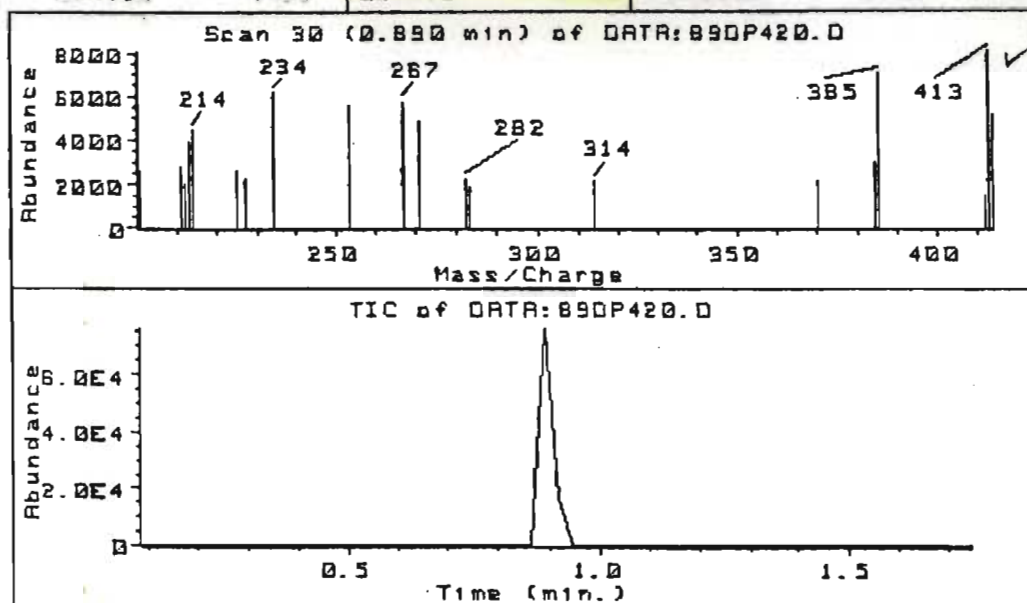


FIGURE 5.5: Chemical shifts from NMR of (A) $R_f 0.5$ and (B) $R_f 0.0$ from green fruits of bugweed.

| A | m/z | abund. | m/z | abund. | m/z | abund. | m/z | abund. |
|---|--------|--------|--------|--------|--------|--------|--------|--------|
| | 200.40 | 2572 | 225.15 | 2627 | 271.15 | 4890 | 384.45 | 2983 |
| | 211.15 | 2759 | 227.15 | 2231 | 282.25 | 2210 | 385.45 | 6984 |
| | 212.15 | 1987 | 234.15 | 6125 | 283.25 | 1879 | 412.25 | 1452 |
| | 213.05 | 3952 | 253.15 | 5621 | 314.30 | 2101 | 413.15 | 8051 |
| | 214.05 | 4428 | 267.15 | 5737 | 370.40 | 2102 | 414.25 | 5091 |



| B | m/z | abund. | m/z | abund. | m/z | abund. | m/z | abund. |
|---|--------|--------|--------|--------|--------|--------|--------|--------|
| | 253.30 | 2121 | 271.30 | 495 | 385.60 | 605 | 414.35 | 337 |
| | 254.20 | 610 | 282.30 | 2819 | 395.60 | 379 | 546.45 | 767 |
| | 255.30 | 372 | 283.30 | 2140 | 396.60 | 5386 | 547.55 | 1532 |
| | 256.30 | 563 | 284.30 | 915 | 397.50 | 1789 | 548.65 | 454 |
| | 267.30 | 1699 | 285.30 | 357 | 398.60 | 491 | 574.55 | 342 |
| | 268.20 | 501 | 376.50 | 988 | 413.25 | 1054 | 575.45 | 1110 |

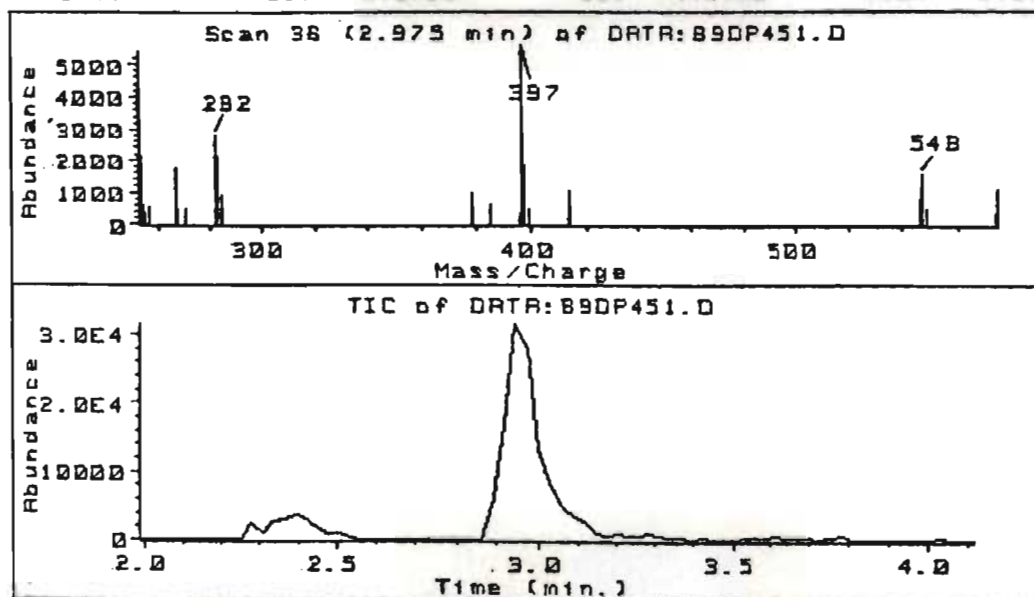


FIGURE 5.6: Mass spectra fragmentation patterns of two alkaloids extracted from green fruits of bugweed. (A) The alkaloid at R_t 0.5, identified as solasodine.

separation had a structure broadly similar to that of solasodine (Figure 5.5B). However, mass spectra showed that the fragmentation patterns of this alkaloid had its most prominent peak at m/z 397 and was therefore different from that of the solasodine fragmentation pattern (Figure 5.6B). High resolution mass spectrometry indicated that the baseline alkaloid had a molecular mass of 575.38600 and most likely corresponds to $C_{36}H_{51}N_2O_4$ (DREWES *pers. comm.*). In the review by RIPPERGER & SCHREIBER (1981) no reference is made to this second alkaloid. In order to determine its structure a more detailed chemical analysis is necessary. Only after this can any predictions be made regarding its possible use as a precursor to corticosteroid hormones.

(b) **Alkaloid yields**

Results indicated that 250 g of green bugweed fruits picked from trees growing under pine yielded 1.55 g of crude alkaloid, which is 0.62 % of the dried fruit material (Table 5.7). Further purification through a column gave yields of 0.41 g of solasodine (0.16 %) and 0.85 g of the unknown alkaloid (0.34 %). The total amount of pure alkaloids obtained was 1.26 g, which is 0.5 % of the dried fruit material (Table 5.7). Thus 81 % of the crude alkaloid extract was alkaloid crystals. This solasodine yield is approximately one third of that reported for *S. auriculatum* (syn. *S. mauritanum*) by MAITI, SIPRA & REBEKA (1965) (Table 5.7).

In the study area, described in Chapter 1, fruit yield was dependent on tree size, with a linear relationship $y = 21x - 513$ (Table 1.5). Thus the larger

TABLE 5.7: Alkaloid yields from green fruits of bugweed. Results are expressed as mass and, in brackets, percentage yield of crude alkaloids, of the purified alkaloid solasodine (R_f 0.5) and the compound at R_f 0.0. Results are compared with those reported by MAITRI, SIPRA & REBEKA (1965).

| Species | Dry weight fruit (g) | Crude alkaloids (g) | Solasodine alkaloid (g) | R_f 0.0 alkaloid (g) | Total purified alkaloids | % of crude extract |
|--|----------------------|---------------------|-------------------------|------------------------|--------------------------|--------------------|
| <i>S. mauritianum</i> | 250.00 | 1.55 (0.62 %) | 0.41 (0.16 %) | 0.85 (0.34 %) | 1.26 % (0.50 %) | 81 |
| <i>S. auriculatum</i> * (Syn. <i>S. mauritianum</i>) | 4 500 | - | 23.04 (0.5 %) | - | - | - |

* MAITI, SIPRA & REBEKA, 1965

the tree, the more fruit is produced. On a per hectare basis, fruit yielding trees would produce 94 539 fruits periodically over 20 months (Table 5.8).

An average-sized green bugweed fruit weighs approximately 1 g, thus approximately 94.5 kg of green fruits would be produced during this time period. However, approximately 60 % of this mass is water, and mass values must therefore be adjusted, since alkaloid yield is related to the dry mass of fruit material. The total alkaloid yield ($\text{g ha}^{-1} 20 \text{ months}^{-1}$) would therefore be $0.5 \% \times 40 \% \times 94.5 \text{ kg} = 189 \text{ g}$. At a price of \$97 for 1 g of solasodine (SIGMA, 1990) a total alkaloid yield of 189 g per hectare would provide a gross income of $\$97 \times 189^6 =$ approximately \$18 000 over a 20 month period.

Solasodine yields from cultivated *Solanum* species are approximately 300 times higher than the yield obtained from *S. mauritianum* growing under a pine canopy (Table 5.9). Assuming the alkaloid extracted by acetone ($R_f 0.0$) is also suitable for conversion to corticosteroid hormones, the total alkaloid yield from *S. mauritianum* is still 100 times lower than solasodine yields from cultivated species (Table 5.9). However, *S. auriculatum* (syn. *S. mauritianum*) in Germany produced a solasodine yield favourably comparable to that of the other species (Table 5.9). Thus low yields obtained from *S. mauritianum* in this present study is apparently due not to limitations of the species but rather to adverse conditions of growth.

⁶Assume the alkaloid extracted by acetone ($R_f 0.0$) is also \$97 for 1 g.

TABLE 5.8: Predicted fruit yield of bugweed growing under a pine canopy. Results are extrapolated from the fruit yield study (Chapter 1).

| | STEM DIAMETER (CM) AT 1 M ABOVE SOIL SURFACE | | | | | | | | | Totals |
|---|--|--------|--------|--------|--------|-------|-------|--------|-------|--------|
| | 2.5 | 3.0 | 3.5 | 4.0 | 4.5 | 5.0 | 5.5 | 6.0 | 6.5 | |
| (a) Tree number per hectare | 133 | 92 | 75 | 50 | 33 | 8 | 8 | 25 | 8 | 432 |
| (b) Fruit number per tree in 20 months | 12 | 117 | 222 | 327 | 432 | 537 | 642 | 747 | 852 | 3 888 |
| (c) Fruit number per hectare in 20 months | 1 596 | 10 764 | 16 650 | 16 630 | 14 256 | 4 296 | 5 136 | 18 675 | 6 816 | 94 539 |

- (a) Results taken from Table 1.22 (Chapter 1).
- (b) Results calculated from the equation $y = 21x - 513$, from Table 1.5 (Chapter 1).
- (c) The product of (a) x (b).

TABLE 5.9: Yield of solasodine from cultivated *Solanum* species (TELEK, DELPIN & GABANILLAS, 1977). Yields are compared with those obtained in this study, based on results from Tables 5.7 and 5.8.

| <i>Solanum</i> species | Country | Yield (kg ha ⁻¹) |
|-------------------------|-------------|------------------------------|
| <i>S. aviculare</i> | India | 21.1 |
| <i>S. laciniatum</i> | Hungary | 20.0 |
| <i>S. marginatum</i> | Ecuador | 32.5 |
| <i>S. mammosum</i> | Puerto Rico | 24.1 |
| <i>S. auriculatum</i> * | Germany | 20.0 |
| <i>S. mauritianum</i> | RSA | 0.061+ |
| | RSA | 0.189** |

* Syn. *S. mauritianum*

+ Solasodine yield based on results from Tables 5.7 and 5.8.

** Solasodine (R_f 0.5) plus R_f 0.0 alkaloid

(c) **Potential uses of fruit material after extraction of alkaloids**

(aa) *Feed for crops*

After extraction of alkaloids, the ground dried fruit material was applied to wheat or radish seedlings, to investigate its effect on growth of these plants. Results were compared to those obtained from using Hoagland's solutions as a nutrient medium. An increasing concentration of Hoagland's solutions tended to increase the root mass and to a lesser extent the shoot mass in the wheat seedlings (Table 5.10). In contrast, increasing concentrations of ground bugweed fruit material did not increase, and even slightly depressed root and shoot growth of wheat (Table 5.10).

In the case of radish seedlings, roots, shoots and tubers were massed separately. Increased concentrations of Hoagland's solutions tended to increase shoot and tuber mass but not root mass. In contrast, application of dried bugweed fruit material resulted in reduced shoot, tuber and root mass, irrespective of the amount applied (Table 5.10).

From this trial, it was seen that neither radish nor wheat seedlings were able to utilize nutrients present, in the sample of bugweed after alkaloid extraction, and suggested that large quantities applied to plants may prove inhibitory to crop growth. Waste plant material after alkaloid extraction could therefore probably not be used as a fertilizer for crop growth, unless modified to release nutrients.

TABLE 5.10: The effect of bugweed fruit material after extraction of alkaloids on dry mass (mean \pm SE) of wheat and radish seedlings

| DRY MASS (mg) | | | | | | | |
|---------------|---------------|-------------------------------------|---------------|-----------------|--------------------------------------|---------------|---------------|
| Test plant | Plant portion | Hoagland's Solutions Concentration* | | | <i>S. mauritianum</i> fruit material | | |
| | | Low* | Medium | High | 0.01 g | 0.1 g | 1.0 g |
| Wheat | Shoot | 341 \pm 63 | 427 \pm 43 | 462 \pm 59 | 282 \pm 26 | 232 \pm 58 | 258 \pm 67 |
| | Root | 572 \pm 187 | 389 \pm 159 | 1 071 \pm 474 | 497 \pm 141 | 383 \pm 191 | 403 \pm 171 |
| Radish | Shoot | 50 \pm 8 | 50 \pm 11 | 88 \pm 13 | 15 \pm 5 | 17 \pm 6 | 21 \pm 4 |
| | Tuber | 24 \pm 8 | 29 \pm 10 | 56 \pm 14 | 8 \pm 3 | 9 \pm 2 | 9 \pm 3 |
| | Root | 83 \pm 22 | 67 \pm 14 | 59 \pm 16 | 42 \pm 7 | 40 \pm 23 | 43 \pm 4 |

* Refer to materials and methods

(bb) *Feed for animals*

One of the *Solanum* weeds which is prevalent in the Cape is *S. elaeagnifolium*. This has been pelleted and successfully fed to animals at Kendrew (WASSERMANN, ZIMMERMANN & NESER, 1988). In Spain, the residue of the industrial process for extraction of steroid glycoalkaloids from *S. laciniatum* was evaluated as roughage for cattle (GONZÁLEZ, FERNÁNDEZ & COSÍN, 1990). An analysis of the chemicals in these species is presented in Table 5.11.

A similar analysis of the dried fruit material of bugweed after alkaloid extraction showed a chemical composition which was favourably comparable to that of *S. elaeagnifolium*, in terms of fat, crude fibre, protein, ash and microelement content (Table 5.11). It was therefore apparent that after extraction of alkaloids, waste plant material of bugweed may potentially be useful as an animal feed. This agrees with the findings of GONZÁLEZ, FERNÁNDEZ & COSÍN, 1990. Large quantities of waste plant material will be available after the extraction of alkaloids, if this is done on a commercial scale, so utilization of this commodity may significantly reduce the costs of the extraction procedure.

TABLE 5.11: An analysis of the chemicals in green fruits of bugweed. The analysis was done on dried fruit material after extraction of alkaloids. Results were compared with those of *S. elaeagnifolium* (reported in WASSERMANN *et al.* 1988) and *S. laciniatum* (GONZÁLEZ *et al.* 1990).

| TEST DATA ON 100 % DRY MATTER BASIS | | | | | | | | | | | | |
|-------------------------------------|--------------|-------|---------------|-----------------|-------|------|------|------|------|--------|--------|--------|
| Sample | % Dry matter | % Fat | % Crude fibre | % Crude protein | % Ash | % Ca | % Mg | % K | % P | ppm Zn | ppm Cu | ppm Mn |
| <i>S. mauritianum</i> | 93.40 | 9.10 | 40.57 | 14.72 | 2.36 | 0.24 | 0.21 | 1.98 | 0.28 | 35.0 | 17.0 | 55.0 |
| <i>S. elaeagnifolium</i> | 93.90 | 14.69 | 28.95 | 14.70 | 4.74 | 0.22 | 0.07 | 0.52 | 0.32 | 22.0 | 12.8 | 25.0 |
| <i>S. laciniatum</i> | 31.59 | - | 36.07 | 13.80 | 8.20 | 1.08 | 0.09 | - | 0.14 | - | - | - |

D. DISCUSSION

(i) THE PROBLEM

In this study it has been established clearly that the reproductive strategy of *S. mauritianum* is the major cause of the success of this species as a weed. Periodic flushes of large numbers of viable seeds are dispersed and added to the soil seed bank. Changing environmental conditions stimulate germination in some of these seeds whereas the same set of conditions may induce other seeds to enter secondary dormancy, and yet leave others totally unaffected. The response of a particular seed to changing environmental conditions is determined by the locality from which it comes, when it was produced, where it is dispersed, and for how long it remains there. The consequent sporadic seedling emergence and also the continual input of seeds from seed-producing trees has rendered current control operations totally inadequate. The result is figures such as those quoted by LE ROUX (1984) where *S. mauritianum* is spreading in pine plantations at a rate of 16 % per annum. It seems then that to curtail this rate of increase the seed component of the plant must be reduced. This has long been recognized by the people who deal with bugweed on a daily basis *i.e.* foresters, and they frequently come up with suggestions such as "shoot all the pigeons!"

(ii) CONTROL OPTIONS FOR THE SEED COMPONENT

(a) Eradication of the soil seed bank

One method of killing seeds is controlled burning. Temperatures during burning operations could reach the levels used in the laboratory trial which

were effective in killing seeds. However, this practice would not be considered for control of bugweed in Natal plantations since it is highly dangerous and may start forest fires which are not easily controlled (HAIGH, *pers. comm.*⁷). This control method would also not be very effective to any great soil depths since the fuel load available in most plantations is patchy which would not allow heat to penetrate deeply into soils. Many seeds would therefore not be exposed to temperatures of 80°C and above and would survive.

Many *Solanaceous* weeds are controlled by means of soil-applied pre-emergent herbicides. A variety of different herbicides are currently in use but rarely prove effective in exhausting a soil seed bank. This is largely due to poor performance of these herbicides under certain conditions concomitant with the dormant fraction of the soil seed bank escaping these generally non-persistent chemicals by delayed emergence. One major physical obstacle to good herbicide performance is soil type. Heavy clay soils and high organic matter content are known to bind herbicides (BESTE, 1983; SENESI & TESTINI, 1984) and so appreciably reduce the concentration that reaches the seeds. Thus choice of herbicide must be suited to the conditions prevalent in the locality in which the weed is to be controlled. Temperature and storage period of seeds is also known to be important in performance of herbicides. For example, dry storage of herbicide-treated *Eupatorium odoratum* L. seeds at -10°C resulted in death after 26 months. An increased storage temperature of 27.5°C however, caused a wider variety of herbicides to be effective in killing seeds in half

⁷Mr H Haigh, Department of Environment Affairs, Private Bag X9029, Pietermaritzburg, 3200

the time (ETEJERE, 1980). It is also possible that seeds might develop a resistance to herbicide application, as was mentioned for *S. nigrum*, or else dormant seeds might be unaffected (ROUAS, 1981; GARCIA-BAUDIN & AGUIRRE, 1983; HIMME, STRYCKERS & BULCKE, 1983; BARLOW & HICKS, 1985; RAPPARINI, 1986).

The problem of dormant seeds escaping herbicides is widely appreciated. According to a recent report in a popular magazine, methyl isothiocyanate (MIT) could act as a "seedicide" by directly killing dormant weed seeds which do not have hard seed coats (ANONYMOUS, 1987). However, the article concluded that application of this chemical would be too costly in certain situations. The ideal herbicide candidate would be one that is cost-effective in killing dormant weed seeds, has an acceptably low level of phytotoxicity to standing timber, is mobile in the soil, is reasonably persistent, does not result in a selection for resistant biotypes, and does not affect non-target species. Further trials in the field situation with new products as they become commercially available may well satisfy most of these ideals in the future.

Use of naturally occurring inhibitors instead of synthetic chemicals has proved ineffective in long-term seed inhibition since leaching away from seeds allowed germination of bugweed to commence. PUTNAM (1988) suggested that these natural products may give rise to a new breed of more effective synthetic chemicals, and this may well be the emphasis of future research.

Products used to stimulate seeds to germinate would require similar attributes to herbicides to be a commercially viable proposition. Substituted phthalimides apparently mimic the germination-stimulating action of gibberellins and would therefore be the most likely candidates for use in bugweed. However, a number of disadvantages have to be surmounted before these chemicals could be considered as a major component of the control strategy for bugweed. These are (1) cost, (2) efficacy, (3) possible phytotoxicity to pine trees, (4) commercial availability to this country, and (5) they must be physiologically more active than GA_3 since bugweed seeds do not always respond to GA_3 (Chapters 3 and 4).

CHANCELLOR (1984) commented that breaking dormancy is one of the most promising approaches to reduce seed numbers in the soil. However, this author believes that this is not applicable in the Natal situation for the control of bugweed. Stimulation of germination after application of chemicals in the agricultural situation has proved a less viable proposition than CHANCELLOR envisaged as is seen in results of UPADHYAYA, HSIAO & BONSOR (1986) and HURTT & TAYLORSON (1986). Since conditions in Natal are more variable than those found in most agricultural situations; the efficacy of such treatments in this province may be even less consistent than results reported in the literature.

The crux of the matter is, however, economic in nature. Extra revenue has to be realized either from increased timber yields or from other sources in order to cover the cost of effective treatments for reduction of the soil seed bank.

(b) **Utilization of fruits to deplete seed reserves**

(aa) *Philosophy of alkaloid production of a weed*

Here, the question may be asked, is it possible to consider bugweed now as no longer the number one weed in forestry, but rather (besides pine trees) as a second crop? Herein lies the concept of agroforestry. Much interest in South Africa is given to this subject. For example, the largest technical gathering ever held in Africa on this subject took place recently at the University of Natal in Durban on 28/29 November 1988 (ANONYMOUS, 1989).

Agroforestry is the term used for the *deliberate* cultivation of trees in association with crops or livestock (ERSKINE, 1989). Potential advantages of agroforestry include:

1. Prevention of environmental deterioration, for example better soil protection.
2. Promotion of rural development in the less developed areas of the world.
3. More effective utilization of nutrients and water.
4. Increased total productivity.
5. Socio-economic advantages. (ERSKINE, 1989)

In infested pine plantations of Natal, bugweed is growing in close association with the pine and is the dominant understory species under a closed canopy. If we can utilize the plant, its status as a weed becomes close to zero, and the system may almost be described as "agroforestry"

(although the occurrence of bugweed is not due to deliberate planting). The last three potential advantages described above could apply to this system, the latter aspect including increased job opportunities for semi-skilled labour.

However, two of the definitions of a weed strongly indicate that bugweed should not be regarded as a crop, but must retain its status as a weed. The first definition, where a weed is considered a plant growing out of place or in a place where it is not wanted, is applicable to the forestry situation. Ideally, nothing should grow under a pine canopy, thus easy access for thinning and felling operations is maintained. Also, competition with pine should be reduced, especially before the first thinning, when growth of trees is most adversely affected (Chapter 1).

The second concept of a weed that is considered here is that of a plant for which man has not yet found a use. It could be argued here that since there is a use for bugweed, in terms of alkaloid extraction, the species is no longer a weed and can be considered a crop. However, a strong possibility will always exist that in the research field of organic chemistry, synthesis of alkaloids from simple molecules may become an economically viable proposition. Alternatively, another genus or species within the Solanaceae may be manipulated genetically or physiologically to produce much higher quantities of alkaloids, and this may result in reduced extraction costs. The status of bugweed as a crop would then definitely not be an economically viable proposition, and it would revert once again to the status of a weed. From this, it can be strongly argued that bugweed is

and always should be considered a weed. Any economic benefits via utilization of the fruits should be considered transitory and incorporated into a weed control programme aimed at the reduction of this species.

(bb) *Alkaloid yields from bugweed fruits*

Yields of solasodine from *S. mauritianum* do not compare favourably to those of species evaluated in large-scale experiments (Table 5.9). However, it must be remembered that *S. mauritianum* in Natal is by no means cultivated. Forest soils have a high acidity and are low in nutrients. In addition to this, bugweed trees are growing in conditions which suppress their reproductive yield (Chapter 1). No input costs are involved in obtaining these solasodine yields from bugweed in Natal, whereas in cultivated crops, continual improvement of agronomic techniques, and both genetic and physiological manipulation of the species is being sought to improve yields. All of these involve direct or indirect input costs. Simply put, we are getting something for nothing. Any improvement of solasodine yield in bugweed growing under pine would come about (initially anyway) by manipulation of what is already there, and not by the addition of costly products such as fertilizer or growth regulators.

(cc) *Proposed strategy for control of bugweed*

Local unemployed labour could be hired on a casual basis to harvest green fruits, with payment made according to mass of fruit collected. This has proved successful for harvesting marula fruits and jointed cactus cladodes (ZIMMERMANN *pers. comm.*)⁸. Initially, workers would be employed to

⁸Dr H G Zimmermann, Plant Protection Research Institute, Private Bag X134, Pretoria, 0001.

fell bugweed. Chopping the softwood stems is easy with a bushknife, and multi-stemmed coppice results from this mechanical damage. Once this is completed, the labour would be directed to neighbouring blocks, under the supervision of the forester. Felled trees which produce multi-stemmed coppice could be repeatedly harvested for fruit, probably on a rotational basis. Repeated chopping of trees to a convenient height for continual harvesting of fruits would probably not impair the growth or fruit output of the plants, although this would require further investigation. It is important also to utilize bugweed trees growing around the perimeter of stands, since fruit production is higher in the sun than under a pine canopy, and high solasodine yield will probably be attained, due to increased light (BHARATI & CHATTERJEE, 1986). Harvesting would not be continued after clearfelling since vigorous growth of bugweed has an adverse effect on the growth of newly planted pine seedlings. Other weeds, notably bramble, which is prevalent in this disturbed situation, would hamper harvesting operations.

MANN (1978) quotes that normally in the commercial production of solasodine, the chemical company contracts with the farmer and supervises harvesting operations to obtain the fruits. For bugweed, green fruits would be picked and delivered to the processing plant without delay, or dried before transportation, since alkaloid yields might be reduced if fermentation begins (MANN, 1978). *Harvesting fruits and their utilization should perhaps be a joint venture between timber growers and a chemical company geared to large scale extraction of alkaloids. This will yield revenue for herbicides and the labour required for follow-up treatments of emergent*

seedlings. This revenue could be enhanced if the waste plant material after extraction of alkaloids could be successfully utilized as a feed supplement for livestock. Utilization of bugweed fruit could have a considerable impact on the control of this weed since fruit and seed production is prolific (7.2 million seeds per hectare over a 20 month period).

The system is illustrated in Figure 5.7. Here, fruits are utilized, thus fewer seeds enter the soil. This will be highly effective, since 90 % of these seeds are viable. The soil seed bank will be gradually depleted, through natural seed or seedling death, where 90 % of the soil seed bank is rendered non-viable. The remaining 10 % of the seeds which are viable will germinate sporadically. This can be enhanced by periodic disturbance of soil during normal forestry procedures. Around 90 % of these seedlings die naturally (Chapter 1). Surviving seedlings must be destroyed by application of herbicides or by hand-pulling. This latter treatment would result in further localized disturbance. Since seeds fall in groups (within fruits or faeces), this localized disturbance would probably affect other seeds situated close to the hand-pulled seedling.

Details of this strategy for control of bugweed via utilization of fruits would need to be discussed to determine whether it is a viable proposition. Discussions would include legislation, economics, market research for requirement of the end product, harvesting management, liaison with the extraction company and the future potential for biological control of this species. These "implementation" aspects are the next stage in planning long-term control of this weed, and this is beyond the scope of this thesis.

E. SUMMARY AND CONCLUSIONS

1. Although 90-98 % of seeds of *S. mauritanum* are viable on trees, a massive mortality occurs in the soil seed bank. However, sufficient viable seed numbers remain to reinfest a cleared area for many years to come (DENNY & GOODALL, *pers. comm.*).
2. Seedling emergence is sporadic, and occurs for at least five years after killing parent trees (DENNY & GOODALL, *pers. comm.*).
3. Heat treatments kill seeds, but this is not applicable under a pine canopy, since fires are potentially uncontrollable and will probably not kill deeply buried seeds.
4. Leachates from bugweed, gum, silver wattle, pine and syringa reduced bugweed germination, but this effect was short-lived and dependent on concentration.
5. The action of most herbicides was reduced by the clay content of the soil, thus not all seeds will be killed in the soil seed bank.
6. Stimulation of germination of all seeds in the soil by chemicals with a similar action to gibberellin is considered highly unlikely, since bugweed seeds do not always respond to GA₃. Costs of these products is also prohibitive and some are not available in South Africa.

7. Solasodine was extracted and purified from green bugweed fruits, with a yield of 0.16 %. This substance was identical to the authentic compound when compared by thin-layer chromatography, high pressure liquid, chromatography, nuclear magnetic resonance and mass spectrometry techniques.
8. A second, low R_f alkaloid was present in twice the amount (0.34 %) which did not correspond to solasodine. NMR spectra were similar to solasodine, but mass spectra fragmentation patterns were different. The product still needs to be identified.
9. The utilization potential of bugweed does not change its status as a weed.
10. Harvesting of bugweed fruits is potentially a commercially viable proposition.

GENERAL DISCUSSION AND CONCLUSIONS

In the successful control of a weed species, three aspects may generally be considered. The first phase usually includes mechanical or chemical control of the vegetative growth of the plant. In the forestry situation, where bugweed is the most economically damaging in terms of access for workers and suppression of young pines after replanting, bugweed trees are generally slashed before thinning or clear-felling operations. Poisoning of stumps only occurs within the limits of the control budget. Where seeds occur in disturbed areas such as roadsides in municipal areas, slashing or spraying herbicides is used to control all weed species, including bramble, bugweed and gum. A range of products has proved highly effective in both situations, either as foliar or stump applications. The problem then is not one of control of the vegetative growth of the species, rather, the current control methods are rendered ineffective, since regeneration of the species occurs in the "cleared area", due to seed germination. Application of herbicides to foliage or stumps of plants does not affect the seeds lying in the soil beneath a stand of bugweed. Up till now, no solution to this problem has been found which is either sound in terms of effectiveness, or as an economically viable proposition.

The second aspect of weed control to be considered is the eradication of the soil seed bank. This is generally a very difficult and costly operation. For hard-coated species like some of the legumes, a mechanical cracking of the seed coat is sufficient to stimulate mass germination of seeds from the soil. Fire is commonly used in situations where hazard is relatively low. Controlled burning operations are then put into use. In the case of forest weed species, however, the fire hazard

is nearly always too high to consider this method of eradicating the soil seed bank (although this would achieve seed death rather than mass germination in the case of bugweed).

The alternatives for exhaustion of the soil seed bank are impractical and uneconomic. Here soil fumigation, microwave irradiation and pre-emergent herbicides to kill seeds in the soil, or the application of chemicals with physiological activity similar to gibberellins to stimulate germination, are either not going to deplete the majority of the seed reserves in the soil, or are simply too costly to apply on a wide scale. In addition to this, such treatments would be non-persistent, and reinfestation by seeds via dispersal of fruits from other areas would add large numbers of viable seeds to the soil.

The third aspect of weed control generally considered would be the rehabilitation of desirable species to suppress germination and establishment of undesirable species. This aspect is not considered applicable in the forestry situation, however, and thus has not been considered a priority for research.

The direction of this dissertation then, has been the investigation of the problem area of control, that is, the seed characteristics of the species, in order to discover any aspect which may be exploited to control the species. An overview of the situation has therefore been presented. This has included the reproductive yield in the species when growing under a pine canopy, the survival of seeds during dispersal, physiological mechanisms controlling germination, and variation of seed germination behaviour in response to different conditions.

Fruit and seed yield was related to bugweed tree size. The larger bugweed trees cause the greatest problem for reinfestation of an area. A total of approximately 7.2 million viable seeds per hectare were produced over a period of 20 months in flushes. This yield will probably be greatly increased for trees growing on forest margins in direct sunlight. Large trees therefore contribute the greatest seed numbers, especially on forest margins.

Positive stimuli for germination include light, alternating temperatures, storage and the application of GA₃. Negative stimuli were incubation at constant temperatures, either in the light or the dark or alternating temperatures in the absence of light. GA₃ overcame the inhibition of germination by these unfavourable conditions. Transfer of seeds from unfavourable conditions to optimum conditions (alternating 15/30°C in the light) also resulted in high germination responses. Thus seeds were in a state of conditional dormancy and apparently did not enter secondary dormancy. More detailed investigation were then carried out in order to determine whether these germination characteristics were constant.

It was found that not only does the prolific viable seed production contribute to the problem, but also variability in germination. The phytochrome system is operant in bugweed seeds, and this introduces variability in germination response for seeds under a pine canopy. Under these conditions, where a high far-red/red ratio exists, germination is inhibited. However, disturbance alters this light regime, and seeds may germinate. Flushes of seedlings may be seen after clear-felling or thinning operations.

Seeds varied in their response to environmental conditions depending on locality, season and year of shedding. These factors influenced the occurrence of primary dormancy, secondary dormancy, conditional dormancy and the response to the application of GA₃. Certain seed batches germinated less readily and entered secondary dormancy more easily than did others. Therefore these seeds would be more difficult to control, due to delayed emergence.

This variability was further compounded by duration, depth and site of storage after dispersal, which could either alleviate conditional dormancy or induce secondary dormancy. Deeply-buried seeds which essentially are stored at constant temperatures in the dark may either lose their conditional dormancy and germinate, or may be brought near the soil surface during disturbance, and this results in exposure to fluctuations of temperature. Again, sporadic seedling emergence will ensue. So, in a given population of seeds, some may be non-dormant, others conditionally dormant, and others in a state of secondary dormancy which may or may not be overcome by the application of GA₃. A sporadic seedling emergence is then assured, but application of chemicals to the soil with an action similar to GA₃ would not be likely to result in mass germination.

Alternatively, the application of pre-emergent herbicides to this soil seed bank would not destroy all seeds, due to ineffectiveness of these herbicides in some soil types. In addition to this, selection of biotypes resistant to such treatments may occur. Germination is so variable that to satisfy the requirements of every seed would need a vast number of combinations of physical factors, depending on the level of dormancy. Since this seems impossible to manipulate, then other options

should be considered for the control strategy for bugweed.

It is evident that although 90 % of the seeds produced on trees are viable, a study of seeds at different depths in the soil showed that approximately 90 % were non-viable. This occurred either due to failed seedling emergence or to seed death. Disturbance will cause germination of some of the viable seeds in the soil, and repeated disturbance will greatly reduce this viable seed fraction. More than 90 % of emerged seedlings die. Thus the viable fraction of 10 % will probably show an exponential decline over time *i.e.* high seedling emergence after the first few disturbances with a concomitant mortality, levelling off over the following years. This depletion of seed reserves in the soil can be accommodated in the control strategy for bugweed during normal forestry procedures. If further input of seeds to a cleared area can be halted, and emergent seedlings destroyed, good control of bugweed may be achieved.

The only chance this can be an economically viable proposition is via utilization of fruits for the production of solasodine, which is a precursor of corticosteroids. Further utilization potential is pelleting of waste plant material (after extraction of alkaloids) as a feed supplement for livestock. Incoming revenue may be used to eradicate trees and seedlings. Further, research to develop this idea is currently under investigation at Cedara State Forest.

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