ACHENE BIOLOGY AND THE CHEMICAL CONTROL OF *CHROMOLAENA ODORATA*

By

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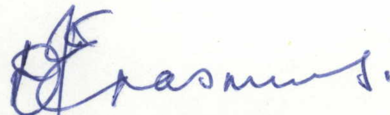
CONTENTS

		Page
PREFACE		i
ACKNOWLEDGEMENTS		ii
ABSTRACT		iv
LIST OF TABLES		viii
LIST OF FIGURES		x
LIST OF PLATES		xii
INTRODUCTION		
1.1	General Introduction	1
1.2	*Chromolaena odorata*: A Review	20
1.3	Study Objectives	50
GERMINATION		
2.1	Introduction	52
2.2	Materials and Methods: General	92
2.3	Optimum Germination Requirements	97
2.4	The Effect of Light Quality on Germination and Endogenous Sugar Levels	114
2.5	Biological Aspects of *C.odorata* Achene Germination	146
2.6	The Effect of Nitrogenous Compounds, Gibberellic Acid, Ethanol and Respiratory Inhibitors on Germination	197
2.7	Summary	232
CHEMICAL CONTROL		
3.1	Introduction	237
3.2	Materials and Methods: General	270
3.3	Screening of Herbicides in Field Trials	276
3.4	Post-Spray Colonisation of Herbicide Treatment Plots	300
3.5	Herbicide and Mixture Volume Reduction Trials	318
3.6	Summary	339
GENERAL DISCUSSION AND CONCLUSION		343
LITERATURE CITED		352

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 June 1983 to July 1985.

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.



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ABSTRACT

Chromolaena odorata (L.) R.M. King and H. Robinson has been recognised as a serious alien invader weed in Natal since the early 1960's. More recently, this weed has also been noticed in the eastern Transvaal. The problems caused by *C. odorata* in South Africa include the widespread encroachment of aesthetically valuable indigenous vegetation and timber plantations where its vigorous growth suppresses that of desirable species; invasion of veld thus resulting in a reduced livestock carrying capacity; and an increased fire hazard in all situations in which it occurs. Notwithstanding the intensity and the extent of the problem, little scientific information pertaining to this weed has been available in South Africa. Therefore information which could be used in combating *C. odorata* was urgently needed.

The achenes are the reproductive propagules and thus form the source for further encroachment and re-infestation of areas in which *C. odorata* has been controlled. Therefore an intensive investigation into the germination of the achenes was carried out. It was found that of the temperatures tested, an alternating temperature of 15/30 °C was optimum for germination. However, exposure to light was found to be a prerequisite for germination; the achenes are therefore positively photoblastic. Elucidation of the light-requirement showed that the germination response was positively affected by red light and that this could be nullified by far-red light. Exposure to red light subsequent to far-red light again pro-

moted germination. This red/far-red light reversibility apparently confirms the operation of a phytochrome-mediated dormancy mechanism. Shifts of imbibed achenes to elevated temperatures, puncturing of the achene coat (pericarp) and the majority of treatments with compounds known to relieve dormancy in seeds generally, failed to substitute for the light requirement. However, removal of the pericarp and, to a far lesser extent, application of gibberellic acid (GA_3) and thiourea partially replaced the light requirement.

The germination of freshly harvested achenes was comparatively low and the percentage germination was initially decreased by dry, dark storage at 25 °C. However, following seven months storage under these conditions, germination was found to be greatly improved thus implicating an after-ripening requirement. A similar trend was observed for achenes stored at - 18°C but in this case seven months storage resulted in only a slight improvement in the percentage germination. By applying various nitrogenous compounds, GA_3, ethanol and respiratory inhibitors, it was found that germination of these dormant achenes could be promoted under light conditions. Further investigations in which azide and salicylhydroxamic acid (SHAM) were applied indicated that dormancy during the after-ripening period might be maintained by the balance in fluxes of the cytochrome oxidase pathway (azide-sensitive) and the alternative pathway (SHAM-sensitive).

The after-ripening requirement was also found to be present in achenes harvested from various localities within the current distribution area. Harvest locality did however

influence the mass of achenes produced and the number of filled (presumably viable) achenes. In areas not well suited to *C. odorata* growth only approximately 30 per cent of the achenes produced were found to be viable. Small differences in the germination and number of achenes produced by plants within an infestation were also noted, while the number of achenes per capitulum and the germination of achenes from individual capitula varied appreciably.

Burial of achenes showed that viability was retained for at least one year. Although unsubstantiated, it was suggested that burial might cause the development of secondary dormancy. It was also observed that during burial, the pericarp became progressively "eroded" and this was thought to be responsible for the improved germination, in the dark, of achenes buried for 12 months.

Soil achene reserve studies showed that the majority of achenes were present on or near the soil surface. Up to 50 per cent of these achenes were found to be germinable and this represents approximately 12 000 seedlings per square metre.

To provide information concerning control of this alien invader, chemical control of *C. odorata* was also investigated. In screening field trials, it was found that mature coppice of this weed was particularly susceptible to tebuthiuron, triclopyr and 2,4,5-T. Subsequent trials showed that immature coppice (< one metre tall) was more susceptible to herbicides. Based on the results, it was recommended that triclopyr be applied to immature coppice as a preferable alternative to

manual and mechanical control methods currently employed.

A technique for target-specific application of herbicides was also investigated. This incorporated the use of a shield to confine the herbicide spray. The results indicated that this might form an important tool for the control of *C. odorata* (and other alien weeds) occurring in close proximity with desirable species. Additionally, the volume of herbicide required for acceptable levels of control was reduced, thereby decreasing control costs and lowering the volume of herbicide released into the environment.

Checklists on the pre- and post-spray species other than *C. odorata* and the plantation species showed that the herbicide trial plots were rapidly colonised and that the species number was greatly increased following herbicide application. The extent of the colonisation was positively associated with the percentage mortality of *C. odorata*.

The two apparently unrelated topics researched in this study, that is germination of achenes and chemical control of *C. odorata* were used to draw up suggested guidelines for reducing the intensity and extent of the problem.

LIST OF TABLES

		Page
Table 2.1.1	The classification of organic seed dormancy patterns	57
Table 2.3.1	The effect of incubation temperature on germination	100
Table 2.4.1	Concentrations of sugars tentatively identified in empty and filled unimbibed achenes	132
Table 2.5.1	Moisture content of the different age groups of achenes	155
Table 2.5.2	Changes in the moisture content during storage of achenes at 25 °C and - 18 °C	157
Table 2.5.3	Initial achene mass, percentage moisture content of achenes, number of achenes per capitulum and percentage filled achenes per capitulum of material harvested at various localities	164
Table 2.5.4	Number of achenes, number of filled and percentage filled achenes per capitulum in material harvested from four individual plants	169
Table 2.5.5	Data obtained from 100 individual capitula	172
Table 2.5.6	The effect of burial duration on germination in the light	175
Table 2.5.7	The effect of burial duration on germination in the dark	177
Table 2.5.8	Achene reserves in the soil	180
Table 2.6.1	The chemicals and treatments used in attempting to stimulate germination in the dark	200
Table 2.6.2	Germination in the dark of achenes incubated on filter paper moistened with either azide, cyanide, nitrate or nitrite	212
Table 2.6.3	Germination in the dark of achenes imbibed for restricted periods on various compounds	213
Table 3.1.1	Methods of weed control and the associated techniques	238
Table 3.1.2	Techniques used for the application of herbicides to woody weeds	244
Table 3.1.3	Common names, chemical names, active ingredient concentration and trade names of herbicides used in screening field trials on *C. odorata*	248

		Page
Table 3.2.1	Monthly means of daily maximum and minimum temperatures and the mean monthly precipitation recorded in the Melmoth and Clansthal regions	271
Table 3.2.2	Analyses of soil samples collected from the Melmoth and Clansthal trial sites	272
Table 3.3.1	Herbicide treatments applied in the Melmoth and Clansthal A and B trials	278
Table 3.3.2	Environmental conditions at the time of herbicide application in the Melmoth and Clansthal A and B trials	283
Table 3.3.3	*C. odorata* mortality in the Melmoth trial	286
Table 3.3.4	*C. odorata* mortality in both Clansthal trial A and B	288
Table 3.3.5	The cost of *C. odorata* control with triclopyr and tebuthiuron	294
Table 3.4.1	The pre- and post-spray species occurring in the Melmoth trial site	303
Table 3.4.2	The pre- and post-spray species occurring in the Clansthal A trial site	305
Table 3.4.3	The pre- and post-spray species occurring in the Clansthal B trial site	308
Table 3.5.1	Environmental conditions at the time of directed-spray herbicide application	324
Table 3.5.2	*C. odorata* mortality following application of triclopyr and glyphosate by the directed-spray technique	325
Table 3.5.3	*C. odorata* mortality following application of triclopyr and glyphosate in the overall-spray trial	334

LIST OF FIGURES

		Page
Figure 1.2.1	The distribution of *C. odorata* in Natal	29
Figure 2.1.1	Absorption spectra of phytochrome	65
Figure 2.1.2	Transformation of phytochrome	67
Figure 2.1.3	Respiratory pathways which might be involved in dormancy breakage	77
Figure 2.3.1A	The effect of the dark imbibition duration on germination of achenes incubated at 15/25 °C	102
Figure 2.3.1B	The effect of the dark imbibition duration on germination of achenes incubated at 15/30 °C	103
Figure 2.3.2	Achene mass increase during dark imbibition	105
Figure 2.3.3	The effect of white light duration on germination	106
Figure 2.3.4	The effect of cumulative white light treatments on germination	108
Figure 2.4.1	The effect of time of exposure to R light on germination	121
Figure 2.4.2	The effect of time of exposure to FR light on germination	122
Figure 2.4.3	The effect of alternating R and FR light on germination	124
Figure 2.4.4	The effect of shortened exposure time to alternating R and FR light on germination	125
Figure 2.4.5	The effect of puncturing the pericarp on germination	126
Figure 2.4.6	The germination response after removal of the pericarp	127
Figure 2.4.7	The effect of elevated temperature shift on germination	128
Figure 2.4.8	Gas chromatographic separation of authentic sugars	130
Figure 2.4.9	Changes in the levels of endogenous sugars following imbibition in the dark or in R light	133
Figure 2.4.10	Changes in the levels of fructose, glucose and sucrose in response to R light	135
Figure 2.4.11	Spectral distribution of radiation under a closed forest canopy	142
Figure 2.5.1	The age groups of achenes harvested at various times	148

Page

Figure 2.5.2 Germination of the different age groups of achenes harvested at various times 159

Figure 2.5.3 Germination response to storage of green, medium and dry achenes at 25 °C and - 18 °C 161

Figure 2.5.4 Final percentage germination of green, medium and dry achenes stored at 25 °C or - 18 °C for various periods 163

Figure 2.5.5A Germination of freshly harvested achenes collected from various localities 166

Figure 2.5.5B Germination of achenes stored for seven months following harvest from four localities 168

Figure 2.5.6 Germination of achenes harvested from four individual plants 171

Figure 2.5.7 Data obtained, including germination responses, of achenes extracted from 100 individual capitula 173

Figure 2.6.1 The effect of nitrogenous compounds, a respiratory inhibitor, ethanol and GA_3 on the germination of dormant achenes 203

Figure 2.6.2A The effect of nitrogenous compounds, on the germination of partially dormant achenes 206

Figure 2.6.2B The effect of hydroxylamine, ethanol and GA_3 on the germination of partially dormant achenes 207

Figure 2.6.2C The effect of azide on the germination of partially dormant achenes 208

Figure 2.6.3A The effect of azide on the germination of achenes stored for two months and incubated (moist) in the light for 24 days 210

Figure 2.6.3B The effect of GA_3 on the germination of achenes stored for two months and incubated (moist) in the light for 24 days 211

Figure 2.6.4 The effect of SHAM on the germination of dormant achenes 215

Figure 2.6.5 The effect of azide, SHAM and nitrite on the germination of dormant achenes 216

Figure 2.6.6 The effect of azide and SHAM on the germination of after-ripened achenes 217

Figure 3.1.1 Classification of herbicides according to use 240

Figure 3.1.2 2,4,5-T 250

Figure 3.1.3 Glyphosate 250

Figure 3.1.4 Tebuthiuron 250

		Page
Figure 3.1.5	Triclopyr	260
Figure 3.1.6	3,6-dichloropicolinic acid	260
Figure 3.1.7	Imazapyr	260
Figure 3.3.1	The Melmoth trial site	280
Figure 3.3.2	The Clansthal A trial site	281
Figure 3.3.3	The Clansthal B trial site	282
Figure 3.5.1	The directed-spray trial site	320
Figure 3.5.2	The shield used in the directed-spray trial	322
Figure 3.5.3	The overall-spray trial site	332

LIST OF PLATES

Plate 1	*Chromolaena odorata* (L.) R.M.King and H. Robinson	19
Plate 2	The glands on the adaxial leaf surface	23
Plate 3	*C. odorata* achene	116
Plate 4	The age groups of achenes tested for germinability	148
Plate 5	The effect of burial on achene coats	178
Plate 6	The shield used in the directed-spray trial	322

INTRODUCTION

1.1 General Introduction

Published literature pertaining to weeds usually includes a section regarding the definition of a "weed". This has resulted in numerous definitions being proposed, some of which have been generally accepted. The most widely used definition is that "a weed is a plant growing where it is not wanted" (PARSONS, 1973; ROBERTS, CHANCELLOR & THURSTON, 1977; KLINGMAN & ASHTON, 1982; STEPHENS, 1982; ANDERSON, 1983). The list of references quoting this definition is extensive. However, even this seemingly simple definition is subjective in that a specific plant species may be classified as a weed by one author but not by another. In addition, a plant species may be a weed in certain situations but not in others. It therefore follows that almost any species may, at sometime, be referred to as a weed or, as stated by WELLS (1978), "all plants are potential weeds if man chooses to make them so".

Initially, only unwanted plant species occurring in arable lands were considered weeds; these are referred to as the "original weeds" (HOLZNER, 1982). But, man has tried to utilise more and more of his environment as a result of the ever-increasing human population. This has brought him into conflict with more and more plant species (WELLS, 1978). Consequently, weed conditions now include a large variety of situations such as pastures, forests, wetlands, rights of way, conservation areas and even aesthetically valuable areas such

as indigenous vegetation communities. BAKER (1965) therefore defined a plant as a weed "if, in any specified geographical area, its populations grow entirely or predominantly in situations markedly disturbed by man (without, of course, being deliberately cultivated plants)".

Weeds occurring in certain situations are distinguished from other unwanted plants by being referred to by a specific title. Of relevance to this dissertation are those weeds which are referred to as "veld invaders" and "alien invaders" in South Africa. The term "veld" is defined as "land which is not being or has not been cultivated and on which indigenous vegetation or other vegetation"..."is or can be utilised as grazing for animals" (Act 43 of 1983, the Conservation of Agricultural Resources Act). "Invader" obviously describes the character of the plant species. Therefore a veld invader is a weed species, indigenous or introduced, which encroaches grazing land, the term is therefore used in an agricultural context. An "alien invader" refers specifically to an introduced (exotic), encroaching plant species but does not specify the situation in which it occurs, although it is generally accepted that cultivated areas are excluded. Therefore it can be assumed that alien invaders may occur in any situation where they are not desired. Alien invaders and veld invaders may therefore be synonymous with regard to a plant species which is introduced and infests, among other situations, the veld. This is the case with *Chromolaena odorata* (L.) R.M. King and H. Robinson, the species studied for this dissertation. Since "alien invader" encompasses a wider range of weed

situations, yet refers to a specific group of plant species, this term will be used hereafter, although "veld" invader will be used to specifically describe indigenous species invading grazing land. These invader weeds are referred to as "environmental weeds" by HOLZNER (1982) and are described as being introduced, aggressive species that colonise natural vegetation and suppress native species to a certain extent (AMOR & STEVENS, 1975).

The situations in which alien invaders occur, are numerous. These include conservation areas such as river catchments, nature and game reserves and indigenous vegetation, rangeland/veld, commercial forest plantations and vacant and/or wasteland in rural and urban areas. These areas consist of either:

(i) disturbed habitats, for example over-grazed or mismanaged veld, areas frequently subjected to burning, commercial forest plantations and wasteland, or

(ii) largely undisturbed habitats, for example indigenous or natural forests and other vegetation which is well managed where veld climax plant communities are present.

The major difference between indigenous veld invaders and alien invaders is that alien invaders encroach both disturbed and apparently undisturbed habitats. Conversely, the indigenous species may dominate a disturbed habitat but not usually an undisturbed habitat. Disturbance in the latter case, generally consists of mismanaged areas in which the natural species diversity becomes unbalanced. A species

particularly adapted to the altered conditions becomes the dominant plant. Mismanagement may consist of a number of activities. For example, overgrazing of an area results in over-utilisation of palatable species until these are completely removed (TOTHILL, MOTT & GILLARD, 1982). The unpalatable species are considered to be weeds and may also lead to the initiation of later stages of succession, in which woody species are likely to predominate. In Australia the woody weeds are by far the most important in native grazing lands (MOORE, 1971) while bush encroachment in many parts of South Africa has become a serious problem. These species therefore become pasture weeds because they "act detrimentally to the animal productivity of a pasture" (TOTHILL, MOTT & GILLARD, 1982). The over-use of fire also results in the shift of succession, but this may favour grass species. Therefore species adapted to the fire regime will become the dominants. It must be realised however, that mismanagement also encourages the encroachment of an area by alien species.

Since undisturbed natural vegetation is very resistant to invaders (SAGAR & HARPER, 1961), colonisation in established communities is usually a sign of disturbance either natural or "unnatural" (human) (HOLZNER, 1982). NUMATA (1982) categorised human interference (or disturbance) of native vegetation into "degraded", "ruderal" and "cultivated". "Degraded" is a habitat where the disturbance of the original community is incomplete and sporadic. "Ruderal" is an area not being used for the production of economic crops, where the original

community is destroyed and a destructive agent is repeatedly applied. "Cultivated" interference occurs in an area being used for crop production. Obviously invader weeds in the present context infest the "degraded" and "ruderal" habitats. The implication from the literature is therefore that although alien species are often thought to invade undisturbed, natural, indigenous vegetation communities, they only do so because some disturbance has occurred. This, together with the weed characteristics common to these species, results in the encroachment of seemingly undisturbed, natural vegetation.

Examination of the Conservation of Agricultural Resources Act (Act 43 of 1983) shows that the most serious and most common of the proclaimed weeds are alien invader species. For example, *Stipa trichotoma* Nees (nasella tussock), a grass native to South America, has infested approximately 70 000 ha of veld in the eastern Cape Province (JOUBERT, 1984). Another serious alien invader is *Opuntia aurantiaca* Lindl. (jointed cactus) which probably originated from central, eastern Argentina and Uruguay in South America (ZIMMERMANN, 1983a). The total area of actual infestation in the Cape Province is apparently in excess of 800 000 ha. Jointed cactus infestations limit the grazing potential of the veld because this species is covered with needle-sharp, barbed spines (ZIMMERMANN, 1983a).

As with alien species problematical in South Africa, species indigenous to South Africa also become serious weed problems when introduced to other countries. For example,

in Australia, *Chrysanthomoides monilifera* (L.) Nordlindh. is a serious invader weed, its dense bushy growth resulting in the suppression of desirable indigenous species (PARSONS,1973). In South Africa however, the protection and cultivation of this species along the Natal coastal belt is encouraged because not only is it a desirable indigenous species, but it is also grown to stabilise dunes and sandy soil along road verges.

The alien invaders have arrived in South Africa in various ways. Many species were intentionally imported for their apparently desirable qualities and have subsequently "escaped" from cultivation. *Rubus cuneifolius* Pursh. (bramble) is a serious invader of agricultural and forestry land. This species was apparently imported for its berries which were used for jam-making (WAGER, 1947) but has subsequently become naturalised and encroached large areas. Another serious alien invader, *Hakea sericea* Schrad. was probably imported for growing hedgerows but has "escaped" cultivation in large areas of the Cape Province (NESER & FUGLER, 1983). Certain species, imported for timber production, have subsequently "escaped" from the plantations and encroached adjoining land where they have become serious invaders, for example *Acacia dealbata* Link in Natal and *Pinus pinaster* Ait. in the Cape Province (KRUGER, 1983).

Other invader species have been imported into South Africa unintentionally. It is thought, for example, that nasella tussock arrived in South Africa by way of seed contaminated hay imported from Argentina for the horses of

the British Army during the South African War in 1899 - 1902 (WELLS, 1983).

Many alien invaders were also imported as ornamental species, which have now become naturalised and have encroached large areas. Examples of these species include *Eichhornia crassipes* (Mort.) Salms. (KLUGE, 1983), *Lantana* species (STIRTON, 1983a) and *Opuntia imbricata* (Haw.) D.C. (ZIMMERMANN, 1983b).

In many cases the escape from cultivation of these species seems to go unnoticed, or is encouraged by continuous, unintentional spreading to remote areas by man. Obviously at this early stage, these species are not considered to be weeds nor is their potential as weeds realised. Nuclear infestations provide a propagule source and, what appears to be suddenly, large areas are infested and the species joins the rank of the alien invaders.

Numerous explanations have been offered for the apparent success of these alien invaders in their new environment. MACDONALD & JARMAN (1984) identified three causal hypotheses for the invasion of the fynbos biome in the Cape Province. These were firstly, that alien plant invasions only occur in ecosystems disturbed by man; secondly, that the ecosystems in the fynbos biome are inherently susceptible to invasion; and the third hypothesis relates to the attributes of the alien invader species themselves.

Regarding the first hypothesis, the situations in which alien invaders occur have already been discussed. From

this previous discussion it can be deducted that invader species occur both in obviously disturbed and apparently undisturbed areas in Natal. However, as was concluded, it is probable that some disturbance had occurred which, in combination with other factors, has resulted in invasion.

The second hypothesis relates to the susceptibility of the naturally occurring communities to encroachment by alien species. One of the major factors which contributes to the susceptibility is the lack of natural competitors which combine to restrict the growth, abundance and distribution of the alien species in their land of origin (HILL, 1977; STIRTON, 1983b). The indigenous plant species however, must contend with natural enemies and are therefore at a disadvantage. For example, jointed cactus native to central South America, invaded vast areas in eastern Cape Province where the natural vegetation is heavily utilised for grazing and browsing. The jointed cactus however, is not eaten. With the introduction of one of its natural predators, *Dactylopius austrinus* (jointed cactus cochineal) which was introduced from South America, the jointed cactus populations were considerably reduced to nearly acceptable levels (ZIMMERMANN, 1983a). It seems that a number of predators may be required to reduce these jointed cactus populations even further.

Another major factor contributing to the susceptibility of natural plant communities to invasion by alien species is the existence of an unfilled ecological niche within the community. The community is thus rendered vulnerable to encroachment by alien species (HILL, 1977). The niche, defined as the

position of a species in a community with regard to its spatial, temporal and trophic relationship to other existing species (OKA & MORISHIMA, 1982), may be such that filling by the alien species results in rapid and extensive invasion of indigenous vegetation. The ecological niche may have arisen as a result of disturbance within the existent indigenous vegetation. The disturbance resulting in a state of imbalance, may be natural or man-mediated. Natural disturbances such as floods or droughts disrupt the usual progression of succession, opening up a niche or niches suitable for colonisation by alien plant species. Man-made disturbances are probably more common and take the form of over-utilisation and exploitation of the land, resulting in habitats vulnerable to rapid infestation by alien species. In both natural and man-made disturbances an "ecological vacuum" (HILL, 1977) is created which is rapidly filled by species most suitably adapted to that particular situation; often alien plant species because although weeds may have different potential niches, their realised niches are opportunistic (OKA & MORISHIMA, 1982). The vacuum may actually result from the eradication of an alien weed species, as has apparently happened in Natal. Here, there are situations where an area was infested by *Lantana camara* L. but on removal, the site has rapidly been colonised by *C. odorata*.

The third hypothesis proposed for the success of alien species relates to the attributes of the alien invader species themselves. The attributes, generally referred to as weed characteristics, will not be discussed in detail since reviews

and articles on this aspect of weed biology abound in journals and manuscripts dealing with weeds in general (HILL, 1977; HOLZNER & NUMATA, 1982; KLINGMAN & ASHTON, 1982; STEPHENS, 1982; ANDERSON, 1983). Furthermore, since as discussed previously, any plant species can become a weed, the weed characteristics are many and varied and generally not in the context of this dissertation. However, because so many of the aforementioned articles and reviews usually apply mainly to the weeds of arable lands (agrestals), it is deemed necessary to emphasise certain characteristics which are of particular relevance to the alien plant invader species. These are discussed below:

(i)a. The majority of problem alien invader species in South Africa are perennials, therefore no gaps exist in the life cycle of these species which would make the infested area vulnerable to colonisation by other plant species. The established plants in the infestations provide a constant competitive presence against other species. In cases where the top growth is seasonal, such as the biennial canes (stems) of bramble plants there is a continual production of top growth, thereby providing the physical presence.

b. Due to the perennial nature of these species, they are usually woody or herbaceous.

(ii) Like most weed species, alien invaders rapidly colonise and infest disturbed areas. In these situations the habitat has been disturbed thus resulting in a habitat

vulnerable to weeds generally. However, the alien invader infestations are semi-permanent or, in cases even permanent, because the natural progression of succession is halted by the presence of these perennial plants which then form the dominants in the climax community.

(iii) The alien invaders usually have an efficient reproductive system providing this group of plants with a characteristic consistent with that of weeds in general. This reproductive system may be by means of sexual and/or asexual propagules.

(iv) Plant invaders are extremely competitive to the point where they are often labelled "aggressive". The encroachment by plant invaders of multi-species plant communities where there is a delicate balance between the species, results in disturbance of the plant community. Consequently a situation develops where the invaders become dominant over the other species (STIRTON, 1983b).

(v) A number of common features, pertaining to the alien invader species, have been identified in those aliens which do become aggressive weeds (NESER, 1984). These features primarily refer to the species in their country of origin and are:

(a) they show a local or temporary tendency to grow in dense stands;

(b) they have a relatively wide distribution as they are not limited to specialised habitats;

(c) they display plasticity in variability; and

(d) seeding is drastically reduced by specialised enemies (predators).

(vi) Alien plant species may form a vigorous hybrid with closely related native or introduced species (OKA & MORISHIMA, 1982; STEPHENS, 1982). An obvious example is *L. camara* which is a native of South America and the West Indies. In South Africa this species apparently consists of a number of vigorous hybrid varieties (STIRTON, 1983a) which may well explain its success as an alien invader. This may also be the case in *R. cuneifolius* and other vigorous growing *Rubus* species in South Africa (STIRTON, personal communication*).

A number of other factors may also explain why alien invader plants often become the dominant species. For obvious reasons, intensively cultivated land is of greater economic importance than (a) areas where extensive agricultural activities are practised and (b) land utilised for nature reserves, conservation and preservation of indigenous vegetation. Commercial forests can also be included in the second category since returns on investments are realised only after a number of years (20 to 30 years). In land of high economic value,

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disciplined production records are kept, providing a means of identifying factors which reduce profit margins. One of the primary causes of decreased production is the occurrence of weeds in these cultivated areas. Production is decreased by weeds through lowered yields as a result of, primarily, competition and other factors such as the possible phytotoxic effect of weeds on the cultivated plant species. The volume of literature, the magnitude of the weed control industry and the effort put into weed control by agriculturalists and silviculturalists is certainly an indication of the economic importance of weeds. Clearly, it is not difficult to provide experimental data which emphasise the detrimental influence of weeds in these situations. However, in more marginal agricultural land, that is the veld, the agricultural activities do not readily lend themselves to calculating the impact of weeds. Furthermore, it is not always possible to attach values to the importance of weeds occurring in conservation areas, nature reserves and tracts of indigenous vegetation. These weeds are the invaders and, because their impact is often thought to be of low economic importance, they attract little attention. Consequently, these invader species are left to dominate such areas unabated. However, there is currently considerable interest being displayed in the control of invader weeds because of the encroachment of aesthetically important areas. Also, the reduction in the value of infested land has promoted some research in the field of invader weeds. Nevertheless, it would appear that researchers have been reluctant to involve themselves with these weeds because, understandably, financial support has been limited.

Closer inspection of the problems caused by the invader weeds reveals that a greater effort should be made in order to successfully control these species. It should be mentioned that "eradication" of weeds is a commonly used expression. Realistically, however, this is usually impossible and therefore control is aimed at; that is, the reduction of the level of infestation to acceptably low limits. The problems caused by invaders are numerous and varied. These will be discussed in order of "economic" importance while the aesthetic importance will be emphasised.

Alien invader infestations reduce food yield in a number of situations. For example, encroachment of grazing by unpalatable invaders results in decreased livestock carrying capacity with a resultant decrease in meat production. Bush encroachment is a problem faced by cattle farmers in many parts of South Africa. In Natal large areas of grazing land have been infested by *L. camara*, bramble and *A. dealbata*. In the Cape Province *Opuntia* species, especially jointed cactus (ZIMMERMANN, 1983a), *Prosopis glandulosa* Torrey (HARDING, 1983) and nasella tussock (WELLS, 1983) have infested large areas. Not only is the productivity of the grassland being reduced, but the species diversity is being irreparably damaged and the scenic beauty of the areas flawed.

Additionally, some invader species are poisonous, notable species being *L. camara* (STIRTON, 1983a), *Senecio isatidens* D.C. (HILLIARD, 1977) and *Sesbania punicea* (Cav.) Benth. (PIENAAR, 1983). Livestock deaths have been reported following ingestion of these plants. Other species, although

not poisonous, also result in decreased meat production through injury to livestock. Jointed cactus leaf pads bear sharp spines which inflict serious wounds to sheep. Wool production can also be reduced by the occurrence of weeds such as nasella tussock and *Xanthium strumarium* L. because the seeds attach to the fur coats and thereby reduce the quality of the wool.

In cultivated areas, alien invaders may also present a problem. During the rotation of crops being produced on a certain section of land, a fallow period is often included in an attempt to restore the organic and nutrient status of the soil. During this time, the fallow land can be invaded by aliens. Preparation of the land prior to subsequent crop production is therefore costly, especially since the aliens are usually woody perennials therefore necessitating destumping the land.

Invader weeds also occur in commercial forest plantations where they suppress seedling growth causing reduced timber yields. In addition, timber production costs are increased since removal of these weeds is necessary to enable normal silvicultural operations to be carried out. *A. dealbata*, bramble, *L. camara*, *Pereskia aculeata* Mill. and *Solanum mauritianum* Scop. are examples of such weeds.

Large sums are spent annually on road verge maintenance in South Africa. Many of the alien invader species are also roadside weeds and the herbaceous and woody species, particularly, hamper operations because they cannot simply be mowed. Manual

control and/or herbicides are required to control infested areas thereby increasing maintenance costs.

In game reserves, invader weeds cause similar problems to those encountered in livestock farming. The problem is further compounded by the fact that many of these weeds are woody perennials which are unpalatable. Infestations result in decreased browsing food for game. Consequently, greater grazing pressure is placed on uninfested areas which are then overgrazed and subsequently provide a disturbed habitat for further weed encroachment. Water catchment areas provide another situation where alien invaders are of economic importance. In South Africa, water is a limiting commodity for many industries. To provide a reliable supply of high quality water, the catchment areas must be well managed to prevent deterioration which could lead to a decrease in water volume and quality. Infestation by invaders could lead to reduced ground cover, for example, under an *A. dealbata* canopy, which could cause erosion and upset the balance between run-off and infiltration. Erosion and decreased penetration could cause drying of perennial springs which feed the river systems.

Of major aesthetical importance is the encroachment of existing vegetation by invaders. Species diversity is decreased by the filling of an ecological niche or niches by invaders which eventually dominate the plant community. The indigenous vegetation eventually disappears and in its place is a profusion of alien weeds. As previously mentioned, it is extremely difficult to attach economic values to these situations. However, an attempt can be made by taking certain

pertinent factors into consideration. A reasonable estimate of the amount spent on conservation activities by such organisations as the National and Provincial Parks Boards, Departments of Forestry and Environment, Department of Agriculture and Water Supply, The Wildlife Society of South Africa and the individual municipalities can be made. If a figure were to be obtained, it seems certain that the annual expenditure would be staggering and would probably put the economic importance of alien invaders into proper perspective. Furthermore, because these amounts are spent willingly and with consent from the public, it must be assumed that there is a great demand for conservation and hence, also for the control of alien invader weeds.

A few of the alien invader species may also have beneficial aspects when they occur in certain situations, or where these species are constructively used. The woody species may provide an invaluable source of firewood where they occur in predominantly grassland regions. In this case, these "weeds" would also provide a supply of building material. Some "weeds" may also comprise a food source for human and animal consumption. For example, the fruits of *Opuntia ficus-indica* (L.) Mill., an invader in the eastern Cape Province, are utilised for food and provide an income where they are sold. Additionally, the young stems of some of the *Opuntia* species provide an untapped food source for human consumption (ZIMMERMANN, personal communication*).

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Some of the cactus species are sometimes used as a stock feed, especially during drought conditions. The pods of *P. glandulosa* are used as fodder in the northern Cape Province (HARDING,1983). Invader weed infestations may also be beneficial where exposed ground is colonised. Due to their apparent hardiness, these species may, for example, prevent erosion by their rapid establishment thereby providing a suitable ground-cover. Invader weed infestations also provide a sanctuary for both small mammals and birds. Conversely however, these infestations may provide cover for vermin.

From the aforegoing discussion, it is clear that although there are many alien invaders in South Africa, the research on these species has been limited by virtue of various factors. In Natal there are a number of serious invader species which need to be controlled urgently. *C. odorata* is one of these and is the subject of investigation in this study. Before stating the objectives of the study however, it is necessary first to review the current state of knowledge of this weed.

Plate 1 *Chromolaena odorata* (L.) R.M. King and H. Robinson (triffid weed). X 0,3 .

1.2 *Chromolaena odorata*: A Review

Until 1970, *Chromolaena odorata* was taxonomically known as *Eupatorium odoratum* L., a species of the family Asteraceae. However, following an investigation into the genus, KING & ROBINSON (1970) classified this species as *Chromolaena odorata* (L.) R.M. King and H. Robinson in the subgenus *Chromolaena*. However, it is still widely known as *Eupatorium odoratum*, suggesting that the classification by KING & ROBINSON (1970) has not been generally accepted. In South Africa, the National Herbarium (Botanical Research Institute, Pretoria) is using *Chromolaena* as the accepted nomenclatural name (GIBBS RUSSELL, 1984) and therefore *Chromolaena odorata* will be used in this dissertation.

In South Africa *C. odorata* is commonly known as triffid weed or paraffin weed. "Triffid weed" is apparently derived from this species' ability to rapidly encroach large areas while "paraffin weed" describes the extraordinary inflammability of the plant and the black oil-like smoke given off during burning. As is often the case with a widely distributed species, *C. odorata* has numerous common names local to specific areas where it is found. For example, it is also known as "Armstrong's weed" and "King's weed", Armstrong and King being farmers who independently drew attention to its presence (EGBERINK & PICKWORTH, 1969). Other common names used throughout the global distribution of *C. odorata* are listed by HOLM, PLUCKNETT, PANCHO & HERBERGER (1977) and by LIGGITT (1983). Since it is desirable to promote the use of

a single common name (WELLS, 1984), it is suggested that "triffid weed" be used because it would seem that this name is most widely used in Natal and in South Africa generally.

C. odorata is a much branched perennial shrub, the growth form of which is dependent on its habitat. In open rangeland the young plants are upright, slender, single-stemmed plants while the mature plants are typically upright, bushy shrubs up to three metres in height with stems of two to eight centimetres in diameter at the base. In rangeland conditions, mature infestations consist of dense stem entangled thickets. In habitats predominated by arborescent and shrubby species, *C. odorata* assumes a scrambling habit with elongated and comparatively thin stems (one to two centimetres and up to four centimetres at the base). These plants reach up to seven metres in length.

C. odorata stems are round and branch in opposite pairs at the nodes. The lateral branches usually only become noticeable towards the end of the first year's growth. The stems and branches are brittle and, at maturity, are fairly woody. The first-year stems are conspicuously green in colour while the older stems are grey-green to grey.

The light-green leaves are opposite at the nodes, alternate at successive nodes and are lanceolate and ovate to triangular in shape. The leaves are up to 10 cm in width and 13 cm long (in shady habitats), have serrated margins and are conspicuously three-veined. The first leaves produced on the seedlings lack these three veins, however. When crushed,

the leaves have a characteristic pungent odour due to the presence of essential oils (MONI & SUBRAMONIAM, 1960) which are probably contained in the numerous small glands present on the adaxial leaf surface (Plate 2).

Although *C. odorata* is shallow-rooted, the root system is fairly extensive, much branched and bearing copious fine roots. No conspicuous tap-root is present.

There are a number of species with which *C. odorata* could be confused if superficially examined. *Mikania natalensis* D.C. (syn. *Mikania cordata* (Burm. f.) B.L. Robinson) which is also a weed in plantation crops in West Africa, India and the Pacific islands (HOLM, PLUCKNETT, PANCHO & HERBERGER, 1977), also belongs to the family Asteraceae. The similarities and differences between *M. natalensis* and *C. odorata* are:

(i) Although the leaves are similar in shape, those of *M. natalensis* are lobulate and lack the pungent smell of those of *C. odorata*.

(ii) Although the flowers of *M. natalensis* are typically composite and similar in colour and size to those of *C. odorata*, only a single row of loosely packed bracts are present as compared to the several rows of tightly packed involucral bracts in the flowers of *C. odorata*.

(iii) *M. natalensis* is a creeper.

Eupatorium adenophorum L. (syn. *Ageratina adenophora* (L.) R.M. King and H. Robinson) is also similar to *C. odorata*.

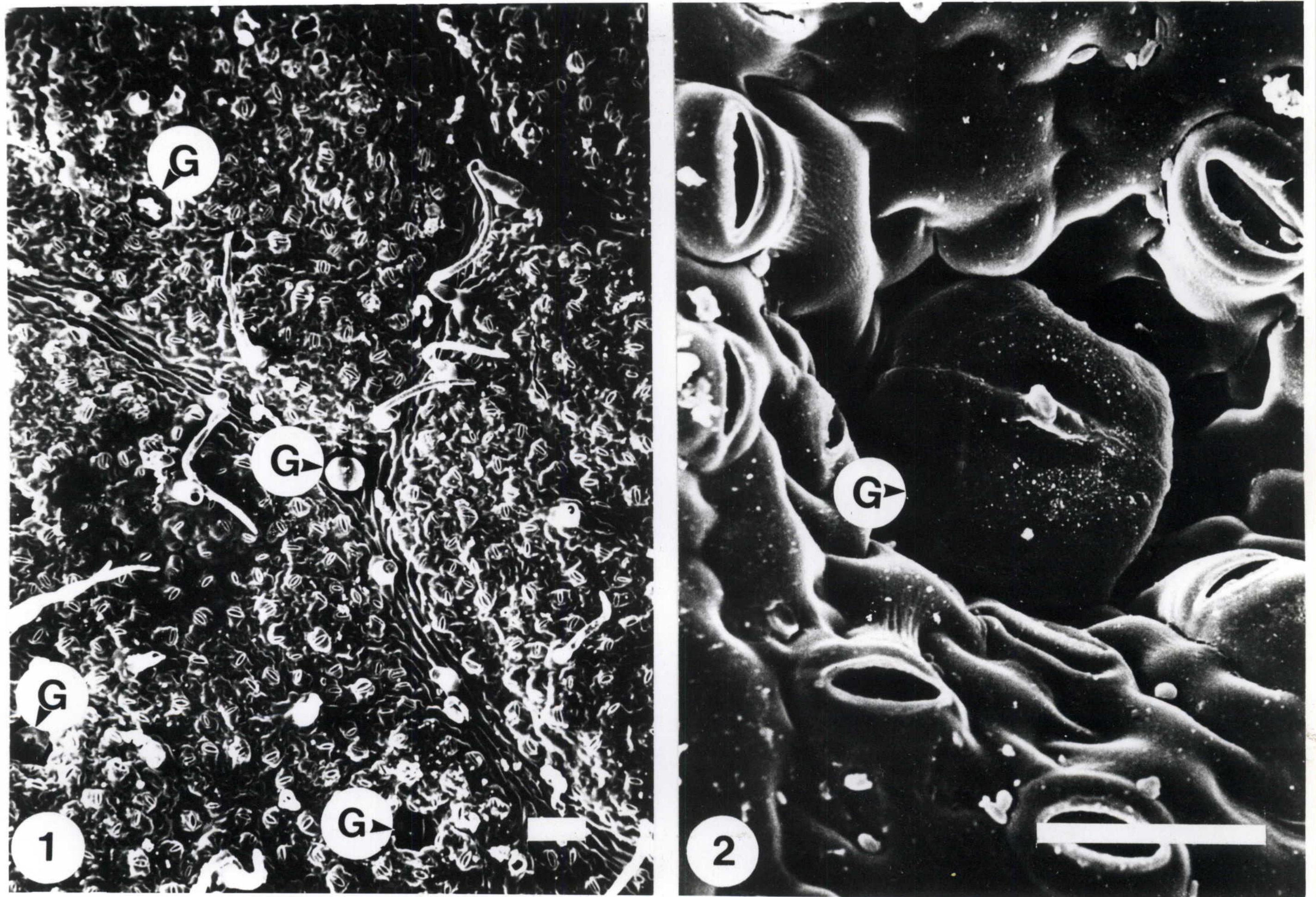

Plate 2 The glands (G) on the adaxial surface of the leaves. (The material was prepared as described for SEM of achenes, in section 2.5).
1. Bar = 100 µm 2. Bar = 50 µm.

The similarities and differences are:

(i) *E. adenophorum* has smaller flowers of the same colour as those of *C. odorata*. Each capitulum bears approximately 60 achenes compared with approximately 18 in *C. odorata*.

(ii) The leaves of *E. adenophorum*, which are similar in shape and morphology to those of *C. odorata*, are an olive-green colour while those of *C. odorata* are light-green. Crushed leaves of *E. adenophorum* do not have the pungent smell of those of *C. odorata*.

(iii) The growth habit of *E. adenophorum* is generally considerably smaller than that of *C. odorata* and mature plants are not as woody as those of *C. odorata*.

(iv) At present the distribution of *E. adenophorum* is largely restricted to the mist-belt region of Natal while *C. odorata* occurs mainly in the coastal region. There is however, some overlap in distribution.

E. adenophorum , a weed in New Zealand (HOY, 1960), California (FULLER, 1981), India, Australia and Thailand (AULD, 1981), is currently considered to be a potentially serious weed in South Africa. The use of biological agents for the control of this species is currently under investigation by Plant Protection Research Institute, Department of Agriculture and Water Supply.

The seedlings of *Ageratum houstonianum* Mill., a cosmopolitan weed of disturbed areas (HOLM, PLUCKNETT, PANCHO &

HERBERGER, 1977), resemble *C. odorata* seedlings in that the leaf morphology is similar. Differences soon become apparent however. *A. houstonianum* reaches a maximum height of only approximately 50 cm and is an herbaceous annual bearing purple flowers.

Although there are a number of species morphologically similar to *C. odorata*, its distinctive feature is the pungent smell of crushed leaves. This characteristic has also contributed to the nomenclatural name in the form of "*odorata*" or "*odoratum*".

C. odorata is a native of Central and Tropical America (HILLIARD, 1977; IVENS, MOODY & EGUNJOBI, 1978) and the West Indies (IVENS, 1975). From these regions it has spread to other continents where it has become a serious weed problem. Countries where it has been recorded as a weed are predominantly located in the tropics, with few exceptions.

This species, as is the case with many of the composites (Family Asteraceae), bears numerous small fruits (achenes, as described later) which are adapted for wind dispersal. Therefore, the spread of the species is rapid following the initial establishment of infestations. Consequently reports of infestations often emanate from several regions concurrently, thereby complicating attempts at identifying the original site of establishment and in providing explanations regarding its introduction. The similarity in the origins and spread of this weed in the various countries where it occurs, is therefore not surprising.

Besides South Africa, *C. odorata* is considered to be a weed in Nigeria, Ghana, Ivory Coast, India, Philippines, Dominican Republic, Thailand and Malaysia.

The introduction of *C. odorata* into countries where it is a serious weed problem, has, without exception, been unintentional. Introduction has been either by way of contaminated seed in Nigeria (ODUKWE, 1965) and Ghana (HALL, KUMAR & ENTI, 1972), or by contaminated clothing in India (MONI & GEORGE, 1959). In other countries infested with *C. odorata*, no explanations for its introduction have been volunteered, implying that it has been unwittingly introduced.

On introduction, the spread of *C. odorata* in the respective countries, has been rapid. Wind dissemination of the pappus-bearing achenes has obviously aided in the rapid and extensive encroachment and man has probably also unintentionally been responsible for the spread of this weed. Suffice it to say that names such as "mile-a-day" plant and "triffid weed" aptly describe the speed with which host countries have become infested.

The literature concerning the origin and distribution of *C. odorata* in South Africa is scant since few published or unpublished reports exist. This situation has probably arisen because the original spread and distribution was inconspicuous as infestations occur predominantly in wasteland of apparently low economic value. However, with encroachment of conservation areas and the relic aesthetically valuable indigenous coastal vegetation, attention has been attracted

to the problem presented by *C. odorata* infestations. As a result information pertaining to the weed is increasing.

The only available literature dealing with the origin and early spread of *C. odorata* in South Africa consists of an information pamphlet published in "Farming in South Africa" by EGBERINK & PICKWORTH (1969) and an unpublished report presented to the Lower Tugela Farmers' Soil Conservation Committee by PICKWORTH (1976). From these reports it would appear that *C. odorata* was accidently introduced into South Africa in achene-contaminated packing material from the West Indies off-loaded at Durban Harbour during World War II. The earliest herbarium specimen was collected from Ndwedwe (± 30 km north-east of Durban) (HILLIARD, 1977) and by 1950 this species had become a conspicuous weed in the Durban area. In 1960 *C. odorata* was well established in the Verulam district north of Durban and by 1962 infestations occurred on the northern side of the Tugela River. It was also present along the coast south of Durban during this time and by 1969 it occurred from Port Shepstone in the south to Gingindlovu in the north. Conspicuous infestations were also present inland as far as Kloof by 1963. During the 1960's infestations reportedly increased in density and extent in waste and non-utilised land. By 1976, *C. odorata* was recorded as far north as Hluhluwe Game Reserve (by 1961 according to MACDONALD (1984)) and as far south as Port Edward and westward spread into the hinterland had also occurred.

In 1982 a comprehensive survey of roadsides was conducted to determine the distribution of *C. odorata* in Natal

and the extent of the area infested (LIGGITT, 1983). The results of the survey are presented in Figure 1.2.1. The dense infestations are predominantly confined to a narrow coastal zone extending along most of the Natal coastal belt from the Transkei border in the south to Mtunzini in the north. Dense infestations also occur in the Lake St. Lucia area. This report also mentions infestations as far north as Mkuze Game Reserve and Sodwana Bay and MACDONALD (1984) reported that, by 1981, *C. odorata* was present in the Kosi Bay area on the Mozambique/Natal border. Spread inland has occurred as far as Ixopo, Pietermaritzburg, Melmoth and Hlabisa. Personal observations have also shown the presence of comparatively new nuclear infestations in the Pietermaritzburg and New Hanover-Wartburg areas, indicating that the spread is continuing. However, the spread has probably been restricted by the recent four drought years. The annual rainfall is likely to limit the distribution in South Africa since, in other countries, distribution is restricted to areas receiving approximately 1250 mm of rain per annum. The spread of *C. odorata* in the past decade has therefore been minimal when compared with the previous two decades and further spread in Natal is likely to be restricted due to the rainfall and possible temperature requirements. However, the density of infestations can be expected to increase; the present nuclear infestations providing a propagule source.

Although *C. odorata* is at present only considered to be a serious weed in Natal, a recent report (MACDONALD, 1984) has drawn attention to large dense infestations of this weed

Figure 1.2.1 The distribution of *Chromolaena odorata* (L.) R.M. King and H. Robinson in Natal (LIGGITT, 1983).

in the north-eastern Transvaal. Apparently an infestation was sprayed in the Tzaneen area as early as 1980 (CILLIERS, personal communication*) indicating that the extent of this weed's distribution in South Africa, can be expected to increase in the eastern Transvaal.

The success of *C. odorata* as a widely distributed weed is due to its many weed characteristics which contribute to its biology. It is a woody perennial which reproduces predominantly by achene production. Flowering, which can occur over a wide range of daylength conditions (10 to 14 hours), is enhanced by the shorter daylengths (SAJISE, PALIS, NORCIO & LALES, 1974). In Natal flowering starts with the development of flower-buds in May and these develop further into white inflorescences which adorn the infestations from June to August. Presumably therefore, shorter daylengths are operative in stimulating flowering of plants in Natal. In south-east Asia (Northern Hemisphere) flowering occurs during the months of November and December (i.e. during the onset of short-day conditions) (SAJISE, PALIS, NORCIO & LALES, 1974). In Nigeria *C. odorata* flowers from November to February (IVENS, 1974), the major peak of flowering occurring during December and January (IVENS, 1975). In Natal, *C. odorata* flowers are white to cream in colour. This is variable however, because in Nigeria the flowers are pale blue (IVENS, 1974).

It has been suggested that reproduction in the *Eupatorium* species is apomictic (GRANT, 1971; AULD & MARTIN, 1975) and

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this may also apply to *C. odorata* (YADAV & TRIPATHI, 1982). Therefore, if no fertilization occurs, the production of the achenes would be by asexual means.

Following flowering, achene filling takes place. In Natal, filled achenes can be obtained in August; these having been produced from the flowers observed in June.

Each flower-head (capitulum), which is borne on a raceme inflorescence, produces varying numbers of achenes which are attached to the receptacle. The number of achenes per capitulum was incorrectly reported as being " a hundred or more" (EGBERINK & PICKWORTH, 1969). In Nigeria 25 to 30 achenes per capitulum are produced (IVENS, 1974). The number of achenes produced per plant varies considerably because the number of capitula produced varies. In Sri Lanka it has been estimated that an annual average of 93 000 achenes are produced per plant (WEERAKOON, 1972). In Nigeria, a mean of 125 000 achenes was estimated, while in South Africa approximately 90 000 (MACDONALD, 1984) to 440 000 (TODD, 1980) achenes are produced. It is not indicated in these reports whether the above figures represent filled, presumably viable achenes, only.

C. odorata propagules are referred to as "achenes" as they have an outer coat consisting of a pericarp and each propagule contains a single embryo. Thus the achenes are single-seeded fruits. The achenes are narrow, linear, approximately four millimetres in length, dark brown to black in colour and closely resemble those of two common South African

annual, composite weeds, *Tagetes minuta* L. and *Bidens pilosa* L.. As is the case with these two species, the achenes of *C. odorata* have a dense pappus at the opposite end to the hilum. This pappus forms a "parachute" which aids wind dispersal in that time in the air is prolonged. Presumably achene distribution is therefore governed by the prevailing wind direction during achene release from the plants. During this time, these winds are predominantly south-westerlies and/or north-easterlies and this may partially explain the rapid spread along the coastal region with fairly limited inland spread.

Few germination tests have been conducted on the achenes of *C. odorata*. In India, YADAV & TRIPATHI (1982) reported that an alternating temperature of 15/25 °C was optimal and light required for germination. EDWARDS (1975) used a constant temperature of 29 °C for investigating the germination of achenes collected from seven localities in the "New and Old World Tropics". In this study highest percentage germination was obtained in the light but up to 40 per cent germination was recorded in the dark. Upon transfer of achenes incubated continuously in the dark to a diurnal light cycle, the percentage germination was considerably increased. EDWARDS (1975) also found that there were differences in the germination characteristics of achenes harvested from the different localities. ETEJERE (1980) used a constant temperature of 30 °C for germination tests involving the effect of various herbicides on germinability of *C. odorata* achenes harvested in Nigeria. Germination was also investigated in Nigeria by IVENS (1974;

1975). It is reported that little or no dormancy was evident, longevity in the soil short (± five months) and that light may be required for germination. A light requirement was also reported by RAI (1976).

In South Africa, it seems that germination occurs from September since seedlings are observed in the field from this time. The dense mat of seedlings which is sometimes observed in exposed situations following warm, moist conditions, has been described as "sward-like" (EGBERINK & PICKWORTH, 1969). Under a closed canopy however, very few seedlings have been observed (IVENS, 1975; RAI, 1976).

Comparatively few seedlings appear to reach maturity. Mortality may be due to a variety of factors including intra- and inter-specific competition and unfavourable environmental conditions. Seedlings which do become established grow vigorously. This growth initially consists of vertical elongation probably due to the strong manifestation of apical dominance but towards the end of summer (March) growth is dominated by profuse lateral-branch production, resulting in a dense canopy. Within a comparatively short period, a dense thicket develops. If arborescent species are present, *C. odorata* plants assume a scrambling habit. Where infestations occur in a shaded area, *C. odorata* has been observed scrambling to seven metres up the trees. In open areas the *C. odorata* plant assumes a shrub-like habit or, in the case of dense infestations, it again assumes a scrambling habit.

Leaves are produced throughout the year, although in winter and in drought conditions leaf production may be sus-

pended. Along the coastal region *C. odorata* remains evergreen while inland most of the leaves senesce. In addition, growth continues throughout the year in the coastal regions albeit less vigorously during winter (May to July) when flowering usually occurs.

Rapid regrowth commences within one month of slashing or burning of mature plants. This regrowth is sometimes referred to as coppice and has been reported previously (IVENS, 1974; SAJISE, PALIS, NORCIO & LALES, 1974; RAI, 1976). Regrowth can be extremely rapid. IVENS (1975) reported that shoots were 0,45 m tall two months after slashing, 0,80 m after three months and 1,75 m after five months. The regrowth occurs from the base of slashed or burnt plants. However, no suckering has been observed (IVENS, 1975). The regrowth from the base often results in the formation of a substantial woody crown (rootstock) (RAI, 1976).

The rapidity with which coppice is produced, indicates that regrowth probably occurs as a result of the removal of apical dominance. Consequently, multi-stemmed plants are produced.

A fairly limited amount of regrowth occurs from roots which remain in the ground after physical/mechanical removal of plants, this occurs when these operations are carried out when soil moisture is high. Re-establishment also occurs from excavated plants whose roots remain in contact with moist soil. It is also reported that branches in contact with the soil occasionally take root (IVENS, 1975; RAI, 1976).

Personal observations have shown that this seldom occurs in South Africa, although stem cuttings propagated in the field situation resulted in 20 per cent rooting after two months, while only 10 per cent survival was recorded after six months.

The major features contributing to *C. odorata* weediness and its widespread distribution in Natal can therefore be summarized as follows:

(i) a copious propagule production in the form of achenes which may be apomictically produced, thereby perpetuating the inherent characteristics. These achenes are also adapted to wind dispersal providing this species with an efficient dissemination mechanism for rapid and widespread distribution;

(ii) upon germination of achenes in safe-sites and the establishment of seedlings; vigorous growth and the production of a dense canopy occurs;

(iii) a flexible growth habit conforming to the habitat;

(iv) an extensive shallow root system from which regrowth occurs if not completely removed;

(v) a perennial nature and thus the ability to rapidly regrow following destruction of the top growth thereby regaining the ascendency over other species.

In addition to these features *C. odorata*, being an alien, lacks its natural competitors and predators, providing this weed with a distinctive competitive advantage especially over indigenous species. It is therefore hardly surprising that *C. odorata* is regarded as one of the most aggresive invader species in Natal.

The problems caused by its presence are discussed below.

C. odorata, although predominantly an environmental weed in South Africa because of the encroachment of natural vegetation, is also a weed of various other situations in which its detrimental effects are manifest.

In south-east Asia, *C. odorata* is closely associated with human activities in forest areas. It is a coloniser in the moist deciduous, semi-evergreen and evergreen forests and grasslands of western Ghats in India (RAI, 1976). In these areas dense *C. odorata* thickets are common. It reportedly competes with leguminous cover crops in rubber plantations and scrambles over small plantation trees, suppressing their growth. *C. odorata* is also a problem in teak plantations where widespread infestations occur. Silvicultural operations are hampered and growth of plantation trees is severely retarded (MONI & GEORGE, 1959; MONI & SUBRAMONIAM, 1960). More recently it was reported that *C. odorata* has become established over very large areas causing the failure of several new plantations of soft-wood and teak (AMBIKA, 1980). This species is also a weed in rice paddy fields where the use of *C. odorata* as a green manure has resulted in the introduction of many achenes (MONI & GEORGE, 1959).

In the Philippines, pasturelands are being encroached by *C. odorata*, resulting in decreased livestock carrying capacity (PANCHO & PLUCKNETT, 1972). It is also reported that while *C. odorata* may be unpalatable, it is apparently sometimes browsed which could result in livestock fatalities

as the plants may contain toxic nitrate levels during stages of active growth (SAJISE, PALIS, NORCIO & LALES, 1974).

Although opinions regarding the weed status of *C. odorata* in Nigeria have sometimes varied, it is generally accepted as being serious (IVENS, 1974). SHELDRICK (1968a) reported that while oil palms free of *C. odorata* bore fruits after four years, the infested plantation trees only started bearing fruit in their sixth year. IVENS (1974) observed that *C. odorata* suppressed the growth of oil palm seedlings but that in mature plantations where a closed canopy was formed, the intensity of the weed was greatly decreased. These remaining plants were therefore only important as a propagule source. The problem in rubber and cocoa plantations is similar to that of oil palm plantations (IVENS, 1974; IVENS, MOODY & EGUNJOBI, 1978) where *C. odorata* is only considered a serious weed during the early phases of plantation establishment. IVENS (1975) also considered *C. odorata* to be a problem in fallow land since destumping was necessary during land preparation. In addition cultivation is initially required to remove *C. odorata* seedling thickets resulting from germination of achenes.

C. odorata is exceptionally inflammable even when green (MACDONALD, 1984). This is apparently due to the high essential oil content of the leaves (MONI & SUBRAMONIAM, 1960). This species therefore increases the fire hazard in all situations in which it is found and reference is often made to this aspect. For example, SHELDRICK (1968a) suggested that fire

in plantations infested with *C. odorata* led to an increase of two to four years non-productivity due to the increased heat of burn.

The status of *C. odorata* as a weed is also elevated by its role as a host to several plant diseases which may affect desirable species (ESURUOSA, 1971; HALL, KUMAR & ENTI, 1972; HOLM, PLUCKNETT, PANCHO & HERBERGER, 1977).

The major problem of *C. odorata* in countries other than South Africa is therefore the infestation of plantations resulting in suppression of seedling growth, an increased fire hazard and the formation of an obstacle to silvicultural operations.

In South Africa *C. odorata* is potentially a serious problem in silviculture in Natal. At present the area infested is small, probably due to judicious weeding operations practised by forestry concerns to reduce the effect of a number of other weed species including *S. mauritianum*, *L. camara* and *P. aculeata*. As is the case elsewhere and mentioned previously, *C. odorata* is only a problem in silviculture during the establishment phase of the plantation since the formation of a dense canopy considerably reduces the extent of the infestation. However, where plantations are neglected during this phase, only a limited canopy is formed by the timber trees. In this situation *C. odorata* thrives and produces an impenetrable thicket of tangled stems. Timber production costs are therefore adversely affected because timber yield is reduced, harvesting operations are costly and slow because

slashing is necessary to provide access and preparation of the land for replanting is an expensive operation. Furthermore the fire hazard in infested plantations is greatly increased. For example, a personal observation has shown that the intensity and extent of fire in a *Pinus taeda* L. plantation appeared to be directly related to the profusion of *C. odorata* present.

In the distribution area, *C. odorata* is a serious weed of road verges, rights of way and vacant land. The presence of this weed increases the cost of weed control due to its woodiness and its rapid regrowth. As a result greater weed control input is required.

Veld encroachment by *C. odorata* results in decreased livestock carrying capacity. The current distribution of *C. odorata* in Natal is such that little livestock farming is practised in the infested region. Consequently the influence on domestic livestock farming is inconsiderable. However, in the game reserves located along the Natal coast, the impact has been dramatic. Although no scientific data are available, the grazing and browsing productivity must be greatly reduced by the vast *C. odorata* infestations. Circumstantial evidence for this can be gathered from the effort being applied to *C. odorata* control in these reserves by the Natal Parks Board. As described by LIGGITT (1983), the infestations result in over-utilisation of the uninfested areas. These areas become disturbed habitats vulnerable to infestation by weeds such as *C. odorata*, *L. camara* and *Psidium guajava* L.. Accordingly the problem is aggravated.

C. odorata is not generally an agrestal weed in South Africa because frequent cultivation is sufficient to exclude its establishment and growth. However, fallow land is rapidly encroached; subsequent preparative actions for replanting are arduous and expensive.

The major problem caused by *C. odorata* in South Africa is the encroachment of aesthetically valuable land: the nature/ game reserves and the wilderness/conservation areas. The magnitude of the infestations has resulted in disruption of normal vegetation succession, the complete inundation of remnant coastal vegetation and the decrease of species diversity. The impact of the invasion on indigenous species has been aesthetically disasterous and may well be further aggravated if environmental conditions favourable for growth of this weed are experienced following the drought in Natal.

The exceptional inflammability of *C. odorata* plants results in an increased susceptibility to fire-destruction of forest and riverine communities which are usually protected by fire-excluding fringe communities (MACDONALD, 1984). As a result, fire-sensitive communities are irreparably damaged due to the presence of *C. odorata* infestations. The situation is aggravated because *C. odorata* rapidly recuperates following fire thereby gaining the ascendency over the indigenous species.

Having examined the extent and severity of the *C. odorata* problem in South Africa, it is not surprising that the "Workshop for Alien Invaders in Natal" (MACDONALD &

JARMAN, 1985), has rated this species the top priority weed in silviculture, conservation areas and utility areas and number four in agriculture. It is disturbing however, that the problem has been allowed to reach the present proportions and intensive measures to reduce the impact of this weed should be implemented without delay.

The various weed control methods generally employed and the corresponding techniques are discussed in Chapter 3. In this section no details of these methods will be provided; only control measures which are reported in the literature regarding *C. odorata* will be related.

The major control methods used in combating *C. odorata* are mechanical, manual or chemical in nature.

The use of mechanical control techniques in Nigeria was reported by SHELDRICK (1968a). Besides physical hand-pulling of seedlings and manual slashing with a cutlass, a Holt Mk.VIIB Weedbreaker was tested in oil palm plantations. The Weedbreaker was tractor-mounted, therefore considerable land preparation prior to the operation was necessary to provide access. The control obtained reduced the *C. odorata* density but with the passage of the equipment through the plantation, bare ground was exposed which provided suitable sites for germination and seedling establishment. A Cambridge ring roller was also used in less dense infestations. This equipment was adjudged superior to the Weedbreaker because, during the rolling operation when *C. odorata* was snapped off near ground level, the leguminous cover crop runners were

pressed to the ground but not injured. Neither the Weed-breaker nor the roller could be used along the palm lines where logs and stumps prevented access. Consequently manual slashing had to be used in these situations.

The experiments described above highlight the difficulty involved in mechanical control of *C. odorata*. That is, mechanical control techniques involving motorised and heavy machinery are of limited value because the infestations are often inaccessible to this equipment. This is not only the case in Nigeria (IVENS, 1975) and India (RAI, 1976) where few plantations are suitable for this type of mechanical control, but also in South Africa. Here accessibility is hampered by the harsh terrain and the vegetation in which *C. odorata* occurs. In commercial forests, stumps, logs and rows of trees themselves impede access. In indigenous vegetation, the desirable species form the obstacle. Therefore manual or other techniques are likely to be used.

Manual control, comprised of manual slashing, digging-up and hand-pulling, is probably the most widely used technique in the struggle against *C. odorata*. Manual control has been practised in Nigeria (SHELDRICK, 1968a; 1968b; IVENS, 1974; 1975), Ivory Coast (DUFOUR, QUENCEZ & BOUTIN, 1979), Ghana (HALL, KUMAR & ENTI, 1972), India (MOHAN LAL, 1960; RAI, 1976; SAXENA & RAMAKRISHNAN, 1984a; 1984b) and in South Africa (EGBERINK & PICKWORTH, 1969; LIGGITT, 1983). The slashed top-growth is either left *in situ* or stacked and burnt or even stacked on racks during drying to prevent re-establishment (IVENS, 1974). IVENS (1975) suggested that slashing was most

productive if carried-out at the end of the rainy season before the onset of achene production. As regrowth occurs following slashing, this can only be considered to be a temporary control measure. Permanency of control can be obtained if plants are uprooted by digging or by hand-pulling of smaller plants.

The disadvantages of manual control, as with mechanical control, are numerous. Labour required for large infestations increases costs, it is a slow, laborious task and re-establishment may occur for three reasons. Firstly, uprooted plants may become re-established if left lying in contact with moist soil. Secondly, regrowth may occur from root segments left in the ground during uprooting and thirdly, soil disturbance may provide conditions conducive to germination of soil-achene reserves. To avoid any of these three situations arising, this control technique should be implemented during the dry season to reduce the occurrence of re-establishment. Although complete removal of the roots is only likely to be achieved when the soil is moist, it would seem logical to conduct manual control during the dry winter months and, in South Africa, before achene filling is complete, that is, from April to June.

Another disadvantage of both manual and mechanical control is that large areas are denuded and are therefore susceptible to erosion during the period before ground cover establishment by other species.

It seems that herbicides have been successfully used for chemical control of *C. odorata* in a number of countries

and this method of control is generally recommended.

The earliest record of chemical control of this species was supplied by MONI & GEORGE (1959) from India. However, they conclude that with herbicides available at that time, large scale applications were impractical. Subsequently GEORGE (1968) conducted trials using 2,4-dichlorophenoxyacetic acid (2,4-D) and 1,1-dimethyl-4,4-bipyridyldiylium ion (paraquat) because it was felt that a suitable and economic technique was required to prevent "enormous" loss in forest and plantation crops. It was concluded that *C. odorata* could be economically controlled with paraquat. Similar research was conducted by RAI (1976), who suggested that of the herbicides tested, a mixture of paraquat + 2,4-D was effective although chemical control was prohibitively costly. MATHEW, PUNNOOSE & POTTY (1977) reported that a mixture of paraquat + 4-(2,4-dichlorophenoxy) butyric acid was ideal for *C. odorata* control in rubber plantations in India.

Following trials in which a number of herbicides were tested SHELDRICK (1968a) reported that Nigerian infestations of *C. odorata* could be controlled by 2,4-D and a mixture of 2,4-D + 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). However, although the aerial parts of the plants were killed, regrowth occurred in some cases. Furthermore, 2,4-D and 2,4,5-T were not recommended for use near palm seedlings since these are very susceptible to the herbicides. Other herbicides recommended were 2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine (atrazine) and 3-(3,4-dichlorophenyl)-1,1-dimethylurea (diuron). SHELDRICK (1968b) suggested that 2,4-D application

would be useful in controlling *C. odorata* during legume cover crop establishment in oil palm plantations. Also in Nigeria, a mixture of 4-amino-3,5,6-trichloropicolinic acid (picloram) + 2,4-D was extremely effective while *N*-(phosphonomethyl)-glycine (glyphosate), atrazine and diuron also gave a high degree of control (IVENS, 1974). In addition to these herbicides, IVENS (1975) also recommended the application of 2,4-D and paraquat.

In the Ivory Coast, DUFOUR, QUENCEZ & BOUTIN (1979) examined the efficacy of glyphosate and a mixture of picloram + 2,4-D on *C. odorata*. They found that both these treatments were highly effective and could be used in oil palm plantations. The cost of glyphosate however, was estimated to be prohibitive. Therefore use of this herbicide was only recommended for situations where there was a labour shortage.

In South Africa, EGBERINK & PICKWORTH (1969) found that picloram and 2,4-D were effective, while 2,4,5-T was moderately effective for *C. odorata* control. It was also reported that although 2,4-D was cheaper than picloram, it did not control other weed species such as *L. camara* and *S. mauritianum*, but on the other hand, picloram was found to be persistent and therefore restricted colonisation of the treated area.

Personal communications with various authorities faced with the problem of *C. odorata* have indicated that 2,4,5-T, glyphosate and picloram are effective against this weed.

Until the beginning of 1985, only one herbicide, 1-(5-*tert*-butyl-1,2,4-thiadiazol-2-yl)-1,3-dimethylurea (tebuthiuron), was registered for *C. odorata* control. Tebuthiuron, in the form of a 20 per cent active ingredient granular formulation, is registered at two to four grammes per metre square. Recently application for registration of (3,5,6-trichloro-2-pyridimyl)oxy-2-butoxy ethyl acetate (triclopyr) was made. This was approved (Registration No. L2351 of the Weeds Act No. 36 of 1947). Triclopyr is registered for application at a concentration of 0,5 per cent formulated product and 400 to 800 $dm^3 ha^{-1}$ on plants 0,5 to 1,0 m in height.

The above summary of the chemical control of *C. odorata* indicates that this weed can be chemically controlled. The most widely recommended herbicides are 2,4-D; 2,4,5-T; picloram + 2,4,5-T; tebuthiuron and triclopyr.

As with other control methods, there are disadvantages to chemical control of *C. odorata*. Chemical control is more sophisticated than the mechanical and manual methods used, therefore training of personnel is a prerequisite. The selectivity of herbicides has to be taken into account. Where *C. odorata* occurs in grassland it can easily be controlled using herbicides selectively toxic to broad-leaved species. However, in the indigenous forest and silviculture situation, the majority of desirable species are also broad-leaved and therefore are susceptible to herbicides which would be effective on *C. odorata*. Consequently herbicide applications must be target specific and precautions to avoid spray-drift onto desirable species need to be taken.

The contamination of the environment by agro-chemicals has recently been highlighted in the media. The use of herbicides, although not as toxic as some of the pesticides used, must also be carried out with due caution. Notably, some herbicides are persistent in the soil due to comparatively long periods required for their degradation. The persistence results in increased periods of denudation, resulting in areas being rendered vulnerable to erosion. Leaching of herbicides into water supplies and estuaries has also caused some concern although fortunately, no notable instances are documented.

The advantages of chemical control are listed below;

(i) it may be cheaper than mechanical and manual control;

(ii) application for effective control is less time consuming;

(iii) although the plants are killed, they remain *in situ* thereby reducing the risk of erosion and although they may present a fire hazard during this time, the risk can be reduced by herbicide application during the rainy season;

(iv) no disturbance of the soil occurs, therefore soil achene reserves are not exposed to light, hence their germination is not promoted;

(v) if effective, no regrowth occurs; therefore re-establishment is only possible by achene germination and seedling establishment, both of which can be appreciably reduced with appropriate management practices.

Intergrated control measures are often adopted for *C. odorata* control. For example, dense thickets can be reduced by manual and/or chemical techniques, firstly, to provide access and secondly, so that when regrowth occurs, the plants are of a size which is suitable for efficient herbicide application (DUFOUR, QUENCEZ & BOUTIN, 1979). Herbicides can also be used to spot-spray regrowth following initial manual and mechanical uprooting of plants (IVENS, 1975). Manual techniques, such as hand-pulling, have also been used as follow-up treatments subsequent to chemical control (SHELDRICK, 1968a). Thus the control method or combination of control methods used, is very much dependent on the situation in which the infestations occur.

Biological control of *C. odorata* would perhaps be the most suitable method because of the selectivity of control and the eradication of plants growing in inaccessible areas. Furthermore, all infestations would theoretically be controlled, not only those forming an immediate problem. Therefore the achene source for re-infestation would be reduced and possibly, eliminated. However, no suitable agents for the control of *C. odorata* are presently available, although research has been initiated in a number of countries and is likely to be initiated in South Africa in the near future.

An initial, preliminary survey of potential biological control agents was conducted by CRUTTWELL (1968). It was subsequently reported that 240 species of insects had been recorded feeding on *C. odorata*. However, since many of the insects were

not specific to *C. odorata* alone, only a few of those screened were found to be potentially suitable (CRUTTWELL, 1968; 1976; 1977).

Biological control using these species in West Africa and south-east Asia and India has been unsuccessful (IVENS, 1975; SYED, 1979). At present, it is not known whether further attempts are being made to combat *C.odorata* by biological control in these areas.

1.3 Study Objectives

During the review of the literature on *C. odorata*, certain aspects requiring investigation were noted.

Firstly, it is clear that only limited information concerning the germination of the achenes is available. As described, the achenes play an extremely important role in the life cycle of this species in that not only are they the reproductive propagules, but they are also responsible for this weed's rapid and extensive encroachment observed in the many countries where it is a problem. A comprehensive investigation into germination is therefore a priority. The information obtained would contribute greatly towards the current state of knowledge regarding *C. odorata*. In addition, it was felt that this knowledge could assist in the formulation of a control strategy aimed at reducing the extent and intensity of the problem in Natal.

Secondly, chemical control of *C. odorata* has received only limited attention in South Africa. Reports from elsewhere have, however, indicated that this weed can be successfully controlled with herbicides. Since urgent control methods are required to restrict further encroachment and to reduce the impact of the *C. odorata* problem in Natal, it was decided that chemical control should be examined. In the first instance, herbicides effective on *C. odorata* needed identification and secondly, techniques for the control of this weed in situations where it has encroached aesthetically valuable indigenous vege-

tation required investigating. Ideally, these techniques should be such that little or no damage to the existent desirable vegetation results.

Thirdly, no detailed *modis operandi* has yet been proposed for the control of *C. odorata*. It was therefore envisaged that with the results and experience gained from this investigation, together with the current state of knowledge, a proposed control strategy could be formulated and in so doing a common approach could be adopted for the removal of this weed and for maintaining a *C. odorata*-free habitat.

It is interesting to note that South Africa is the only area in the southern hemisphere where *C. odorata* occurs outside the tropics. In the northern hemisphere, *C. odorata* only occurs outside the tropics in Assam Province, India. The infestations in Natal are therefore unique in the southern hemisphere. A study on this weed in Natal would thus provide a basis for comparison with other infestations in the tropics and with those outside the tropics in the northern hemisphere.

GERMINATION

2.1 Introduction

Chromolaena odorata (L.) R.M. King and H. Robinson reproduces almost entirely by "seed"*. Seed production is usually associated with sexual reproduction. However, as discussed previously (Chapter 1, section 1.2), *C. odorata* may be apomictic in which case reproduction is by asexual means. Nevertheless, in *C. odorata* the achenes fulfil the function of seeds and are thus discussed in this context.

Almost without exception, plant species are immobile thus dispersal is usually by way of propagule dissemination which, in many cases including *C. odorata* is the dispersal of the seed. The seed, in its various forms, contains the embryo of the new plant and is therefore the reproductive propagule, ensuring perpetuation of the species. Additionally, the seed is often equipped with structural and physiological devices to prepare it for its role as the dispersal unit. In addition, seeds form an important and persistent stage in the life cycle of plants because they usually survive adverse environmental conditions since the embryo is usually protected within coverings such as the testa and/or pericarp. Food reserves are present which sustain the embryo and eventually, the immature seedling. Only after establishment does the seedling become autotrophic (BEWLEY & BLACK, 1978). In addition, seeds possess two unique

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* Except for *C. odorata* where the term "achene" is used, the term "seed" is uniformly employed to describe the dispersal propagule which, for example, may technically be an achene, caryopse etc.

physiological and biochemical properties for survival (BEWLEY & BLACK, 1978). Firstly, seeds of most species remain alive through a state of dehydration during which the water content may drop to about 10 per cent of the total mass. Concurrently, the cellular organelles become inactive and even disorganised. However, with the onset of suitable conditions, the seed can resume full metabolic activity, growth and development. How the embryo and its associated structures withstand the desiccated conditions which would usually be fatal to other parts of the plant, is not known (BEWLEY & BLACK, 1978). Secondly, while seeds may be provided with conditions apparently suitable for stimulating the resumption of full metabolic activity, often no further development occurs, the germination process being incomplete. This is referred to as dormancy (BEWLEY & BLACK, 1978).

One of the aims of this study was to investigate the germination of *C. odorata* achenes. Due to the lack of published information and a paucity of research on this specific topic in South Africa, it was necessary to investigate all facets which may be of importance in the germination of this species. Consequently, it is pertinent to review the literature and to extract information which may be of relevance to *C. odorata* achene germination.

Before discussing seed dormancy, it is necessary to discuss briefly the basic requirements for germination. These are referred to as the "germination agents" by JANN & AMEN (1977).

Germination consists of those processes which begin with water uptake and which terminate with the emergence of the radicle or hypocotyl through the seed coverings (BEWLEY & BLACK, 1978). Water therefore plays an important role, being essential for rehydration of seeds, in the initial step towards germination. The water potential of a mature, dry seed is considerably lower than that of the surrounding moist substrate and consequently water moves into the seed. This initial uptake of water is known as imbibition (BEWLEY & BLACK, 1978), and results in a rapid mass increase of the seeds. This phase is independent of metabolic activity since dead seeds also imbibe water. Following imbibition, a plateau phase is reached where little further increase in mass occurs. It is during this phase that major metabolic events take place in preparation for germination (BEWLEY & BLACK, 1978). A third phase of water uptake follows, resulting in a further mass increase. This phase incorporates the visible stage of germination (radicle emergence), the mass increase resulting from water uptake required for processes such as food reserve hydrolysis and cell expansion and growth.

Two other factors are generally considered to be necessary for germination. Although the seeds of certain aquatic species germinate better under conditions of reduced oxygen tension (SIFTON, 1959), oxygen is required for aerobic respiration and therefore is of considerable importance in germination. The initial rapid rate of respiration is attributed partially to hydration and the associated activation of mitochondrial enzymes of the citric acid cycle and electron transport chain

(BEWLEY & BLACK, 1978). The rate of respiration is therefore governed by oxygen availability. The other important basic requirement for germination is a suitable temperature or, often, an alternating temperature. The temperature obviously governs the rate of respiration and metabolism through its action on enzyme reactions and physical states.

A seed provided with sufficient water and oxygen and a suitable temperature should germinate. However, this only occurs in seeds whose growth has been suspended by unfavourable environmental conditions; these seeds being referred to as quiescent (JANN & AMEN, 1977; BEWLEY & BLACK, 1978). Therefore, when supplied with the non-specific germination agents discussed above, quiescent seeds germinate readily. These seeds are said to be "germinable" which is defined as "the capacity of an embryo to resume the growth activities which were suspended earlier" (JANN & AMEN, 1977) by the unfavourable environmental conditions.

Besides quiescent seeds, there are those in which the suspension of growth is imposed by active endogenous inhibition (JANN & AMEN, 1977). These seeds are dormant. A dormant seed is one which will not germinate even under conditions which are usually favourable. Dormancy, as defined by VILLIERS (1972), is "the failure of otherwise viable seeds to recommence development immediately when supplied with water and oxygen at temperatures recognised as normally favourable for plant growth . This delay may last for varying lengths of time under constant conditions and in some cases may even continue indefinitely until some special condition is fulfilled". Therefore, even

though a dormant seed may be viable, it is not germinable until provided with the stimulus required to overcome the dormant state. Stimulation of a dormant seed to germinate requires a specific environmental stimulus which is not constant but which triggers germination (JANN & AMEN, 1977). The trigger is not required for germination itself but only to prime the seed to respond subsequently to conditions which support germination, or as described by BEWLEY & BLACK (1982), the trigger "potentiates" germination. Trigger agents include mechanical disruption of seed coats, stratification, repeated temperature shifts, treatments with oxidants and with red (R) light (JANN & AMEN, 1977). These will be discussed later.

There are a number of seed dormancy categories which have been identified. However, the terminology used to describe these is often confusing. For example, the non-germination of quiescent seeds is sometimes referred to as "imposed dormancy" but, as discussed earlier, quiescence is not strictly a state of dormancy.

The classification of seed dormancy types was initiated in 1916 by CROCKER. Subsequently, the most detailed classification was proposed by NIKOLAEVA (1977) and is presented in summarised form in Table 2.1.1. A considerably simplified classification is favoured by some authors. For example BEWLEY & BLACK (1982) used three broad categories of dormancy. The first is "dormancy" or "primary dormancy" and describes a seed which, from the time of dispersal from the parent plant is dormant over a range of normal temperatures, but germinates

Table 2.1.1 A classification of organic seed dormancy patterns (NIKOLAEVA, 1977).

Types of dormancy	Factors causing dormancy	Conditions breaking dormancy
Types of exogenous dormancy		
Physical	Impermeability of seed coat to water	Scarification
Chemical	Inhibitors of pericarp	Removal of pericarp or leaching of the fruits
Mechanical	Mechanical resistance of covers to embryo growth	Various methods for destroying the covers
Types of endogenous dormancy		
Morphological	Underdevelopment of embryo (UE)	Warm stratification
Physiological	Physiological inhibiting mechanism (PIM) of germination	
Nondeep	PIM weak	Short cold stratification, light, dry-storage, injury of covers, growth stimulators
Intermediate	PIM intermediate	Long cold stratification and several other influences
Deep	PIM strong	Long cold stratification only
Morpho-physiological	Combination of UE and PIM	
Intermediate, simple	Combination of UE and intermediate PIM of germination	First warm, then cold stratification, growth stimulators
Deep, simple	Combination of UE and strong PIM of germination	First warm, then cold stratification
Deep, simple, epicotyl	Combination of UE and strong PIM of epicotyl growth	Same
Deep, simple, double	Combination of UE and strong PIM of hypocotyl and epicotyl growth	Warm followed by cold stratification for hypocotyl growth then another combination of warm and cold stratification for epicotyl growth
Intermediate, complex	Combination of UE and intermediate PIM of post-development and germination	Cold stratification or growth stimulators
Deep, complex	Combination of UE and strong PIM of post-development and germination	Cold stratification only

after a period of prechilling. The second category "relative dormancy", refers to a seed which, for example, does not germinate in the dark when temperatures are elevated above a certain value. At the higher temperatures however, the dormancy can be broken and germination initiated by exposing the seeds to a short period of light. Thirdly "secondary dormancy" refers to seeds which, for example, germinate only in darkness, but when returned to the dark following exposure to light germination no longer occurs. Therefore seeds in this category do not germinate in conditions which were previously promotive. The terminology used by BEWLEY & BLACK (1982) will be adhered to in this dissertation.

The above discussion indicates, therefore, that there are a number of known dormancy types in seeds. Suffice it to say that the presence of these dormancy types can only be established by means of germination tests which then contribute to the understanding of the life cycle of a particular species.

BEWLEY & BLACK (1982) also emphasised the importance of the "dormancy mechanisms" that is, the nature of the blocks in the seed which prevent germination under apparently favourable conditions and how these operate. This is in contrast to the environmental factors causing dormancy and what is required to break dormancy. Obviously, the answers to these questions are inter-related.

According to BEWLEY & BLACK (1982) there are two types of dormancy which involve different mechanisms, emphasis being given to the site where the imposition of dormancy resides.

(i) Embryo dormancy, where the control of dormancy resides within the embryo itself; and

(ii) Coat-imposed dormancy, where dormancy is maintained by the structures enclosing the embryo.

Both types may exist concurrently or consecutively.

Embryo dormancy can be demonstrated by the failure of the viable, mature embyro to germinate, even when it is isolated from the remainder of the seed. The inability to germinate is therefore a deficiency in the axis and/or metabolic blocks within the cotyledons.

An obvious reason for embryo dormancy is morphological immaturity (BEWLEY & BLACK, 1982). Seeds with immature embryos therefore require a period of further development before germination can occur.

Where mature embryos fail to germinate, it is likely that either the cotyledons and/or the axis and/or germination inhibitors control dormancy (BEWLEY & BLACK, 1982). In cotyledonary control of dormancy, the removal of the cotyledons results in germination. The physiological and biochemical basis for the action of cotyledons in dormancy is unknown although it is often ventured that chemical inhibitors are involved (BEWLEY & BLACK, 1982). It is possible that these inhibitors are leached out of the cotyledons into other tissues thereby inhibiting the germination processes. Should the inhibitors leach out of the seeds altogether, the imposed dormancy may be relieved.

Seed coat-imposed dormancy may result from one of the following:

(i) Interference with water uptake;

(ii) Interference with gaseous exchange;

(iii) Exertion of mechanical restraint on embryo expansion;

(iv) Prevention of inhibitor escape from the embryo;

(v) Presence of chemical inhibitors; and

(vi) Modification of light reaching the embryo.

As described earlier, both water and oxygen are required for germination. Prevention of water uptake and gaseous exchange by the seed coat may therefore prevent or inhibit germination. Exertion of mechanical restraint on embryo expansion is generally found in species where hard-seededness is characteristic (ROLSTON, 1978) and therefore not likely to be operative in *C. odorata* achenes where the achene-coat (pericarp) is comparatively soft.

As has been mentioned earlier germination inhibitors may be present within the inner tissues of a seed. It is possible that the seed coat may restrict the escape of these inhibitors by either forming a completely impermeable barrier or by reducing the rate at which outward diffusion of inhibitors occurs (BEWLEY & BLACK, 1982). Consequently a high concentration of inhibitor is maintained in the embryo which prevents germination. In addition to these inhibitors, it has also been shown that inhibitors occur in the seed coats (BLACK & WAREING, 1959; DEL TREDICI & TORREY, 1976; JUNTILLA, 1976). The

most common inhibitors in the seed coat are the aromatic acids (BARTON, 1965). It is generally accepted that leaching in water aids in, or results in, the removal of these inhibitors.

The final factor pertaining to seed coat-imposed dormancy is that the covering structures enclosing the embryo serve to intercept light, thereby forming a light shield or filter. The thickness and nature of the seed coat thus is likely to influence the amount and/or quality of light reaching the embryo. According to BEWLEY & BLACK (1982) dormancy, in the majority of light-requiring seeds, is coat-imposed since isolated embryos of these seeds can germinate in complete darkness. However, as will be discussed later in this section, the photoreceptor pigment (phytochrome) is probably located in the hypocotyl of the embryo of light-requiring seeds. Therefore, since it is the action of the active form of phytochrome which is responsible for the stimulation of germination in light-requiring seeds, dormancy should be embryo-imposed. This aspect will be discussed further in section 2.4.

The role of light in the removal of dormancy has attracted considerable attention ever since it was discovered that seed dormancy in a large number of species could be terminated when hydrated seed was irradiated. The seeds of species in which germination is governed by light are referred to as being photoblastic (EVENARI, 1965). Positively photoblastic seeds require light for germination while the germination of negatively photoblastic seeds is inhibited by light. The physiology of the photo-control of dormancy is varied and complex, but these responses are all attributed to the mode of

operation of a pigment system called phytochrome, so named by BUTLER, NORRIS, SIEGELMAN & HENDRICKS (1959). The role of phytochrome in seed germination will be discussed in detail since preliminary experimentation showed that *C. odorata* achenes were positively photoblastic.

Although ROLLIN (1972) states that the influence of light on germination was first observed in 1860, it is reported by BEWLEY & BLACK (1982) that awareness of the effect of light on germination dates back to the 1780's but that detailed investigations were only conducted in 1903 and 1904. Following these, continued research led to the finding that R light promoted germination and far-red (FR) light inhibited germination (FLINT & McALISTER, 1937). Eventually in 1952, BORTHWICK, HENDRICKS, PARKER, TOOLE & TOOLE were first to describe the mechanism for the control of germination by light namely the phytochrome system. In this and subsequent investigations (BORTHWICK, HENDRICKS, TOOLE & TOOLE, 1954) the R and FR reversibility in *Lactuca sativa* L. var. Grand Rapids (lettuce) seed germination was demonstrated. The R/FR light reversibility in seed germination apparently confirms phytochrome as the photoreceptor in light-requiring seeds (TOOLE, 1973; MOHR & SHROPSHIRE, 1983).

In 1959, BUTLER, NORRIS, SIEGELMAN & HENDRICKS isolated phytochrome, from dark-grown *Zea mays* L. (maize) seedlings for spectrophotometric detection. Phytochrome extraction and partial purification was effected in 1964 by SIEGELMAN & FIRER. At this stage however, very little was known about phytochrome as reviewed by SIEGELMAN & BUTLER (1965). Hereafter the interest

in phytochrome increased considerably as is reflected by the number of general reviews concerning this topic (HILLMAN, 1967; SIEGELMAN, 1969; SMITH, 1970; BRIGGS & RICE, 1972; MITRAKOS & SHROPSHIRE, 1972). Numerous articles have been published more recently (SMITH, 1975; 1976; DE GREEF, 1980; PRATT, 1982; SHROPSHIRE & MOHR, 1983). Many of these articles abound with the chemical and physical properties, isolation, detection and identification of phytochrome. However, in the present discussion attention will be given to the mechanism of phytochrome action in seed germination. To do this, the photo- and other transformations of phytochrome first need explanation.

In 1940 it was known that three spectral regions of light were active in seed dormancy and germination (BEWLEY & BLACK, 1982). These wavelengths were in the vicinity of 450 nm (blue), 650 nm (red) and 750 nm (far-red). This information had been produced by FLINT & McALISTER (1937) who showed that, in lettuce seeds, R light promoted germination and that following potentiation with a R light treatment, germination was reduced by exposure to blue and FR light. This also showed that the promotive effect of R light could be reversed. Later, BORTHWICK, HENDRICKS, TOOLE & TOOLE (1954), also using lettuce seeds, showed peak germination promotive activity at 660 nm and peak activity for re-imposition of dormancy at 730 nm. These researchers are credited with having "rediscovered" FR light inhibition (BORTHWICK, 1972a).

It seems that initially it was thought that two pigment systems were involved, the one being activated by R light and

resulting in germination and the other being activated by FR light and causing inhibition of germination. Evidence that a single pigment, which exists in two interconvertible forms, is operative in the antagonistic effects of R and FR light was provided by BORTHWICK, HENDRICKS, PARKER, TOOLE & TOOLE (1952) and BORTHWICK, HENDRICKS, TOOLE & TOOLE (1954). In these investigations lettuce seeds were exposed to alternations of R and FR light before transference to darkness. It was found that dormancy was only removed if the final irradiation was R light. On the other hand, if the final irradiation was FR light, dormancy was maintained regardless of the number of previous R light irradiations. BORTHWICK (1972a) reported that lettuce seeds removed after the 99th (FR) and 100th (R) irradiations reached the same final germination percentage as those seeds removed after the first (FR) and second (R) irradiations. This confirmed that the response to R and FR light was repeatedly reversible without loss of effect. It was therefore concluded that the photoreaction itself was reversible which meant that the photoreceptive pigment must exist in two forms which are interconvertible by irradiation (BORTHWICK, 1972a). It was also suggested that one form of phytochrome is active and the other inactive. The formation of the active form, Pfr, is promoted by R light absorbed by the inactive form Pr, and the inactive form Pr is promoted by FR light absorbed by the Pfr form.

The absorption spectra of purified phytochrome has been determined (HARTMANN, 1966; HANKE, HARTMANN & MOHR, 1969) and is shown graphically (Figure 2.1.1).

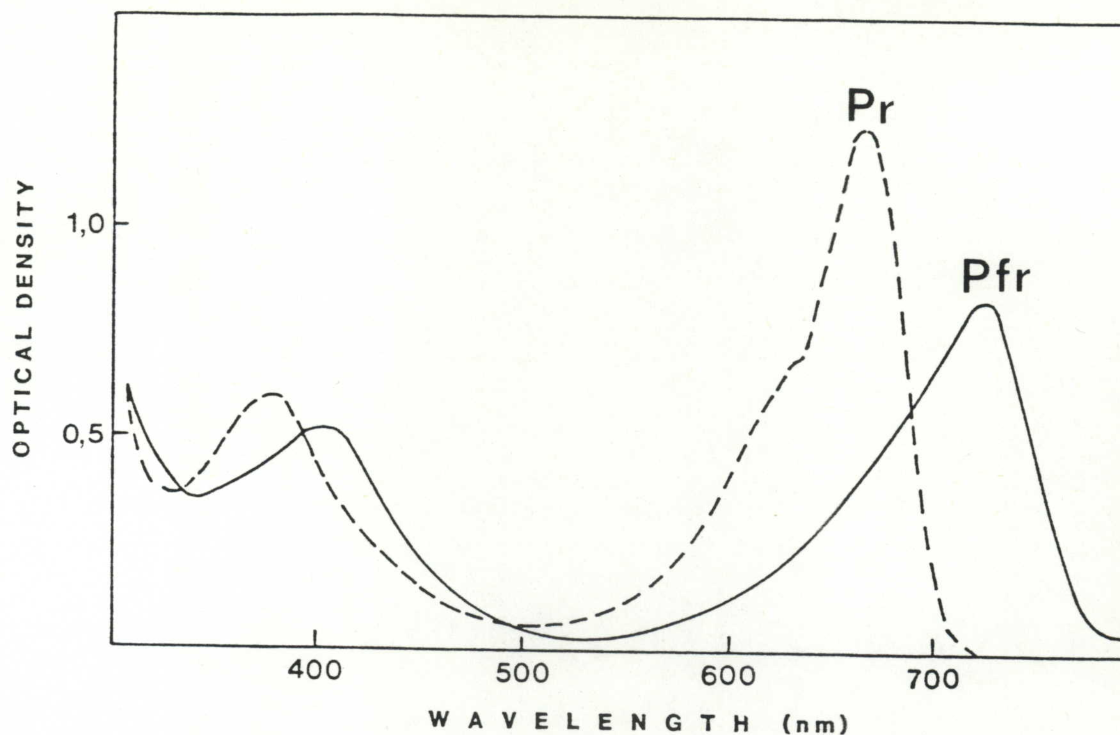


Figure 2.1.1 Absorption spectra of phytochrome (After HARTMANN, 1966).

As illustrated, Pr has peak absorption at or near 660nm. This closely coincides with the wavelength required to break dormancy of lettuce seeds (BORTHWICK, HENDRICKS, TOOLE & TOOLE, 1954). Following conversion of Pr to Pfr by exposure to R light, the peak absorption shifts to 730 nm, which previously had been found to be the most effective wavelength for inhibiting germination of R light-potentiated lettuce seeds (FLINT & McALISTER, 1937; BORTHWICK, HENDRICKS, PARKER, TOOLE & TOOLE, 1952; BORTHWICK, HENDRICKS, TOOLE & TOOLE, 1954). It is pertinent to note that overlap in wavelength absorbance occurs in the 700 nm region (Figure 2.1.1). In this spectral region photochemical transformations take place in both directions simultaneously (BEWLEY & BLACK, 1982). However, because the rate of the transformations is likely to be different a photoequilibrium is eventually established. This photoequilibrium, or photostationary state, is the ratio of

Pfr/P_{total} and is symbolised by the Greek letter Φ (phi) (BEWLEY & BLACK, 1982). Therefore, in light of mixed wavelength the phytochrome exists in a ratio (Φ), the level of which governs the termination of dormancy. The seed is thus able to detect the light quality of its environment through the Φ value (BEWLEY & BLACK, 1982) thereby restricting germination to environments possessing a particular light quality, and presumably, germination would be stimulated by a particular threshold level of Φ. This threshold level is however, likely to be graded in a population of seeds of a particular species because of the different levels of phytochrome present in the individual seeds and other factors.

The various models proposed to explain phytochrome transformation can be summarised diagrammatically (Figure 2.1.2) Several of these transformations are still tentative (KENDRICK & SPRUIT, 1977) and the molecular changes are still poorly understood (BEWLEY & BLACK, 1982). As illustrated in Figure 2.1.2, there are thought to be several phytochrome intermediates between Pr to Pfr conversion and *vice versa*. It is important to note however, that the progression from one intermediate to the next is dependent on either:

(i) the light quality, and/or

(ii) temperature and/or

(iii) the state of hydration of the seed.

For example, Pr cannot be converted to Pfr in a dry seed although suitable light and temperature conditions prevail. However, phytochrome in the Meta-Rb state can be thermally converted

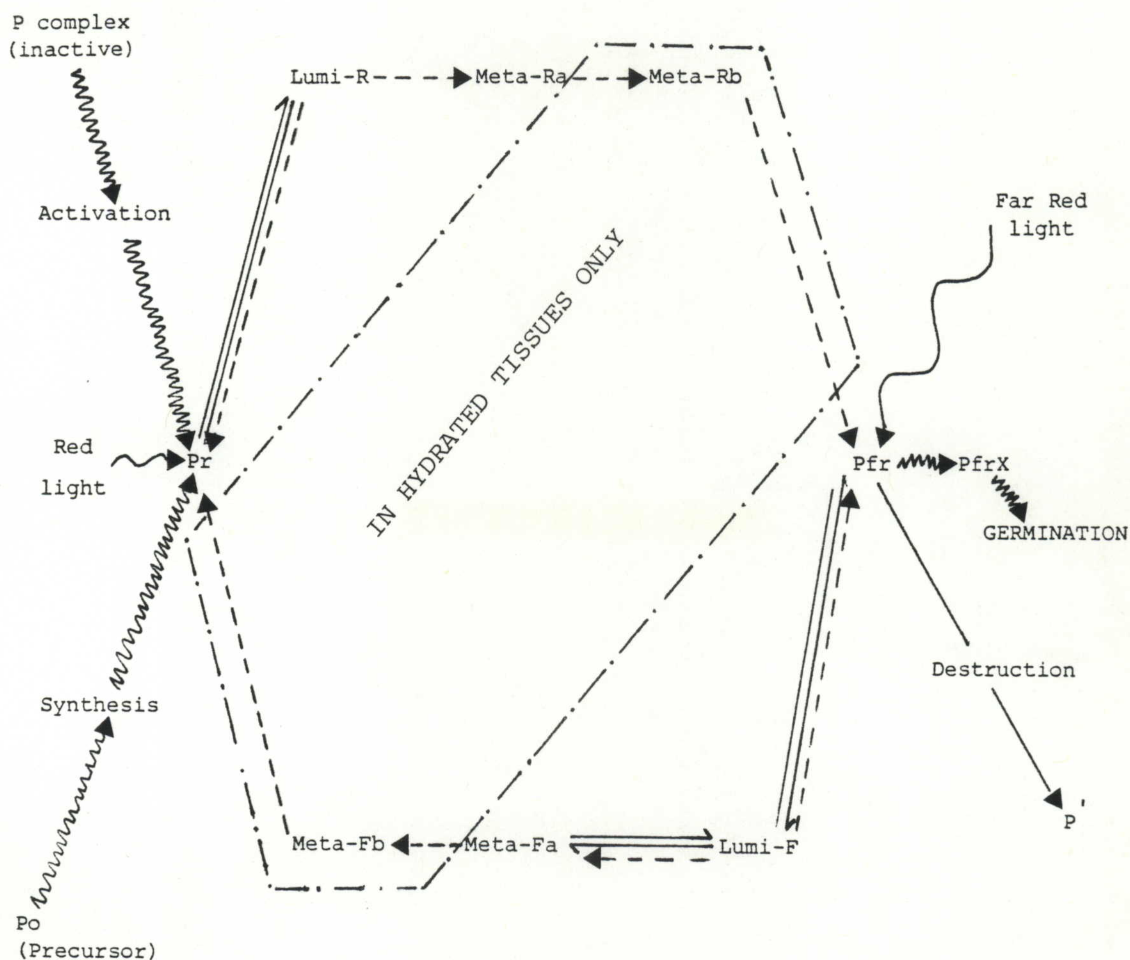


Figure 2.1.2 Transformation of phytochrome (Adapted from ROLLIN, 1972; KENDRICK & SPRUIT, 1977; BEWLEY & BLACK, 1982; MAYER & POLJAKOFF-MAYBER, 1982).

(Solid lines represent photo-transformations and broken lines represent dark thermal transformations).

to Pfr in the absence of light, providing the seed is hydrated. In Pfr conversion, the initial step to lumi-F requires illumination. Reversion to Pfr can however occur, this being stimulated by both light and temperature. Both thermal- and photo-promotion of lumi-F to Meta-Fa occur, but further steps are dependent on the seed being in an hydrated state. As a result Meta-Fa may accumulate in seeds which dry out following the initial steps of Pfr to Pr conversion. During the dehydrated state, Meta-Fa can be thermally degraded back to Pfr. Therefore, upon hydration, sufficient phytochrome may be in the Pfr state, resulting in germination in the absence of a suitable irradiation stimulus.

Due to the various intermediates in the conversion between the Pr and Pfr states of phytochrome and *vice versa*, it is reasonable to assume that the actual state of phytochrome in a seed is likely to fluctuate, depending on the light, temperature and hydration experienced by the seed. Presumably therefore, the state of phytochrome in a seed is likely to include a number of the intermediates and end products in varying amounts. Although the total phytochrome in dry seeds is about 10 times less than that in etiolated seedlings (ROLLIN, 1972), measurements of phytochrome in seeds have been made. A few examples will suffice. In dry *Cucumis sativus* L. seeds, 66 to 75 per cent of the phytochrome was found to be in the Pfr form (SPRUIT & MANCINELLI, 1969). In dry *Pinus nigra* Arn. seeds, all the phytochrome measured was in the Pr form (ORLANDINI & MALCOSTE, 1972). In both these species, total phytochrome was found to increase with water

uptake. This increase of phytochrome recorded may be due to hydration of previously existing chromoprotein (BEWLEY & BLACK, 1982) and to *de novo* synthesis as suggested by KENDRICK, SPRUIT & FRANKLAND (1969). SMITH (1973) suggested that dark-germinating seeds contain relatively large quantities of Pfr compared to limiting amounts in light-requiring seeds. In a further attempt to explain dark germination it has been proposed that Pfr is continually produced in the dark (KHAN, 1977).

As indicated in Figure 2.1.2 and described above, the complete conversion of Pr to Pfr can only occur in hydrated seeds and this explains why seeds are usually only fully responsive to light when in the imbibed condition. BORTHWICK, HENDRICKS, TOOLE & TOOLE (1954) first showed that longer periods of imbibition increased the photosensitivity of lettuce seeds. Similar results have been obtained in other species (KOLLER, SACHS & NEGBI, 1964; DUKE, EGLEY & REGER, 1977). Increased photosensitivity may be due to increased hydration of existing phytochrome as already discussed and/or a gradual accumulation of substances upon which phytochrome acts (KOLLER, SACHS & NEGBI, 1964; DUKE, EGLEY & REGER, 1977). It does seem, however, that full imbibition is not essential for Pr $\rightleftharpoons$ Pfr conversion (HSIAO & VIDAVER, 1971 ; TAYLORSON & HENDRICKS, 1972a) although FRANKLAND & TAYLORSON (1983) maintained that the proportion of photo-responsive seeds in a population increases with increasing dark imbibition time. This may indicate merely that imbibition is also required for the subsequent germination processes to proceed. Conversely, prolonged dark imbibition may result in a decreasing sensitivity

to light. With excessively prolonged imbibition, a form of secondary dormancy known as skotodormancy, may develop. In this case seeds which are initially responsive to light no longer germinate when exposed to light subsequent to prolonged dark imbibition. The physiological basis of skotodormancy is not understood (BEWLEY & BLACK, 1982).

In addition to light-stimulated interconversion of phytochrome, BORTHWICK, HENDRICKS, TOOLE & TOOLE (1954) showed that Pfr could revert thermally to Pr in the dark. This was demonstrated by potentiating the lettuce seeds with R light and then subjecting them to temperatures too high to allow germination. On return to the optimum germination temperature, little or no germination was obtained. However, germination could again be promoted by short exposure to R light. Subsequently, this dark reversion of Pfr to Pr has been confirmed spectrophotometrically (ORLANDINI & MALCOSTE, 1972).

As is also implied in Figure 2.1.2, production of Pfr stimulates germination in light-requiring seeds and therefore overcomes the dormancy mechanism. Pfr activation of the process of germination and other phytochrome mediated phenomena such as flowering and vegetative growth, consists of a two-part response in time (BORTHWICK, 1972b). During the first part, Pfr produced as a result of R light absorbed by Pr, performs a certain action or actions which must proceed for a specific period of time, this being dependent on the species. Therefore, in light-requiring seed germination, if Pfr is removed by the conversion of Pfr to Pr by FR light before

sufficient time has elapsed for Pfr to activate the dormancy breaking events, the response fails. However, if the delay in Pfr removal is long enough, germination results. Subsequent exposure of seeds to FR light at this stage no longer nullifies R light stimulated germination. In this second part of the response to the initial Pfr produced, the germination process is no longer governed by phytochrome, although Pr and/or Pfr are present. The time period required for successful germination stimulation by Pfr, that is, the period after which FR light no longer inhibits germination, is known as the escape time. The escape time is therefore an indication of the duration of Pfr presence required to terminate dormancy in positively photoblastic seeds.

The duration of the escape time varies for different species. In lettuce seeds, the escape time is four to five hours (BEWLEY, BLACK & NEGBI, 1967), 30 hours in *Chenopodium album* L. (KARSSEN, 1970a) and about 50 hours in *Paulownia tomentosa* Kanitz seeds (BORTHWICK, TOOLE & TOOLE, 1964). Besides duration, Pfr action is also dependent on temperature (BORTHWICK, HENDRICKS, PARKER, TOOLE & TOOLE, 1952; BEWLEY, BLACK & NEGBI, 1967). It has been postulated that during this time Pfr interacts with some "reaction partner" or "co-effector" "X" (DUKE, EGLEY & REGER, 1977). Escape time may therefore also indicate the time taken for threshold levels of "Pfr X" complex to develop which are required to induce germination. The "Pfr X" complex, or reacted phytochrome, according to ELDABH, FREDERICQ, MATON & DE GREEF (1974) is formed following the initial rapid photo-conversion of Pr to Pfr. The trans-

formation of Pfr to the reacted form (also symbolised as Pfr*) is reported to be considerably slower than the initial photo-conversion but is not reversible by brief FR irradiation.

The events which lead to the breaking of dormancy by light probably occur in the radicle region and therefore it is likely that phytochrome is present here (BEWLEY & BLACK, 1982). By covering different areas of the seed IKUMA & THIMANN (1959) found that the photo-receptor in lettuce seed is probably located in the tip of the hypocotyl. In *Citrullus calocynthus* (L.) Schrad. the site of light perception was found to be located in the radicular portions of the embryo (KOLLER, POLJAKOFF-MAYBER, BERG & DISKIN, 1963). However, if the phytochrome-induced effect is transmitted, the receptor and response site would be separate. This was investigated by BOISARD & MALCOSTE (1970). The photo-sensitive site was determined by direct irradiation of selected regions of the seed of *Cucurbita pepo* L. with R light microbeams from a laser. They found that the radicle/hypocotyl region was the photo-receptive site and that no promotive effect on germination was transmitted from the cotyledons. Additionally, it was found that the phytochrome content was highest in the radicle/hypocotyl region.

Besides the well documented general characteristics of the phytochrome/germination relationship already discussed, there are various response types in phytochrome controlled germination. As mentioned, a single short exposure to R light is sufficient to overcome dormancy in lettuce seeds. In certain other species however, a long period of R light

illumination is necessary. For example in *Kalanchoë bloss-feldiana* Poelln. several days of R irradiation was found to be necessary to promote germination (ELDABH, FREDERICQ, MATON & DE GREEF, 1974). However, it was found that this long exposure could be replaced with intermittent exposures. The same response has been found in other species also apparently requiring a prolonged exposure to R light as in *P. tomentosa* (BORTHWICK, TOOLE & TOOLE, 1964) and in *Hyptis sauveolens* Poit. (WULF & MEDINA, 1971).

Another aspect of note in the examples outlined above is that there was considerable heterogeneity in the response of the seed population to R light. Some seeds only required a single brief period of irradiation, while others in the population required prolonged or intermittent irradiation. BEWLEY & BLACK (1982) suggested that these differences observed may be partly genetic and partly environmental. Differences in seed dormancy have been found in various genetic lines of *Avena fatua* L. (ANDREWS & SIMPSON, 1969; NAYLOR & FEDEC, 1978; ADKINS, SIMPSON & NAYLOR, 1984a). GLOBERSON, KADMAN-ZAHAVI & GINZBURG (1974) found that factors controlling light-induced germination in three distinct lines of lettuce seeds were transmitted in a normal Mendelian manner, thereby implicating genetic involvement in seed dormancy.

Environmental conditions to which the parent plants are subjected have been found to influence dormancy in, for example *Syringa vulgaris* L. (JUNTILLA, 1973), lettuce (KOLLER, 1962), *A. fatua* (PETERS, 1982a) and *Amaranthus retroflexus* L. (KIGEL, OFFIR & KOLLER, 1977). It seems therefore, that

although the environmental effect on the seed dormancy operates predominantly through its direct action on the seeds, it also acts through its action on the mother plant (BEWLEY & BLACK, 1982).

The above discussion clearly demonstrates that although the germination of an individual seed is an all-or-nothing response (FRANKLAND & TAYLORSON, 1983), there is considerable heterogeneity within a seed population. This emphasises the importance of a large seed sample requirement in determining the germinability of seeds under investigation. The response of the seeds to, for example, light treatments, is therefore only an indication of the mean response of seeds in the population.

Although there is considerable published information concerning light/temperature interactions, temperature has an influence over the light-requirement (BEWLEY & BLACK, 1982). Generally these seeds have an absolute requirement for Pfr and therefore must be illuminated. It has however, been found that temperature shifts may induce high germination of light-requiring seeds in the dark. TAYLORSON & HENDRICKS (1972b) found that *Rumex crispus* L. seeds germinated in the dark following a temperature shift. They concluded that this germination resulted from the interaction of pre-existent Pfr in the mature seeds with the temperature shift. Pre-existent Pfr was also implicated in dark germination of light-requiring *Rumex obtusifolius* L. seeds (TAKAKI, KENDRICK & DIETRICH, 1981). It was suggested that the temperature shift increases the sensitivity of the seeds to a low level of pre-existing active

form of phytochrome. It was also proposed that the temperature shift may induce the appearance of Pfr in the dark. According to the model presented in Figure 2.1.2 thermal production of Pfr could occur from the intermediates Meta-Ra, Meta-Rb, lumi-F and Meta-Fa. HAND, CRAIG, TAKAKI & KENDRICK (1982) conducted a series of germination tests on *R. obtusifolius* and concluded that germination induction in the dark by a temperature shift results from increased sensitivity to pre-existing Pfr in the seeds. In lettuce seed it was found that dark germination could be promoted by a temperature shift. TAKAKI & ZAIA (1984) again implicated pre-existing Pfr and suggested that the high temperature acts by decreasing the threshold of Pfr needed to promote germination.

Numerous light and temperature interactions have been found in the seeds of many species. These interactions, which are often complex, are discussed in detail by BEWLEY & BLACK (1982) and VANDERWOUDE (1983).

The "depth" of dormancy in seeds can change with time. The most common change is the gradual reduction in dormancy with time, a process called after-ripening. After-ripening apparently only occurs in seeds with a low moisture content (BEWLEY & BLACK, 1982). In these seeds, dormancy can often be broken by various treatments including moist chilling (stratification) or by the application of various growth stimulators such as gibberellins, cytokinins and nitrate (NIKOLAEVA, 1977). Presumably in nature, dormancy is eventually overcome by low temperature and/or with time.

The germination of dormant seeds can often be induced by the application of various compounds. This information has sometimes been used to elucidate dormancy mechanisms and associated aspects of seed physiology. The compounds include respiratory inhibitors, nitrogenous substances, plant growth regulators and anaesthetics.

Several inhibitors of normal cytochrome-mediated mitochondrial electron transport are known to break dormancy in a number of species (HENDRICKS & TAYLORSON, 1972; FAY & GORECKI, 1978). The mode of action of these inhibitors in seed germination is unclear.

ROBERTS & SMITH (1977) proposed that the activity of the pentose phosphate (PP) pathway is responsible for the loss of dormancy and the hypothesis described may be stated as follows. The covering structures of the seed form a barrier to the entry of oxygen, and thus the availability of oxygen to the embryo within the intact seed is limited. Within the tissues at least two respiratory pathways compete for oxygen: the first is conventional respiration involving glycolysis, the Krebs cycle and the electron transport chain culminating in cytochrome oxidase; the second is the oxidative PP pathway. These are illustrated in Figure 2.1.3. The terminal oxidase of the PP pathway, although unknown, can be expected to have a much lower affinity for oxygen than cytochrome oxidase. According to ROBERTS & SMITH (1977) it is the operation of the PP pathway which is essential for dormancy removal. They suggested that the rate-limiting step of the PP pathway is the re-oxidation of NADPH which is the co-enzyme

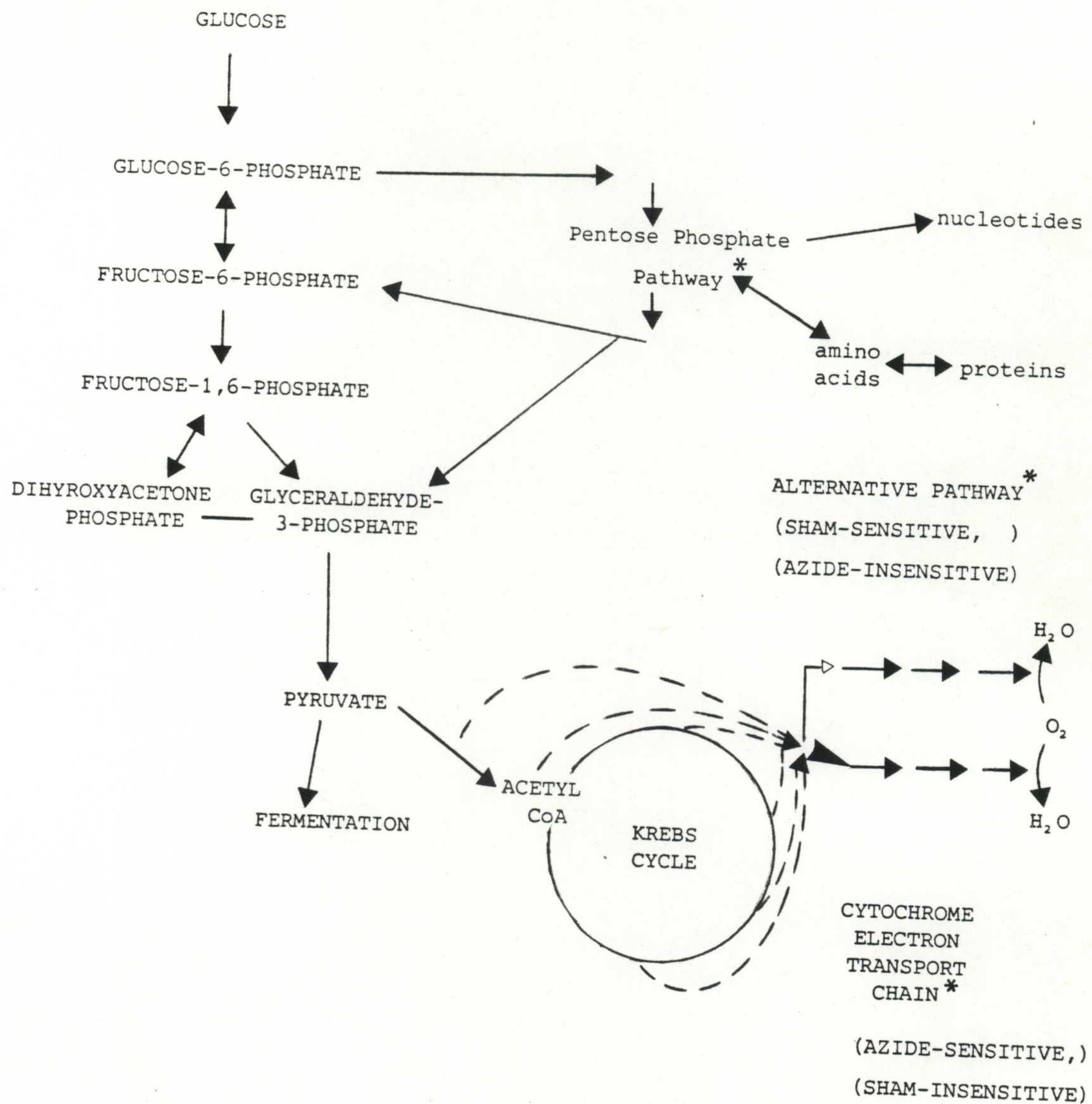


Figure 2.1.3 A diagrammatic representation of the respiratory pathways which may be involved during seed dormancy breakage. (* pathways which may be regulated by various compounds as discussed in the text).

involved in two dehydrogenation steps of this pathway, namely glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. They also suggested that the re-oxidation of the co-enzyme would not affect conventional respiration where NAD was the major co-enzyme. Therefore, any "agent" which stimulated re-oxidation of NADPH would be expected to remove dormancy.

Evidence confirming the hypothesis detailed by ROBERTS & SMITH (1977) is lacking however (BEWLEY, 1979). It seems that the involvement of the PP pathway in dormancy is difficult to confirm since parameters measured, such as enzyme activity may, in fact, be involved in normal germination as opposed to dormancy breakage. Also, the relative contributions of glycolysis and the PP pathway to total carbohydrate oxidation requires investigation to determine the importance of the PP pathway in dormancy removal. Measurements of the relative contributions of these two pathways have attracted more attention than any other aspect of respiratory flux (AP REES, 1980). Methods used to measure the activities of glycolysis and the PP pathway include: measurement of $^{14}CO_2$ production from specifically labelled hexose supplied to replicate samples of plant tissue; estimates based on labelling of intermediates; and measurement of the contribution of the PP pathway to total metabolism of glucose-6-P. These are reviewed by AP REES (1980). This author concludes that there is insufficient data to establish the relative contributions of glycolysis and the PP pathway to plant respiration, especially since the methods used are generally inadequate and therefore of no real value.

A similar attitude towards the involvement of the PP pathway has been adopted by BEWLEY & BLACK (1982). These authors suggested that insufficient evidence was available to, for example, separate the activity of enzymes in the actual dormancy breaking process and that of the normal germination process. It has been suggested by KOVACS & SIMPSON (1976) that enzyme activity of the PP pathway is increased during dormancy breaking, but YAMAMOTO (1963) demonstrated the importance of the PP pathway in the tissues of seedlings during their establishment. Therefore, records of increased PP pathway enzyme activity may be associated with development and establishment of seedlings and not with dormancy breakage.

In some investigations evidence has been produced implicating the lack of PP pathway involvement during dormancy breakage. ADKINS & ROSS (1981) monitored the activities of cytosolic dehydrogenase enzymes of the PP pathway in *A. fatua* seeds. They found no obvious connection between dormancy breakage and increased activity of the PP pathway dehydrogenases. FUERST, UPADHYAYA, SIMPSON, NAYLOR & ADKINS (1983) found that there was decreased activity of the PP pathway relative to glycolysis and the Kreb's cycle which is contrary to the hypothesis proposed by ROBERTS & SMITH (1977). With the various discrepencies in mind, alternate hypothesis to the PP pathway proposal have been investigated by various researchers.

These investigations have led to at least one alternate hypothesis for the removal of dormancy. This involves what is referred to in the literature as the alternative respiratory pathway. However, to avoid confusion this pathway

will be referred to as the SHAM-sensitive pathway. The SHAM-sensitive pathway hypothesis was developed from research findings which demonstrated that mitochondria isolated from the tissues of a number of species showed incomplete inhibition of respiration by cyanide (HACKETT, 1957; BENDALL, 1957). Subsequently, it was also found that azide yielded similar effects to those of cyanide. BENDALL & BONNER (1971) critically evaluated the various hypotheses proposed to explain these findings and concluded from their results and those of STOREY & BAHR (1969), that the apparently confusing results indicated a branched electron transport pathway from substrate to oxygen through cyanide/azide-sensitive cytochrome oxidase and a cyanide/azide-insensitive alternate oxidase as demonstrated in Figure 2.1.3. In subsequent investigations it was found that the SHAM-sensitive pathway could be inhibited by the application of hydroxamic acids (SCHONBAUM, BONNER, STOREY & BAHR, 1971). These authors reported that the hydroxamic acids are inhibitors specific to the respiratory pathway which was insensitive to cyanide and azide. It is therefore possible to isolate the two respiratory pathways and to study their mode of action separately with the application of hydroxamic acid or azide/cyanide as shown in Figure 2.1.3.

In addition, WILSON & BONNER (1971) investigated the mitochondrial activity during germination of *Arachis hypogea* L. seeds. Here it was found that Cytochrome c was lacking until about 16 hours after the start of imbibition. This led to the suggestion that respiratory activity before this time was via an alternate pathway which was insensitive to

cyanide and azide. Using this information YENTUR & LEOPOLD (1976) investigated the germination of *Glycine max* Merr. seeds. By making use of hydroxamic acid, they found that germination was dependent on the activity of alternative respiration (SHAM-sensitive) during the early stages of germination. Similar results were obtained for six other species (lettuce, *Avena sativa* L., *Phaseolus aureus* L., maize, *Lycopersicon esculentum* L. and *Pisum sativum* L.). It was concluded that SHAM-sensitive respiration provided something essential for the completion of the earliest stages of seed germination.

Extensive use has been made of hydroxamic acids and cytochrome oxidase inhibitors, such as cyanide and azide, for investigating dormancy mechanisms in the seeds of several species. Evidence obtained in such research (ESASHI, OHHARA, OKAZAKI & HISHINUMA, 1979; ESASHI, WAKABAYASHI, TSUKADA & SATOH, 1979; YU, MITCHELL, YENTUR & ROBITAILLE, 1979; ESASHI, KUSUYAMA, TAZAKI & ISHIHARA, 1981; ESASHI, SAKAI & USHIZAWA, 1981; UPADHYAYA, NAYLOR & SIMPSON, 1982; 1983) has intimated that the SHAM-sensitive respiratory pathway may be involved in the regulation of dormancy in the seeds of some species.

The dormancy breaking effect of nitrogenous compounds has been reported for several plant species (ROBERTS & SMITH, 1977; ESASHI, OHHARA, OKAZAKI & HISHINUMA, 1979; ADKINS, SIMPSON & NAYLOR, 1984a; 1984b; EGLEY, 1984). ROBERTS & SMITH (1977) suggested that the application of nitrogenous compounds, especially nitrate and nitrite, ensures the re-oxidation of NADPH making NADP available for reduction by the PP pathway dehydrogenases. In this way, the activity of

the PP pathway is supposedly increased, thereby stimulating germination. HENDRICKS & TAYLORSON (1975) proposed that nitrogenous compounds indirectly promote the activity of the PP pathway by inhibiting catalase activity which spares hydrogen peroxide (H_2O_2) for re-conversion of NADPH to $NADP^+$ More recently, it has been proposed that, in *A. fatua* germination, nitrogenous compounds function as electron acceptors and may therefore stimulate SHAM-sensitive respiration and, consequently, germination (ADKINS, SIMPSON & NAYLOR, 1984a). (These authors (ADKINS, SIMPSON & NAYLOR, 1984b) subsequently found that although the nitrogenous compounds did stimulate germination, the increased germination response was not exclusively dependent on increased activity of the SHAM-sensitive pathway. It was, however, stated that the operation of either the normal cytochrome pathway (azide-sensitive) or the SHAM-sensitive respiratory pathway was required to stimulate germination.) ESASHI, SAKAI & USHIZAWA (1981) reported that the higher germination potential of the lower seeds of *Xanthium pensylvanicum* Wallr. (cocklebur) may be related to their high capacity for the SHAM-sensitive respiratory pathway while ESASHI, KUSUYAMA, TAZAKI & ISHIHARA (1981) concluded that an appropriate balance between the azide-sensitive and SHAM-sensitive path fluxes was required to induce the germination of secondarily dormant cocklebur seeds.

Brief mention should be made of the importance of the PP pathway and glycolysis (incorporating the azide- and SHAM-sensitive pathways) in plants. The two systems are the major pathways of carbohydrate oxidation in higher plants (PURVIS &

FITES, 1979). Metabolism of the carbohydrate substrate provides energy and NADH, mainly by the glycolysis pathway (AP REES, 1980), while the PP pathway is important for supplying NADPH for synthesis of fatty acids for membrane formation (PRYKE & AP REES, 1976; PURVIS & FITES, 1979) and nucleotides and amino acids (see Figure 2.1.3).

Both nitrogenous compounds and respiratory inhibitors have been implicated in the stimulation of germination, in the dark, of light-requiring seeds. TAYLORSON & HENDRICKS (1973) found that both azide and cyanide improved germination in *Amaranthus albus* L. seeds and lettuce seeds but not in *Lepidium virginicum* L. seeds. Nitrogenous compounds including the respiratory inhibitors azide and cyanide have been found to stimulate dark germination of seeds of a number of species (HENDRICKS & TAYLORSON, 1974; ZAGŌRSKI & LEWAK, 1983). HILTON (1984) found that nitrate application did stimulate germination in *A. fatua* in the light but was not effective in the dark. Therefore the Pfr requirement persisted. Dark-dormant seeds of *Portulaca oleracea* L. (common purslane) could be stimulated to some extent by nitrite but not by nitrate (EGLÉY, 1984). It was suggested that nitrate exerted its effect via conversion to nitrite within the seed and that the rate of nitrate conversion could be a limiting factor in the dark germination of common purslane seeds.

Other compounds have also been found to stimulate germination of positively photoblastic seeds in the dark and, of particular interest, is the suggested involvement of plant growth regulators (hormones), especially the gibberellins.

It has been claimed that gibberellins substitute for R light requirement in positively photoblastic seeds and promote germination of negatively photoblastic seeds (JONES & STODDART, 1977). BIANCO & BULARD (1981) for instance, found that the amount of certain gibberellins in lettuce seeds maintained in the dark was very low. Following white or R light treatment there was a significant increase of free gibberellic acid (GA) in the seeds. However, the evidence, as reviewed by BEWLEY & BLACK (1982) suggests that gibberellins do not substitute the Pfr requirement. Rather, they inferred, gibberellins complement low levels of Pfr present, thereby inducing germination. This is supported by the fact that concentrations of GA which break dormancy of lettuce seeds are unable to stimulate germination of seeds which had previously been treated with FR light (NEGBI, BLACK & BEWLEY, 1968). Three major response types namely: seeds insensitive to gibberellins, seeds showing synergism between light and gibberellins and seeds whose response in darkness utilises residual Pfr, have been identified (BEWLEY & BLACK, 1982). Together, these responses indicated that gibberellins do not substitute the Pfr action but co-operate with it. Therefore it is unlikely that Pfr breaks seed dormancy simply by activation of gibberellin synthesis. Other plant hormones have also been implicated, in association with gibberellins, in Pfr substitution. For example, a study conducted by SPEER, HSIAO & VIDAVER (1974) showed the following relationships: Ethylene could substitute for GA but not for R light in breaking the secondary dormancy induced by extended dark-storage of fully hydrated lettuce seeds.

Applied together, some germination was induced in the dark, but singly applied in the absence of R light, neither promoted germination. Also, ethylene did not promote germination by regulating GA synthesis, nor did GA exert its effect by promoting ethylene synthesis or activity. In addition, R light (that is, Pfr) did not promote germination by stimulating either ethylene or GA activity or synthesis. It was concluded that the effect of neither ethylene nor GA is analogous to that of the phytochrome system.

Further evidence supporting the scepticism in gibberellin substitution of Pfr is obtained from other researchers. FREDERICQ, RETHY, VAN ONCKELEN & DE GREEF (1983) found that GA alone does not induce germination of *K. blossfeldiana* seeds, but that it may increase the physiological activity of Pfr.

There are similar uncertainties with consideration to cytokinins in relation to dormancy and light substitution. It has been found, for example, that cytokinin levels rise following R light treatment (VAN STADEN & WAREING, 1972; VAN STADEN, 1973). However, it is suggested by BEWLEY & BLACK (1982) that these changes probably occur within the escape time therefore Pfr can be removed by FR light to prevent termination of dormancy.

Endogenous ethylene is also unlikely to play a major role in breaking seed dormancy during light treatment, although it has been found that it enhances the light effect and interacts with other growth regulators (SPEER, HSIAO & VIDAVER, 1974; THOMAS, PALEVITCH, BIDDINGTON & AUSTIN, 1975; KARSSEN, 1976a).

Therefore, although ethylene may stimulate the germination of dormant seeds, it does not substitute for the light requirement.

Considerable evidence has been generated to underline the importance of the role of hormones in seed dormancy. It is thought that via these compounds specific environmental conditions are detected resulting in biochemical responses within the seed. Therefore environmental triggers can be translated into the potentiation of germination of dormant seeds and the exogenous applications of hormones can be used to regulate germination (JANN & AMEN, 1977). It is therefore necessary to briefly examine those phytohormones thought to be of consequence in maintenance and removal of seed dormancy.

Applied abscisic acid (ABA) is a strong inhibitor of germination. This compound is a notable component of dormant seeds where it is thought to prevent premature germination (WALTON, 1981). In mature seeds, ABA may be located in the covering structures from where it is probably transported into the embryo (JARVIS, 1975) resulting in coat-imposed dormancy as previously described. However, the importance of ABA in seed dormancy is not conclusive. In the dormant seeds of some species, the levels of ABA are higher than the corresponding non-dormant seeds (SONDHEIMER, TZOU & GALSON, 1968; ISAIA & BULARD, 1978) but not so in other species (BERRIE, BULLER, DON & PARKER, 1979). In addition, some non-dormant seeds apparently contain very high levels of ABA (McWHA & HILLMAN, 1974). Further scepticism concerning the role of ABA in seed dormancy was provided by KARSSEN (1976b) who demonstrated that applica-

tion of ABA only prevented the final stages of germination in *C. album* seeds and was therefore not involved in dormancy *per se*. In their review BEWLEY & BLACK (1982) concluded that there is little definitive evidence that dormancy generally is imposed by an inhibitor such as ABA.

Applied gibberellins have been found to stimulate germination in the seeds of a wide variety of species. Gibberellins may be important in the earlier phases of dormant-seed germination if the embryos lack key enzymes or adequate nutrients (JANN & AMEN, 1977). The effect of gibberellins is on a wide range of dormancy mechanisms including embryo growth, mechanical coat-imposed dormancy, high inhibitor levels and physiologically incompetent embryos (JONES & STODDART, 1977).

Gibberellins substitute for the chilling requirement of some seeds. It is possible that chilling of seeds serves to activate the gibberellin-producing mechanism which results in actual synthesis when seeds are transferred to elevated temperatures (WILLIAMS, BRADBEER, GASKIN & MACMILLAN, 1974). After-ripening in seeds of some species can also be replaced by gibberellin application and therefore storage may also activate gibberellin synthesis.

In germination, cytokinins are reported to affect the function of proteins (THOMAS, 1977) and membrane permeability (MILLER, 1956). THOMAS (1977) suggested that compounds which affect membrane permeability also stimulate a cytokinin-gibberellin mixture response on seed germination. Therefore a cytokinin-inhibitor interaction could be a mecha-

nism whereby membrane permeability could be controlled, that is, cytokinins regulate movement of gibberellins between various compartments within seeds (THOMAS, 1977). The inhibitor governing cytokinin-induced responses may be ABA, which has been demonstrated to have an antagonistic effect on cytokinins (WEBB, VAN STADEN & WAREING, 1973). KHAN (1971) developed a model for hormonal control of dormancy and dormancy breakage. Roles of primary, preventative and permissive were assigned to gibberellins, inhibitors (e.g. ABA) and cytokinins respectively. This model was based on the assumption that cytokinins compete with ABA for a common site of action whereas non-competitive interaction exists between ABA and gibberellins. This model has been used to explain the wide variety of gibberellin-mediated responses which are inhibited by ABA and to demonstrate that, in many cases, cytokinins are essential for the completion of gibberellin-induced germination, especially where this has been blocked by inhibitors.

The involvement of auxins in dormancy has long been under dispute. NIKOLAEVA (1975) found that indole-acetic-acid (IAA) decreased during germination of some deeply dormant seeds. MAYER & POLJAKOFF-MAYBER (1982) concluded, in a review of the information available at that time, that auxins will only stimulate germination under "very special conditions". The involvement of auxins in seed dormancy is therefore regarded to be of minor importance.

Ethylene and seed dormancy have long been associated and this topic is well documented. Ethylene has been found

to relieve the dormancy in seeds of several species (KETRING, 1977). For some species, for instance cocklebur, a substantial body of evidence has been produced to implicate ethylene control of dormancy and germination (ESASHI & KATOH, 1975; ESASHI, OHHARA, KOTAKI & WATANABE, 1976; ESASHI, WATANABE, OHHARA & KATOH, 1976; KATOH & ESASHI, 1975a; 1975b).

Compounds referred to as anaesthetics by TAYLORSON & HENDRICKS (1981) which include the alcohols, are usually considered inhibitors of germination (REYNOLDS, 1977). However, promotive effects have sometimes been noted. In positively photoblastic lettuce seeds, for example, relatively brief treatment of imbibed seeds with low concentrations of ethanol (± one per cent v/v), promoted germination (PECKET & AL-CHARCHAPCHI, 1978). Ethanol has also been found to increase the dark germination of light-requiring seeds of several grass species (TAYLORSON & HENDRICKS, 1979). Again brief ethanol treatment was necessary to elicit an improved germination response since failure to reduce the ethanol concentration inhibited germination.

The precise mode of alcohol action in the replacement of light in seed germination, is not known. It has been suggested that ethanol affects membranes (REYNOLDS, 1977; PECKET & AL-CHARCHAPCHI, 1978). PECKET & AL-CHARCHAPCHI (1978) implicated ethanol action on cellular membranes since treatment with Ca^{2+}, a membrane stabilizer (TOPOVER & GLINKA, 1976), markedly reduced ethanol promotion of seed germination. TAYLORSON & HENDRICKS (1979) suggested that anaesthetics, through their action on seed membranes, interfered with phyto-

chrome-like action causing germination in the absence of irradiations or by increasing responsivity to irradiations. In later studies TAYLORSON (1984) found that ethanol prevented the action of Pfr in *R. crispus* seeds, thereby inhibiting germination usually induced by R irradiation. Again, the mode of action of ethanol was attributed to its effect on membranes, resulting in perturbations which are thought to prevent Pfr from acting.

A review of the literature has clearly revealed that in addition to the importance of seeds as reproductive and dispersal units, they often also form a crucial stage in the life cycle of many species. Not only do seeds provide plants with the means to survive adverse environmental conditions, but seeds also have delicately controlled inherent mechanisms which ensure that further development, germination in the natural situation, occurs at a time when environmental conditions are likely to be favourable for the survival of the seedling. The elucidation of the mechanisms operative in seed germination aid greatly in the understanding of the biology of a species and may explain the situations observed in the field.

Seeds also play an important role in the success of certain plants as weeds, a fact which is often highlighted in the literature since several components of seed biology are referred to as weed characteristics. It is therefore not surprising that investigations on weed seed germination form an integral part of weed science. Bearing in mind the importance of seeds to plants in general and specifically to weeds,

it was considered pertinent to investigate the germination of *C. odorata* achenes. Additionally, as described in section 1.2, there is a severe lack of knowledge of *C. odorata* achene germination generally; for instance, although a small number of articles dealing with *C. odorata* has been published, none has been found describing the basic optimum germination conditions for the achenes. The importance of the achenes is often emphasised yet little information is available to support these views. The high reproductive potential by means of achenes and the adaptation of these to wind dispersal is a well established fact yet little effort appears to have been made to elucidate the germination requirements of the achenes and how these may contribute to this weed's survival/success strategy. Local information pertaining to this weed in South Africa is also minimal, even though it has been recognised as a serious invader in Natal since the early 1960's. Furthermore, it was also known that an investigation into the chemical control of *C. odorata* was to be commenced (see Chapter III) and therefore information was required to determine the potential for re-infestation of areas cleared of this weed. Since the achenes are the reproductive propagules likely to be responsible for re-infestation, germination studies would complement the *C. odorata* control project.

All the factors described above contributed to the decision to initiate a comprehensive study on *C. odorata* achenes.

2.2 Materials and Methods: General

In this investigation into the germination of *C. odorata* achenes experimentation was conducted in a sequential pattern. Consequently, procedures used for the specific germination tests were often dependent on the results of the preceding experiment(s). It is therefore not possible to present in one section, all the information pertaining to the materials and methods. However, certain aspects were standard throughout and these are reported here.

For the majority of experiments, the achenes used were collected from a large infestation (Plate 3) in Virginia Bush, a conservation area in Durban, located at 29°49'S 31°1'E. This site was selected as the achene source because it is within a conservation area managed by the Durban City Council (Department of Parks and Recreation) and is thus protected from fire and man-made disturbances. The site therefore provided a reliable source of experimental material. In addition, the site was conveniently located for frequent harvesting. In certain experiments, achenes were acquired from other sites; the locality of these is detailed for the relevant experiments.

Harvesting consisted of cutting capitula-bearing shoots from *C. odorata* plants in the infestation, ensuring that material collected was from a large number of plants. This material was then taken to the laboratory where the achenes were removed from the involucral bracts and receptacles of the capitula. The achenes were then hand-sorted under a dissecting microscope and separated into filled and empty achenes. The

status of the achenes was determined by applying slight pressure with fine forceps to the individual achenes. Only those containing an embryo (filled achenes) were retained for the germination tests.

The total number of achenes and percentage filled and empty achenes per capitulum were determined for various achene batches harvested. These data provided an indication of the reproductive potential since the filled achenes were assumed to be viable; this was confirmed by the high percentage germination obtained with these achenes in certain tests.

The achenes retained for experimentation were stored dry in the dark at 25±2°C until required. Where possible, the moisture content of the achenes was determined prior to storage and again prior to utilisation for experimentation, thereby providing an indication of the change in moisture status during storage. For the moisture content determinations, 10 replicates of 100 achenes each were used where plentiful material was available. In some cases, where the amount of material was limited, only five replicates of 100 achenes each were used. The mass of each replicate was determined before and after drying at 103 °C. Percentage moisture content was then determined as follows:

$$\frac{\text{Initial mass - Mass after drying}}{\text{Initial mass}} \times 100 = \text{Percentage moisture content}$$

Germination tests, unless stated otherwise, were conducted in petri dishes lined with a double layer of Whatman's No. 1 filter paper. The filter paper in each petri dish was

moistened with three cubic centimetres of double-distilled water or with three cubic centimetres of the various test solutions as specified for certain experiments. The achenes in the petri dishes were examined every two or four days at which time the number of germinated achenes was counted and removed and, if necessary additional water was added to prevent drying-out of the filter paper and therefore, desiccation of the achenes.

The achenes in the petri dishes were incubated in Labex cabinets in which the required light and temperature regime could be set. The chamber temperature was adjusted and allowed to equilibrate prior to commencement of experimentation. The temperature was continuously monitored with a maximum/minimum thermometer to ensure maintenance of the correct temperature.

In experiments where continuous dark incubation was required or where achenes were exposed to light for restricted periods, the achenes, in the petri dishes, were enclosed in light-tight boxes which were then placed in the respective incubators. It was found that the temperature inside the boxes was the same as that of the incubator chambers. The boxes were only opened under green safelight conditions for examination of the achenes.

As subsequently described, different treatments were used for individual germination tests. However, the same sources of irradiation were used throughout. These were as follows:

all white light was provided by six 30 cm-long fluorescent tubes supplying 0,5 $W m^{-2}$ in the wavelength range 600 to 700 nm. R light was provided by two one metre-long red fluorescent tubes supplying 0,9 $W m^{-2}$ in the wavelength range 600 to 700 nm. FR light was provided by six incandescent bulbs filtered to supply 1,6 $W m^{-2}$ in the wavelength range 700 to 800 nm. The FR light was also filtered through a two centimetre layer of water. For examination of achenes incubated in the dark, a green safelight (KENDRICK, SPRUIT & FRANKLAND, 1969) supplying 0,2 $W m^{-2}$ in the wavelength range 450 to 550 nm, was erected in a darkroom. In addition, a similar green safelight filter was fitted to a dissecting microscope to facilitate examination of the achenes. The influence of the green safelight on the germination of the *C. odorata* achenes was investigated prior to experimentation. It was found that exposure to this light had no effect on germination percentage.

Although the number of replicates per treatment in each individual germination test was constant, the number for different experiments was varied, these being dependent on the availability of material. The number of replicates used is detailed in each of the experiments.

Germination, protrusion of the radicle through the pericarp, was recorded as a cumulative percentage. Germinated achenes were removed from the petri dishes. For all the germination tests, the value of the final percentage germination was analysed by analysis of variance (L.S.D. p = 0,05). It was found that no transformation of the raw data was necessary because these did not fit a normal binomial distribu-

tion curve. That is, the standard errors of high, medium and low percentages were similar. To provide a measure of the variability of the means at each day germination was recorded, the standard errors of the means are included in all the figures as vertical bars.

2.3 Optimum Germination Requirements

Prior to an investigation of the germination biology of seeds, it is first necessary to determine the conditions required for germination. In this way the optimum conditions for most germination can be identified and used as the standard conditions for germination tests. In addition, the optimum conditions for germination also provide a basis for comparison with various treatments applied to seeds during germination studies.

Materials and Methods

Achenes harvested from Virginia Bush in August 1983 were used to determine the optimum conditions for germination. These achenes had been stored for at least six months prior to use.

To identify the optimum temperature for germination, achenes were imbibed for 48 hours in the dark at the various temperatures and, since it was found in preliminary experiments that light was required for germination, were then exposed to one hour white light. Thereafter the achenes were returned to the respective temperatures and incubated in the dark. The temperatures tested were 15, 20, 25 and 30 °C constant and diurnal alternating temperatures of 10/20, 10/25, 15/25 and 15/30 °C (10 hours : 14 hours). At each of these temperatures 12 replicates of 20 achenes each were used. The same number of imbibed achenes was retained continuously in the dark to

serve as a control.

Based on the results obtained for the temperature experiment, only two temperature regimes, 15/25 °C and 15/30 °C, were used to determine the optimum imbibition duration for sensitisation of the achenes to light. Achenes imbibed in the dark for three, six, 12, 48 and 144 hours at both 15/25 °C and 15/30 °C (10 hours : 14 hours) were irradiated with one hour white light. A comparison was made with the germination of achenes incubated (i) continuously in the dark, and (ii) in a diurnal light/dark cycle (14 hours : 10 hours) following two days dark imbibition at both the temperature regimes. In this experiment 20 replicates of 10 achenes each were used for each treatment.

Concurrently with the imbibition experiment, the water uptake of achenes imbibed in the dark at 15/30 °C was measured. The information was obtained by recording the mass of the achenes after zero, one, three, six, nine, 12, 15, 24, 48 and 142 hours imbibition. For the mass determinations, the achenes were carefully removed from the moist filter paper by clasping the pappus (thereby avoiding damage to the pericarp and enclosed structures) and then blotted dry on tissue paper. Ten replicates of 25 achenes each were used and all the manipulations were executed under green safelight conditions.

In both the temperature and imbibition experiments it was found that germination was stimulated by exposure of the achenes to white light. Consequently, an experiment to determine the optimum white light duration for germination

was conducted at 15/30 °C since this temperature had resulted in the highest final percentage germination in the earlier experiments. For this (and subsequent experiments) a diurnal 12 hour dark : 12 hour light cycle (diurnal light cycle) was used which coincided with the 15/30 °C temperature regime. Achenes were imbibed for 48 hours and then exposed to either five minutes, one hour, six hours or 12 hours white light. On completion of the exposure periods, dark incubation was continued. Additional treatments included dark and diurnal light controls. Twelve replicates of 20 achenes per treatment were used.

Simultaneously with the white light duration experiment, the effect of cumulative white light on germination was investigated. Achenes were imbibed for 48 hours and then exposed to white light for either six hours on two consecutive days, three hours on four consecutive days or two hours on six consecutive days. Again, 12 replicates of 20 achenes per treatment were used.

Results

Germination of *C. odorata* achenes was influenced by the incubation temperature (Table 2.3.1). Of the temperatures tested, the three highest germination percentages were obtained from achenes incubated at alternating temperatures. Of these, 15/30 °C yielded the highest ($p = 0{,}05$) final percentage germination (42 per cent), followed by 15/25 °C (34 per cent). At all constant incubation temperatures tested germination was low

Table 2.3.1 The effect of incubation temperature on the percentage germination of *C. odorata* achenes which had been imbibed for 48 hours in the dark prior to one hour white light treatment.

Temperature (°C)	Percentage Germination					
	Days after light treatment					
	4	8	12	16	20	24
15	0	0	0	0	0	0
20	1	6	6	6	6	6
25	10	17	17	21	21	21
30	4	7	11	11	11	11
10/20	1	1	3	5	5	5
10/25	4	4	13	26	28	28
15/25	8	15	28	32	33	34
15/30	16	25	36	40	41	42
LSD p = 0,05	7	6	6	5	6	5

and at 15 °C, no germination occurred. Consequently, the 15/30 °C and 15/25 °C incubation temperatures were used for the imbibition experiment.

A notable feature of the temperature experiment was that at all temperatures, germination of the achenes retained in the dark was low (< 10 per cent). These results are not presented.

At an incubation temperature of 15/25 °C, a dark imbibition period of either 48 or 144 hours followed by one hour white light resulted in similar germination percentages ($p = 0,05$) (Figure 2.3.1A). Both these imbibition times significantly improved germination in comparison to the other imbibition times which all resulted in a similarly low promotive effect. The lowest percentage germination (5,5 per cent) was however, obtained in the dark control (Figure 2.3.1A).

At the 15/30 °C incubation temperature, a dark imbibition period of greater than 24 hours prior to light treatment was sufficient to significantly promote germination (Figure 2.3.1B). The difference in the final percentage germination of achenes imbibed for 24, 48 or 144 hours was not marked although that of achenes imbibed for 144 hours was significantly higher than that of achenes imbibed for 24 hours. Final percentage germination in the dark was again low (Figure 2.3.1B).

The highest percentage germination in both incubation temperature regimes was obtained for the achenes experiencing a diurnal light cycle (Figure 2.3.1A; 2.3.1B); the final germination percentage at 15/30 °C (92 per cent) was higher

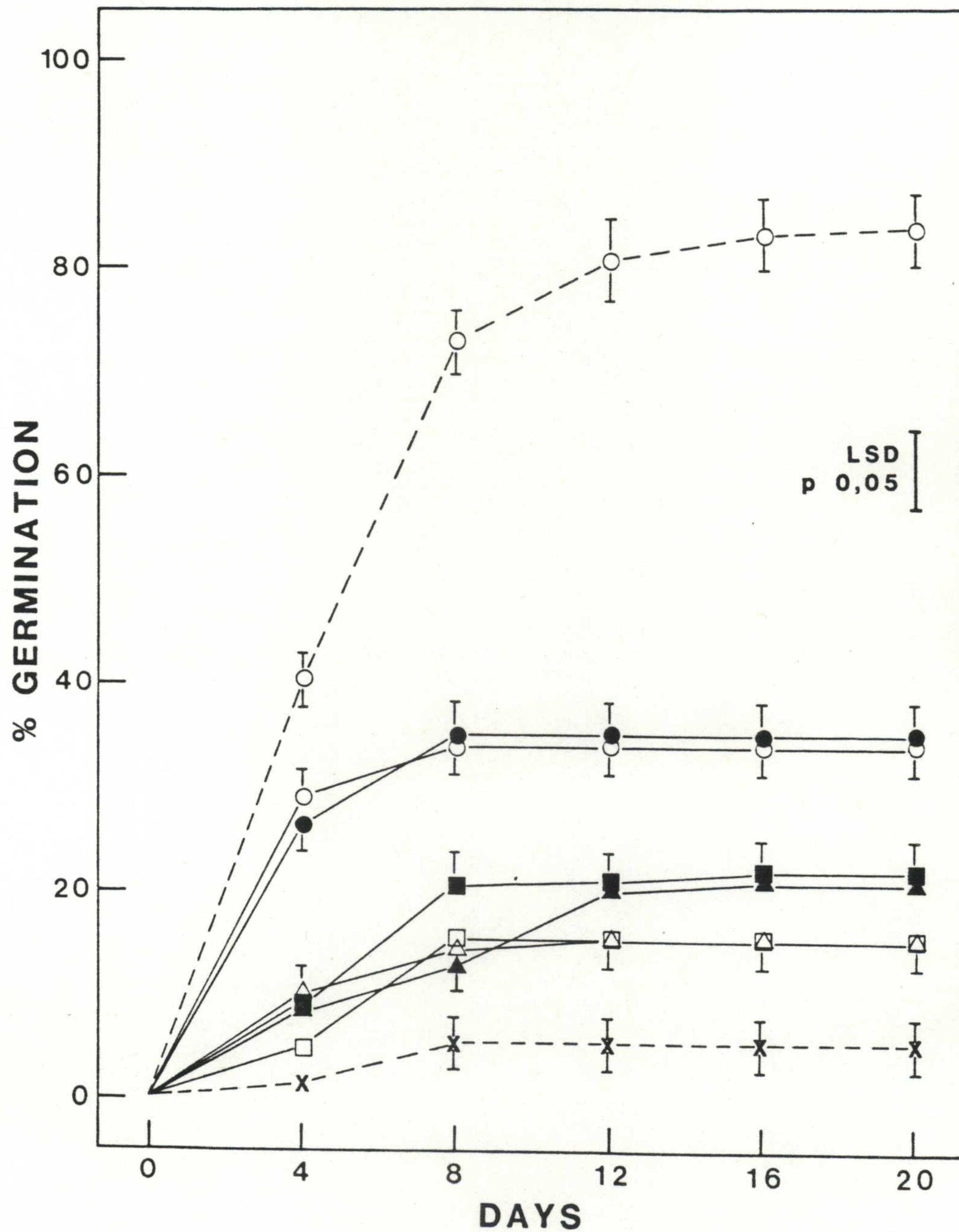


Figure 2.3.1A The effect of dark imbibition duration prior to exposure to one hour white light, on the germination of achenes incubated at 15/25 °C. (—○— 144h; —●— 48h; —□— 24h; —■— 12h; —△— 6h and —▲— 3h dark imbibition; --x-- dark; --○-- diurnal light).

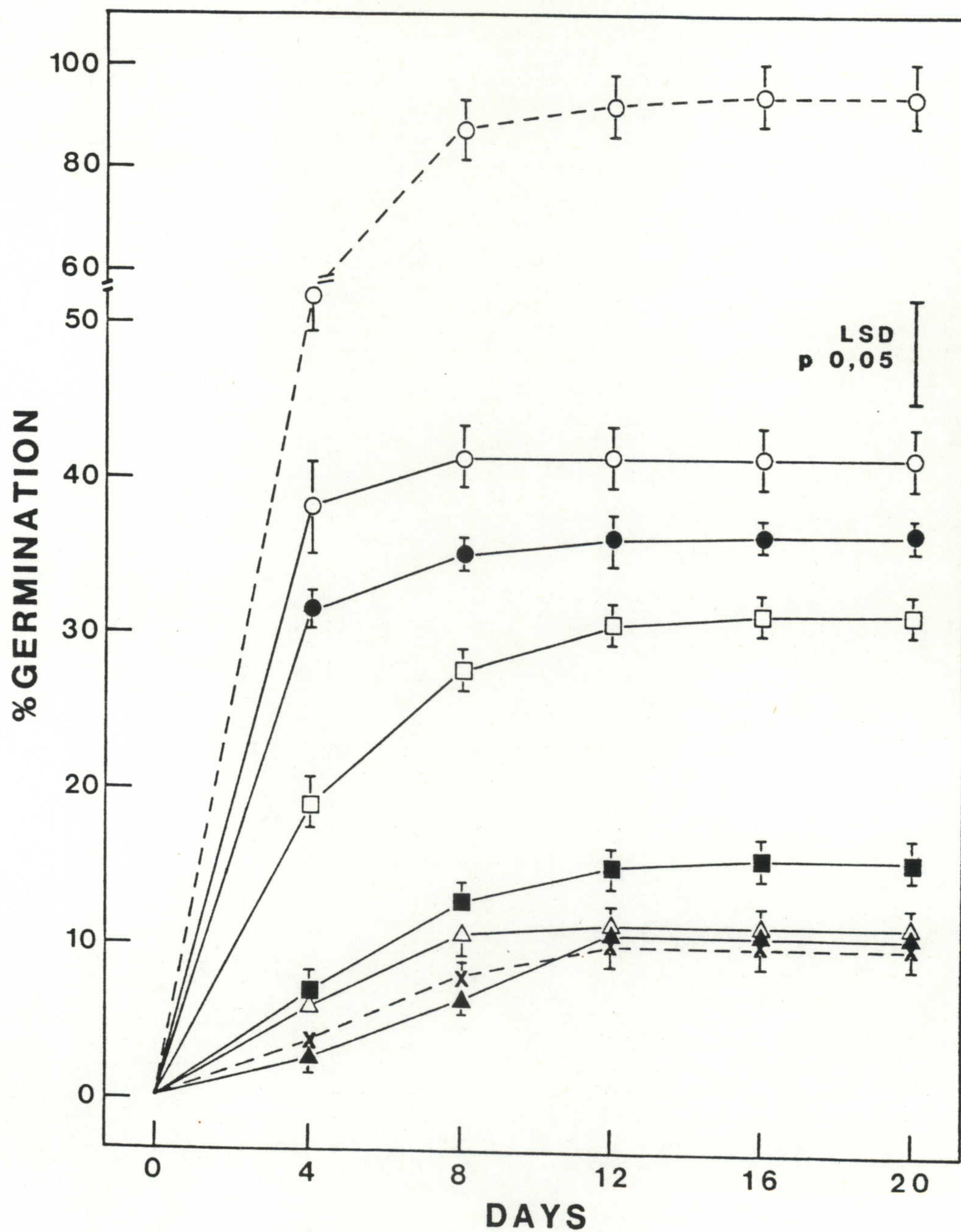


Figure 2.3.1B The effect of dark imbibition duration prior to exposure to one hour white light, on the germination of achenes incubated at 15/30 °C. (—○— 144h; —●— 48h; —□— 24h; —■— 12h; —△— 6h and —▲— 3h dark imbibition; --x--dark; --○-- diurnal light).

than that at 15/25 °C (84 per cent) thereby providing confirmation that the former temperature regime was more favourable for germination.

In the water uptake experiment, the mass of achenes increased rapidly in the first 15 hours of imbibition (Figure 2.3.2). At this time the mean achene mass was 168 per cent of the mass of the achenes at time zero. For the remainder of the imbibition period, the mass increased only slightly and when the experiment was terminated after 142 hours, the achene mass was 184 per cent of the initial mass.

Based on the results of the imbibition experiments, a dark imbibition period of two days was used prior to light treatments in subsequent experimentation. Furthermore, experiments were only conducted at an incubation temperature of 15/30 °C because of the consistently higher percentage germination obtained in this temperature regime.

Duration of illumination with white light influenced germination. Illumination of achenes for six or 12 hours resulted in higher percentage germination ($p = 0,05$) than the shorter irradiation periods of one hour or five minutes (Figure 2.3.3). Again the percentage germination of achenes in the diurnal light cycle was considerably higher than any of the other treatments while germination in the dark was again low (six per cent).

In the cumulative light experiment, the total time of exposure was 12 hours. Therefore the results of the 12 hour treatment presented in Figure 2.3.3 are again presented

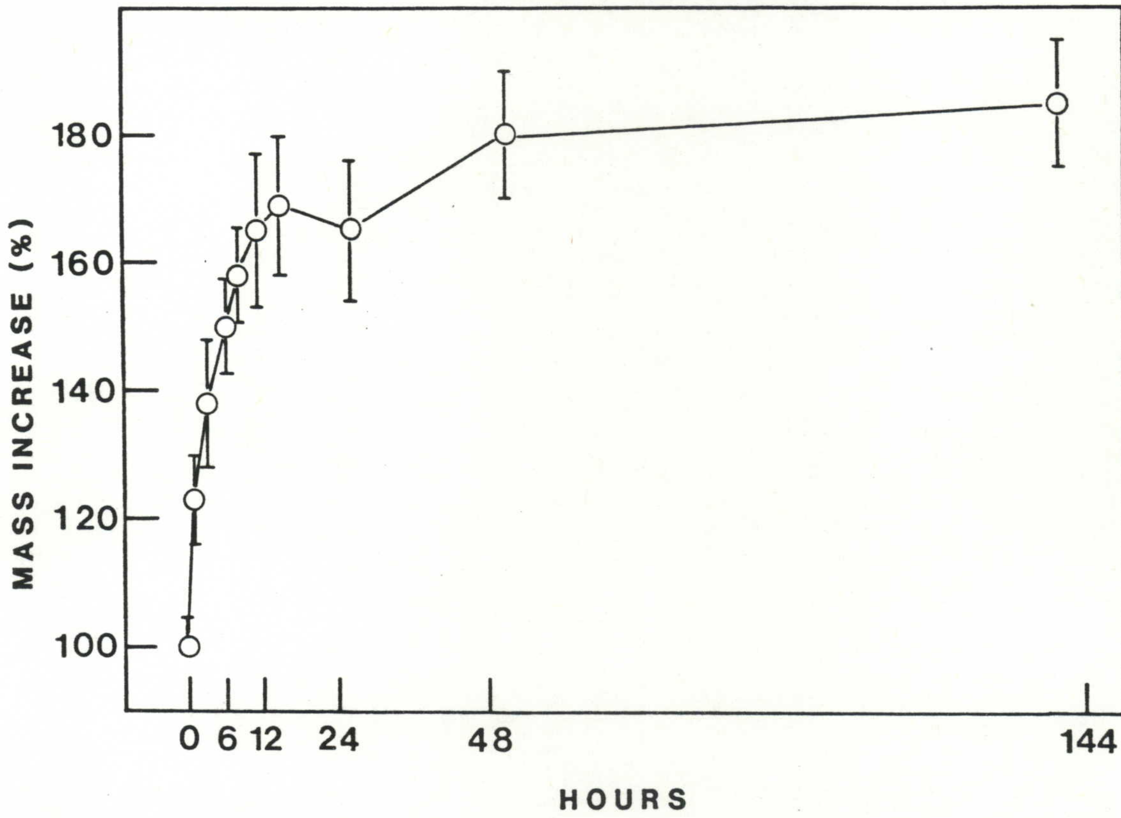


Figure 2.3.2 The mass increase of achenes of *C. odorata* during dark imbibition at 15/30 °C.

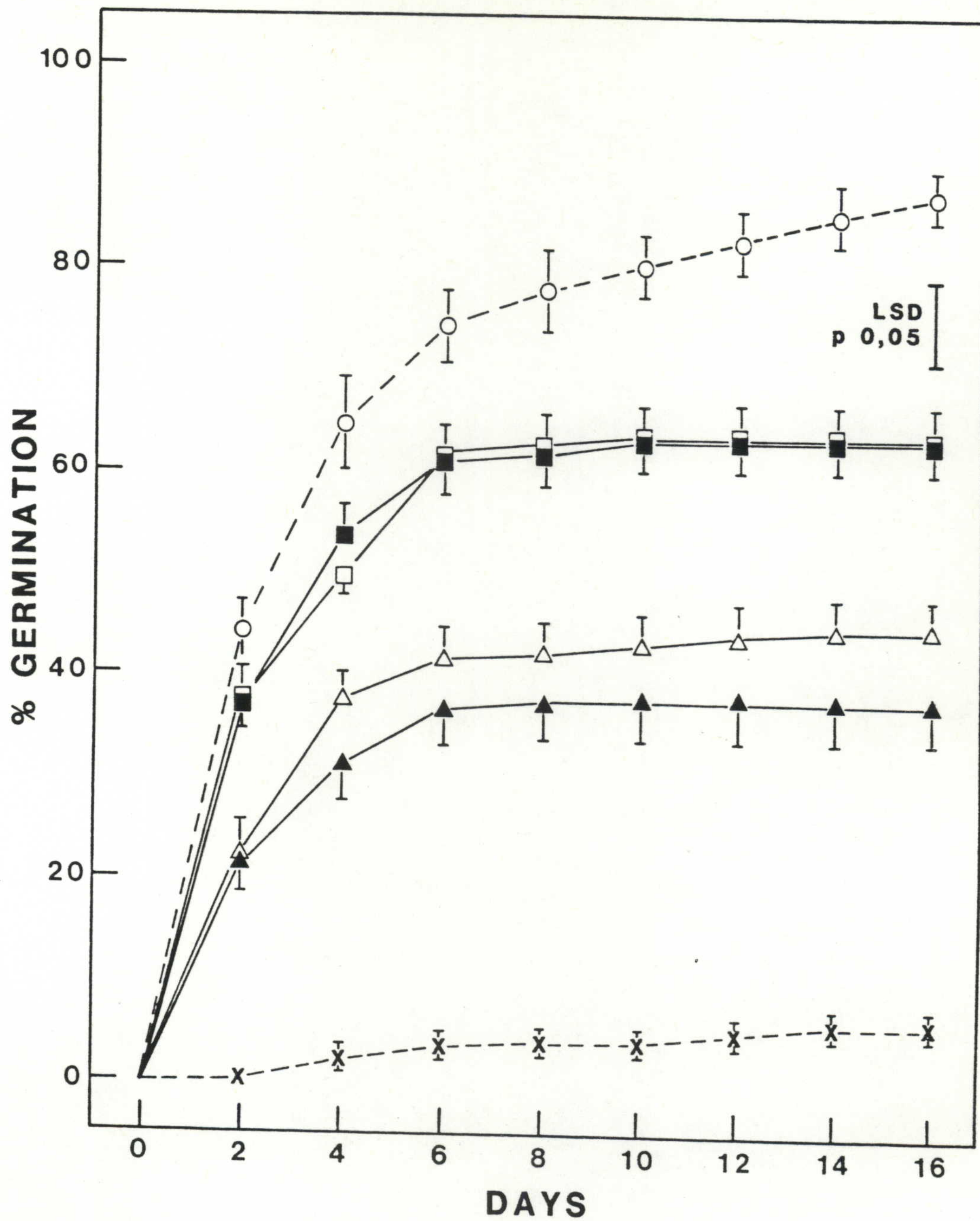


Figure 2.3.3 The effect of the duration of white light on the germination of pre-imbibed achenes. (—□— 12h; —■— 6h; —△— 1h and —▲— 5 min white light; --x-- dark; --○-- diurnal light).

in Figure 2.3.4 together with the results of the cumulative white light treatments. A single irradiation of 12 hours was more favourable than the cumulative light treatments, while six consecutive exposures of two hours each resulted in a similar percentage germination to that of four consecutive exposures of three hours each. Although two consecutive exposures of six hours each promoted germination over that of the continuous dark treatment, the percentage was lower than that of the other light treatments (Figure 2.3.4).

Discussion

In identifying the optimum temperature for germination, highest percentage germination was found to occur at an alternating temperature of 15/30 °C. The second and third most optimum temperatures (15/25 °C and 10/25 °C) which were also alternating, indicate that constant temperatures, which in any case are unlikely to occur in the field, are not favourable for germination of *C. odorata* achenes. The induction of germination by alternating temperatures is not an uncommon phenomenon (THOMPSON, 1974; THOMPSON, GRIME & MASON, 1977; VINCENT & ROBERTS, 1977; TOTTERDELL & ROBERTS, 1980; THOMPSON & GRIME, 1983; THOMPSON & WHATLEY, 1984). The mode of action of such alternating temperatures is, however, not clear. A considerable amount of research has been conducted on the genus *Rumex*. In a review article, ROBERTS & TOTTERDELL (1981) identified nine characteristics of an alternating temperature requirement which may stimulate loss of dormancy

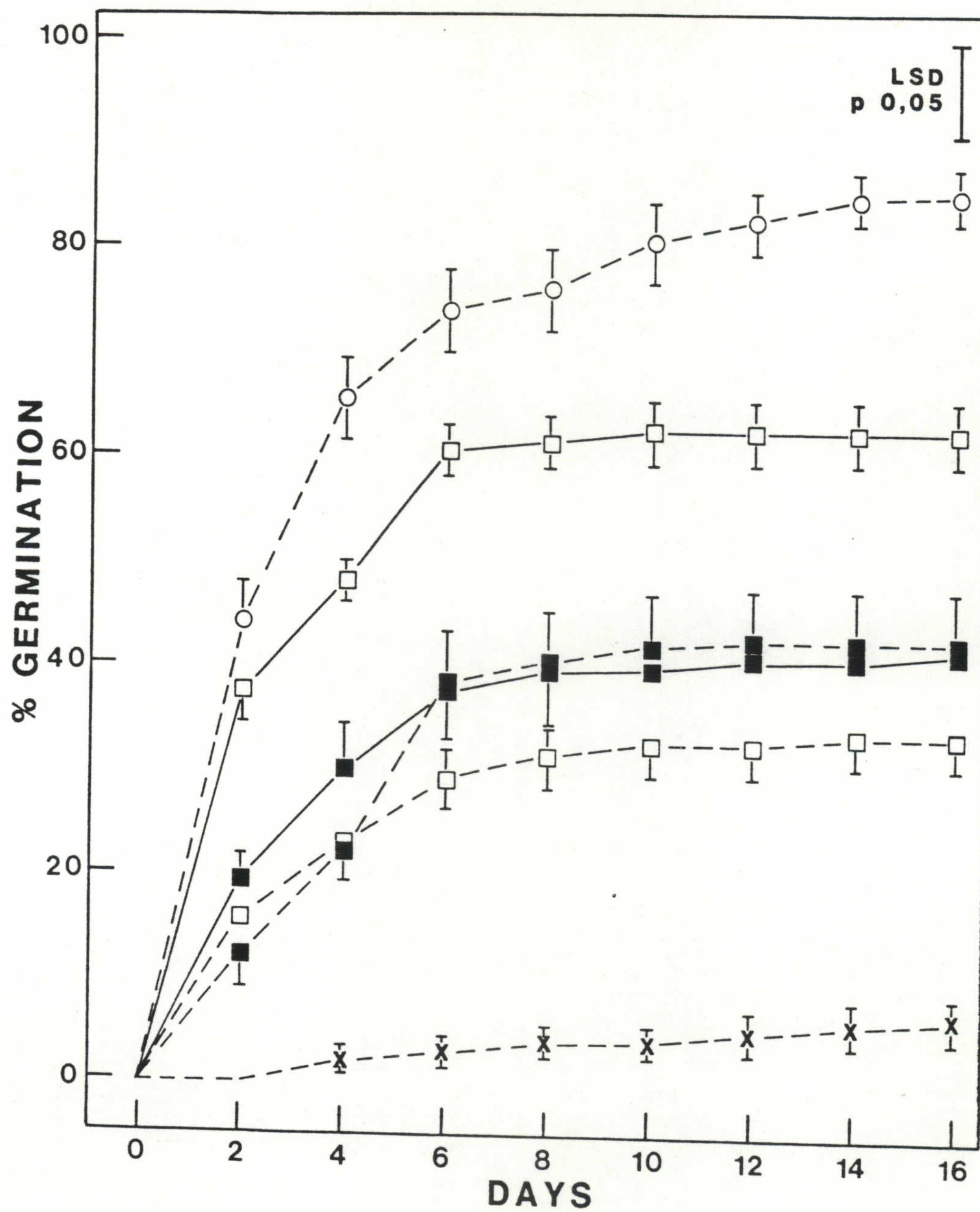


Figure 2.3.4 The effect of cumulative white light treatments on the germination of pre-imbibed achenes. (—□— 12h; --□-- 6h+6h; —■— 3h+3h+3h+3h; --■--2h+2h+2h+2h+2h+2h; --×--dark; --○--diurnal light).

in the seeds of *Rumex* species. It was suggested that these characteristics greatly contributed to the flushes of germination which occur in undisturbed soil particularly in spring, and the flush of germination which follows soil disturbance. It has also been suggested that ecologically, a fluctuating temperature requirement for germination is of adaptive value as a soil-depth sensing and canopy-gap detecting mechanism (THOMPSON & GRIME, 1983; THOMPSON & WHATLEY, 1984). These factors (in combination with photocontrol of germination) may well be of relevance to *C. odorata* especially since nuclear infestations in existing vegetation occur beneath gaps in the canopy and seedling emergence occurs in bare, disturbed soil during summer. In addition, the optimum germination temperature is one which is prevalent during spring and summer in the regions currently infested by *C. odorata*. Since this is also a predominantly summer rainfall area, the promotive temperatures occur at a time when soil moisture would usually be available for imbibition and seedling survival.

The temperature experiment also confirmed results from preliminary experiments that the germination of *C. odorata* achenes is photo-controlled; the achenes are positively photoblastic since light is required for germination (EVENARI, 1965). This photo-control of germination is at least partially governed by temperature because of the differences in the germination percentages at the various temperatures tested. Various explanations for the probable temperature-photo-control link have been proposed. It may be that: the intermediates of Pr and Pfr are temperature dependent (KENDRICK & SPRUIT, 1977) thereby governing the production of Pfr required for

germination; temperature may regulate the formation of "products" upon which or with which light-promoted germination mechanisms act or interact (DUKE, EGLEY & REGER, 1977); and/or imbibition at the lower temperatures may have been insufficient to fully sensitise the achenes to the irradiation (TAYLORSON & HENDRICKS, 1972a).

Although the dry seeds of several species are perceptive to light quality (BARTLEY & FRANKLAND, 1984), *C. odorata* achenes were only perceptive to light following imbibition. This was confirmed by exposing the achenes to either white or R light prior to dark imbibition. No effect of the light treatments on germination was noted.

The achenes appeared to be fully imbibed 15 hours after commencement of the imbibition period since an asymptote in the mass increase was reached at this time. This time corresponds to the imbibition period required to illicit a significantly promotive germination response by exposure to light at 15/30 °C (Figure 2.3.1B). At 15/25 °C however, germination was significantly promoted only after an imbibition period of 48 hours. The limited response of achenes imbibed for the shorter durations is unlikely to have arisen as a result of insufficient hydration for Pr to Pfr conversion since, as stated by TAYLORSON (1982) cellular metabolic systems also require hydration to manifest the phytochrome stimulus. It was suggested that factors such as a substrate, adequate energy or a certain organisation of cellular apparatus (especially functional membranes) are required for interaction with Pfr. Although germination was improved by longer imbibition periods,

the highest percentage germination, excluding the control treatments, was only 40 per cent, while in the controls up to 92 per cent germination was recorded. It is therefore probable that the irradiation supplied was suboptimal.

Upon investigation, the above assumption was confirmed since increased time of exposure to white light improved germination. An exposure period of six hours significantly increased the percentage germination over that obtained for one hour white light treatment. However, a 12 hour exposure did not provide for a further increase of germination over that of the six hour treatment and in addition, both these exposure periods were insufficient to illicit a response similar to that of the control in the diurnal light cycle.

Although prolonged or continuous illuminations may be inhibitory to some species (TOOLE, 1973) there are several accounts of an apparently prolonged light requirement (ELDABH, FREDERICQ, MATON & DE GREEF, 1974). It has also been found that this long exposure could be replaced with short, intermittent exposures (BORTHWICK, TOOLE & TOOLE, 1964; WULF & MEDINA, 1971; ELDABH, FREDERICQ, MATON & DE GREEF, 1974). In *C. odorata* achenes a single 12 hour light treatment resulted in significantly higher percentage germination than shorter, intermittent exposures equalling a total of 12 hours illumination. The achenes in the different treatments therefore received the same energy (irradiation period x intensity = energy; TOOLE, 1973). Since the phytochrome reaction is, within certain limits, dependent on energy rather than the duration of irradiation (TOOLE, 1973), the percentage germina-

tion should have been similar with all treatments, or possibly even greater with the intermittent exposure treatments. It may be that the energy of the irradiation supplied was too low and therefore adequate energy for the phytochrome reaction was only obtained from the single long illumination period. Examples are cited by BEWLEY & BLACK (1982) and TAYLORSON (1982) indicating that in some species, brief exposure to R light may be insufficient to stimulate germination since rapid thermal reversion of Pfr to Pr may occur. Therefore time of Pfr presence is inadequate for activation of the germination process. This aspect may also have played a role in reducing percentage germination of *C. odorata* achenes treated with white light for shorter periods.

The results obtained in this set of experiments can be summarised as follows:

(i) an alternating temperature was required for maximum promotion of germination;

(ii) of the temperatures tested, a 15/30 °C temperature regime was optimum for germination;

(iii) the achenes were positively photoblastic;

(iv) imbibition, for at least 12 hours at a temperature of 15/30 °C, prior to irradiation was necessary to sensitise achenes to light;

(v) percentage germination increased with increased exposure duration up to a maximum of six hours; neither six nor 12 hours white light was sufficient to stimulate germination to the same extent as a diurnal light

cycle; and

(vi) intermittent illuminations did not promote germination to the same extent as a single prolonged (12 hour) illumination period.

Based on these results, an incubation temperature of 15/30 °C and a diurnal light cycle of 12 hours dark : 12 hours light (the dark period corresponding to the lower temperature) was used for all subsequent germination tests. To gauge the effectiveness of the various treatments applied to the achenes, two control treatments were also included in each germination test. These were distilled water controls incubated (i) in the diurnal light cycle and (ii) in continuous darkness.

2.4 The Effect of Light Quality on Germination and Endogenous Sugar Levels

During the experiments aimed at defining the optimum conditions for germination, it was confirmed that *C. odorata* achenes required light for germination. Experiments were therefore initiated to further elucidate the light requirement and the effect of light quality on germination. Additionally, the effect of light quality on endogenous sugar levels was investigated to determine whether utilisation of the respiratory substrates was enhanced following R irradiation of imbibed achenes.

Materials and Methods

The batch of achenes used for the experiments conducted in the previous section (2.3) was again used in the first three experiments.

In the first experiment the effect of the duration of exposure to R or FR light was examined. Achenes imbibed in the dark for two days were exposed to either a flash, 10 minutes, one hour, four hours or eight hours of R or FR light. Following irradiation, incubation in the dark at 15/30 °C was continued.

Concurrently with the experiment described above, the effect of sequential treatments of R and FR light was examined. Initially, the following treatments were used:

(i) one hour R light,

(ii) one hour FR light,

(iii) one hour R light + one hour FR light, and

(iv) one hour R light + one hour FR light + one hour R light.

To confirm the R/FR light response, the duration of the exposures was shortened. The treatments were:

(i) one hour R light,

(ii) ten minutes R light,

(iii) five minutes FR light,

(iv) ten minutes R light + five minutes FR light,

(v) ten minutes R light + five minutes FR light + ten minutes R light, and

(vi) ten minutes R light + five minutes FR light + one hour R light.

Further experiments were conducted in an attempt to substitute the distinct light-requirement of the achenes. In these, the effect of puncturing the achene coat, removal of the pericarp and the effect of various periods of elevated temperature were examined.

The achenes used had been harvested from Virginia Bush on 5th September 1984 and stored dry in the dark at 25 ± 2 °C for at least five months. In the first experiment the pericarp of filled achenes was carefully punctured with a needle in the region where the pappus is attached. As shown in the inset in Plate 3 the embryo does not extend for the full

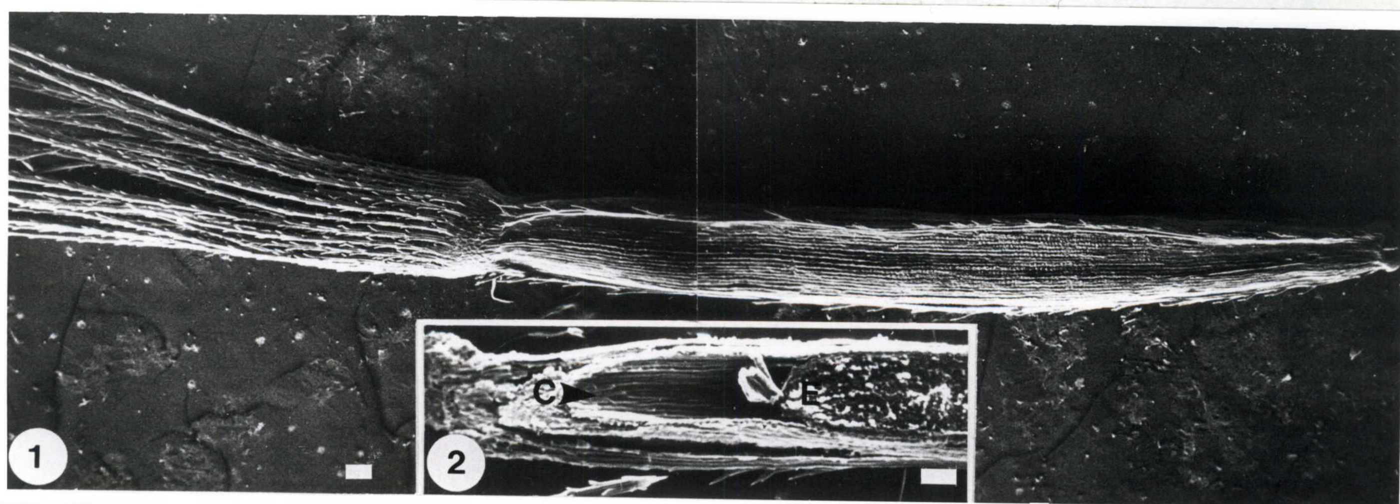


Plate 3 SEM of achene. (1) Whole achene. Bar = 100 µm
(2) L.S. to show cavity (C) in the region of the pappus which is not filled by the embryo (E). Bar = 100 µm.

length of the achene and hence no damage to the embryo was likely to occur from puncturing the pericarp in this region. The germination of these punctured achenes in both the dark and the light (diurnal light cycle) was compared to that of intact achenes. Twenty replicates of 10 achenes each were used for each treatment.

In the second experiment, the pericarp was carefully removed from each of the achenes. The germination of these embryos, enclosed only by the endosperm, was tested in the light and in the dark. Due to the difficulty experienced in removal of the pericarp only five replicates of 10 embryos each were used.

For the elevated temperature experiment, the achenes were imbibed for two days in the dark at 15/30 °C, and then placed, still in the dark, at either 35 °C or 40 °C for either 15 minutes, 30 minutes, one hour, two hours or four hours. After the elevated temperature treatment dark incubation at 15/30 °C was continued. Sufficient replicates were treated with the elevated temperatures to allow 10 replicates of 10 achenes of each of the treatments to be exposed to the diurnal light cycle following the elevated temperature treatments.

The effect of imbibition on achene sugar content after dark incubation and after exposure to R light was also investigated. Achenes were imbibed either in the dark or R light for nine hours, 48 hours, 96 hours or 120 hours prior to analysis of sugars. For comparison, unimbibed, filled achenes and achenes not containing an embryo were also analysed.

Analyses of the soluble sugar content of the achenes treated as described above were conducted using a modified procedure of that used by SWEELEY, BENTLEY, MAKITA & WELLS (1963) and TANOWITZ & SMITH (1984). For the determination of the endogenous sugar levels of unimbibed achenes, 0,3 g of dry achenes were extracted. Where imbibition was necessary, a mass of 0,3 g of dry achenes was again used, the sample being placed on moistened filter paper to imbibe for the required time period prior to extraction.

On completion of the imbibition period, the imbibed achenes were frozen in liquid nitrogen, ground with a mortar and pestle and then extracted for six hours in 12 cm^3 80 per cent redistilled ethanol in distilled water (v/v). This mixture was then filtered through Whatman's No.1 filter paper which had been washed with 80 per cent redistilled ethanol. The ground material and filter paper were washed with a further 18 cm^3 80 per cent redistilled ethanol. The filtrate was taken to dryness at 40 °C *in vacuo* and redissolved in three cubic centimetres 80 per cent redistilled ethanol and stored in sealed pill vials at 5 °C.

A 0,5 cm^3 aliquot of the redissolved filtrate for each of the extract samples was then treated as follows:

Add 0,5 cm^3 trehalose (five milligrammes analytical grade trehalose (Merck) dissolved in five cubic centimetres 80 per cent redistilled ethanol) for the internal standard

Reduce to dryness at 40 °C under nitrogen (N_2) gas

Convert sugars to oximes by addition of 0,5 cm^3 hydroxylamine hydrochloride (BDH Chemicals Ltd., Poole, England) (25 mg dissolved in one cubic centimetre pyridine)

Incubate for 20 minutes at 40 °C

Cool to room temperature

Remove 0,1 cm^3 aliquot into reacti-vial

Reduce to dryness at 40 °C under N_2 gas

Cool to room temperature

For trimethylsilyl (TMS) derivatisation, add 0,1 cm^3 Sigma Sil-A (containing trimethylchlorosilane, hexamethyldisilizane and pyridine in the ratio of 1:3:9; Sigma Chemical Company) and incubate for 15 minutes at room temperature

Resolve a 0,001 cm^3 aliquot on a Chromosorb 80/100 glass column (1,875 m long; 0,65 cm inside diameter) by temperature programming as follows:

(i) initial temperature, 125 °C for three minutes,

(ii) increase temperature by 2 °C per minute,

(iii) final temperature, 270 °C for five minutes.

A Varian 3700 Gas Chromatogram fitted with a flame ion detector (FID) was used for the sugar analyses. The FID was set at 300 °C and the injector temperature at 200 °C. The carrier gas was high purity nitrogen.

For identification of the sugars, a range of standards was prepared by dissolving five milligrammes of each in five cubic centimetres of 80 per cent redistilled ethanol. Initially these were made separately in order to determine the retention time (or temperature at which elution occurred) using the procedure already described. Once the retention time of each of the standards used had been identified, the standards were combined in a single sample to which the internal standard (trehalose) was added and resolved together with the achene extracts.

The standards used, as listed below, were all of analytical grade and supplied by either BDH Chemicals Ltd. or Merck: arabinose, fructose, galactose, glucose, inositol, maltose, ribose, sorbitol, sucrose, xylose and trehalose. Trehalose was used as the internal standard because the achene extracts were found not to contain this compound.

Results

Exposure to increasing duration of R light improved germination (Figure 2.4.1). The germination of achenes exposed to a flash of R light was similar ($p = 0,05$) to that of the dark treatment. However, a 10 minute R light treatment resulted in an appreciable increase in percentage germination. Longer exposure (one and four hours) further improved germination and, of the R light treatments, eight hours R light resulted in the best germination. The percentage germination of the eight hour R light-treated achenes, was however, significantly lower than that of achenes experiencing a diurnal light cycle.

Exposure to eight hours FR light promoted germination over that of the continuous dark treatment (Figure 2.4.2). However, exposure to one and four hours of FR light resulted in a percentage germination similar to that of the dark control. The ten minute and flash of FR light results are omitted from Figure 2.4.2 since the curve was similar to that of the dark and one and four hour FR light treatments.

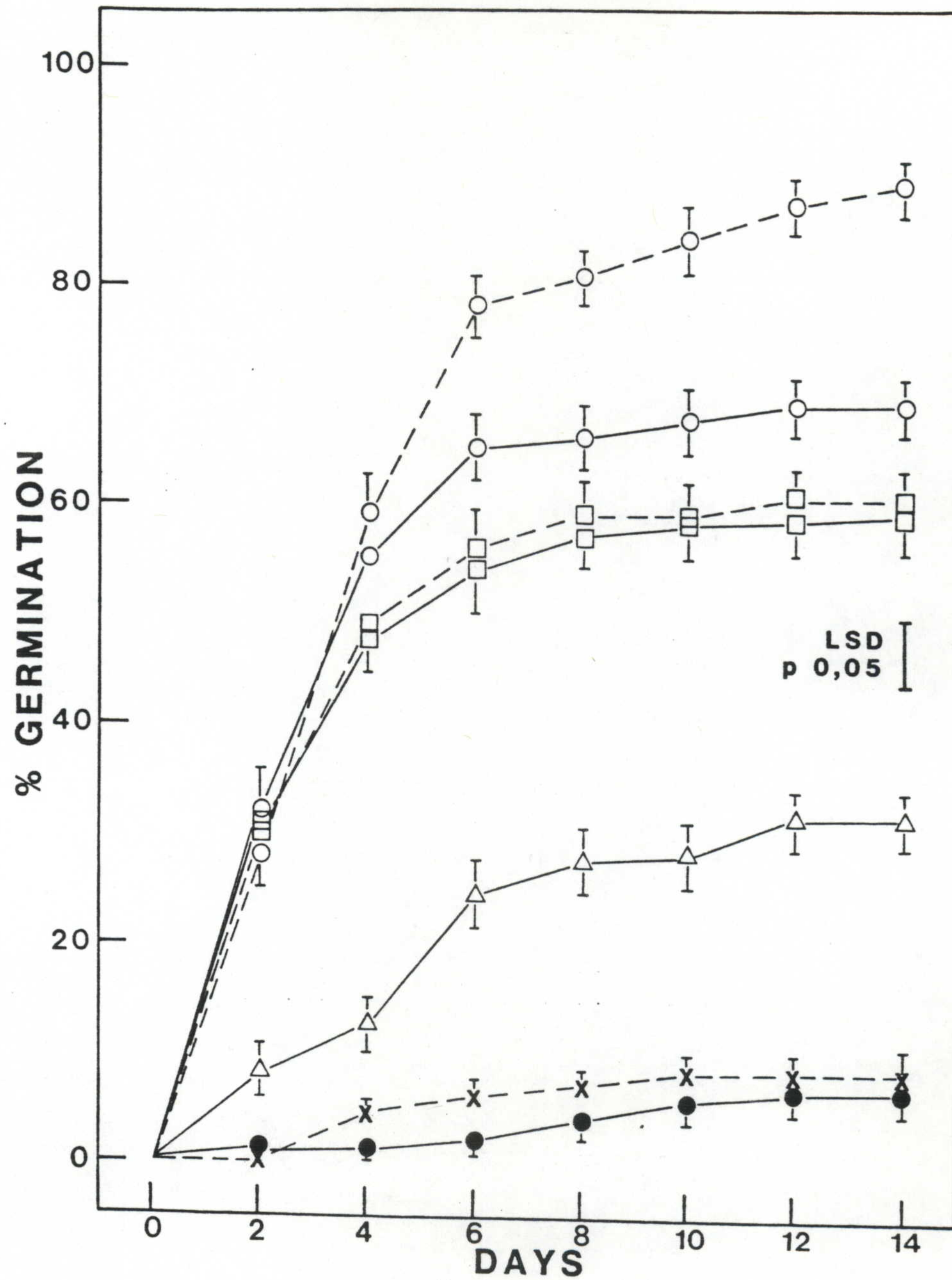


Figure 2.4.1 The effect of time of exposure to R light on the germination of achenes imbibed for two days prior to treatment. (--○--12h light:12h dark, diurnal; —○— 8h R; --□--4h R; —□— 1h R; —△— 10 min R; —●— flash R and --x-- dark).

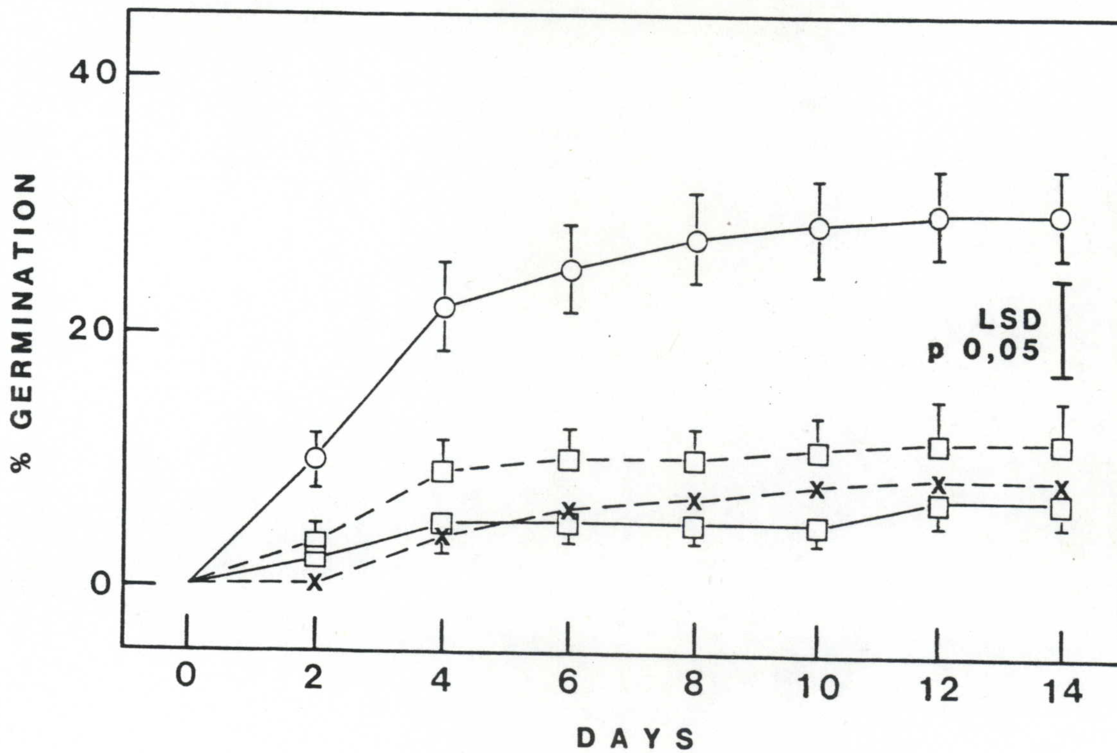


Figure 2.4.2 The effect of time of exposure to FR light on the germination of achenes imbibed for two days prior to treatment. (—○— 8h FR; - -□- - 4h FR; —□— 1h FR; - -×- - dark).

The results presented in Figure 2.4.3 show that the promotive effect of one hour R light on germination, was nullified if followed immediately with FR light. Where the FR light exposure was followed by a subsequent R light exposure, there was only a slight increase in percentage germination, this was similar to that of the dark control. However, if the duration of exposure to FR light was reduced from one hour, as used in the aforementioned experiment, to five minutes, the promotive effect of R light on germination was still nullified as shown in Figure 2.4.4. But, if this was then followed by a subsequent R light exposure of either one hour or even ten minutes, the percentage germination was improved and was similar ($p = 0,05$) to that of achenes exposed to one hour R light (Figure 2.4.4).

Although not significant, puncturing of the achene pericarp resulted in a higher percentage germination of achenes incubated in the dark (Figure 2.4.5). In the diurnal light cycle the germination rate was almost identical for intact and punctured achenes. However, the final percentage germination of the punctured achenes was significantly higher than that of intact achenes (Figure 2.4.5). Removal of the pericarp (excised embryos) resulted in depressed germination in the light, but in the dark, germination of the excised embryos was significantly greater than that of intact achenes (Figure 2.4.6). None of the intact achenes germinated in the dark.

The effect of temperature shift on germination in the diurnal light cycle is shown in Figure 2.4.7. A temperature shift to 35 °C following both a two and four day imbibition

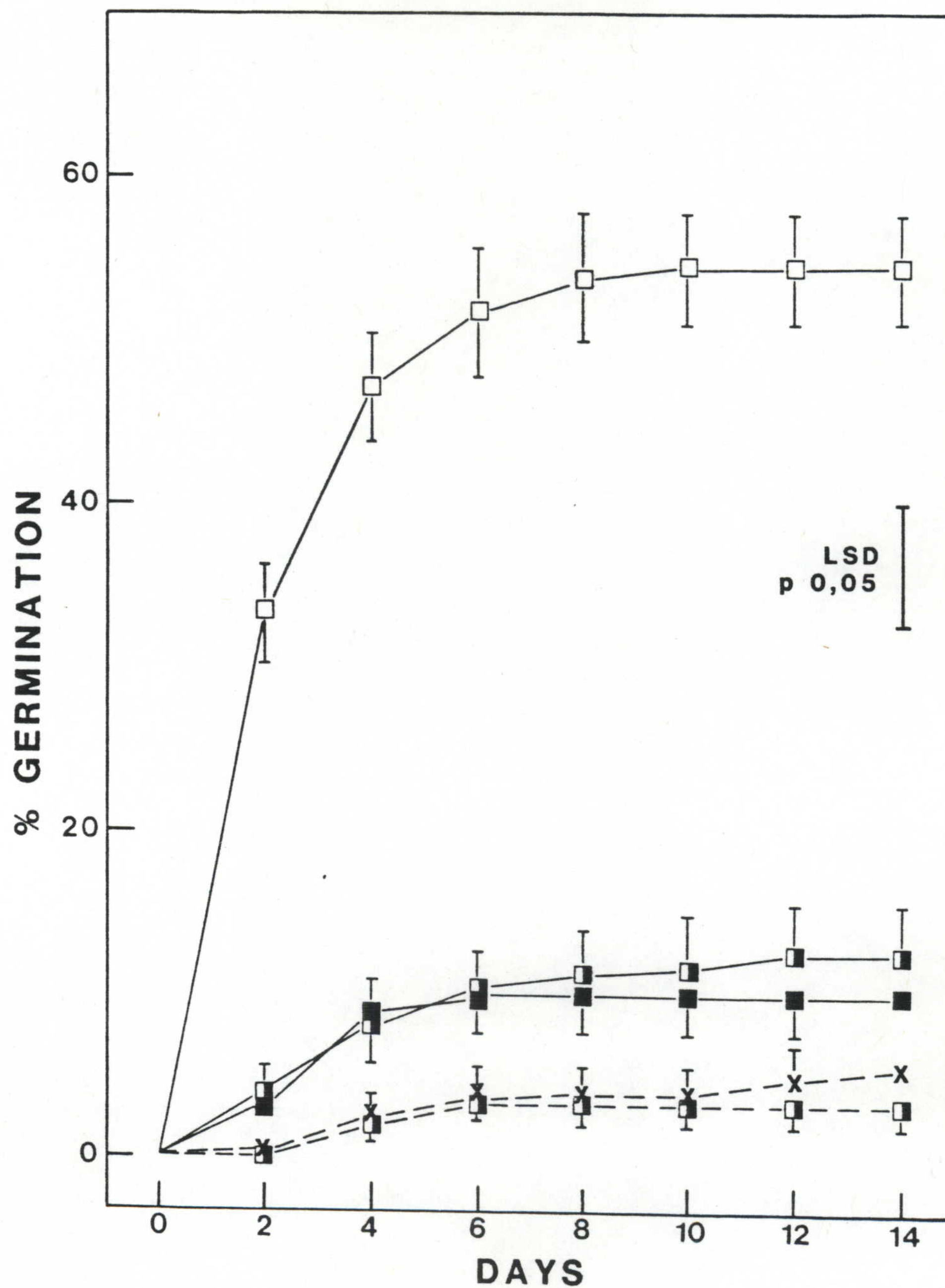


Figure 2.4.3 The effect of alternating R and Fr light on the germination of achenes imbibed for two days prior to treatment. (—□— 1h R; —■— 1h FR; —◧— 1hR + 1h FR + 1h R; - -◧- - 1h R + 1h FR; - -x- - dark).

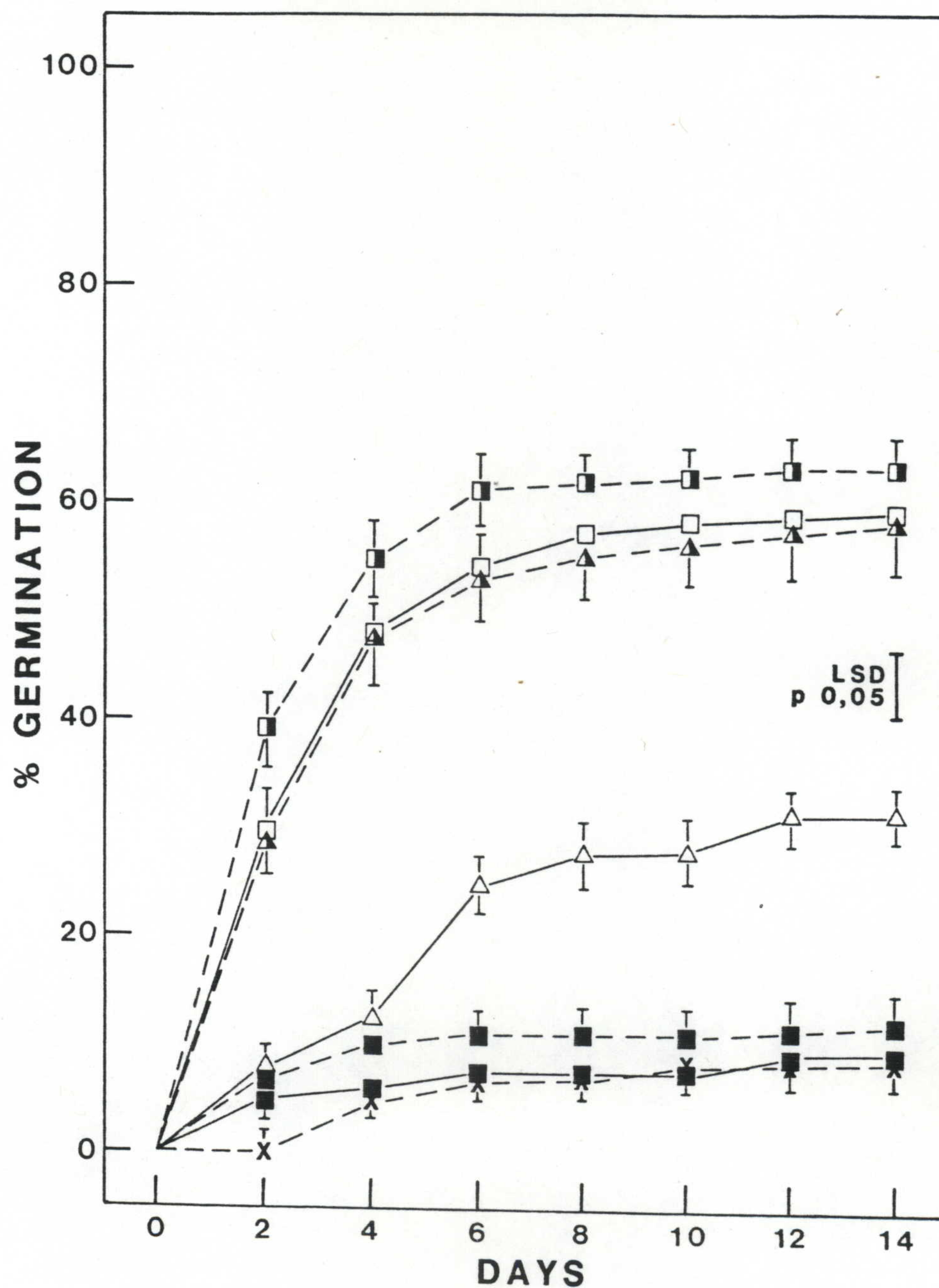


Figure 2.4.4 The effect of shortened exposure time to alternating R and FR light on the germination of achenes imbibed for two days prior to treatment. (—□— 1h R; —△— 10 min R; --◧-- 10 min R + 5 min FR + 1h R; --▲-- 10 min R + 5 min FR + 10 min R; --■--10 min R + 5 min FR; —■— 5 min FR; --X--dark).

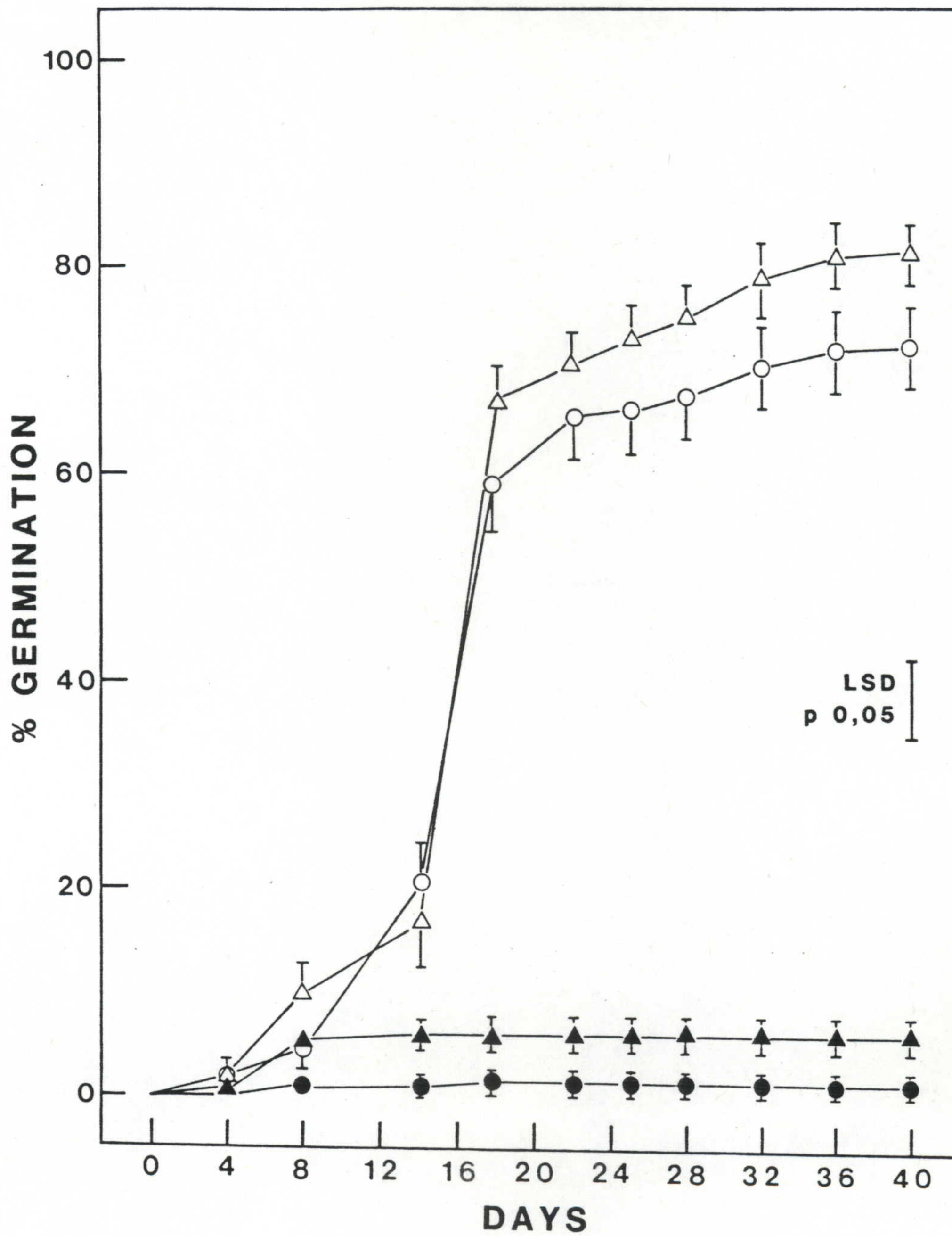


Figure 2.4.5 The effect of puncturing the achene pericarp on germination. (○ light control; ● dark control; △ light punctured; ▲ dark, punctured).

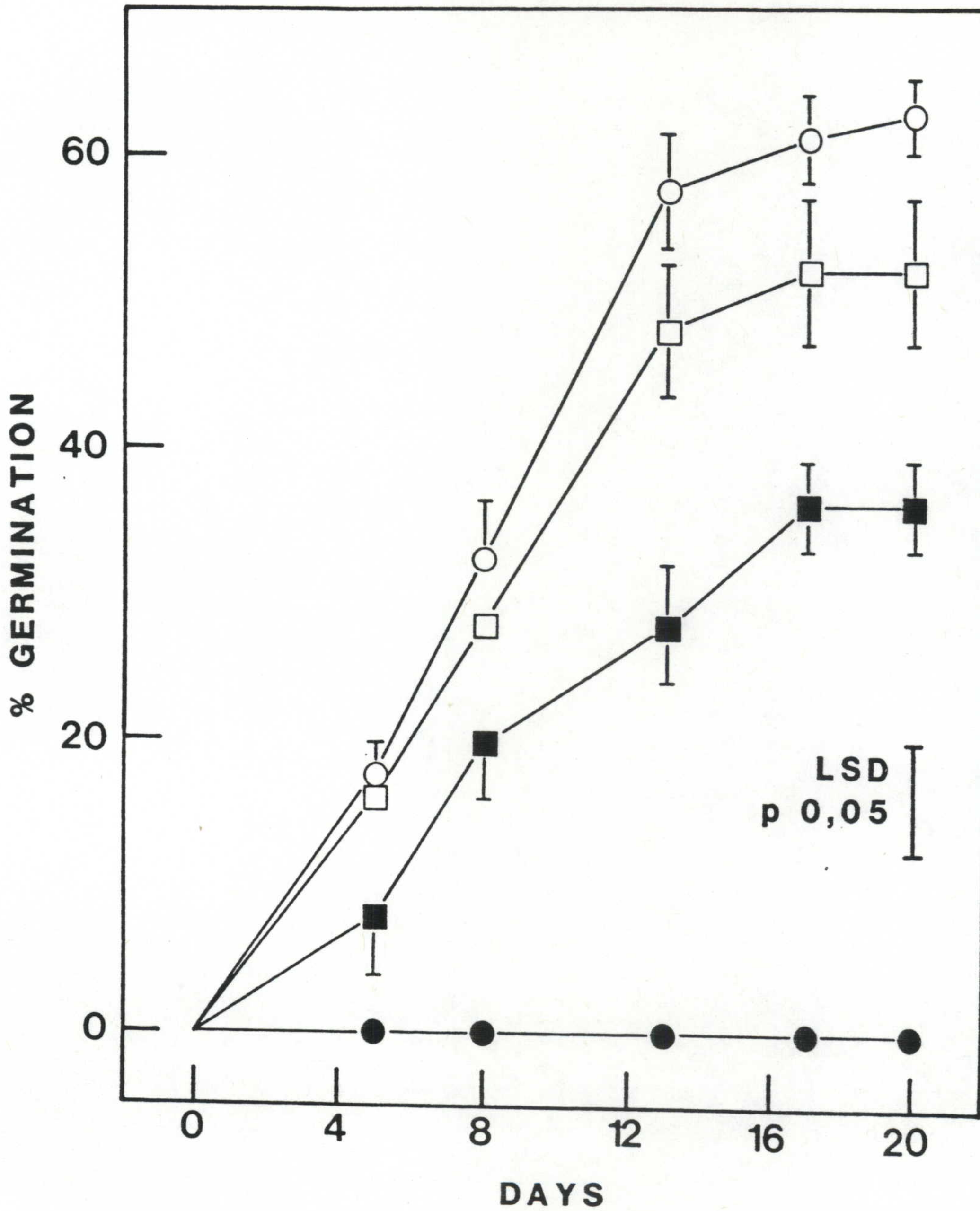


Figure 2.4.6 The germination response in the light and in the dark to the removal of the pericarp. (○ light control; ● dark control; □ excised, light; ■ excised, dark).

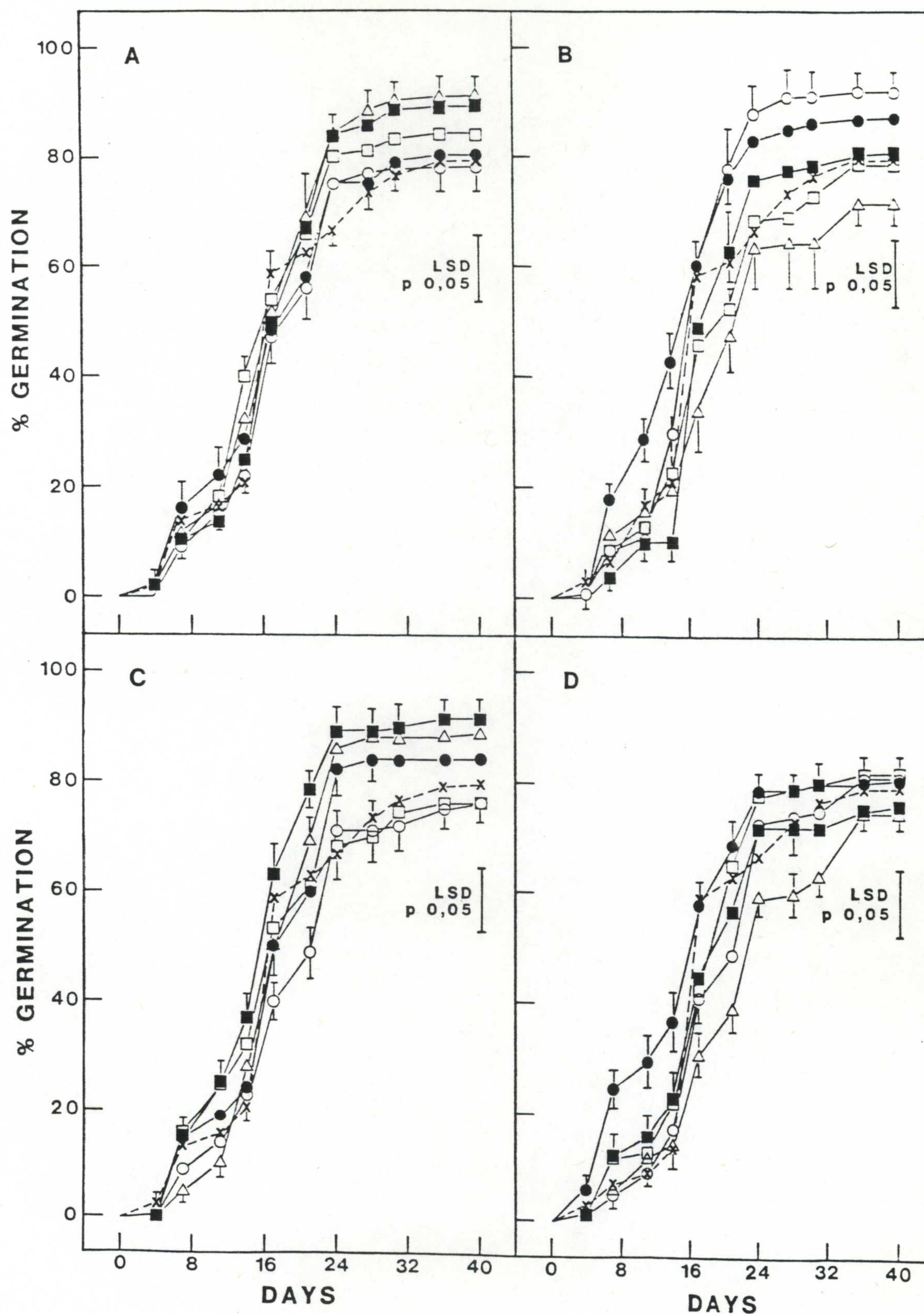


Figure 2.4.7 The germination response in the light to various periods of elevated temperature following dark imbibition for two or four days at 15/30 °C. (A) Two days dark imbibition followed by a shift to 35 °C. (B) Two days dark imbibition followed by a shift to 40 °C. (C) Four days dark imbibition followed by a shift to 35 °C. (D) Four days dark imbibition followed by a shift to 40 °C. (○ 15 min; ● 30 min; □ 1h; ■ 2h and △ 4h elevated temperature;--x-- control).

period slightly increased the percentage germination (Figure 2.4.7A; Figure 2.4.7C). A similar result was obtained for the temperature shift to 40 °C following a four-day imbibition period (Figure 2.4.7B) while a shift to 40 °C following a four-day imbibition period slightly depressed germination (Figure 2.4.7D).

For achenes retained in the dark, the percentage germination for all the treatments was low (< 10 per cent). As no significant differences were obtained, these results are not presented.

Gas chromatographic separation of the sugars used as standards is illustrated in Figure 2.4.8. By resolving achene extracts and the standards sequentially, tentative identification of the peaks obtained for the achene extracts could be made on the temperature (retention time) at which the peaks occurred. A quantitative measure of the sugars was made by comparing the respective peak heights with that of the internal standard, trehalose.

Before reporting on the sugars tentatively identified, and the respective concentrations, it should be pointed out that in the majority of achene samples an unidentified peak was detected at a temperature of approximately 128 °C. None of the sugars available for standards were detected at or near this temperature. However, since this peak was apparently consistent throughout the samples, it is assumed that this compound formed part of the achene coat and was therefore disregarded for the purpose of this study.

The endogenous sugar levels, as detected by gas

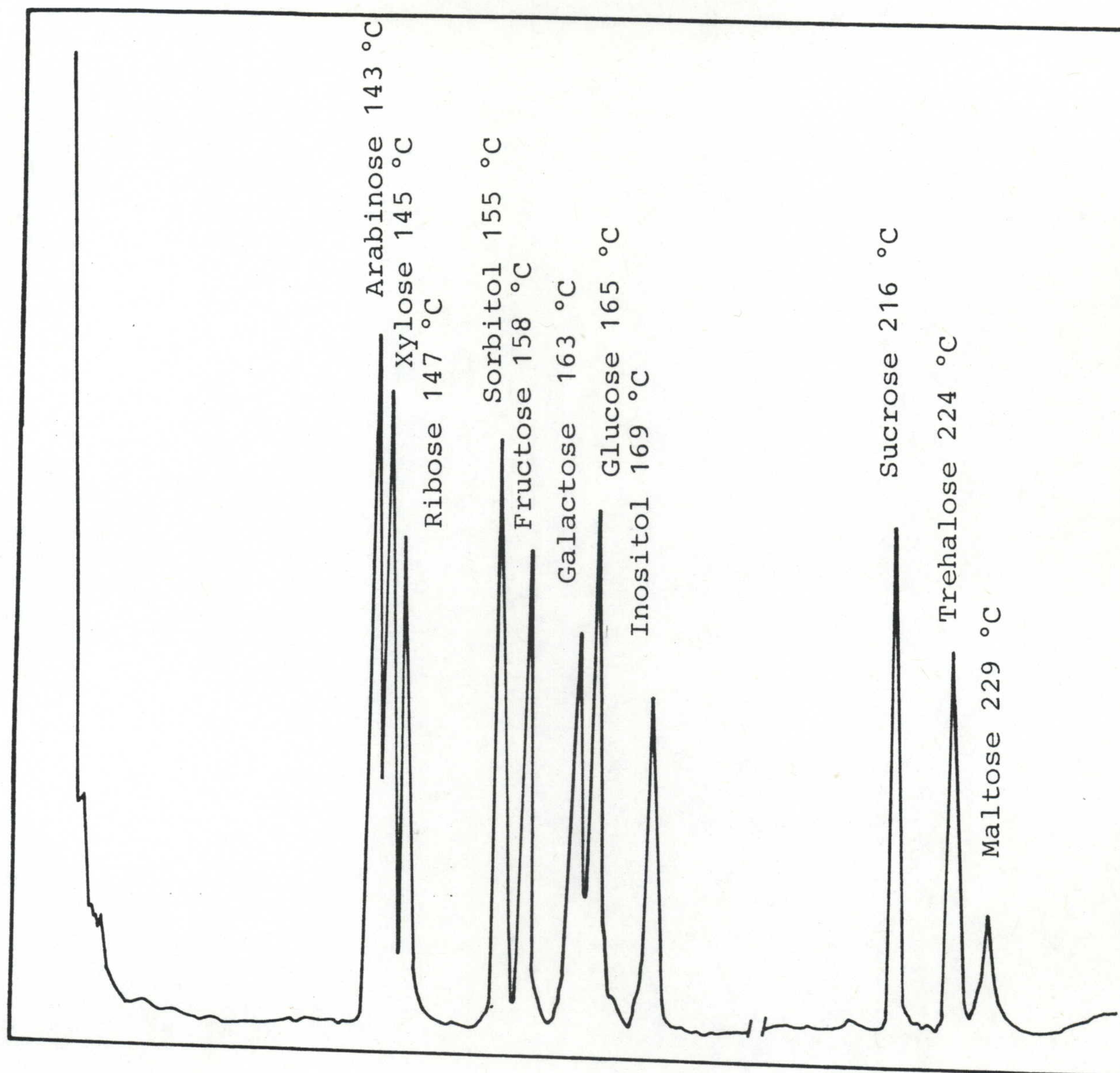


Figure 2.4.8 An example of the separation by gas chromatography, of a number of authentic sugars. The temperature at which each sugar was detected is indicated.

chromatography, in unimbibed empty and filled achenes is presented in Table 2.4.1.

Five sugars were tentatively identified in the empty achenes and six in the filled achenes. Ribose and glucose were detected in the filled achenes but not in the empty achenes while galactose and inositol were detected in the empty achenes but not in the filled achenes. All the sugar levels in the empty achenes were low (Table 2.4.1). In the filled achenes comparatively high levels of fructose, glucose and sucrose were detected.

Where samples of achenes imbibed in the dark or in R light were extracted at various times after commencement of imbibition, certain noticeable changes in the levels of certain sugars were observed (Figure 2.4.9). Nine hours after the start of imbibition there were no distinctive differences in the levels of the sugars (Figure 2.4.9A). After 48 hours of imbibition, the levels were again similar with the exception of sucrose (Figure 2.4.9B). The level of sucrose in the achenes imbibed in R light had increased appreciably as compared with that of the achenes imbibed in the dark. The level of sucrose detected in the extract from achenes imbibed in R light for 96 hours was even higher than in the previous extraction time (Figure 2.4.9C). At this time the fructose and glucose content has also increased in the achenes imbibed in R light. A slight increase in fructose, glucose and sucrose was also recorded in the achenes imbibed in the dark. At the final sampling time (120 hours imbibition), the sugar levels in both the dark- and light-imbibed achenes were similar to those

Table 2.4.1 Concentrations of sugars tentatively identified in empty and filled achenes.

Sugars	Concentration (mg/g dry material)	
	Empty	Filled
Unknown	2,8	3,2
Arabinose	3,2	4,7
Ribose	-	4,8
Sorbitol	-	0,8
Fructose	0,7	10,0
Galactose	1,6	-
Glucose	-	8,7
Inositol	3,2	-
Sucrose	0,9	10,6

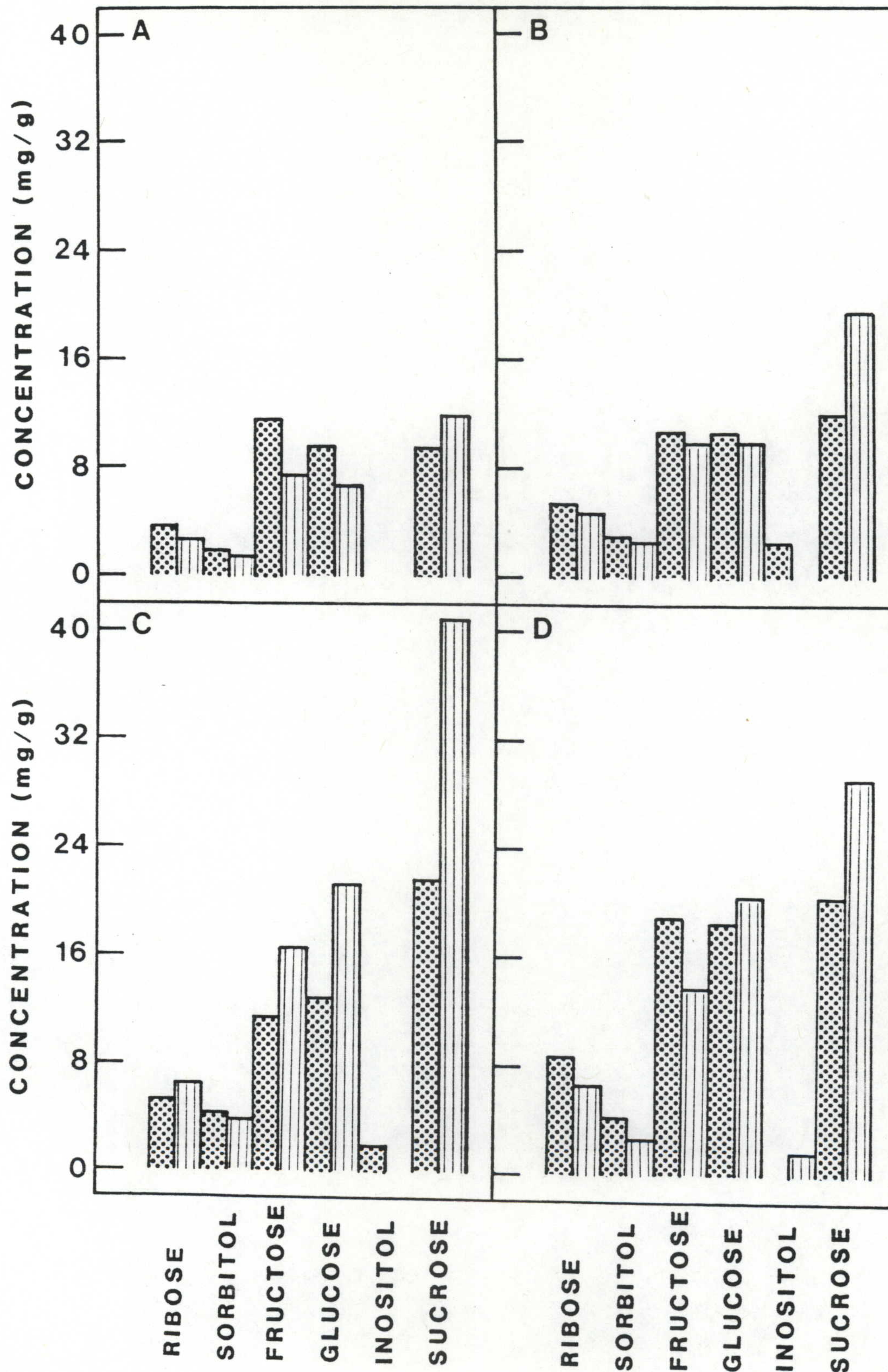


Figure 2.4.9 The effect of incubation of achenes in the dark ( ) or under R light conditions ( ) on the levels of tentatively identified endogenous sugars. (A) 9h, (B) 48h, (C) 96h and (D) 120h incubation.

of the earlier sampling time, with the exception of the sucrose which had decreased in the achenes imbibed in R light (Figure 2.4.9D).

For clarity, the major changes in the levels of the sugars with time, as described above, are illustrated in Figure 2.4.10. In the achenes retained in the dark, the levels of fructose were highest in the samples imbibed for 120 hours (Figure 2.4.10A). In the R light-treated achenes, the level of fructose was highest after 96 hours imbibition, and after 120 hours, the level had decreased. The glucose levels increased in dark and R light-treated achenes at 96 hours imbibition; after 120 hours the level in both samples had increased further (Figure 2.4.10B). The most noticeable change was that recorded for sucrose. In the achenes retained in the dark, the level of sucrose rose at the 96 hour sample time and remained constant (120 hour sample time) (Figure 2.4.10C). The sucrose levels in achenes treated with R light increased at the 48 hour sample time and increased markedly at the 96 hour sampling time whereafter a decrease was recorded.

Discussion

Photo-control of germination is usually attributed to a phytochrome-mediated system. Since the phytochrome reaction is controlled by light quality, the involvement of phytochrome can be confirmed by the germination response to irradiation with different light spectra. In *C. odorata* achenes, it was found that there was a positive germination response to R light

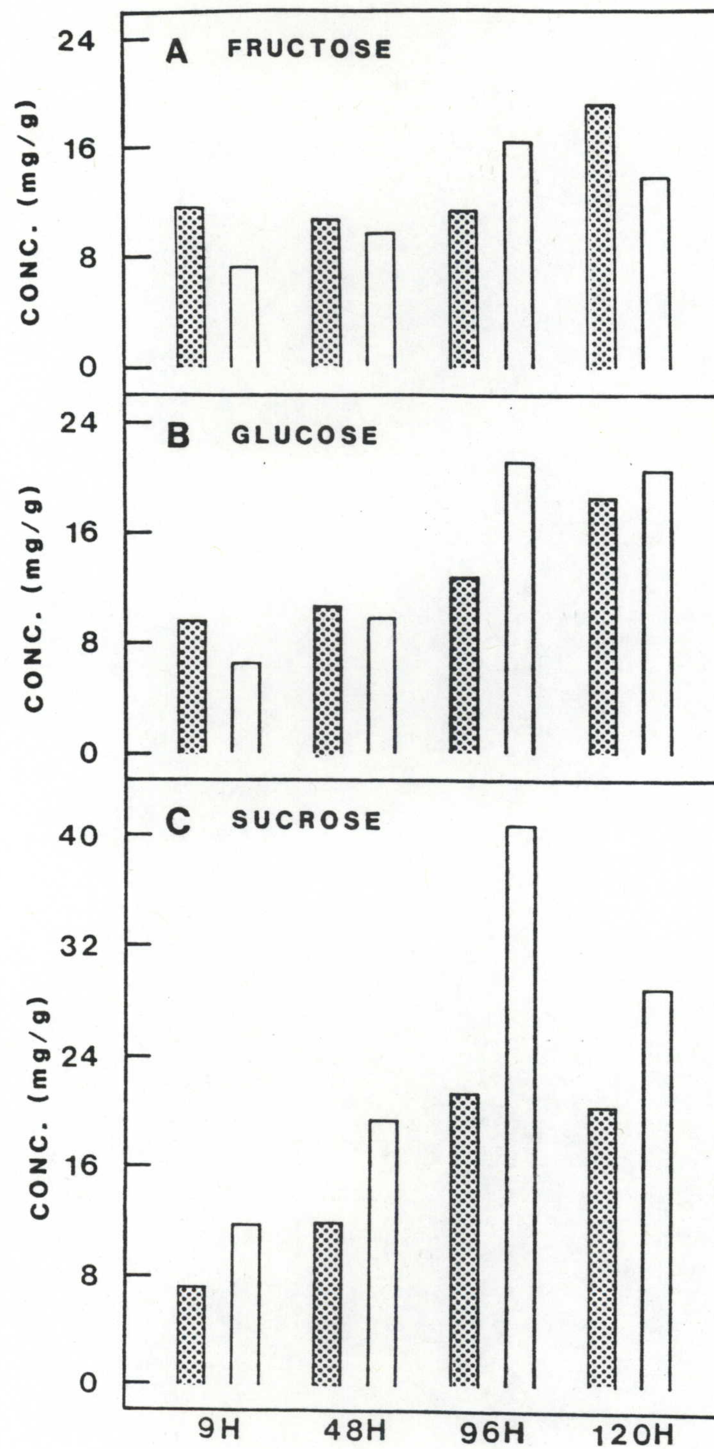


Figure 2.4.10 The changes in the levels of (A) fructose, (B) glucose and (C) sucrose within achenes incubated in the dark (▩) or under R light conditions (□).

exposure time. Illumination of imbibed achenes for 10 minutes resulted in a significantly higher percentage germination than either a flash of R light or continuous dark incubation. Both the one and four hour R light treatments significantly improved germination over that of the 10 minute R light treatment. Although not significant, eight hours of R light resulted in a higher percentage germination than all the other treatments. These results clearly implicated phytochrome involvement in the achene's germination response. However, it was necessary to demonstrate the R/FR reversal of the germination response in order to confirm phytochrome as the photo-receptor (TOOLE, 1973; MOHR & SHROPSHIRE, 1983).

In the first experiment in which the R/FR reversal of germination was investigated, the promotive effect of R light was reversed by an immediate FR light treatment. However, the inhibitive effect of FR light was not reversed (Figure 2.4.3). It is possible that this arose as a result of the high-energy or high irradiance reaction (HIR) which is reported to over-ride the reversible, low-fluence phytochrome effects (BORTH-WICK, HENDRICKS, SCHNEIDER, TAYLORSON & TOOLE, 1969; MOHR, 1969). In seeds the FR HIR is thought to act on events which occur very late in the germination process, that is, the HIR stops cell elongation in the radicle (KARSSEN, 1970b). Although germination of seeds in which germination has been inhibited by the HIR (as a result of prolonged FR light treatment) can generally be promoted by R light treatment (BEWLEY & BLACK, 1982); HARTMANN (1967) reported that as a result of prolonged FR light, the sensitivity to subsequent R light may be reduced by 50 per cent. It has also been found that in *Nemophila isignis* L.

seeds, which become photo-dormant during the HIR, germination cannot be promoted by establishing a Φ value (Pfr/P total) usually favourable for germination (ROLLIN, MALCOSTE & EUDE, 1970). A similar condition apparently developed in *C. odorata* achenes where germination was not stimulated by R irradiation following the one hour FR irradiation treatment.

Since the HIR is dependent on light energy (ROLLIN, 1972), it was decided to repeat the R/FR light germination response by reducing the duration of FR light exposure. The results obtained showed that the germination response was dependent on the quality of the final irradiance treatment. It would therefore seem that the results obtained for the previous experiment were a reflection of the difference in the quanta of the R and FR irradiance. (This is supported by the higher intensity of the FR light ($1,6\ W\,m^{-2}$) than that of the R light ($0,9\ W\,m^{-2}$)).

The results obtained in this second experiment showed a typical low-fluence phytochrome-controlled germination response in *C. odorata* achenes. It is therefore probable that the imbibition requirement identified in section 2.2.3, is neccesary for, amongst other reasons, the provision of hydrated tissue essential for the conversion of Pr through the intermediates to Pfr as detailed by KENDRICK & SPRUIT (1977). In addition, the incomplete stimulation of germination by short exposure to R light may indicate that the irradiation was insufficient to promote adequate conversion of Pr to Pfr in order to increase the photo-equilibrium (Φ) to the level required to stimulate germination of all achenes. This may

result from the likelihood that the state of the phytochrome in the individual *C. odorata* achenes is different and therefore different quanta of irradiation would be required by the individual achenes to ensure a ϕ value favourable for germination.

Attempts to substitute the R light requirement either by puncturing the achene coat or with elevated temperatures failed. BEWLEY & BLACK (1982) reported that in the majority of light-requiring seeds, dormancy is coat-imposed. Therefore isolated embryos of most light-requiring seeds germinate in the dark. In *C. odorata* puncturing the pericarp either did not substitute for complete removal of the coat or, the achenes possessed dormant or immature embryos. The latter is the less likely because a high percentage germination was obtained with a diurnal light cycle. MAYER & POLJAKOFF-MAYBER (1982) reported that merely puncturing the endosperm of lettuce seeds is sufficient to abolish the light requirement. It is therefore possible that by puncturing the *C. odorata* achene coat, the endosperm was not punctured.

In the experiment where the pericarp was removed from the achenes, germination in the dark was considerably improved over that of intact achenes. This result apparently confirms the view of BEWLEY & BLACK (1982) that photo-controlled dormancy is coat-imposed. However, removal of the pericarp did not promote germination to a similar level as that of the intact and excised embryos experiencing the diurnal light cycle, thus light was still required, albeit to a lesser extent. It is possible that removal of the endosperm enclosing the

excised embryos would further improve germination in which case both the pericarp and endosperm play a role in imposing the light-requirement. KARSSEN (1970b) found that in *C. album*, removal of the seed coat also resulted in improved germination in the dark.

Besides forming a light filter, it is not clear what the role of the seed coat is in maintaining the light requirement. As described in section 2.1, the photo-receptive site resides in the embryo. Why then is light (Pfr action) no longer required upon removal of the embryo-covering structures? Does the removal of the seed coat potentiate germination of light-requiring seeds in the dark by way of lowering the threshold value of Φ or, as proposed by KARSSEN (1970b), does the seed coat in some way interfere with the synthesis of the reaction component of Pfr? Clearly this aspect requires further investigation to elucidate the mechanism(s) involved.

Temperature shift treatment (that is, a restricted period of temperature change for fewer than eight hours (TAYLORSON & HENDRICKS, 1972b)) has been found to promote germination of light-requiring seeds in the dark (TAYLORSON & HENDRICKS, 1972b; 1972c; TAKAKI, KENDRICK & DIETRICH, 1981; HAND, CRAIG, TAKAKI & KENDRICK, 1982; TAKAKI & ZAIA, 1984). It has generally been concluded, in these studies, that germination in the dark was induced by an interaction of pre-existent Pfr with the temperature shift. As a result the sensitivity of the seeds to a low level of pre-existent Pfr may be increased or Pfr production may be induced by the elevated temperature. In the former possibility it is thought that the threshold of

Pfr needed to promote germination could be decreased by the elevated temperature (TAKAKI & ZAIA, 1984). The failure of the various elevated temperature shifts to induce germination of *C. odorata* achenes in the dark may be due to various factors. There may be an extremely low level of Pfr in the achenes which is insufficient to stimulate germination in the dark regardless of the elevated temperature or, the elevated temperatures used were too high/low or too short/long. In these experiments, an attempt was made to simulate possible field conditions occurring in the areas infested by *C. odorata*. Although further investigations are necessary for a categorical confirmation, the results obtained in this study suggest that dark germination in the field situation is unlikely to occur as a result of an elevated temperature. Further confirmation was obtained from achene burial studies in which minimal germination occurred during the burial period (section 2.5).

Phytochrome has been found in every major taxonomic group of plants (BORTHWICK, 1972b). Besides its role in seeds phytochrome mediates, through light, the induction of and release from dormancy of various structures such as buds, bulbs and spores. In the most evolutionarily advanced taxa, phytochrome plays the decisive role in mediating photomorphogenesis (MOHR & SHROPSHIRE, 1983). This pigment is therefore of great importance in the plant kingdom and has resulted in a considerable volume of published literature on the topic. Although it is realised that pitfalls exist in extrapolating results obtained in laboratory experiments to the natural environment, it is necessary to discuss the importance of phytochrome and

its possible role in *C. odorata* achenes since, according to TAYLORSON (1982), the subject of phytochrome is too often approached only as a laboratory phenomenon.

Sunlight is comprised of a broad spectrum of light including light in the R and FR wavelengths. Interception of sunlight by seeds therefore results in the establishment of a Φ value of phytochrome. Full sunlight is generally accepted as resulting in a Φ value favourable for the germination of positively photoblastic seeds. Therefore, in *C. odorata* achenes, sunlight was expected to stimulate germination. For fear of over-extrapolating incubator-obtained results to the natural situation, *C. odorata* achenes were imbibed in an incubator at 15/30 °C for two days in the dark and then exposed to sunlight for a period of approximately 30 minutes every second or third day to confirm the above assumption. It was found that 88 per cent of the achenes germinated. Therefore, it seems reasonable to assume that *C. odorata* achenes are likely to germinate in situations where there is sufficient moisture for imbibition, a suitable alternating temperature and unfiltered sunlight. Field observations have, in fact, shown that dense carpets of *C. odorata* seedlings emerge in disturbed bare earth in open areas during spring and summer.

A further extrapolation of data from laboratory results to the field situation suggests that *C. odorata* achene germination is likely to be minimal in conditions where the incident light is predominantly in the FR wavelength. It is well known that the R light in sunlight is removed by chlorophyll in the leaves of canopy vegetation (COOMBE, 1957; HOLMES & SMITH,

1977) and consequently natural leaf canopies result in a much higher proportion of the incident light energy being transmitted in the FR wavelength than the shorter wavelengths (JORDAN, 1969). This was clearly demonstrated by LEE (1985) who measured the spectral distribution of radiation under a closed forest canopy as illustrated in Figure 2.4.11.

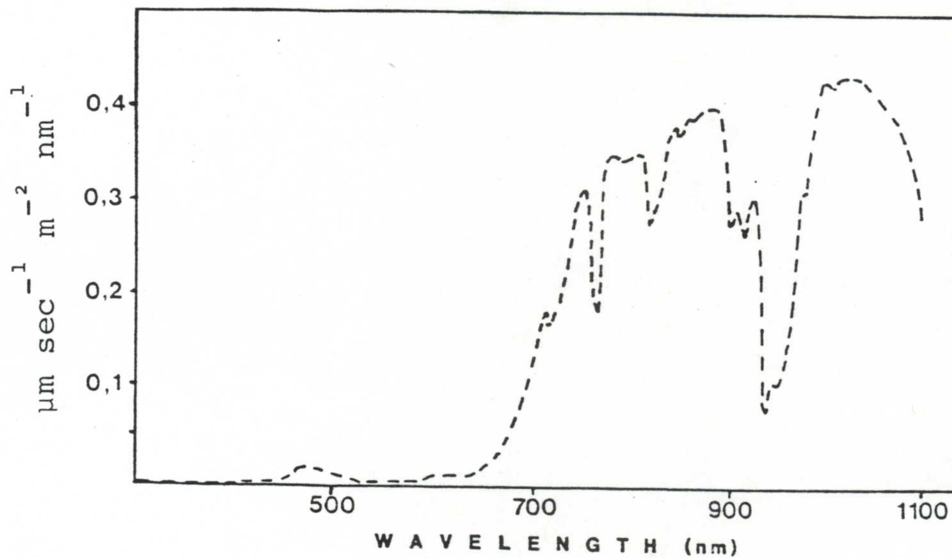


Figure 2.4.11 Spectral distribution of radiation under a closed forest canopy (LEE, 1985).

Several studies have been conducted to investigate the effect of light filtered through leaves on the germination of positively photoblastic seeds. TAYLORSON & BORTHWICK (1969) found that appreciably more R than FR energy was absorbed by leaves. This leaf-filtered light was generally found to inhibit the germination of seeds potentiated by pre-irradiation with R light and it was suggested that light filtered through a leaf canopy could depress the germination of positively photoblastic weed seeds in the natural situation (TAYLORSON & BORTHWICK, 1969). KING (1975) obtained similar results with seeds of three species. It was proposed that there was an absolute selection pressure in shaded habitats for a mechanism

which would impose dormancy on seeds on the soil surface until the vegetation cover was removed. Subsequent germination would thus improve the likelihood of vigorous seedling growth and eventual seed production. Similar studies have yielded comparable results (GORSKI, 1975; FENNER, 1980a; 1980b). Although this particular aspect has not been investigated with *C. odorata* achenes, the laboratory results obtained, together with field observations, strongly implicate an analogous mechanism in these propagules.

As already mentioned, the HIR might also be operative in *C. odorata* achenes. The low Pfr/Ptotal value resulting from the HIR in seeds at or near the soil surface in shaded areas would influence the dormancy of seeds (TAYLORSON, 1982). Therefore in *C. odorata* achenes it is probable that the HIR will, together with the low-fluence phytochrome reaction, contribute to restricted germination in shaded habitats. Prolonged exposure to FR-enriched radiation might also induce secondary dormancy in the achenes since in the laboratory experiments, R irradiation treatments failed to stimulate germination following the longer periods of FR irradiation.

The precise mode of action of Pfr in stimulation of germination is not known. Possible modes of action include activation of enzymes, controlled gene action and changes in membrane properties (BORTHWICK, 1972b; TAYLORSON, 1982). The possibility that the primary Pfr action is on membranes is well supported by available evidence (TAYLORSON, 1982) and, in seeds, circumstantial evidence has been provided to this end (TAYLORSON, 1982; 1984). The events subsequent to Pfr action on

membranes have, however, not been satisfactorily explained. One possibility investigated in this study, is the effect of R light on the endogenous sugar levels since it is possible that one of the consequences of increased membrane permeability might involve the utilisation of respiratory substrates. Obviously, energy is required for the germination process and therefore Pfr action might directly influence substrate utilisation.

The results obtained showed that during early incubation (up to 48 hours), the levels of the free sugars remained fairly constant and comparatively low, although a small but noticeable rise in the level of sucrose was evident in the R light-treated achenes at the 48 hour extraction time. At a time when germination was first recorded (96 hours) there was a marked increase in the sucrose level and a smaller rise in the glucose and fructose levels in the R light-treated achenes. These results are difficult to interpret since it is not clear whether the changes observed were due to dormancy breakage or to the germination process *per se*.

Sucrose is an important sugar in plants since it is the major form in which carbohydrates are transported (BEWLEY & BLACK, 1978). The increase noted at the 48 hour extraction time in *C. odorata* achenes might reflect the increased metabolic activities associated with stimulation of germination by R light and therefore Pfr action. The comparatively high level detected after 96 hours however, was probably associated with later visible stages of the germination process including cell division and cell enlargement.

Glucose and fructose seldom accumulate in seed tissues as they are relatively unstable (BEWLEY & BLACK, 1978). Since the levels of these were only found to increase during the visible stage of germination it is probable that this accumulation had little to do with the initial triggering of the germination process. METIVIER & PAULILO (1980) found a similar accumulation during the later stages of *Phaseolus vulgaris* L. seed germination.

There are obvious limitations to the study conducted on *C. odorata*. Firstly, intact achenes were extracted for the analyses. To provide clarification of the Pfr action on the levels of endogenous sugars, it would be necessary to analyse achene fractions comprised of cotyledonary tissue separate from the embryonic axis. However, this is impractical for *C. odorata* achenes because of their small size, thus a species with larger seeds is required. Secondly, the extraction times were too widely spaced and therefore it is of dubious value to draw conclusions concerning Pfr action since other transient changes in the sugar levels might have occurred which would not have been detected. Finally, to implicate the association between the primary action of Pfr and substrate utilisation, it is necessary to examine the changes in relation to R/FR reversibility of phytochrome.

2.5 Biological Aspects of *C. odorata* Achene Germination

In the previous experiments (sections 2.3 and 2.4) it was found that a high percentage germination (> 70 per cent) could be obtained under certain incubation conditions, namely an alternating temperature of 15/30 °C and a diurnal light/dark cycle. These conditions were used to investigate various aspects pertaining to the biology of *C. odorata* achenes, thus providing a greater understanding of factors involved in the reproduction of this weed.

Materials and Methods

In weeds, the time of seed production is sometimes crucial for the survival of the species and its role as a weed. Production of germinable seed should theoretically coincide with environmental conditions favourable for germination and seedling establishment, particularly of seeds in which longevity is likely to be restricted, that is, in soft-coated seeds. Hence the time of achene production in *C. odorata* was investigated. In previous seasons, observations had shown that although flowering in this species occurs from May to June, fully formed achenes are only produced from August to November. Thus the germination of achenes harvested throughout the latter period in 1984 was investigated. The harvest dates were: 21st August; 5th September; 21st September; 5th October; and 25th October. After the last harvest date, no further achenes were found on the plants. At each of

these dates, achenes were collected from the same infestation of plants in Virginia Bush, Durban, Natal. On return to the laboratory, the achenes were separated into age groups based on the maturity of the capitula and the axes bearing the capitula. The age groups for each harvest date are described below and are illustrated in Plate 4 and Figure 2.5.1 for clarity.

On the 21st August, some capitula bore green involucral bracts; the axes bearing these capitula were also green (Figure 2.5.1(a)). The styles, stigma and corollas of the individual achenes contained in these capitula had senesced. The achenes which were removed from these capitula were kept separate and are hereafter referred to as "green" achenes. This formed the most "immature" achene group. The second group, "medium" achenes, were removed from capitula in which the involucral bracts were brown. Only a small part of the capitula-bearing axes had turned brown (Figure 2.5.1(b)). The third group of achenes, termed "dry", were the most mature achenes at this harvest time. These were removed from capitula in which the bracts were brown and the capitula-bearing axes had senesced past the first axis joint as indicated by browning of the axes in Figure 2.5.1(c)).

On the 5th September no capitula containing green achenes were present due to progressed ripening. Both medium and dry achenes, as described above, were obtained. An additional group was however, present. These achenes, referred to as "ultra-dry" were removed from capitula from which some involucral bracts had dropped (Figure 2.5.1(d)).

Plate 4 The four age groups of achenes tested for germination during the harvest date experiment. ((a) green, (b) medium, (c) dry and (d) ultra-dry.) X 1,5.

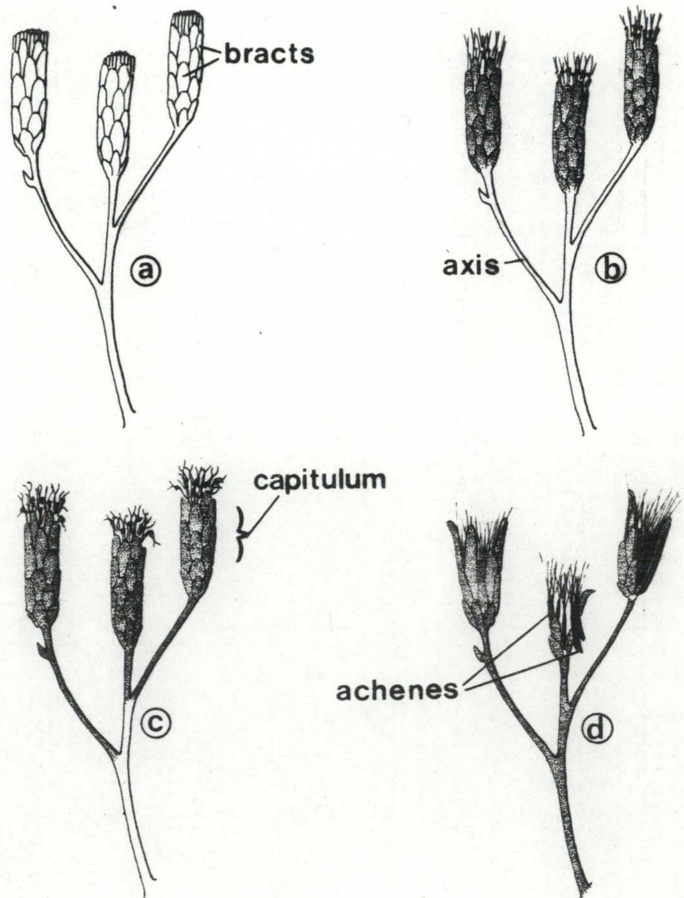


Figure 2.5.1 An illustration of the criteria used for differentiating different age groups of achenes. ((a) green, (b) medium, (c) dry and (d) ultra-dry.) (Stippling represents extent of browning of each age group). X 1,25.

The browning of these capitula-bearing axes had progressed even further than that of the capitula bearing dry achenes. The ultra-dry achenes were however, still attached to the receptacle although in some instances, achenes had already been released and dispersed.

The achenes harvested on the 21st September and 5th October consisted only of dry and ultra-dry achenes while on the 25th October only ultra-dry achenes were obtained.

The germination of each of the achene groups collected at the respective harvest dates was examined by incubating achenes at 15/30 °C either continuously in the dark or in a diurnal light cycle (12 hours dark : 12 hours white light). Sixteen replicates of 10 achenes each were used. In addition, the moisture content of the achenes in each of the classes (five replicates of 100 achenes each) was determined at each harvest date at the commencement of incubation.

It has been found that in many species, the seeds require an after-ripening period to potentiate germination (MAYER & POLJAKOFF-MAYBER, 1982). After-ripening in *C. odorata* achenes was investigated using the three groups of achenes harvested on 21st August, that is, the green, medium and dry achenes. As soon as possible after harvest, batches of achenes in each of the groups were placed in both

(i) dry, dark storage at 25 ± 2 °C and

(ii) dry, dark storage at -18 ± 2 °C, in sealed containers.

Germination of these achenes at 15/30 °C in a diurnal light

cycle and continuous darkness was tested after one, two, four and seven months storage. Sixteen replicates of 10 achenes each were used in the germination tests. In addition, the moisture content of each of the achene groups stored at 25 °C and at -18 °C was determined at the start of the germination tests for each storage time.

In Chapter I, the distribution of *C. odorata* in Natal was outlined (Figure 1.2.1). As shown, the area infested is large and is therefore likely to include diverse environmental conditions. It was decided that an investigation of the germination of achenes produced in different localities should be instigated to determine whether germination of achenes is influenced by locality.

Achenes were harvested from six localities as listed below. The harvest dates are included:

(i) New Hanover (29°25'S 30°31'E), 11th September, 1984;
(ii) Melmoth (28°45'S 31°29'E), 12th September, 1984;
(iii) Empangeni (28°50'S 31°52'E), 13th September, 1984;
(iv) Stanger (29°27'S 31°14'E), 13th September, 1984;
(v) Durban (Virginia Bush) (29°49'S 31°1'E), 13th September, 1984;
(vi) Pietermaritzburg (29°35'S 30°25'E), 14th September, 1984.

Only achenes of the dry category (as previously described), were used. During sorting, the number of filled and empty achenes for 50 capitula from each harvest site, was recorded. The moisture content of filled achenes (10 replicates of 100 achenes each) from each site was also determined to provide an indication of the moisture status of the achenes.

The germination of the filled achenes was tested in both the light (diurnal cycle, 12 hours dark : 12 hours light) and the dark at 15/30 °C. These germination tests were all commenced on the same day (17/9/84), within one week of achene harvest. The germinability of these achenes was again tested after seven months dry, dark storage at 25$\pm$2 °C. At this time the germination of the achenes harvested at Pietermaritzburg was not tested due to lack of material and that of the Virginia Bush site was excluded because extensive tests had already been carried out on these achenes. Twenty replicates of 10 achenes each were used for each germination test.

On 13th September when achenes were collected from Virginia Bush for the aforementioned experiment, achenes were harvested from four separate plants to test the germinability of achenes produced by individual plants. Again, the achenes of 50 capitula from each plant were sorted into filled and empty achenes and the numbers recorded. Germination of achenes from the individual plants (20 replicates of 10 achenes each) was tested at 15/30 °C in the dark and in the light (diurnal cycle 12 hours dark : 12 hours light).

Although all the capitula from each of the four plants were collected, only those from one of the plants were counted and the number of achenes, filled and empty, estimated using the data obtained from the counts made for 50 capitula.

The germination of achenes from individual capitula was also investigated. A sample of 100 capitula was removed from a large amount of well mixed capitula. Only capitula

from which no achenes were obviously missing were used. For each capitulum, the total number and the number of filled and empty achenes were recorded. The germination of each group (filled or empty) was tested in the diurnal light cycle for each individual capitulum. A mean percentage germination was obtained for (i) the total number of achenes per capitulum, (ii) the number of filled achenes and (iii) the number of empty achenes.

Another important biological aspect of *C. odorata* achene germination is the effect of burial on germinability. To investigate this, fine mesh plastic gauze pockets were made into which filled achenes (dry, harvested 24th August, 1983) were placed. The pockets were buried at a depth of five centimetres at three localities within Virginia Bush conservation area. Pockets, containing the achenes, were retrieved after one, two, six and 12 months from each of the three localities. Germination was tested at 15/30 °C in the diurnal light cycle and in the dark. At least 10 replicates of 10 achenes each were used.

At time zero and six month and 12 month retrieval times achenes were examined using a Scanning Electron Microscope (SEM) to establish the state of the embryo-covering structures. The preparation procedure is detailed below.

Initially various preparative procedures were used to obtain examples of the achene-coat morphology and transverse sections of the coat layers. However, the results proved unsatisfactory due mainly to excessive rupturing

of the cell walls during cutting. It was therefore decided that the intact achenes would first require critical point drying prior to sectioning and coating. The following preparative procedure was used:

(i) Soak overnight in six per cent gluteraldehyde in 0,05 M cacodylate buffer (pH 7,2);

(ii) Wash in 0,05 M cacodylate buffer (twice for 10 minutes each);

(iii) Immerse in two per cent osmium tetroxide in 0,2 M cacodylate buffer (two hours);

(iv) Wash in 0,05 M cacodylate buffer (twice for 10 minutes each);

(v) Pass through graded ethanol series (10 per cent to 100 per cent), for dehydration (10 minutes in each concentration);

(vi) Pass through 25, 50, 75 and 100 per cent amylacetate in absolute alcohol solutions (10 minutes in each).

The achenes were then critical point dried with carbon dioxide on an Hitachi HCP-2 Critical Point Dryer, mounted on stubs and coated with gold palladium in a Polaron SEM Coating Unit (E5100) and viewed with either a Joel JSMT 200 or an Hitachi 5570 SEM.

The presence of achenes on and in the soil was also examined. In this study soil core samples were collected from the three sites where achenes had been buried for the burial study. Each core (20 cm in diameter) was sectioned horizontally into the following layers:

(i) the loose surface layer (± one centimetre deep) including the litter;

(ii) the soil to a depth of five centimetres;

(iii) the soil to a depth of 10 cm; and

(iv) the soil to a depth of 15 cm.

The respective soil layer samples were sieved through a graded mesh-size series of sieves, and the achenes removed from the soil on each of the sieves with the aid of a large diameter magnifying glass. The achenes obtained were counted and their germination tested at 15/30 °C in a diurnal light cycle.

The method used for recovering achenes from the soil had previously been tested using similar soil samples obtained from an area where no *C. odorata* plants were present. A pre-determined number of achenes was added to the samples which were then well mixed and sieved. It was found that 80 to 90 per cent of the achenes were recovered.

Results

The percentage moisture content of the groups of achenes used in the harvest-date experiment is presented in Table 2.5.1. The moisture content of the green achenes as compared with that of the other achene groups, was notably high. Although there was no apparent trend in the moisture content of the other groups, the mean percentage moisture content was, with two exceptions (medium, 5th September and ultra-dry, 5th October), below 10 per cent. The lowest percentage moisture content was recorded for the ultra-dry achenes harvested on 21st September. Only the dry achenes appeared

Table 2.5.1 Moisture content of the achenes of different ages harvested at various dates from Virginia Bush (± S.E. of five replicates of 100 achenes each).

Harvest Date	Moisture Content (Percentage)			
	Green	Medium	Dry	Ultra-dry
21/8/84	19,2±0,7	8,8±1,2	7,9±0,9	-
5/9/84	-	10,4±0,7	9,5±0,3	9,8±0,9
21/9/84	-	-	9,9±0,4	6,5±0,5
5/10/84	-	-	9,9±1,4	10,0±0,7
25/10/84	-	-	-	10,0±0,9

to have attained a consistent moisture content.

Regarding the germination of achenes harvested at the various dates, the percentage germination of these achenes in the dark was low (< 10 per cent) for all the harvest times (results not presented).

The percentage germination in the light cycle is shown in Figure 2.5.2. The germination of achenes harvested on 21st August is shown in Figure 2.5.2A. The medium achenes germinated best (p = 0,05); 50 per cent of these achenes having germinated during the incubation period. The percentage germination of the green achenes was similar to that of the dry achenes (± 38 per cent).

For the achenes harvested on 5th September, the highest percentage germination (70 per cent) was obtained for the dry group (Figure 2.5.2B). At this harvest time ultra-dry achenes were also obtained. The germination of these was initially rapid but the eventual percentage germination was similar to that of the medium achenes (± 55 per cent) as illustrated in Figure 2.5.2B.

On 21st September, only dry and ultra-dry achenes were obtained. The rate of germination of both these groups was initially slow but increased appreciably between day 16 and day 24 of incubation (Figure 2.5.2C). The percentage germination after 28 days incubation was 80 per cent for the ultra-dry achenes and 74 per cent for the dry achenes. Due to the apparently delayed germination response of both these achene groups, incubation was continued. After 32 days the

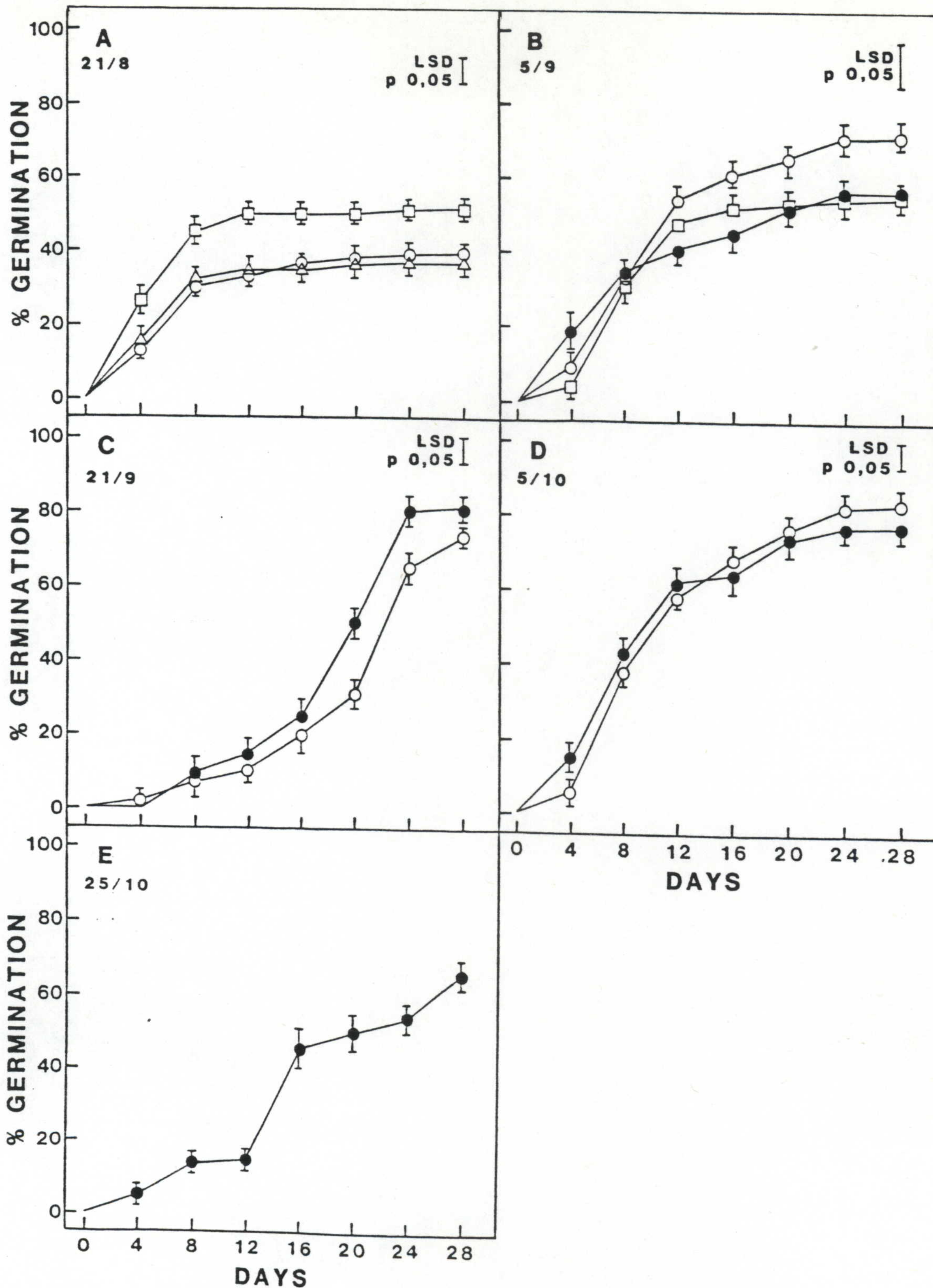


Figure 2.5.2 Germination in the light of the different age groups of achenes harvested at various times during achene-filling and ripening. (A) 21st August, (B) 5th September, (C) 21st September, (D) 5th October and (E) 25th October, 1984. (△ green; □ medium; ○ dry; ● ultra dry).

percentage germination of the ultra-dry achenes had risen to 86 per cent and that of the dry achenes to 74 per cent (not shown in Figure 2.5.2C). Thereafter, no further germination occurred.

The final percentage germination of the dry and ultra-dry achenes (83 and 77 per cent respectively) harvested on 5th October (Figure 2.5.2D) was similar to that of the achenes harvested at the preceding harvest time. However, the rate of germination of the achenes harvested on the later date was considerably more rapid (± 65 per cent after 16 days as compared to ± 25 per cent for the achenes harvested on 21st September).

The germination rate of the ultra-dry achenes harvested on the 25th October was slow initially with only 15 per cent having germinated after 12 days incubation (Figure 2.5.2E). After 28 days incubation, 66 per cent had germinated. Since achenes were still germinating at this time, incubation was continued (results not presented in Figure 2.5.2E). It was found that after 32 days incubation the percentage germination had risen to 82 per cent and after 36 days to 84 per cent. Thereafter no further germination occurred.

The effect of storage at 25 °C and -18 °C on the moisture content of achenes is presented in Table 2.5.2. The most notable change in the percentage moisture content was that of the green achenes stored at 25 °C. In the first month, the moisture content decreased from ± 19 per cent to eight per cent. In no other group did any appreciable decrease

Table 2.5.2 The effect of storage time at 25 °C and -18 °C on the percentage moisture content of green, medium and dry achenes (± S.E. of 10 replicates of 100 achenes each).

Storage Time (Months)	Moisture Content (Percentage)					
	Green		Medium		Dry	
	25 °C	-18 °C	25 °C	-18 °C	25 °C	-18 °C
0	19,2±0,7	19,2±0,7	8,8±1,2	8,8±1,2	7,9±0,9	7,9±0,9
1	8,0±0,5	14,9±0,7	6,4±0,4	9,0±0,9	5,4±0,3	8,5±0,8
2	8,2±0,9	20,9±0,7	6,6±1,0	10,1±0,8	7,8±0,8	9,0±0,7
4	8,1±0,8	17,0±0,7	7,7±1,3	9,1±1,2	7,7±0,6	8,2±0,6
7	7,0±0,4	23,5±1,8	7,1±0,5	11,4±1,0	7,7±0,7	8,3±0,5

in moisture content occur although the percentages recorded appeared to fluctuate. This was especially evident in those seeds stored at -18 °C.

The effect of storage duration and conditions on germination is shown in Figure 2.5.3. The germination of freshly harvested green and dry achenes was similar, while that of the medium achenes was more rapid (Figure 2.5.3A). An asymptote in precentage germination was reached after eight days incubation whereafter little further germination was recorded. After one month storage a similar trend in germination was observed (Figure 2.5.3B) but the percentage germination was lower than that of freshly harvested achenes. The rate of germination was similar for all groups except the green achenes stored at -18 °C. Germination percentages of the respective groups of achenes stored for either two or four months were similar (Figure 2.5.3C and Figure 2.5.3D respectively). Storage for seven months resulted in a distinctive difference in the germination of achenes stored at 25 °C and -18 °C (Figure 2.5.3E). Although the asymptote for the percentage germination of all achenes was reached after 12 days incubation, the germination of achenes stored at 25 °C was considerably more rapid than that of the respective groups stored at -18 °C. There was also a difference between the groups stored at -18 °C. The medium and dry achenes germinated at a similar rate while that of the green achenes remained low.

For clarity, the final percentage germination obtained for the various groups after each storage time is

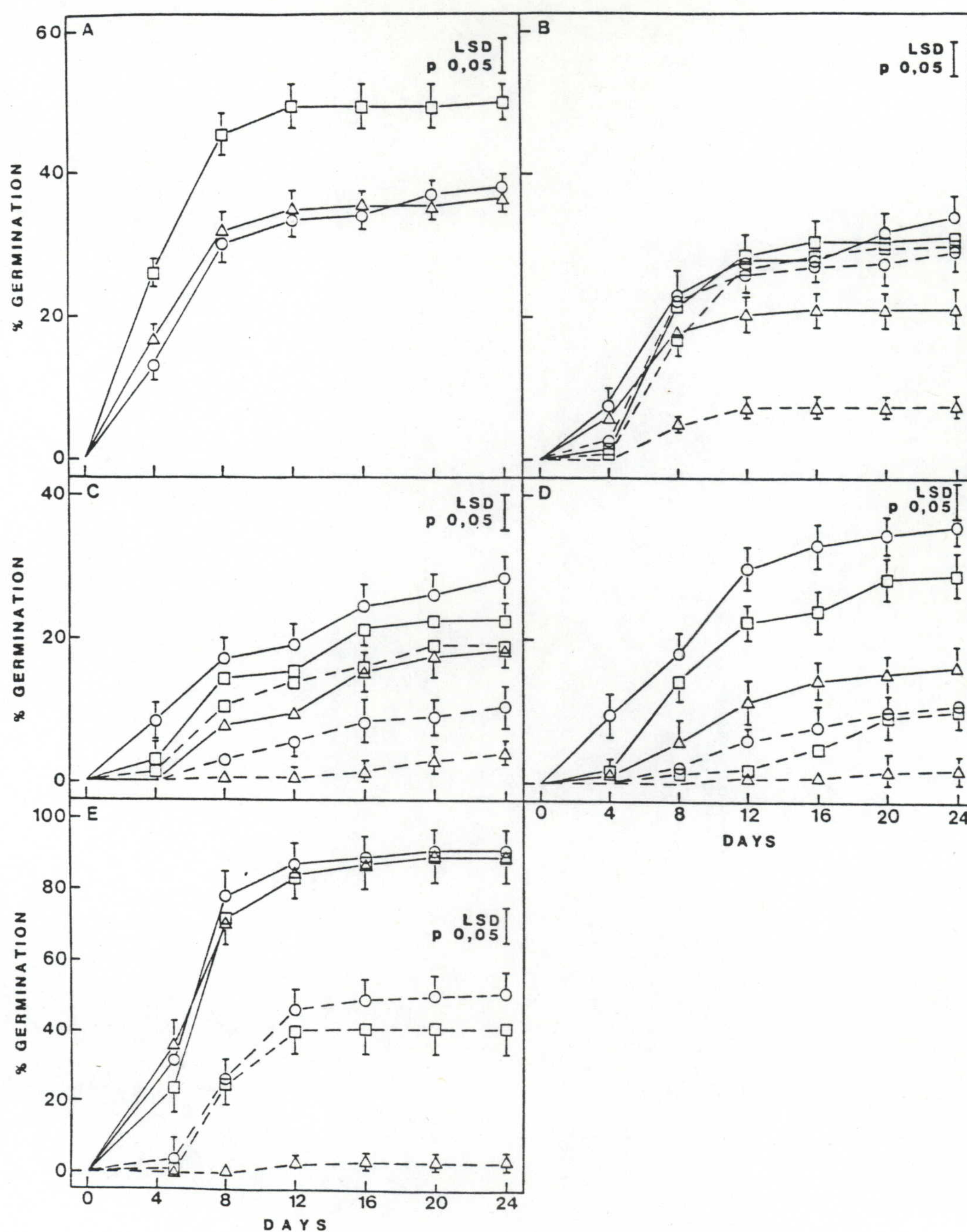


Figure 2.5.3 Germination response following storage of green, medium and dry achenes at 25 °C (solid lines) and - 18 °C (broken lines) for (A) zero, (B) one month, (C) two months, (D) four months and (E) seven months. (△ green; □ medium; ○ dry).

shown in Figure 2.5.4. For achenes stored at 25 °C, the final percentage germination decreased with increased storage time up to two months (Figure 2.5.4A). Thereafter the percentage germination remained fairly constant up to four months storage. However, after seven months storage, the percentage germination of all three groups was markedly higher. A similar trend in percentage germination was obtained for the achenes stored at -18 °C (Figure 2.5.4B). There were however, two distinct differences. Firstly, the percentage germination of the green achenes stored at -18 °C decreased markedly and remained low for the duration of the investigation. Secondly, although the percentage germination of the medium and dry achenes stored at -18 °C also increased after seven months storage, the increase was not as marked as that of the achenes stored at 25 °C.

The data pertaining to the achenes harvested from various localities are presented in Table 2.5.3. The mean mass per achene was similar ($p = 0,05$) for the achenes harvested from New Hanover, Empangeni and Pietermaritzburg. The mass per achene for these three batches of achenes was significantly lower than that of achenes harvested at the other localities. The Virginia Bush and Melmoth achenes were of a similar mass and the heaviest achenes were collected from Stanger.

The percentage moisture content of achenes harvested at Melmoth and Empangeni was significantly lower than that of achenes harvested from Virginia Bush (Table 2.5.3) while that of the Melmoth achenes was also significantly

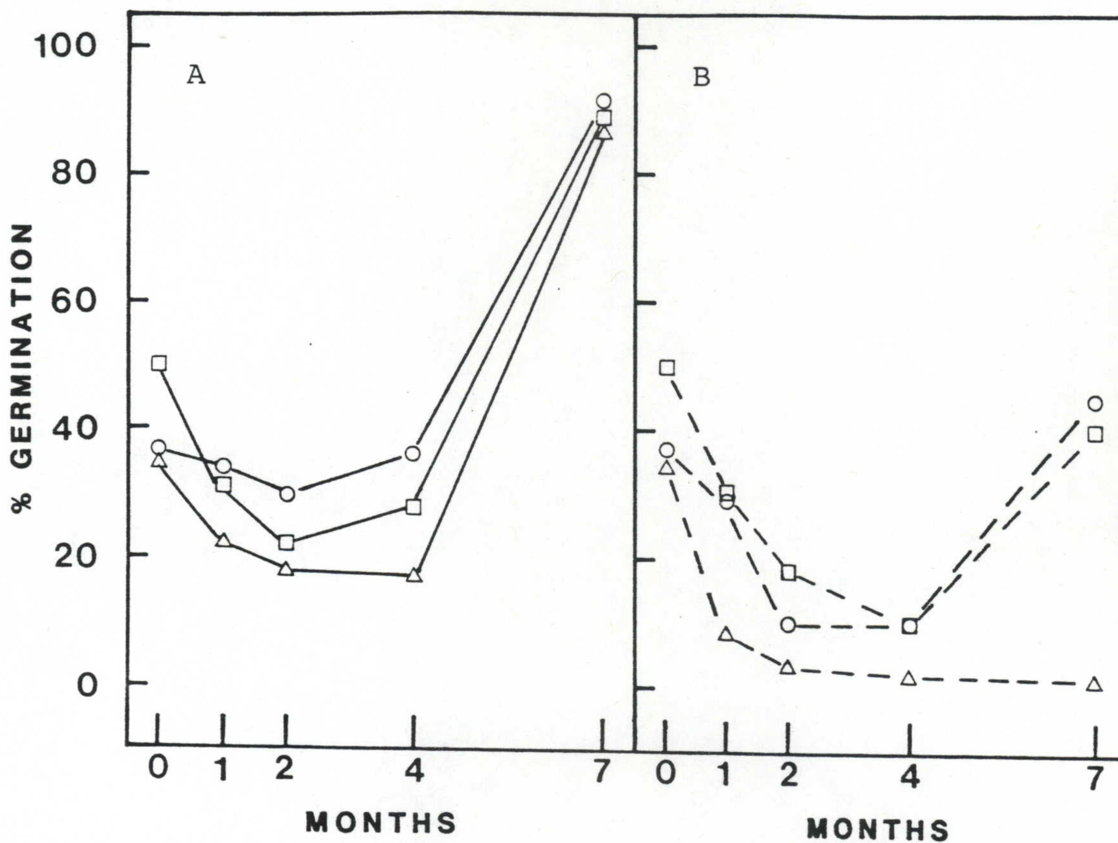


Figure 2.5.4 The final percentage germination (in the light) of achenes stored for one, two, four and seven months at 25 °C (A) or - 18 °C (B). (△ green; □ medium; ○ dry).

Table 2.5.3 Initial mass of achenes, percentage moisture content, number of achenes per capitulum and percentage filled achenes of material collected from six localities.

Harvest locality	Mean initial mass/achene[1]	Moisture content[2]	No. achenes/ capitulum[3]	Filled achenes/ capitulum[4]
New Hanover	$2,31 \times 10^{-4}$ [a]	$9,0 \pm 0,7$ [ab]	$18,4 \pm 2,0$ [ab]	$30,5 \pm 15,8$ [a]
Melmoth	$2,59 \times 10^{-4}$ [b]	$7,2 \pm 0,5$ [c]	$18,8 \pm 0,2$ [a]	$59,1 \pm 1,8$ [b]
Empangeni	$2,34 \times 10^{-4}$ [a]	$7,7 \pm 0,3$ [bc]	$18,2 \pm 0,2$ [ab]	$62,6 \pm 1,9$ [b]
Stanger	$3,02 \times 10^{-4}$ [c]	$8,3 \pm 0,8$ [abc]	$18,8 \pm 1,6$ [a]	$75,5 \pm 2,0$ [c]
Virginia Bush	$2,68 \times 10^{-4}$ [b]	$9,5 \pm 0,8$ [a]	$18,3 \pm 0,3$ [ab]	$70,8 \pm 1,4$ [c]
Pieter-maritzburg	$2,29 \times 10^{-4}$ [a]	$8,8 \pm 0,6$ [ab]	$18,0 \pm 1,4$ [b]	$48,2 \pm 16,7$ [d]

[1] mean mass per achene before drying; calculated from 10 replicates of 100 achenes each.

[2] mean percentage moisture content ± S.E. of 10 replicates of 100 achenes each.

[3] mean number of achenes ± S.E. of 50 capitula.

[4] mean percentage filled achenes ± S.E. of 50 capitula.

[abcd] values in vertical columns with the same letter are not significantly different ($p = 0,05$).

lower than that of the achenes harvested from New Hanover and Pietermaritzburg. The percentage moisture content was similar for achenes harvested from New Hanover, Empangeni, Stanger, Virginia Bush and Pietermaritzburg.

The major difference in the number of achenes per capitulum occurred between the samples harvested at Pietermaritzburg and Melmoth (Table 2.5.3), the number being significantly lower for the Pietermaritzburg material. The number was similar for all other localities.

There were distinct differences in the percentage filled achenes per capitulum for the samples from the six harvest localities (Table 2.5.3). The percentage filled achenes per capitulum for the New Hanover sample was the lowest, followed by the Pietermaritzburg sample which was lower (p = 0,05) than the Melmoth and Empangeni samples; the latter two were similar. The highest percentage of filled achenes per capitulum was obtained for the Stanger sample (75,5 per cent) which was similar to that recorded for the Virginia Bush sample (70,8 per cent).

The effect of harvest locality on germination of freshly harvested achenes is shown in Figure 2.5.5A; apparent differences were obtained. The percentage germination of achenes harvested from Stanger was low, only 10 per cent having germinated after the 24 day incubation period. Although the rate of germination of achenes harvested from Empangeni was initially rapid, an asymptote in percentage germination (19 per cent) was reached after 12 days incubation and by 24

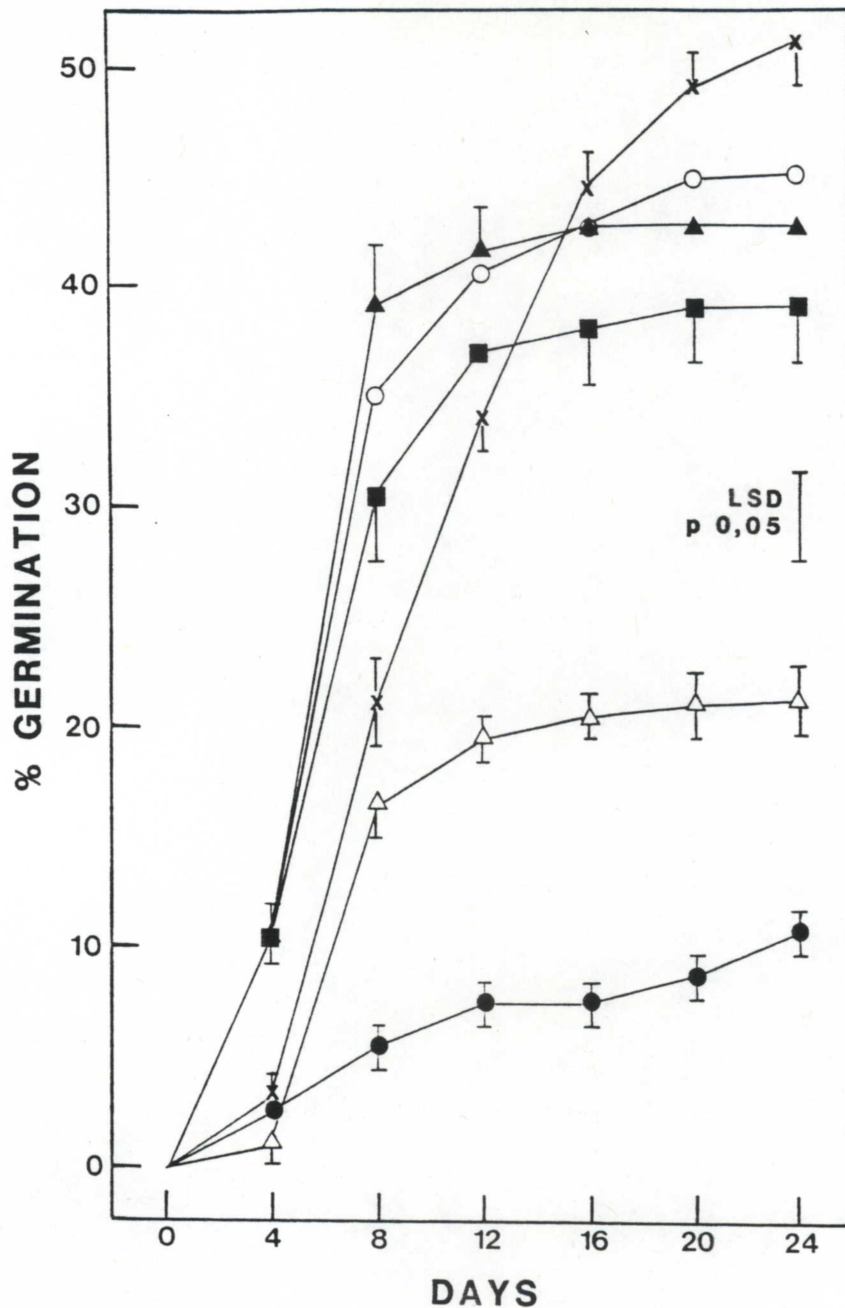


Figure 2.5.5A Germination of freshly harvested achenes collected from various localities in Natal. (○ New Hanover; △ Empangeni; ● Stanger; ▲ Melmoth; ■ Pietermaritzburg; x Virginia Bush).

days, only 23 per cent of the achenes had germinated. The germination of achenes harvested from Pietermaritzburg, Melmoth and New Hanover was similar. Initially the percentage germination was rapid and by 12 days an asymptote had been reached for the achenes from these three localities. The final percentage germination was 45 per cent for the New Hanover achenes, 43 per cent for the Melmoth achenes and 39 per cent for the Pietermaritzburg achenes. The achenes harvested from Virginia Bush also initially germinated fairly rapidly, but no distinct asymptote was reached and the final percentage germination was nearly 51 per cent.

After seven months of storage the germination of achenes from the four localities tested, was considerably higher (Figure 2.5.5B) than that obtained with the freshly harvested achenes. At the time that the experiment was terminated, the percentage germination was similar ($p = 0,05$) for all four harvest localities (± 75 per cent).

In the samples harvested from four individual plants, there was some variation in the number of achenes per capitulum (Table 2.5.4). The highest number was recorded for plant C (18,2) which was significantly higher than the 16,9 obtained for plant B. The mean number of filled achenes also varied; here the number for plant B was the highest recorded (13,1) which was significantly higher than the 11,6 recorded for plant D. Due to a comparatively low total number of achenes per capitulum found for plant B, which was associated with the comparatively high number of filled achenes, the mean percentage filled achenes per capitulum was significantly

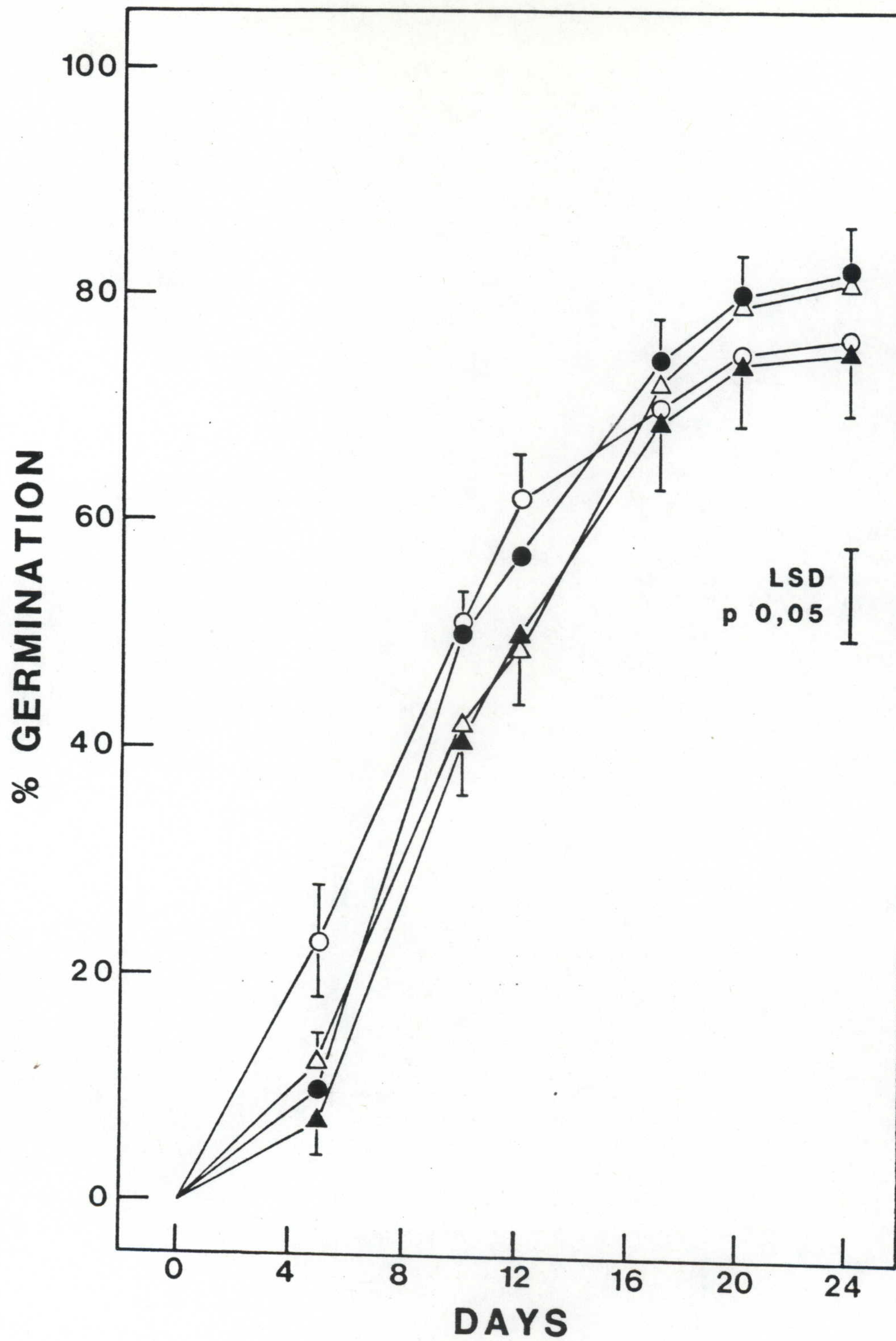


Figure 2.5.5B The effect of storage for seven months on the germination of achenes harvested from New Hanover (○); Empangeni (△); Stanger (●); and Melmoth (▲).

Table 2.5.4 The number of achenes, number of filled achenes and percentage filled achenes per capitulum (± S.E. of 50 capitula harvested from four individual plants).

Plant	Mean no. achenes/ capitulum	Mean no. filled achenes capitulum	Mean % filled achenes/ capitulum
A	17,7±1,9[ac]	12,2±2,3[ab]	68,6±11,1[a]
B	16,9±1,5[b]	13,1±2,3[a]	77,6±11,3[b]
C	18,2±2,0[c]	12,5±3,1[ab]	68,2±14,4[a]
D	17,3±1,5[ab]	11,6±2,6[b]	66,7±13,1[a]

[abc] Values within vertical columns with the same letter are not significantly different (p = 0,05).

higher for this plant. The percentage filled achenes per capitulum was similar for plants A, C and D.

The germination of achenes from the individual plants is shown in Figure 2.5.6. Initially, (day 0 to 10) it appeared as though the germination rate of achenes from plants A, C and D was similar while that of plant B was more rapid. After 16 days incubation, the percentage germination of achenes from plant C was far lower (30 per cent) than that of the other plants ($\pm$ 48 per cent). After 24 days it seemed that no further germination was occurring. At this time the percentage germination of plant D was 65 per cent, plants A and B 50 per cent and plant C 35 per cent. However, the duration of the experiment was extended to 48 days. Between 24 and 48 days incubation a gradual increase in the percentage germination occurred, that of plant C being the most rapid. When the experiment was terminated the percentage germination of plant C achenes was similar ($p = 0,05$) to that of plant B achenes, but lower than that of plants A and D. The percentage germination of plants A, B and D achenes was similar ($p = 0,05$).

The data obtained for the individual capitula are presented in Table 2.5.5 and in Figure 2.5.7. The mean number of achenes (filled plus empty) was 17,6 and ranged from 12 to 21 achenes per capitulum (Table 2.5.5). The mean number of filled achenes per capitulum was 11,8 or 67 per cent of the total number of achenes per capitulum. The number of filled achenes ranged from 6 to 17 per capitulum (Table 2.5.5). The percentage germination of the filled achenes was nearly 50 per cent of the total number of achenes that is, filled

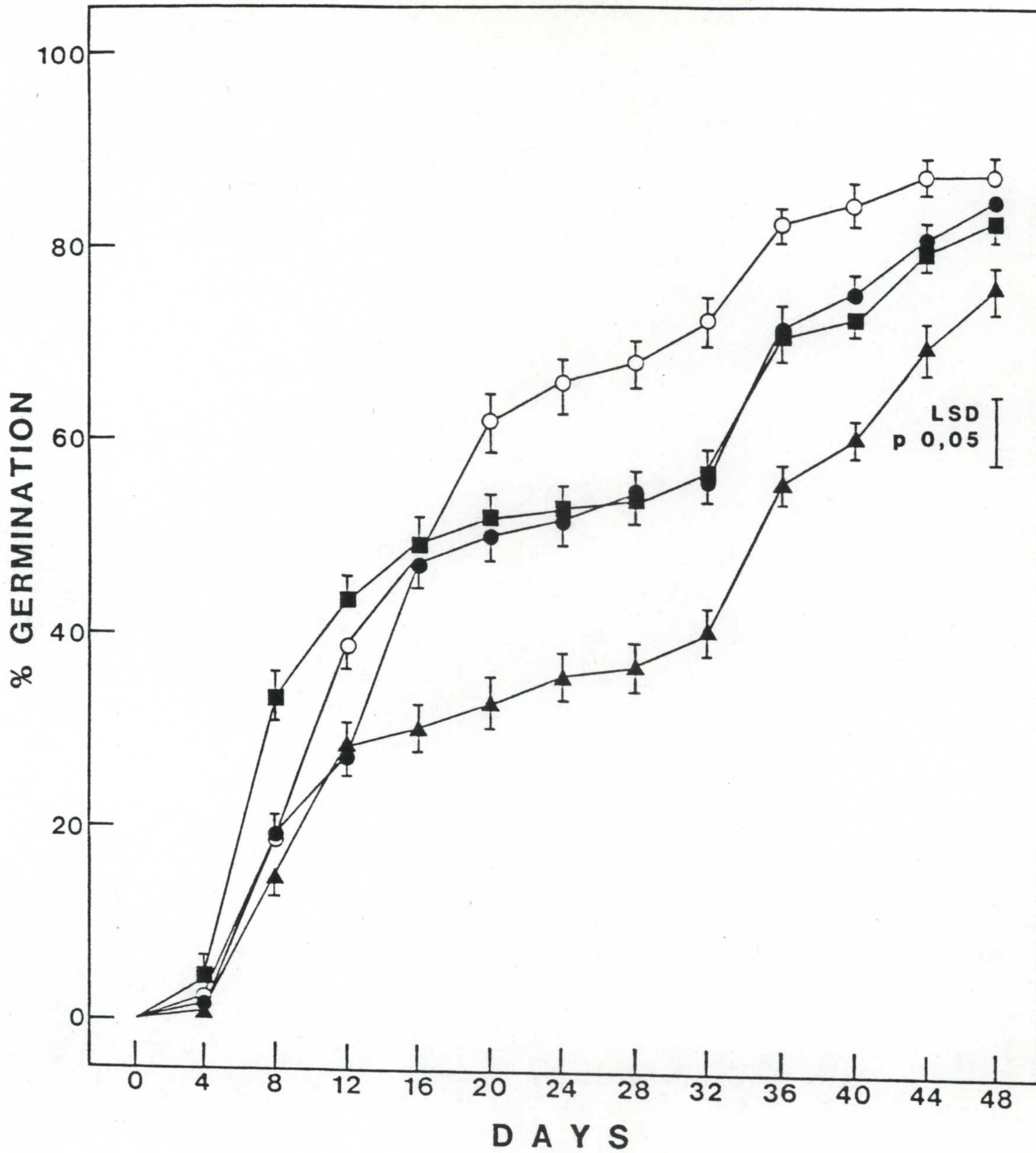


Figure 2.5.6 Germination in the light of achenes harvested from four individual plants. (● plant A; ■ plant B; ▲ plant C; ○ plant D).

Table 2.5.5 The data obtained for 100 individual capitula (± S.E. of mean).

Per Capitulum	No. or %	Range (No. or %)
Mean total no. of achenes	17,6±0,2	12 - 21
Mean no. of filled achenes	11,8±0,3	6 - 17
Germination of filled achenes[1]	47,5±1,6	11 - 83
Germination of empty achenes[1]	0,7±0,1	0 - 13
Germination of filled achenes[2]	71,4±2,1	15 - 100
Germination of empty achenes[2]	2,1±0,1	0 - 40
Mean total % germination[3]	48,2±1,6	-

[1] Germination calculated as a mean percentage of the total number of achenes per capitulum.

[2] Germination calculated as a mean percentage of either the number of filled or empty achenes.

[3] Percentage germination of the total number of achenes, that is, filled plus empty.

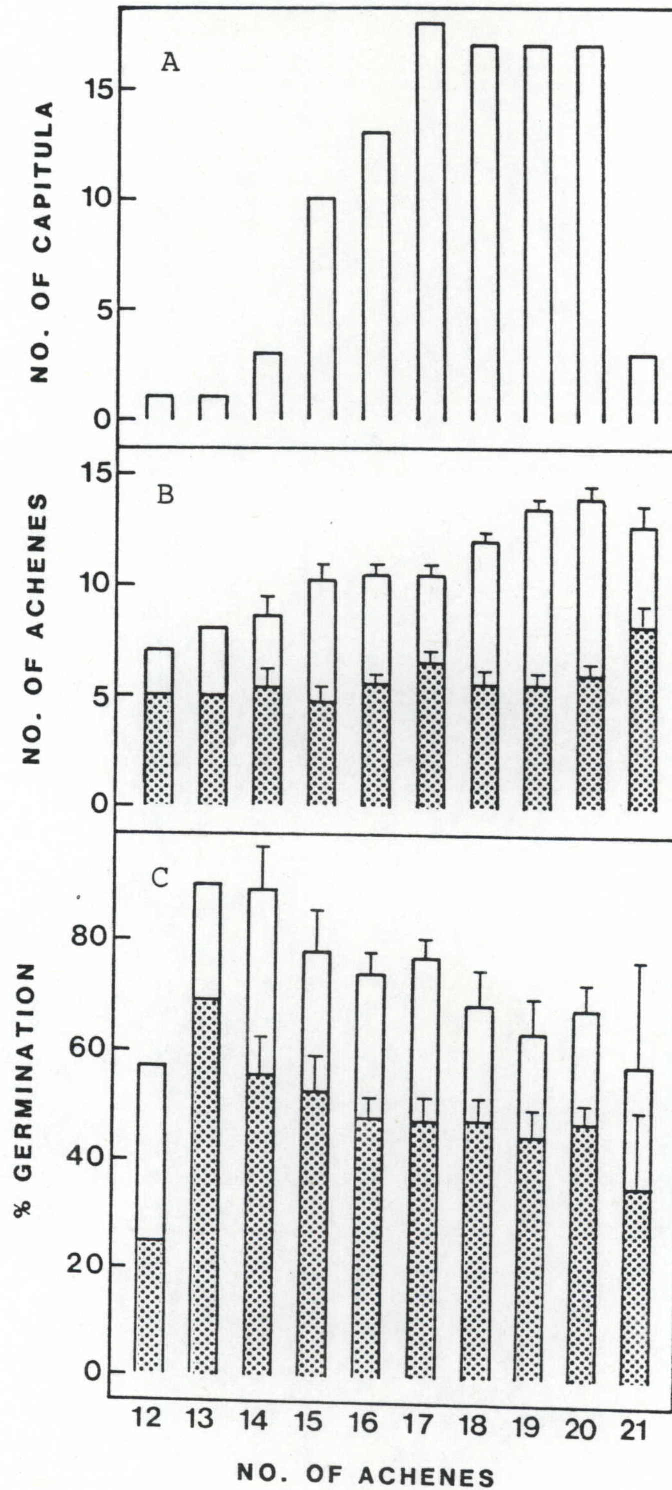


Figure 2.5.7 Data obtained from counting and testing the germination of achenes from 100 individual capitula. (A) the number of capitula bearing a specific number of achenes; (B) the mean number of full (⊓) and empty (▨) achenes comprising the total number of achenes; and (C) the germination of full achenes calculated as a mean percentage of the total number of achenes (▨) and as a mean percentage of the number of full achenes (⊓). Vertical bars respresent the S.E.s of the means.

plus empty achenes. The germination of empty achenes was 0,7 per cent when calculated as a percentage of the total number of achenes or 2,1 per cent when calculated as a percentage of the number of empty achenes. Germination of filled achenes was 71,4 per cent when calculated as a percentage of the number of filled achenes.

As shown in Figure 2.5.7A, the majority of capitula contained 17 to 20 achenes; very few contained 12, 13, 14 or 21 achenes. The mean number of empty achenes per capitulum remained fairly constant regardless of the total number of achenes per capitulum (Figure 2.5.7B). However, the mean number of filled achenes increased with an increase in number of achenes per capitulum.

Although it appeared as if the percentage germination of filled achenes, calculated either as a percentage of the total number or as a percentage of filled achenes, decreased with increasing number of achenes per capitulum (Figure 2.5.7C), the trend was not distinctive.

Upon retrieval of buried achenes, these were examined to establish whether any germination had occurred during burial. It was found that three achenes (one per cent) of those originally buried had germinated in the sample retrieved after one month from site C and one achene (0,3 per cent) of those retrieved after six months from site B.

The effect of burial duration and site on germination of achenes in the diurnal light cycle is shown in Table 2.5.6. The percentage germination of achenes stored in the

Table 2.5.6 The effect of burial duration at three sites, and of dry storage in the laboratory, on the germination of achenes incubated in a diurnal light cycle (15/30 °C) (± S.E.).

Burial Duration	Percentage Germination				
	Lab.[1]	Site A	Site B	Site C	L.S.D. p = 0,05
1 month	74±4	26±5	46±7	47±5	7
2 months	80±6	37±5	44±4	40±6	7
6 months	79±4	43±5	41±8	43±6	7
12 months	77±6	37±4	30±5	47±6	7
L.S.D. p = 0,05	8	8	8	8	

[1] Achenes stored dry in the dark at 25±2 °C.

laboratory remained constant throughout the study period. Burial for one month significantly reduced the percentage germination of achenes for all three sites. The reduction was greatest at site A. After two months burial, the percentage germination for the site A achenes had increased and thereafter remained constant. The percentage germination of achenes buried at site B remained constant until the 12 month burial time. At this stage germination was significantly reduced. The germination of achenes buried at site C remained constant for the duration of the study. At the final retrieval time the percentage germination of achenes buried at site C was significantly higher than that of the other two sites.

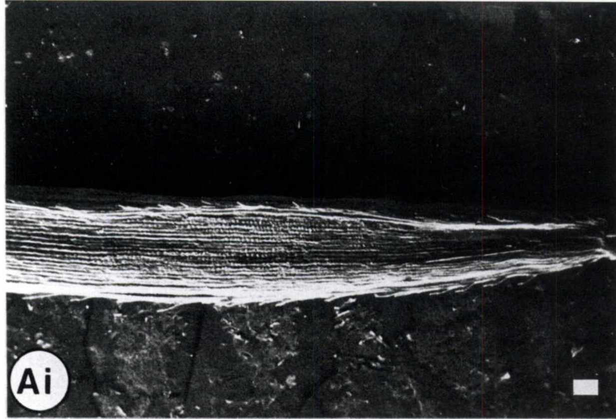
Dark germination of retrieved achenes was positively influenced by burial duration. At the one, two and six month retrieval times, percentage germination in the dark was low (Table 2.5.7). However, percentage germination of achenes which had been buried for 12 months was significantly higher for two of the three sites than that of achenes buried for shorter periods and than that of achenes stored dry in the dark at 25 °C.

Burial of achenes resulted in progressive degradation of the achene coat as illustrated in Plate 5. After six months burial the outer layer of the pericarp had been "eroded" away (Plate 5B). In parts, it appeared as though the achene coat (pericarp and endosperm) had been completely punctured (Plate 5B(i)). Achenes retrieved after 12 months burial exhibited "peeling" of the pericarp (Plate 5C(i)) and

Table 2.5.7 The effect of burial duration at three sites, and of dry storage in the laboratory, on the germination of achenes incubated in the dark (15/30 °C) (± S.E.).

Burial Duration	Percentage Germination				
	Lab.[1]	Site A	Site B	Site C	L.S.D. p = 0,05
1 month	6±2	2±1	3±1	2±1	7
2 months	3±1	0	5±3	5±3	7
6 months	4±2	3±1	3±1	4±1	7
12 months	4±2	17±5	10±3	13±4	7
L.S.D. p = 0,05	8	8	8	8	

[1] Achenes stored dry in the dark at 25±2 °C.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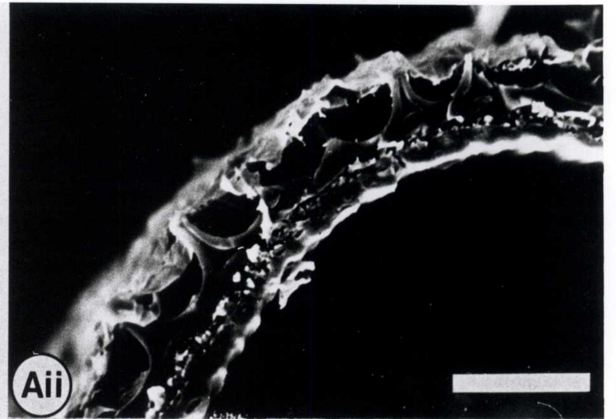
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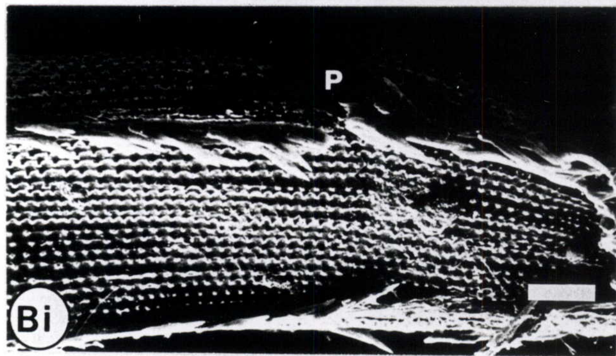
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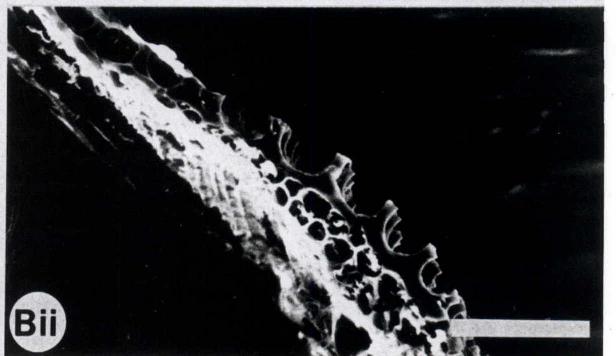
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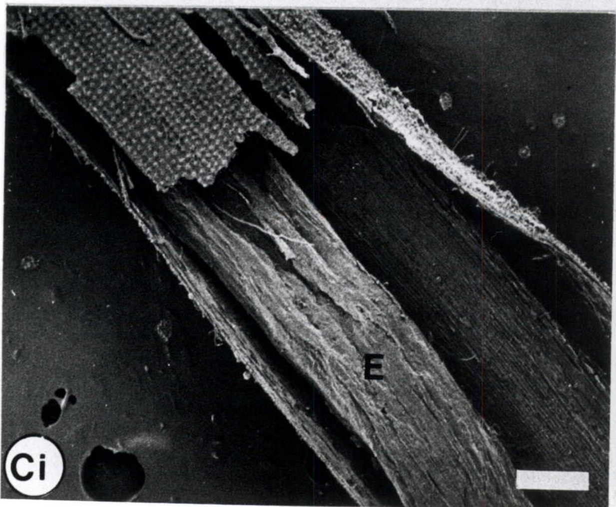
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in some instances, embryos, covered only by the endosperm (Plate 5C(ii)), were present.

The number of achenes retrieved from the various soil depths at the three sites is presented in Table 2.5.8. There were large numbers of achenes in the surface samples (zero to one centimetre depth) at all three sites, but especially at site A. The numbers in the two to five centimetre depth soil samples were similar for the three sites. No achenes were obtained from soil samples below a depth of five centimetres.

The number of retrieved achenes which germinated and the respective germination percentages after 28 days incubation are also shown in Table 2.5.8. At site A, 400 (49,8 per cent) of the 804 achenes retrieved, from the surface germinated. The number of achenes (and percentage germination) retrieved from the surface at the other two sites was lower. Achenes retrieved from the two to five centimetre soil depth samples also germinated (Table 2.5.8). The highest percentage germination was recorded for the site B samples. Few (six per cent) of the achenes in the two to five centimetre soil depth sample collected at site A germinated.

Discussion

In the harvest-date experiment the highest percentage moisture content, was recorded for the green achenes harvested on the 21st August (Table 2.5.1). This high moisture content was due to these achenes apparently being immature,

Table 2.5.8 The numbers of achenes retrieved from soil samples collected at three sites in Virginia Bush. The number of achenes which germinated (diurnal light cycle; 15/30 °C) and the respective percentage which germinated, are also indicated.

Soil depth (cm)	No. achenes retrieved			Germination					
				Site A		Site B		Site C	
	Site A	Site B	Site C	No.	%	No.	%	No.	%
0- 1	804	330	357	400	49,8	77	23,2	52	14,6
2- 5	50	49	65	3	6,0	32	65,3	28	43,1
6-10	0	0	0	-	-	-	-	-	-
10-15	0	0	0	-	-	-	-	-	-

no *in situ* drying-out having occurred. There was no obvious difference in the moisture contents of medium, dry and ultra-dry achenes, indicating that the moisture content of the achenes decreased rapidly (that is, from the green to the medium stage) whereafter little further moisture loss occurred. The relatively small fluctuations which were recorded for the moisture contents of the latter three stages were probably due to two factors. Firstly, the prevailing weather conditions immediately prior to harvest are likely to have influenced the moisture content since moisture was clearly visible in the capitula at certain harvest times following recent rain. Secondly, associated to the first factor, is the time taken to sort the achenes. During sorting under a dissecting microscope, the heat generated by the microscope light is likely to have resulted in moisture loss from the achenes. A certain amount of the fluctuations recorded may therefore merely reflect the difference in time taken to execute the sorting process.

As illustrated in Figure 2.5.2A, achenes which were green and contained a high moisture content (earlier referred to as immature) were in fact sufficiently well developed to germinate. Therefore, if the parent plants were to be felled at a time when the achenes had reached the "green" stage, re-infestation could occur from germination of these achenes. The reason for the comparatively high germinability of the medium achenes is unclear. It may be that the environmental conditions experienced by the parent plants at the time that "seed-filling" occurred were optimum. It is pertinent

to note that the highest percentage germination was obtained for the dry achenes harvested on 5th September. This group was probably representative of the medium achenes at the earlier harvest time and of the ultra-dry achenes harvested on 21st September. Here the percentage germination was again high (80 per cent). The rate of germination of the ultra-dry achenes harvested on 25th October was erratic but generally low. Although the eventual percentage germinated achenes was high, it is likely that these achenes were produced at a time when environmental conditions were sub-optimal for achene-filling and hence the erratic germination response observed.

The effect of environmental conditions experienced by the parent plants on seed germination, has been the topic of numerous investigations. PETERS (1982a; 1982b) showed that moisture stress and temperature influenced both the production of viable seeds and the germinability of seeds produced by *A. fatua*. Examples cited by KOLLER (1972) demonstrated that germination is decreased by longer photoperiods under which seeds matured. KARSSEN (1970c) observed that germination of *C. album* seeds produced by plants under long-day photoperiods was lower than that of seeds from plants grown under short-day conditions. Furthermore, McCULLOGH & SHROPSHIRE (1970) found that light quality during seed maturation also influenced subsequent germination and several examples were cited by GUTTERMAN (1977) in which it has been shown that daylength during seed ripening on the mother plant, influences germinability.

The influence of the environmental conditions may also act directly on the achenes since HAYES & KLEIN (1974)

proposed that light quality may directly influence the phytochrome status of the developing seeds independently of the parent plants. This has been substantiated in *C. sativus* seed (GUTTERMAN, 1977). More recently BARTLEY & FRANKLAND (1984) showed that perception of light by dry seeds also occurs.

The evidence in the literature therefore indicates that there are numerous factors which influence the germination of seeds harvested or produced at different times of the year. The results obtained for *C. odorata* are thus not uncommon and may merely reflect the conditions experienced by the plants and directly by the developing achenes. As stated by VIDAVER (1977), the environmental conditions prevailing at the time of seed development may be of such importance that reproducible results with seeds of different lots are essentially unobtainable.

The results obtained for *C. odorata* achenes clearly show that percentage germination varied for achenes harvested at different times. It is therefore of dubious value to compare the results with those obtained for this species in other countries (EDWARDS, 1975; IVENS, 1975) especially since no indication was made of the time of harvest and the age of the achenes used in these studies. However, the importance of the results of the present study is that regardless of the time at which achenes were harvested, germination occurred. Therefore, it can be assumed that even apparently immature achenes are germinable.

The most noticeable feature of storage on moisture content of the achenes was that that of the green achenes stored at -18 °C remained comparatively high. Storage of the green achenes at 25 °C for one month resulted in an appreciable decrease in the percentage moisture content, but thereafter no apparent changes occurred. In the other achene samples, there were minor fluctuations in the percentage moisture content; the probable reasons for these were discussed earlier. The moisture content is therefore unlikely to have played a major role in the germination results obtained.

The effect of storage time at either 25 °C or -18 °C on germination was distinctive. Immediately after harvest, highest percentage germination was obtained for the medium achenes. However, within one month of storage, the percentage germination had decreased from 50 per cent to 30 per cent, regardless of the storage conditions. The most notable effect of storage was the marked decrease in percentage germination of the green achenes. Throughout this investigation, the germination of the green achenes stored at -18 °C was extremely low (± two per cent). This may have been due to internal membrane damage as a result of freezing of these achenes which had a high moisture content. Or, as was probably the case with the medium and dry achenes, storage at the lower temperatures retarded the after-ripening process. (Evidence for this is provided in section 2.6 where green achenes stored at -18 °C could be induced to germinate with GA_3 and azide). There was however, an inexplicable increase in the percentage germination obtained for the medium and dry achenes stored at

-18 °C. This increased germination occurred concurrently with the improved percentage germination obtained with achenes stored at 25 °C for seven months. The percentage germination of the achenes stored at the higher temperature was significantly higher than that of the achenes stored at -18 °C.

The results obtained in the storage experiment clearly show that the initially low germination percentages recorded were due to an after-ripening requirement. Therefore the achenes were primarily dormant at the time of harvest. An after-ripening period of approximately seven months was required for removal of the primary dormancy. The after-ripening requirement found for these achenes is contrary to results reported by IVENS (1974) where it was suggested that the achenes showed no dormancy although only a maximum of 68 per cent germination was obtained. It is possible that in fact the relatively low percentage may have been due to a lack of after-ripening. The after-ripening requirement may also have contributed to the low percentage germination (33 per cent) obtained by YADAV & TRIPATHI (1982) in India.

The process of after-ripening apparently only occurs in seed with a low moisture content (five to 15 per cent) (BEWLEY & BLACK, 1982). After-ripening is a common phenomenon in the seeds of many species, especially in the family Poaceae (BRECKE & DUKE, 1980; TAYLORSON, 1980; LODGE & WHALLEY, 1981; ERASMUS & VAN STADEN, 1983a). After-ripening is defined by MAYER & POLJAKOFF-MAYBER (1982) as the changes which occur in seeds during storage as a result of which

germination becomes possible or is improved. Although it has been found that treatments such as prechilling or long term stratification can stimulate germination of seeds during the after-ripening period (VIDAVER, 1977), these were not tested in *C. odorata* achenes because, in the majority of the areas infested, such conditions are highly unlikely to occur. However, an attempt was made to elucidate the nature of the dormancy mechanism present in the primarily dormant achenes prior to the fulfilment of the after-ripening period. This aspect is discussed in section 2.6.

The necessity for a period of after-ripening may be due to a number of factors. In the case of an immature embryo further anatomical and morphological changes may occur such as have been observed in *Malus domestica* Borb. embryos DAWIDOWICZ-GRZEGORZEWSKA & LEWAK, 1978). In the seeds of many species however, no visible anatomical or morphological changes occur in the embryo during after-ripening and thus it is assumed that the process of after-ripening is the result of chemical or physical changes which occur within the seed or seed coat (MAYER & POLJAKOFF-MAYBER, 1982). Although unsubstantiated, it is possible that restricted germination of *C. odorata* achenes may be due to inhibitors which, after storage, are released from the pericarp. This statement is based on the observation that a brown coloured substance was leached from achenes stored for long (seven months) periods, but generally not from freshly harvested achenes. Clearly, this aspect requires further investigation.

The obvious importance of an after-ripening requirement is the prevention of germination of seeds still attached to the parent plant. There are two further important implications of this finding in *C. odorata* achenes. Firstly, in a research context, the improved germination following storage must be borne in mind during the interpretation of results obtained for achenes which have been stored and in comparing results obtained by other researchers. The second implication is in a management context, namely, after-ripening can also be expected to occur in the field situation. Although the storage conditions used for this study did not in any way resemble field conditions, the fact that after-ripening, even of achenes which appeared distinctly immature, did occur, is an indication that this may happen to achenes should the parent plants be slashed/cut or sprayed following achene production. Observations have shown that a large proportion of weed control, especially that of *C. odorata*, is executed during the "off-season", that is, winter, at a time when achene production is occurring or has occurred.

An additional factor requiring attention is that the achenes left on growing plants apparently "after-ripen" more rapidly than was the case for achenes stored dry in the dark at 25±2 °C. This conclusion is drawn from the germination results obtained for the freshly harvested achenes harvested on 21st August (Figure 2.5.2A and Figure 2.5.3A) and achenes harvested on 25th October (Figure 2.5.2E) and those stored for two months (Figure 2.5.3C). The trend observed for laboratory-stored achenes was that a period of at least four months

was required for increased germination. However, for achenes left on the plants and harvested at a time when the initial batch had been stored for two months, germination was appreciably higher.

There were differences in the achenes harvested from different localities. Firstly, the mass of individual achenes harvested from Stanger was highest. This area is severely infested by *C. odorata*. The next heaviest achenes were harvested from Virginia Bush and Melmoth; both these areas are also severely infested. Achenes harvested from marginal areas , that is those areas on the edge of the current distribution area and where *C. odorata* has not flourished, were the lightest. These were the Pietermaritzburg and New Hanover sites. An apparent anomaly is the low mass of the Empangeni achenes which were of a similar mass to those harvested from marginal areas. Empangeni, situated near the coast, falls in an area which could be expected to be well suited to *C. odorata* growth. However, as previously illustrated in Figure 1.2.1, there appears to be a definite reduction in the density of *C. odorata* infestation in this region, indicating that, for unknown reasons, this area is not particularly well suited to *C. odorata*. In a closely related species, *Eupatorium adenophorum* Spreng. it was also found that the mass of achenes varied from site to site, with the smallest being produced by plants growing in marginal areas in New South Wales, Australia (AULD, 1981). It was suggested that the differences observed in the mass were probably due to differences in rainfall during flowering and achene-filling.

The moisture content of *C. odorata* achenes produced at the harvest localities did vary. This however, is considered to be of little importance and may merely reflect different climatic conditions immediately prior to harvest or, a slight difference in the maturity of achenes harvested.

The only significant difference in the number of achenes per capitulum was between the material harvested from the Pietermaritzburg site (marginal area) and that of material harvested from Melmoth and Stanger (optimum areas). The suitability of the area for *C. odorata* growth was therefore again refected in the achene numbers produced. The numbers recorded are lower than those recorded for the same species in India (28,4) (YADAV & TRIPATHI, 1982) and in Nigeria (25 to 30) (IVENS, 1974).

The most conspicuous difference between the achenes collected from the six localities was the large variation in percentage filled achenes per capitulum. At New Hanover and Pietermaritzburg, approximately 30 per cent and 48 per cent respectively, of the achenes produced were filled as compared to 70 per cent and 75 per cent of achenes produced at Virginia Bush and Stanger respectively. YADAV & TRIPATHI (1982) found that a large number of non-viable achenes were produced by *C. odorata* plants in India. It was suggested that this was probably due to their apomictic mode of reproduction reported to occur in the genus previously known as *Eupatorium*. However, in Natal it is likely that the relative number of non-viable achenes (empty) was due to environmental conditions governing growth of this species.

The differences obtained in the germination of achenes freshly harvested from the various localities was misleading since the germination after seven months storage was similar; at least for the achenes of the four localities tested. The initial differences were therefore probably the result of differences in maturity of the achenes harvested from the various sites. Following seven months storage, high percentages of germination were obtained, confirming the requirement for an after-ripening period in *C. odorata* achenes. The relatively high germination percentages recorded following the after-ripening period is an indication that even in marginal areas such as New Hanover, viable achenes are produced. The present infestations therefore form a source of propagules for further encroachment in these areas.

EDWARDS (1975) also found that there were differences in the germination of *C. odorata* achenes harvested from different localities. As has already been suggested, the differences observed may have been due to differences in the maturity of the achenes at time of collection or, different post-harvest storage times and conditions.

The results obtained for the tests conducted on achenes from four individual plants show that, in addition to differences at different localities as discussed above, there are also differences between plants within an infestation. Clearly, a range of achenes per capitulum and mean number of filled achenes per capitulum were obtained for the four randomly selected plants. The mean percentage filled achenes

per capitulum for the four plants did not vary markedly although the highest percentage of filled achenes was obtained for the plant with the lowest mean number of achenes per capitulum (plant B, Table 2.5.4) which resulted in the mean number of filled achenes per capitulum being relatively high.

The initial differences obtained for the germination of achenes produced by the four plants may, as has been previously mentioned, have been due to differences in the maturity of the achenes. It is pertinent to note that the method used for dividing achenes into different age groups was based on external, visual features which may have had little influence on the state of maturity of the embryos. Eventually however, the percentage of achenes which germinated was not appreciably different for the four plants.

The estimation of the number of filled achenes (presumably viable) for one of the plants revealed that approximately 87 000 filled achenes had been produced. This figure concurs favourably with figures suggested by MACDONALD (1984).

Differences have already been noted for achenes produced at different localities and plants within the same infestation. Further distinctive differences were observed in achenes produced in different capitula.

Although the mean number of achenes per capitulum (17,6) was similar to those obtained in previous studies (approximately 18), it can be seen (Table 2.5.5) that the mean was calculated from a wide range of achenes per capitulum

(12 to 21). Similarly, the mean number of filled achenes per capitulum is also comprised of a wide range (6 to 17) as were the other recorded parameters. However, due to the large number of readings (100) the standard errors presented in Table 2.5.5 were deceptively low.

The data presented in Figure 2.5.6 also indicate that the number of filled achenes is higher in the capitula bearing a number of total achenes similar to the mean. Consequently, the capitula bearing a total of 18 to 20 achenes made the greatest contribution to the number of filled achenes. It is possible that these capitula are produced either at a time which is optimal for achene production or on a position on the plant which is optimal for achene production. Although it is generally known that position on the parent plant influences the germinability of seeds (HARPER, 1977), these finer details were not investigated in *C. odorata* achenes because these factors are likely to be of little importance to their germination.

Seeds of many species are able to survive for long periods in the soil. Inhibition of germination during burial in the soil is evidently a prerequisite for survival (KARSSEN, 1980/81a). It has been shown that temperature, absence of light or oxygen, presence of volatile or allelopathic inhibitors, and moisture storage conditions are among factors contributing to inhibition of germination (KARSSEN, 1980/81b). In weed species, the seeds in the soil play an important role in population dynamics and weed problems exist for as long as seeds remain viable (BASKIN & BASKIN, 1983a;

EGLEY & CHANDLER, 1983). Many investigators have attempted to determine the longevity of weed seeds in the soil (TAYLORSON, 1970; LEWIS, 1973; STOLLER & WAX, 1974; ROBERTS & LOCKETT, 1975; ROBERTS & NEILSON, 1982; BASKIN & BASKIN, 1983a; FROUD-WILLIAMS, DRENNAN & CHANCELLOR, 1984). In determining the longevity, meaningful data can only be obtained from studies in which the seed is buried in the natural situation; results are therefore likely to provide a true reflection of events which occur in the field.

The achenes of *C. odorata* are the propagules for encroachment or re-infestation by this species. It was therefore necessary to make an attempt to determine the longevity of the achenes in the soil. Hence soil-burial studies were initiated. It was found that there was an initial steep decline in the percentage of achenes which germinated. Whether this was due to achene mortality or the development of secondary dormancy is not known. Although the number of achenes buried was sufficient to implement further investigations, the numbers recovered were low. This situation arose as a result of loss of achenes from the pockets, since following the rapid breakdown of the pappus during burial, the achenes were small enough (due to their shape) to fit through the pores of the gauze used for the pockets. Thus, insufficient material was obtained to investigate the relatively low germinability of buried achenes in comparison to that of achenes stored in the laboratory. However, since no marked decline in percentage germination occurred subsequently, it can be speculated that secondary dormancy, and not mortality, was responsible for

the relatively low percentage germination obtained. Support for this view is provided by YADAV & TRIPATHI (1982) who found that "induced" dormancy (secondary dormancy) occurred in achenes which had been buried. Cognizance should be taken of the fact that the achenes used had been after-ripened prior to burial and thus the restricted germinability was not due to primary dormancy. The development of secondary dormancy, as reviewed by KARSSEN (1980/81b) has been observed in the seeds of many species and since these often follow cyclical changes, seasonally induced changes confer an adaptive mechanism for survival as germination is prevented at periods unfavourable for seedling establishment (FROUD-WILLIAMS, DRENNAN & CHANCELLOR, 1984). *C. odorata* seedling emergence, as observed in the field, suggests that similar events may be involved. However, this was unsubstantiated in the present study.

The data obtained do however, reveal two important factors. Firstly, achenes survive for at least one year in the soil even though distinct erosion of the achene coat occurred (Plate 5). These results are contrary to those obtained by IVENS (1975) in which it was suggested that the achene life-span in the soil is relatively short (± five months). Plate 5C provides evidence for the second notable factor, the increased percentage germination which occurred under dark conditions after prolonged burial. It seems that the removal of the pericarp may be responsible for the higher percentage germination. Support for this proposal is gleaned from the experiment in which the pericarp was removed from achenes (section 2.4). Removal of the pericarp significantly improved germination in the dark in these experiments.

In summary, the results obtained for the burial study suggest that:

(i) the longevity of *C. odorata* achenes exceeds one year;

(ii) induction of secondary dormancy may occur as a result of enforced dormancy (that is, burial and thus, lack of light);

(iii) the erosion of the pericarp (probably microbial degradation) may promote germination in the dark but since germination is also governed by temperature, germination of achenes in the soil is likely to be restricted to periods of suitable temperature and sufficient moisture, and

(iv) site differences were noticed and thus the effect of burial may be dependent on locality.

YADAV & TRIPATHI (1982) found that, in India, no achenes were present at a depth greater than two centimetres. In the present study, no achenes were found below a depth of five centimetres. As evidenced by the numbers of achenes found in the soil samples, there is a large propagule reserve in the soil. Although the majority of these were on the soil surface (zero to one centimetre depth) a few achenes were present at a depth of two to five centimetres in the soil profile. The percentage germination of these achenes was low, probably due to both a large proportion of non-viable achenes (many were obviously empty) and possibly also a fairly large proportion of secondarily dormant achenes for the reasons

described for the achene-burial study. If indeed some of the achenes were secondarily dormant, then it is likely that achenes exhumed at various times would show a difference in germinability due to the cyclical changes in secondary dormancy observed in other species such as *Veronica hederifolia* L. (ROBERTS & LOCKETT, 1978); *Veronica arvensis* L. (BASKIN & BASKIN, 1983b); *Lamium amplexicaule* L. (BASKIN & BASKIN, 1981); *Aphares arvensis* L. (ROBERTS & NEILSON, 1982) and *Arabidopsis thaliana* (L.) Heynh. (BASKIN & BASKIN, 1983a), to mention a few. Although in the majority of these studies annual, agrestal weeds were investigated, similar results have been obtained for weeds, including perennials, of other habitats (ROBERTS, 1979; ROBERTS & CHANCELLOR, 1979; ROBERTS & NEILSON, 1981; ROBERTS & BODDRELL, 1984). Nevertheless, the germination obtained confirms that *C. odorata* achenes in and on the soil, provide an important propagule source for re-infestation. For example, the five per cent germination obtained for the zero to one centimetre layer of soil collected from site A is equivalent to approximately 12 000 seedlings per square metre nearly triple the 4 500 seedlings per square metre reported by IVENS (1974).

The presence of germinable achenes on the soil surface is also confirmation of the importance of phytochrome in controlling germination. The soil samples were collected from under a dense canopy where, as discussed in section 2.4, the incident radiation is likely to consist predominantly of light in the FR wavelength. Thus the germination of achenes likely to encounter severe intra-specific competition is restricted.

2.6 The Effect of Nitrogenous Compounds, Gibberellic Acid, Ethanol and Respiratory Inhibitors on Germination

The results obtained in section 2.5 showed that freshly harvested *C. odorata* achenes are generally primarily dormant or at least partially dormant. Therefore, a dormancy mechanism other than that of phytochrome as discussed in section 2.4, was operative during the after-ripening period which effectively inhibited germination. The nature of this dormancy mechanism was investigated in an attempt to elucidate its operation. In addition, the effect of the treatments applied on the germination of dark-incubated achenes was examined concurrently to provide information concerning the possible operation of phytochrome in these achenes.

Materials and Methods

For these experiments dry achenes harvested from Virginia Bush on 5th September 1984, were used. At the commencement of these experiments, the percentage germination of these achenes in apparently optimum conditions was low. The germinability was monitored continually by way of untreated controls (double-distilled water) which provided a basis for comparison with the various treatments in each of the experiments.

In the first experiment solutions of the following compounds were used to moisten the filter paper in the petri dishes:

(i) Potassium nitrite (KNO_2, nitrite);

(ii) Potassium nitrate (KNO_3, nitrate);

(iii) Thiourea ($CS(NH_2)_2$, thiourea);

(iv) Hydroxylamine hydrochloride ($NH_2OH.HC\ell$, hydroxylamine);

(v) Sodium azide (NaN_3, azide);

(vi) Ethanol (CH_3CH_2OH, ethanol); and

(vii) Gibberellic Acid (GA_3).

Four concentrations of each compound were prepared; these were, with the exception of GA_3, 0,1; 1,0; 10,0 and 100,0 mM. For GA_3 the concentrations were 0,001; 0,010; 0,100 and 1,000 mM. Where necessary, the pH of the solutions was adjusted with hydrochloric acid ($HC\ell$) or sodium hydroxide (NaOH) to 6,8. The achenes were incubated at 15/30 °C in the light (diurnal 12 hour cycle) or in the dark. Each treatment was comprised of 20 replicates of 10 achenes each.

This experiment was repeated at a later date (four months storage) when the dormancy of the achenes had been partly reduced by after-ripening. The same treatments and concentrations were applied and the achenes incubated under the same conditions. However, after 32 days when no further germination was recorded, all the dark treatments were transferred to the diurnal light cycle for an additional 32 day incubation period. Twenty replicates of 10 achenes each were used per treatment.

In another experiment which was aimed primarily at substituting the light requirement, achenes were imbibed in the dark on various solutions for restricted periods before

transfer under green safelight conditions, to petri dishes containing filter paper moistened with double-distilled water. The imbibition times were one, three and six hours. The treatment solutions were 0,1, 1,0 and 10,0 mM concentrations of nitrite, nitrate, azide and potassium cyanide (KCN, cyanide). Additional treatments consisted of achenes which were first imbibed with water for 24 hours in the dark before transfer, again under green safelight conditions, to petri dishes containing filter paper moistened separately with each of the treatment solutions. For comparison achenes were also incubated continuously on each of the treatment solutions in the dark. Ten replicates, each of 10 achenes, were used per treatment.

In a further attempt to substitute for the light requirement, the effect of various combinations of compounds on germination of achenes in the dark was tested. The treatments consisted of restricted and continuous imbibition on the solutions listed in Table 2.6.1. The treatments, comprised of solutions of single compounds or combinations of compounds, are also presented in Table 2.6.1. The concentrations of the treatments, as listed in Table 2.6.1, reflect the actual concentration of the solution on which the achenes were imbibed. The concentrations of the stock solutions were therefore prepared accordingly. Ten replicates, of 10 achenes each, were used per treatment.

The effect of salicylhydroxamic acid (SHAM) on germination was also investigated. Achenes were imbibed continuously on filter paper moistened with either 0,01; 0,10;

Table 2.6.1 The chemicals and treatments used in an attempt to stimulate germination of achenes in the absence of light.

Treatment soln. and conc. (mM)	Treatments*																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
KNO_2 10,0	xc																		
KNO_3 1,0	xc																		
CH_3CH_2OH 1,0	xc																		
$NH_2OH.HC\ell$ 0,1	xc																		
NaN_3 0,1	xc																		
GA_3 0,1	xc	xc							x1	x3	x6				xt		xt		
GA_3 1,0						x1	x3	x6				xt		xt					
$CS(NH_2)_2$ 0,1	xc																		
$CS(NH_2)_2$ 10,0		xc							x1	x3	x6					xt	xt		
$CS(NH_2)_2$ 100,0			x1	x3	x6							xt	xt						
dH_2O																		xc	xc

* N.B. x denotes solutions included in the treatment;

c denotes continuous incubation on the treatment solution(s) for the duration of the experiment;

1 achenes were imbibed on the treatment solution(s) for one hour and then transferred to double-distilled water for the remainder of the experiment;

3 similar to (1) but with a three hour imbibition period;

6 similar to (1) but with a six hour imbibition period;

t achenes were imbibed on double-distilled water for 24 hours and then transferred to the treatment solution(s) for the duration of the experiment.

1,00 or 10,00 mM solutions. The SHAM was dissolved in an alkaline solution of NaOH which was adjusted to pH 6,8 with dilute HCℓ before use. Germination was compared to that of distilled water controls incubated either in the light or in continuous dark. Twenty replicates, of 10 achenes each, were used per treatment.

Based on the results obtained for germination following treatment with the various chemical compounds, further germination tests were conducted to investigate the effect of combinations of azide, SHAM and nitrite on germination of primarily dormant achenes. The concentrations used were those found to promote the highest percentage germination in the previous germination tests. The concentrations and treatments used were:

(i) KNO_2 - 10,0 mM;

(ii) NaN_3 - 0,1 mM;

(iii) SHAM - 0,1 mM;

(iv) treatments (i)+(ii) - 10,0 mM KNO_2 + 0,1 mM NaN_3;

(v) treatments (i)+(iii) - 10,0 mM KNO_2 + 0,1 mM SHAM;

(vi) treatments (ii)+(iii) - 0,1 mM NaN_3 + 0,1 mM SHAM; and

(vii) treatments (i)+(ii)+(iii) - 0,1 mM KNO_2 + 0,1 mM NaN_3 + 0,1 mM SHAM.

The concentrations of the stock solutions were such that the concentrations listed above, were obtained in three cubic centimetres applied to wet the filter paper in the petri dishes. Where necessary, the solutions were adjusted to pH 6,8 using HCℓ or NaOH. Each treatment was comprised of 20 replicates of 10 achenes each.

In a further experiment, the effect of NaN_3 and SHAM on the germination of apparently non-dormant achenes (dry, 21/8/84; stored dry, in the dark, for six months at 25 °C) was investigated. The treatments applied to these achenes and the concentrations used are listed below:

(i) 0,1 mM NaN_3;

(ii) 1,0 mM NaN_3;

(iii) 10,0 mM NaN_3;

(iv) 0,1 mM SHAM;

(v) 1,0 mM SHAM;

(vi) 10,0 mM SHAM;

(vii) 0,1 mM NaN_3 + 0,1 mM SHAM;

(viii) 0,1 mM NaN_3 + 1,0 mM SHAM;

(ix) 0,1 mM NaN_3 + 10,0 mM SHAM;

(x) 1,0 mM NaN_3 + 0,1 mM SHAM;

(xi) 1,0 mM NaN_3 + 1,0 mM SHAM;

(xii) 1,0 mM NaN_3 + 10,0 mM SHAM;

(xiii) 10,0 mM NaN_3 + 0,1 mM SHAM;

(xiv) 10,0 mM NaN_3 + 1,0 mM SHAM;

(xv) 10,0 mM NaN_3 + 10,0 mM SHAM; and

(xvi) distilled water control.

Twenty replicates of 10 achenes each were used per treatment.

Results

The effect of application of various chemicals on the germination of primarily dormant achenes is shown in Figure 2.6.1A to 2.6.1G as compared to that of the distilled water

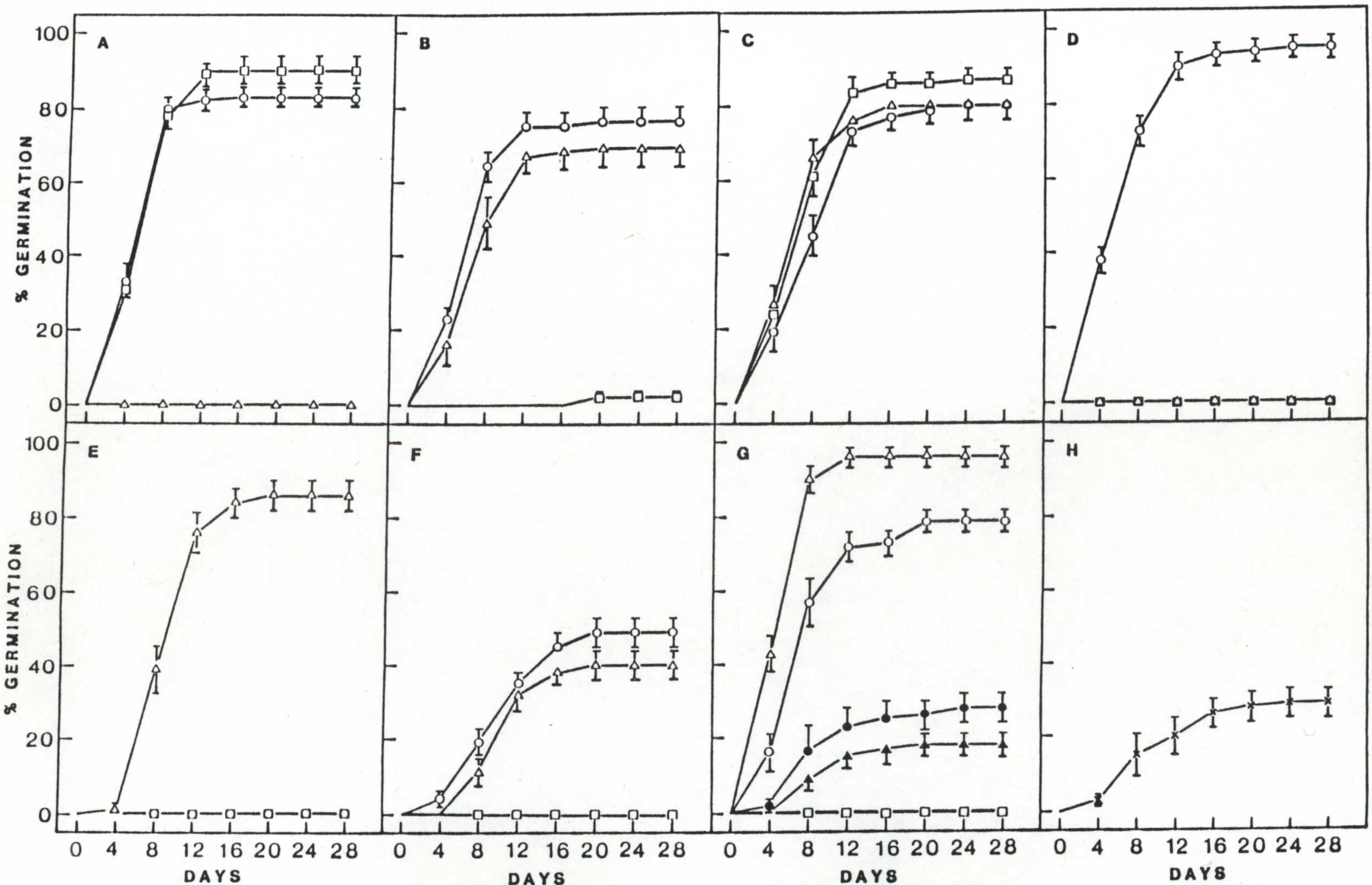

Figure 2.6.1 The germination response of dormant achenes to (A) nitrite, (B) nitrate, (C) thiourea, (D) hydroxylamine, (E) azide, (F) ethanol, (G) GA_3 and (H) double-distilled water. Open symbols represent germination in the light and closed symbols germination in the dark. (△ 0,1 mM; ○ 1,0 mM; □ 10,0 mM and ▽ 100 mM solutions; × H_2O. NB. GA_3 concentrations were △ 0,001 mM; ○ 0,01 mM; □ 0,1 mM and ▽ 1,0 mM).

control (Figure 2.6.1H). Firstly, it is pertinent to note that all the 100 mM concentrations of the various chemicals completely prevented germination (this was also the case for 10 mM GA_3); to avoid confusion, none of these are displayed in Figure 2.6.1. and will not be referred to again. Secondly, the only germination recorded in the dark was with the 0,001 and 0,010 mM concentrations of GA_3 (Figure 2.6.1G, closed symbols). Thirdly, many of the treatments greatly improved germination over that of the distilled water control (Figure 2.6.1H). The results for each chemical will be discussed in relation to the control.

Nitrite concentrations of 1,0 and 10,0 mM resulted in greater than 80 per cent germination; no germination was recorded for the 0,1 mM treatment (Figure 2.6.1A). Nitrate, at a concentration of 0,1 or 1,0 mM improved germination while the 10,0 mM concentration appreciably inhibited germination (Figure 2.6.1B). The 0,1; 1,0 and 10,0 mM concentrations of thiourea improved germination and resulted in greater than 80 per cent germination. Only the 1,0 mM hydroxylamine treatment promoted germination (95 per cent after 28 days incubation); no germination was obtained with the 0,1 and 10,0 mM treatments (Figure 2.6.1D). Although 1,0 and 10,0 mM concentrations of azide completely inhibited germination, the 0,1 mM treatment provided for 90 per cent germination (Figure 2.6.1E). With ethanol, 0,1 mM and 1,0 mM concentrations slightly promoted germination but no germination was obtained with the 10,0 mM treatment (Figure 2.6.1F). GA_3 applied at a 0,001 or 0,010 mM concentration stimulated germination but

no germination was recorded in the 1,0 mM treatment.

In a repeat of the above experiment but with achenes stored for four months, similar trends in the germination were observed (Figures 2.6.2A, 2.6.2B and 2.6.2C). In the initial incubation period, the highest concentrations of the chemicals again generally inhibited germination although in certain instances some germination was obtained. As in the earlier experiment, germination in the dark was slightly promoted by GA_3 (Figure 2.6.2Biii) and additionally, by thiourea (Figure 2.6.2Aiii). The promotive effect on germination by certain compounds during the initial incubation period was however, not as noticeable as was the case in the earlier experiment. This was largely as a result of the improved percentage germination obtained in the distilled water control and consequently, inhibition of germination occurred in some treatments.

Upon transfer of achenes to the light following the initial 32 day incubation period, certain notable results were obtained. Firstly, percentage germination of the distilled water control was lower than that of the control experiencing the diurnal light cycle during the initial incubation period. Secondly, certain treatments provided for significantly higher germination percentages of achenes transferred to the light than the control subjected to the light transfer. These treatments included: 0,1 mM nitrite (Figure 2.6.2Ai); 0,1 mM thiourea (Figure 2.6.2Aiii); 0,1 and 1,0 mM hydroxylamine (Figure 2.6.2Bi); 0,1 mM ethanol (Figure 2.6.2Bii); 0,1 mM GA_3 (Figure 2.6.2Biii) and 0,1 mM azide (Figure 2.6.2C)

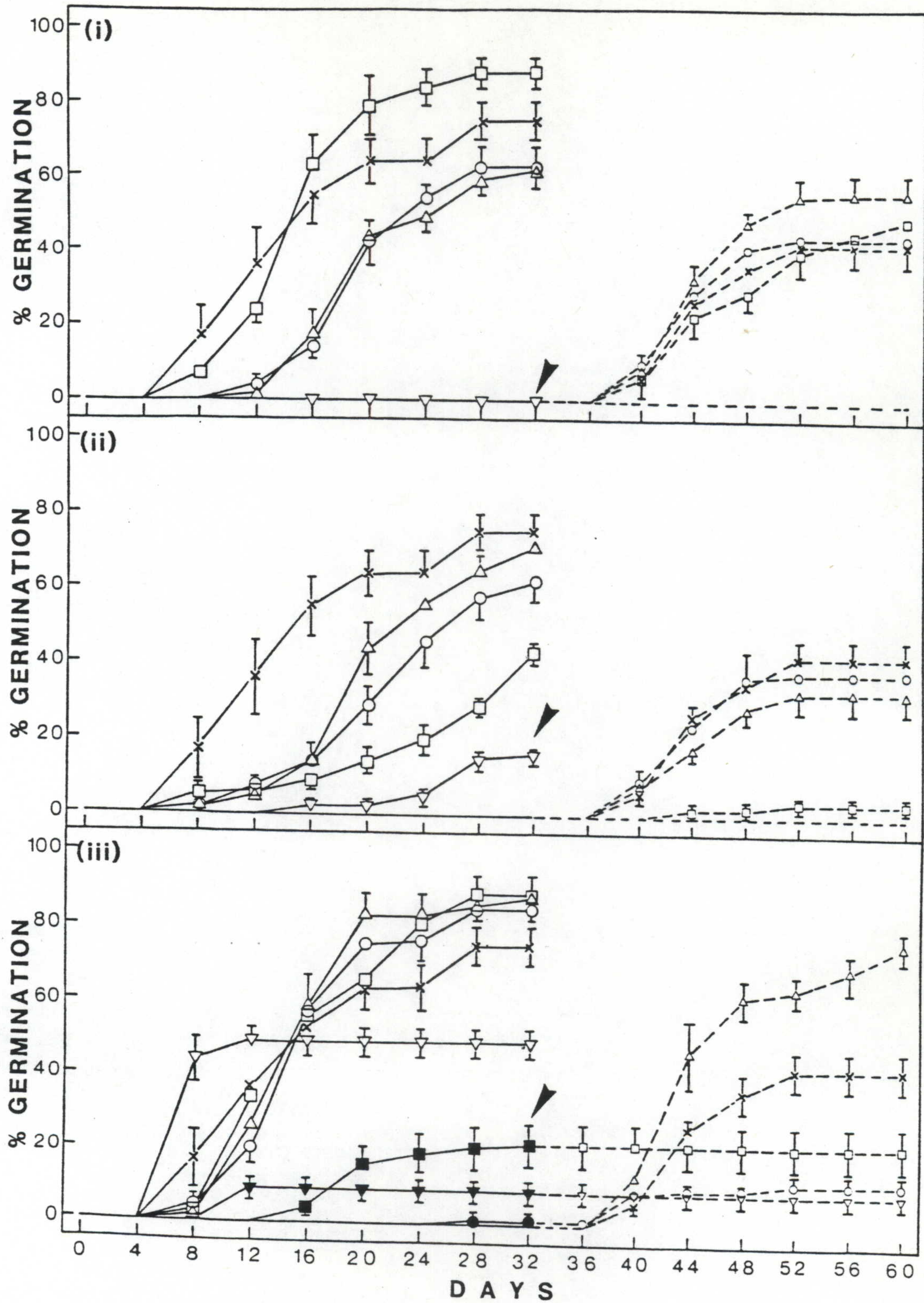

Figure 2.6.2A The effect of (i) nitrite (ii) nitrate and (iii) thiourea on the germination of partially dormant achenes in the light (open symbols) and in the dark (closed symbols). On day 32, achenes incubated in the dark were transferred to the light (broken lines). (△ 0,1 mM; ○ 1,0 mM; □ 10,0 mM and ▽ 100,0 mM solutions; X H_2O).

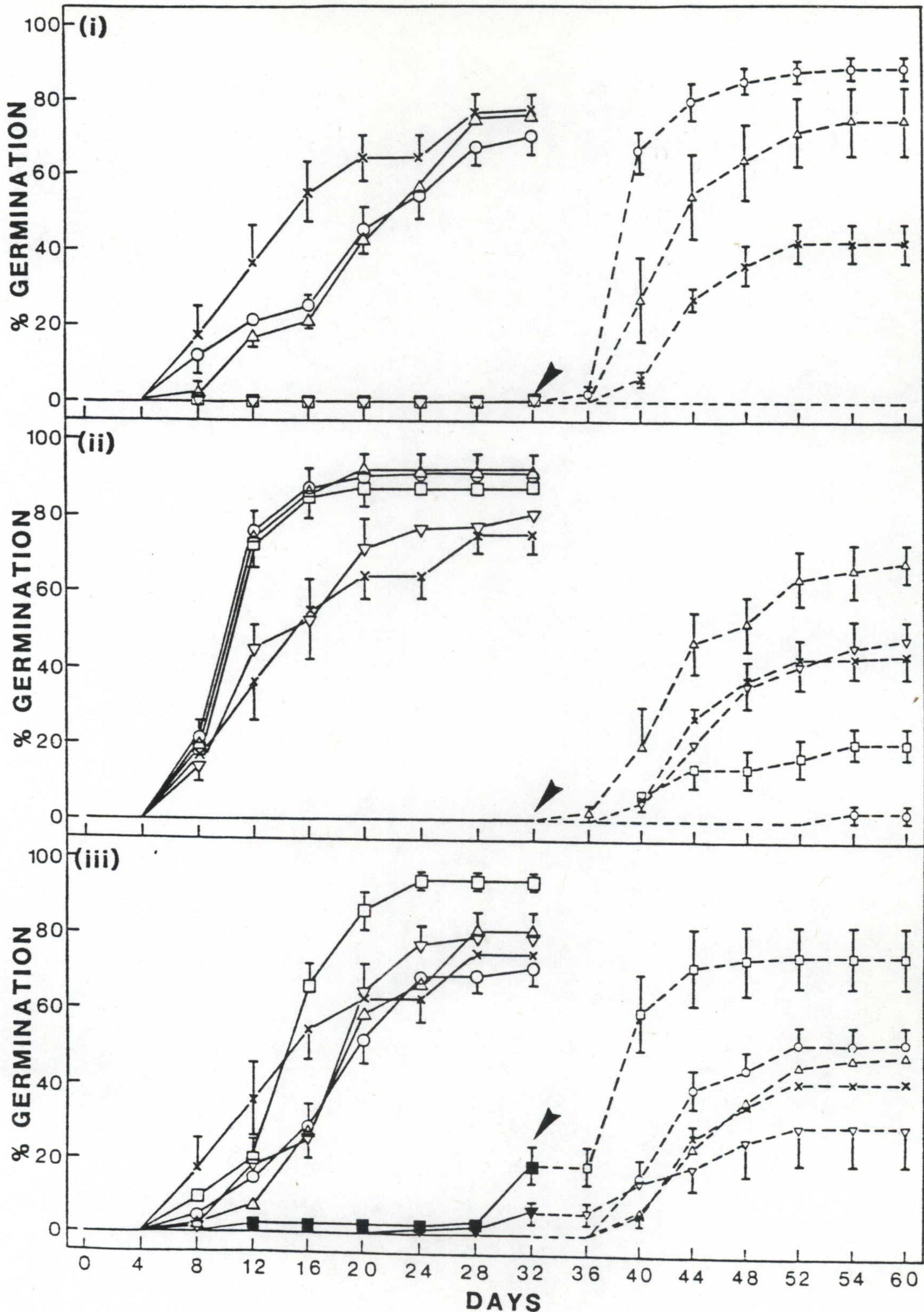

Figure 2.6.2B The effect of (i) hydroxylamine (ii) ethanol and (iii) GA_3 on the germination of partially dormant achenes in the light (open symbols) and in the dark (closed symbols). On day 32, achenes incubated in the dark were transferred to the light (broken lines). (△ 0,1 mM; □ 1,0 mM; ○ 10,0 mM and ▽ 100,0 mM solutions; × H_2O. NB. GA_3 concentrations were △ 0.001 mM; ○ 0,01 mM; □ 0,1 mM and ▽ 1,0 mM).

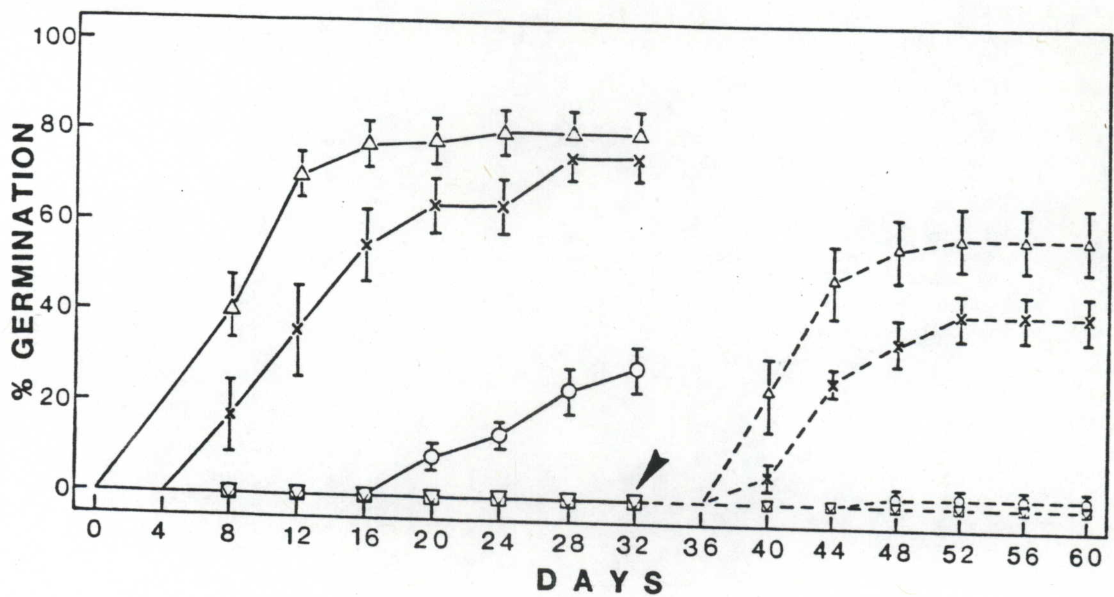

Figure 2.6.2C The effect of azide on the germination of partially dormant achenes in the light. On day 32, achenes incubated in the dark were transferred to the light (broken lines). (△ 0,1 mM; ○ 1,0 mM; □ 10,0 mM; ▽ 100,0 mM).

Having confirmed the promotive effect of some of the compounds, two (0,1 mM azide and 0,1 mM GA_3) were selected for testing on the germination of dormant achenes in the storage experiment described in section 2.5. On completion of the incubation period (24 days) of the achenes which had been stored for two months (Figure 2.5.3B), half the replicates of each group were tranferred to filter paper moistened with three cubic centimetres of 0,1 mM azide and the other half to filter paper moistened with three cubic centimetres of 0,1 mM GA_3. The results obtained are shown in Figure 2.6.3A and Figure 2.6.3B. The azide treatment markedly promoted germination of all three groups of achenes which had been stored at 25 °C (Figure 2.6.3Ai) and the dry and medium achenes stored at -18 °C (Figure 2.6.3Aii). A slight improvement in percentage germination of the green achenes stored at -18 °C also occurred. Transfer to GA_3 only slightly improved germination of all achenes (Figures 2.6.3B).

Imbibition of achenes on the various solutions for restricted periods did not improve germination in the dark (Table 2.6.2). Only one per cent germination resulted from some of the solutions and thus this was not considered to be significant. In the water controls, 49 per cent of the achenes had germinated after 24 days incubation.

The results obtained for the treatments outlined in Table 2.6.1 are presented in Table 2.6.3. As in the previous experiment, little noticeable germination occurred in the majority of treatments. Only one treatment provided significantly higher germination than the dark controls. This

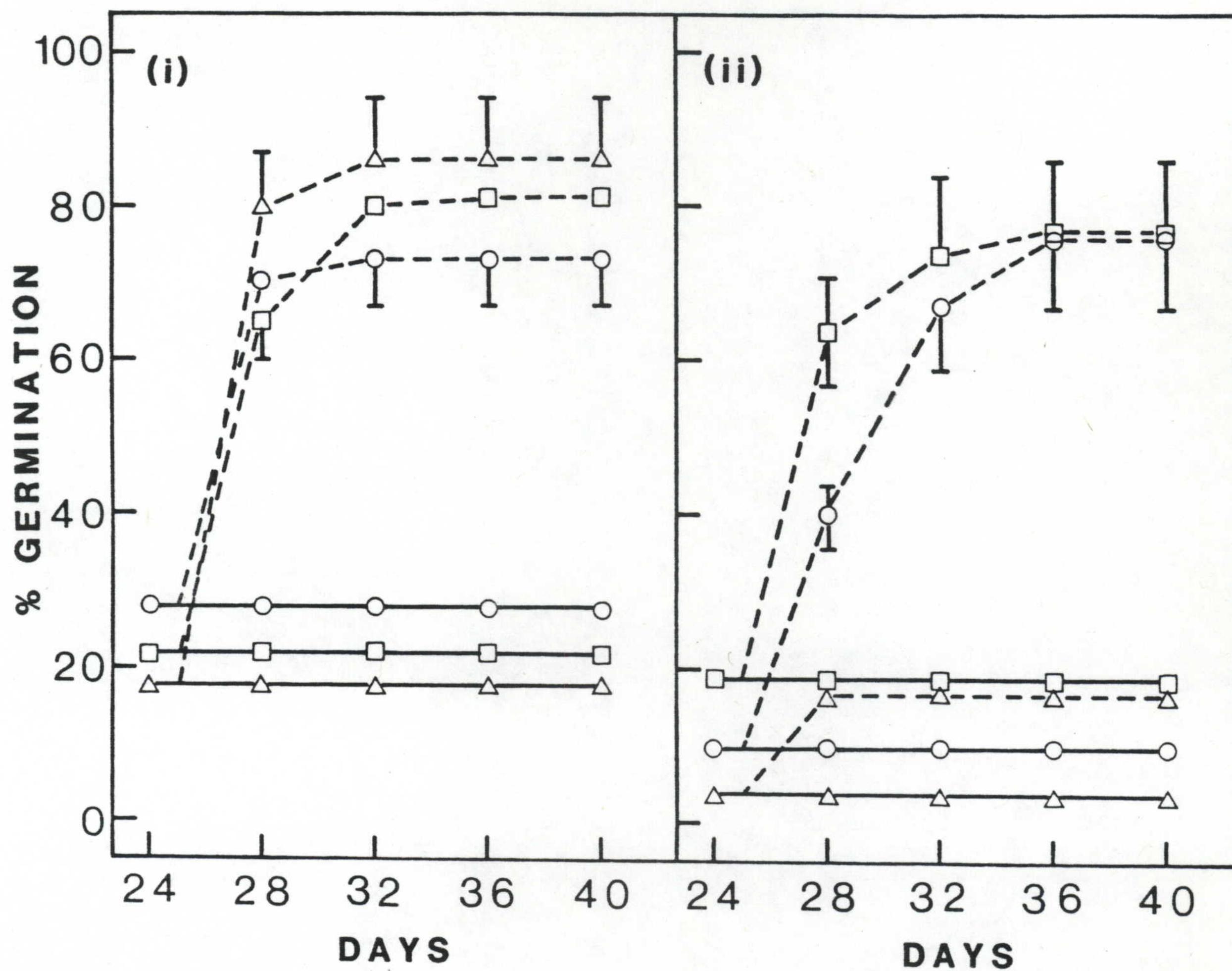

Figure 2.6.3A The effect of 0,1 mM azide application (broken lines) on the germination of green, medium and dry achenes incubated in the light for 24 days subsequent to two months storage in (i) 25 °C and (ii) - 18 °C. Solid lines represent germination of achenes not treated with azide.
(△ green; □ medium; ○ dry).

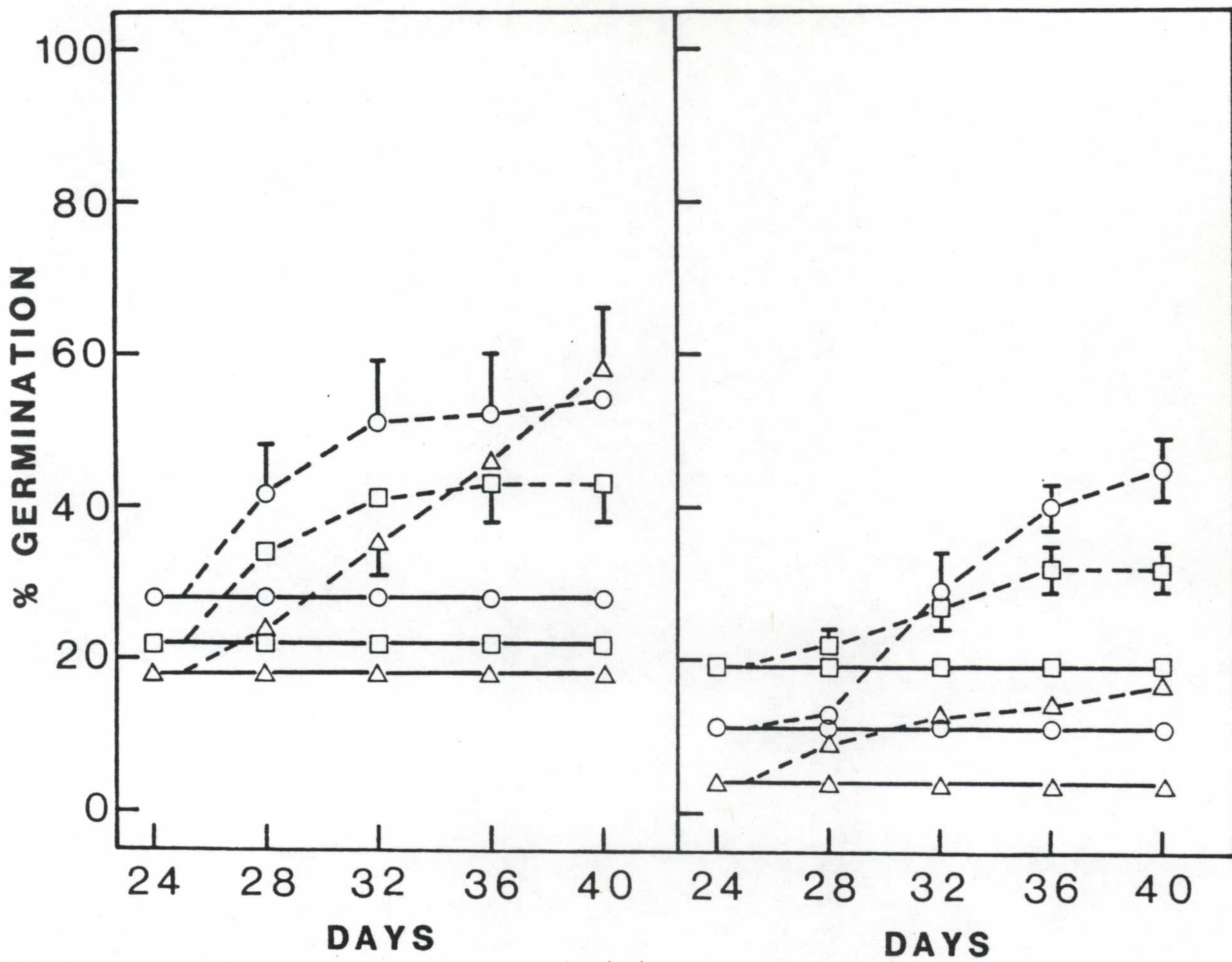

Figure 2.6.3B The effect of 0,1 mM GA_3 application (broken lines) on the germination of green, medium and dry achenes incubated in the light for 24 days subsequent to two months storage in (i) 25 °C and (ii) - 18 °C. Solid lines represent germination of achenes not treated with GA_3.
(△ green; □ medium; ○ dry).

Table 2.6.2 Cumulative percentage germination of achenes imbibed for restricted periods on filter paper moistened with either azide, cyanide, nitrate or nitrite and incubated in the dark at 15/30 °C.

	Percentage Germination					
	Days Incubation					
Treatment	4	8	12	16	20	24
Light, H_2O	2	24	40	48	48	49
1h imb. 0,1 mM NaN_3 H_2O		1	1	1	1	1
3h imb. 10,0 mM KNO_2 H_2O				1	1	1
3h imb. 1,0 mM KCN H_2O				1	1	1
3h imb. 0,1 mM KNO_2 H_2O		1	1	1	1	1
6h imb. 1,0 mM KNO_3 H_2O				1	1	1
6h imb. 10,0 mM KNO_2 H_2O	1	1	1	1	1	1
24h imb. H_2O 10,0 mM KCN				1	1	1

Table 2.6.3 The effect on achene germination of restricted imbibition periods on various compounds applied separately or in combination (mean±S.E.). Achenes were incubated at 15/30 °c in the dark unless otherwise indicated.

Treatment[1,2]	Percentage Germination
1. $KNO_2+KNO_3+CH_3CH_2OH+NH_2OH.HC\ell+NaN_3+GA_3+CS(NH_2)_2$	3±2
2. $CS(NH_2)_2+GA_3$	6±3
7. GA_3	4±2
8. GA_3	4±2
11. $GA_3+CS(NH_2)_2$	3±2
12. $GA_3+CS(NH_2)_2$	17±3
13. $CS(NH_2)_2$	1±1
19. Control, diurnal light cycle	70±6
L.S.D. (p = 0,05)	9

[1] the treatment number according to Table 2.6.1 is provided to enable clarification of the exact treatment applied.

[2] only treatments in which germination occurred are presented.

treatment consisted of transferring the achenes onto a solution containing 1,0 mM GA_3 and 100,0 mM thiourea following an initial imbibition period of 24 hours on distilled water. The effect of the two compounds was synergistic since little or no germination occurred where they were applied separately (zero per cent for 1,0 mM GA_3 and one per cent for 100,0 mM thiourea).

Imbibition of achenes on a solution of 10,00 mM SHAM totally inhibited germination of achenes (Figure 2.6.4). A 1,00 mM concentration of SHAM improved the percentage germination over that of the control, but this improvement was not significant. Both the 0,01 and 0,10 mM concentrations resulted in significantly improved germination of achenes.

As illustrated in Figure 2.6.5A, azide, nitrite and SHAM when applied separately, resulted in germination percentages significantly higher than that of the control achenes. Germination in the dark (results not presented) was less than five per cent in all treatments.

Imbibition of achenes on solutions consisting of mixtures of azide, SHAM and nitrite also resulted in germination percentages which were significantly higher than that of the control (Figure 2.6.5B). Again, germination in the dark was less than five per cent in all treatments (results not presented).

The effect of azide and SHAM, separately and in combination, on the germination of after-ripened achenes is shown in Figure 2.6.6. Only treatments in which germination occurred are included. The other treatments which were applied com-

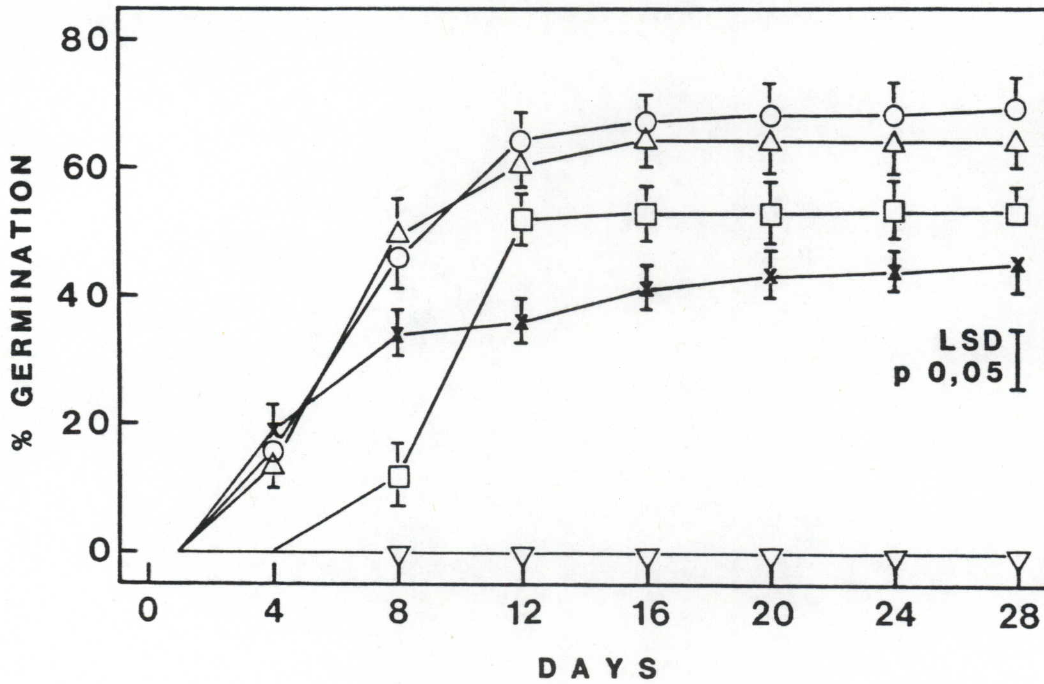

Figure 2.6.4 The effect of a range of SHAM concentrations on the germination of dormant achenes in the light. (x control; △ 0,01 mM; ○ 0,10 mM; □ 1,00 mM; ▽ 10,00 mM).

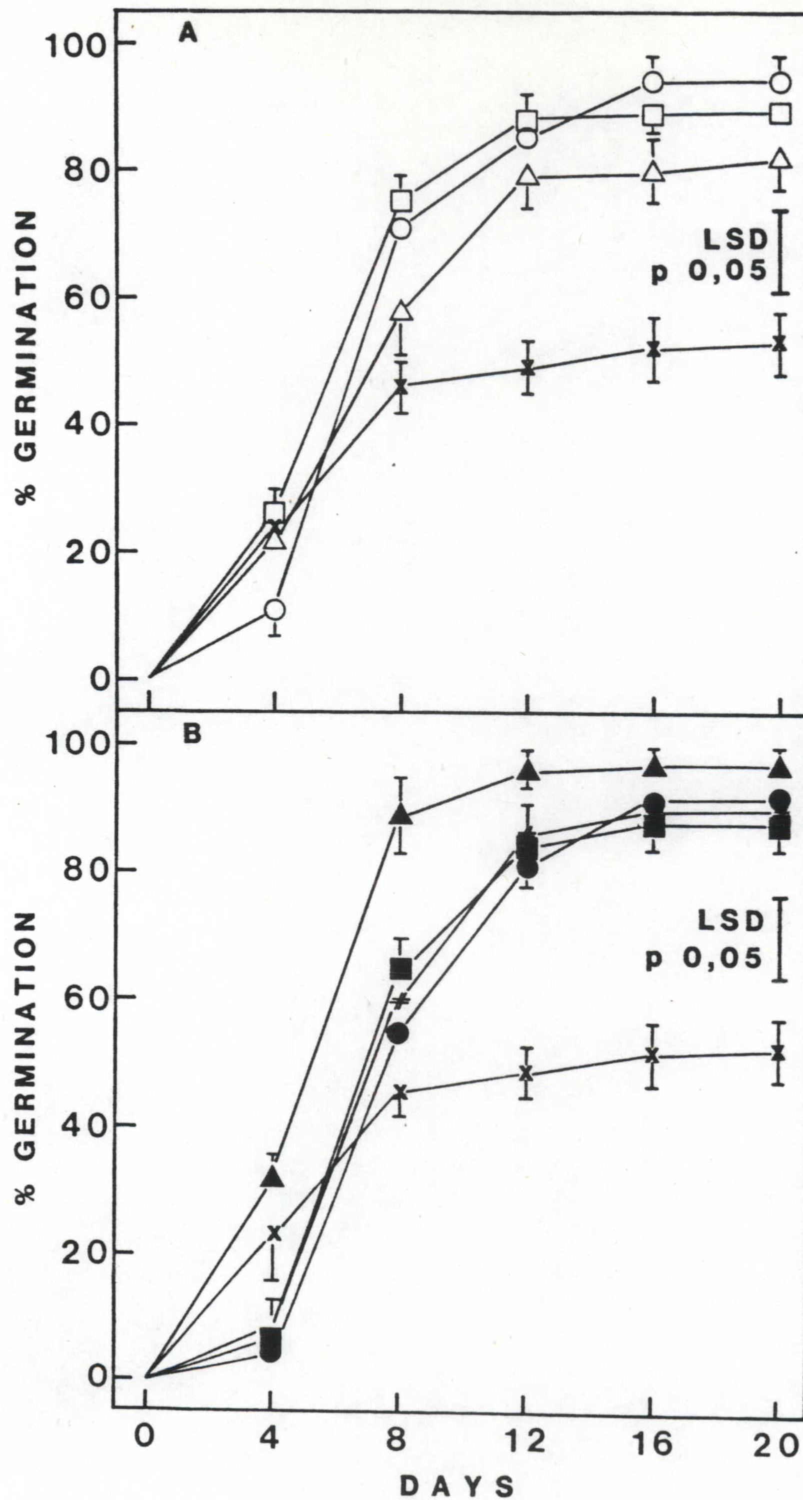

Figure 2.6.5 The effect of azide, SHAM and nitrite on the germination of dormant achenes in the light; (A) compounds applied separately; (B) compounds applied in combination. (x dH_2O; ○ azide; △ SHAM; □ nitrite; ▲ SHAM + nitrite; ● SHAM + azide; ■ nitrite + azide; # nitrite + azide + SHAM).

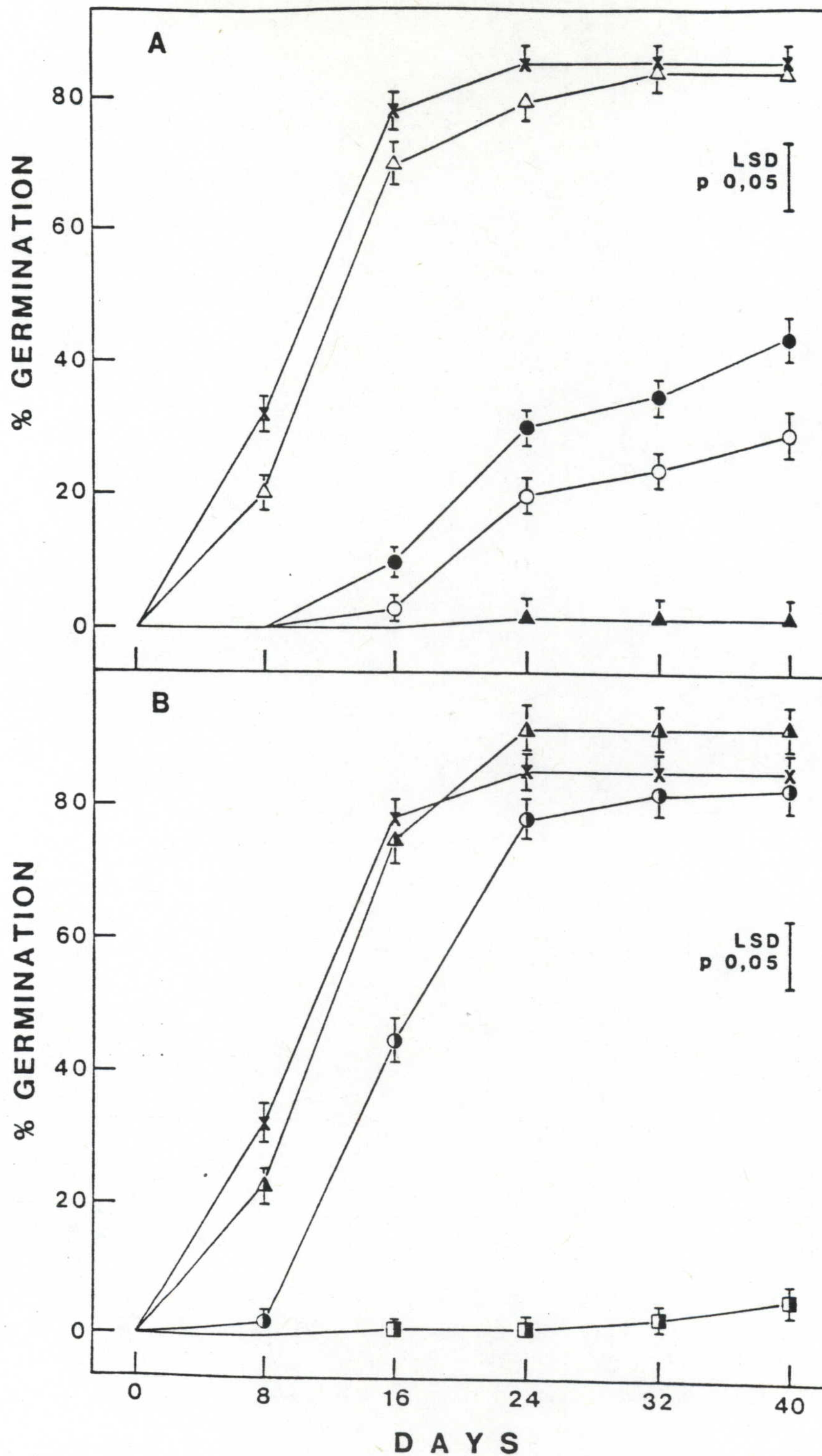

Figure 2.6.6 The effect of azide and SHAM on the germination of after-ripened, non-dormant achenes. (A) Compounds applied separately (X control; △ 0,1 mM azide; ▲ 0,1 mM SHAM; ○ 1,0 mM azide; ● 1,0 mM SHAM). (B) Compounds applied in combination (x control; ▲ 0,1 mM azide + 0,1 mM SHAM: ◑ 0,1 mM azide + 1,0 mM SHAM; ◨ 0,1 mM azide + 10,0 mM SHAM).

pletely inhibited germination. The 0,1 mM azide treatment did not significantly alter the germination of the achenes (Figure 2.6.6A) while the other concentrations severely inhibited germination. All concentrations of SHAM inhibited germination; the least inhibitory treatment was the 0,1 mM SHAM concentration (Figure 2.6.6A).

Combined application of 0,1 mM azide and SHAM did not inhibit germination. With the 0,1 mM azide + 1,0 mM SHAM solution, germination was inhibited initially but final percentage germination was similar to that of the distilled water control. The 0,1 mM azide + 10,0 mM SHAM solution significantly inhibited germination; only six per cent of the achenes germinated. It is pertinent to note that with the combined azide - SHAM treatments, any solution containing a concentration of greater than 0,1 mM azide completely inhibited germination (results not presented).

Discussion

As described in section 2.1 considerable use has been made of various compounds said to stimulate the germination of dormant seeds; this information has also been used to elucidate the dormancy mechanisms operative in controlling germination. In a number of cases, these compounds have also been found to promote germination of light-requiring seeds in the dark. The effect of the compounds on the germination of *C. odorata* achenes in the dark will be discussed before examining the possible dormancy mechanism in non-after-ripened achenes.

Germination in the dark was only obtained with the following treatments: GA_3 in two experiments, thiourea in one experiment and thiourea combined with GA_3 in another experiment. However, in all these cases the percentage germination was low.

The stimulation of germination of light-requiring seeds in the dark with gibberellins has been demonstrated for lettuce seeds (IKUMA & THIMANN, 1963) and for *Asteracantha longifolia* Nees (CHAWAN & SEN, 1970). Although these results may indicate that gibberellins substitute for the light requirement and therefore for Pfr action, it has been suggested that in fact, the action of gibberellins is synergistic with suboptimal levels of pre-existing Pfr in the seeds (FREDERICQ, ELDABH, DE GREEF & MATON, 1975). Consequently, the threshold levels of Pfr required to induce germination are lowered in much the same way as described for the promotive effect of elevated temperature shifts. A similar effect of GA_3 in the stimulation of *C. odorata* achene germination may have been operative in the experiments where improved germination occurred. Although GA_3 did not stimulate germination in some instances it is possible that the pre-existing Pfr levels were too low to elicit a response. Another possibility is that, had a different gibberellin been applied, germination may have occurred since BIANCO & BULARD (1981) found a significant increase of free GA_9 levels in lettuce seeds following short irradiation of the seeds with fluorescent or R light. Furthermore, DEDONDER, RETHY, FREDERICQ & DE GREEF (1983) found that a combination of GA_4 and GA_7 increased the sensitivity of

K. blossfeldiana seeds to R irradiation thereby implicating the involvement of other gibberellins in the stimulation of germination of light-requiring seeds. In addition, these authors found that only a small fraction of GA_3 penetrated the seed coat and thus only a limited amount reached the embryo, the site of expected physiological activity. JONES & STODDART (1977) questioned interpretations based on applied gibberellins because the effects may be pharmacological and, therefore, unrelated to the normal developmental processes in seeds.

It seems that the precise mode of action of gibberellins in promoting germination is not clear. Several sites of possible gibberellin action have been identified which may be of importance in dormancy breakage. Initially it was believed that dormancy breakage was due to rapid hydrolysis of starch and the formation of reducing sugars due to production of α-amylase in response to GA_3 application (MAYER & POLJAKOFF-MAYBER, 1982). However, POLLARD (1969) found that GA_3 stimulated the production of a number of enzymes prior to its effect on α-amylase. On the basis of the current state of knowledge it is likely that the effect of gibberellin on mobilisation of storage material in the endosperm is not the mechanism whereby germination of dormant seeds is initiated (MAYER & POLJAKOFF-MAYBER, 1982) especially since, in some species, low concentrations of GA_3 induce amylase formation but fail to break dormancy (CHEN & PARK, 1973). In reviews concerning gibberellins and dormancy breakage, both JONES & STODDART (1977) and MAYER & POLJAKOFF-MAYBER (1982) concluded

that although a great number of responses to gibberellins in seeds have been observed, the primary events in their action are still unknown.

Concerning the possible substitution of the Pfr requirement by gibberellins in light-requiring seeds, the evidence provided in the literature on the topic strongly opposes the likelihood that Pfr merely promotes the production of gibberellins which are then responsible for actual triggering of germination. This is because the promotive effect of the gibberellins can be reversed with FR light at a time after increases in endogenous gibberellin levels have occurred (BEWLEY & BLACK, 1982). However, TAYLORSON & HENDRICKS (1976) found that gibberellin-enhanced germination of *Lamium amplexicaule* L. seeds could be partially reversed by FR irradiation. A possible common mode of action of endogenous gibberellins and Pfr is that gibberellins have been found to modify membranes, for example, by changing the permeability of membranes towards glucose (WOOD, PALEG & SPOTSWOOD, 1974); similarly, Pfr action may partially be on membranes (TAYLORSON, 1982; 1984). Therefore an interaction between gibberellin and the triggering of germination of light-requiring seeds, cannot be excluded. WAREING (1982) suggested that the effect of R light on the endogenous hormone levels of seeds may be a "necessary but not sufficient" condition for germination, especially since evidence has been provided for the action of phytochrome on membrane permeability. WAREING (1982) further suggested that the action of phytochrome on membranes may affect compartmentation in the cell and that Pfr may bring enzymes and substrates

together where they were previously separated.

To clarify the gibberellin/Pfr interaction in seeds generally, certain specific aspects require further detailed investigation, for example, the mode of action of Pfr, especially at the membrane level. However, for *C. odorata* achenes no further investigations were embarked upon, because of the relatively indistinctive response to GA_3. But, because thiourea was also found to improve slightly germination in the dark, the combined effect was briefly tested. Thiourea alone has been found to stimulate germination of light-requiring seeds in the dark (MAYER & POLJAKOFF-MAYBER, 1982). Although not yet established, these authors also suggested that thiourea might act by changing the level or activity of the growth promotors or inhibitors. Thiourea has also been found to promote germination of dormant seeds of many species as described in section 2.1. The promotive effect of GA_3 and thiourea (in combination) on dark germination of *C. odorata* achenes showed a synergistic response. The particular treatment in which improved germination occurred, consisted of a 24 hour water imbibition period prior to transfer to the treatment solutions. In other treatments using similar concentrations of both compounds in combination, achenes were imbibed on the treatment solutions before transfer to water; no improvement in percentage germination occurred. It would therefore seem that these compounds are not necessary in the early stages of imbibition. The response obtained is suggestive of a system in which hydration of phytochrome, of intermediates and/or pre-existent Pfr, was first necessary; there-

after an interaction between sub-optimal levels of Pfr and the applied compounds resulted in triggering of germination of a small percentage (17 per cent) of the achenes.

The attempts aimed at promoting germination of *C. odorata* in the dark were based on the hypothesis that Pfr, produced in reaction to a suitable light stimulus, acted on or with certain factors which triggered germination and that the application of either one, or a combination of compounds known to improve germination of dormant seeds, would result in a set of circumstances within the *C. odorata* achenes, resembling the Pfr trigger. In so doing it would thus be possible to provide further information concerning the mode of action of phytochrome in stimulating germination of positively photoblastic seeds. However, such results were not forthcoming although a comparatively broad selection of treatments was employed. It therefore seems that, in *C. odorata* achenes, the mode of action of Pfr is dissimilar to that of the compounds tested, which, as discussed below, resulted in the removal of dormancy of the achenes. Although no further information has been provided to clarify the mode of action of Pfr, the results obtained indicate that either (a) Pfr does not operate by way of the respiratory pathways or that (b) an additional trigger prior to changes in the respiratory pathways is necessary.

As described in the previous section (section 2.5), a definite after-ripening period was required to promote germination of certain categories of freshly harvested achenes. The nature of the block restricting germination

was investigated to identify the reason(s) for the low percentage germination obtained during the after-ripening period.

Clearly, dormancy could be removed with any one of a number of compounds if applied at the required concentration. The possible role of these compounds in breaking of dormancy of *C. odorata* achenes will be discussed. As illustrated in Figure 2.1.3 in section 2.1, there are three possible outlets for substrate (usually glucose) metabolism, namely the PP pathway, the cytochrome electron transport chain (azide-sensitive pathway) and the alternative respiratory pathway (SHAM-sensitive pathway). The majority of the compounds tested on *C. odorata* achenes in this study have been implicated in at least one of these pathways by various authors.

Although several researchers have questioned the likelihood of the PP pathway being responsible for dormancy removal, this pathway cannot yet be excluded as one of the possibilities. Even though it is reported that convincing or confirmatory evidence supporting the involvement of the PP pathway has not been forthcoming (BEWLEY, 1979), evidence against its involvement has been limited. Considerable support for the PP pathway involvement in dormancy breakage was provided by the interpretation of results obtained by HENDRICKS & TAYLORSON (1975). The results obtained indicated that inhibition of catalase activity spared metabolically derived hydrogen peroxide from decomposition . The hydrogen peroxide is thought to result in oxidation of NADPH which is required as the oxidant in stimulating PP pathway activity. The proposed mechanism is especially attractive since it is

promoted by such compounds as thiourea, nitrates and even the respiratory inhibitor cyanide. In addition, this hypothesis has many similarities to the hypothesis proposed by ROBERTS & SMITH (1977).

Some evidence has been provided which has reduced support for the involvement of the PP pathway during dormancy removal. Firstly, the measurements used for determining increased PP pathway activity during dormancy breakage have been questioned (AP REES, 1980); secondly, some of the results obtained have implicated a decrease in PP pathway activity during germination (ADKINS & ROSS, 1981; FUERST, UPADHYAYA, SIMPSON, NAYLOR & ADKINS, 1983); and thirdly, the changes in PP pathway activity observed by some researchers might, in fact, be due to PP pathway involvement in the actual germination process. That is, the PP pathway might only come into operation following an initial trigger and is therefore a result of, rather than a cause of, dormancy breakage.

In section 2.1 an hypothesis more recently proposed than the PP pathway was discussed, namely the azide-sensitive and/or SHAM-sensitive pathways (Figure 2.1.3). Briefly, this hypothesis refers to the balance in respiration occurring via these two pathways and is based on the assumption that the former is inhibited by azide (and cyanide) whilst being insensitive to the hydroxamic acids, and the latter is inhibited by hydroxamic acids but insensitive to azide (and cyanide).

The germination tests conducted on *C. odorata* achenes

neither prove nor disprove the PP pathway or the azide-sensitive and/or SHAM sensitive pathway hypothesis. However, circumstantial evidence implicates the involvement of the azide-sensitive and/or SHAM-sensitive pathway hypothesis. The results showed that at low concentrations, either azide or SHAM stimulated germination of dormant achenes, as did several nitrogenous compounds (thiourea, nitrate, nitrite and hydroxylamine). The precise site of action of the nitrogenous compounds is debatable (that is either as promoters of the PP pathway or electron acceptors in the SHAM-sensitive pathway) and they were therefore omitted from further experiments aimed at elucidating which respiratory pathway might be involved in the removal of dormancy of non-after-ripened *C. odorata* achenes.

Both the azide-sensitive and SHAM-sensitive pathways of respiration appear to be involved in the removal of dormancy in *C. odorata*. High concentrations (> 0,1 mM) of either compound (azide or SHAM) inhibited germination, while the lower concentration (0,1 mM) significantly promoted germination. When applied in combination at low concentrations (0,1 mM) no significant improvement in percentage germination was observed over that of the achenes to which the compounds were applied separately, probably because of the already high percentage germination obtained in this case. Additionally, a low concentration of either compound plus a high concentration of the other, resulted in a reduced percentage germination, which seems to support the view that both the cytochrome oxidase (azide-sensitive) and the alternate (SHAM-sensitive) pathways

are involved in dormancy removal.

Further confirmation of the combined action of the two compounds was obtained where these were applied to non-dormant achenes (Figures 2.6.6A and 2.6.6B). The previously promotive concentration of 0,1 mM azide did not inhibit germination but SHAM, at the same concentration, and one which previously promoted germination of primarily dormant achenes, did inhibit germination. However, when applied in combination, the percentage germination obtained was even higher than that of the control treatment.

The results obtained in the two experiments in which the combined effect on germination of azide and SHAM was investigated suggest that a balance in the activity of both the pathways (azide-sensitive and SHAM-sensitive) results in dormancy breakage. Furthermore, it seems that regulation of the SHAM-sensitive pathway performs the major role since, 0,1 mM SHAM inhibited germination of non-dormant achenes while the response to 0,1 mM azide was similar to that of the control.

This hypothesis, based on circumstantial evidence, for the control of *C. odorata* achenes is similar to that proposed for both *A. fatua* and for cocklebur seeds. In *A. fatua* seeds, UPADHYAYA, NAYLOR & SIMPSON (1982) suggested that SHAM-sensitive respiration was necessary for the stimulation of germination of dormant seeds in the presence of azide. These results were confirmed by investigating the oxygen uptake following application of SHAM and/or azide (UPADHYAYA, NAYLOR & SIMPSON, 1983). ESASHI, KUSUYAMA, TAZAKI & ISHIHARA (1981)

concluded that in secondarily dormant cocklebur seeds, an appropriate balance between the cytochrome (azide-sensitive) and alternative (SHAM-sensitive) fluxes was required to induce germination.

The discovery and involvement of the SHAM- sensitive respiratory pathway was described in section 2.1. There are however, certain factors concerning this pathway which need clarification. Firstly, YENTUR & LEOPOLD (1976) found that in *G. max* seeds there was a transition from predominantly SHAM-sensitive respiration to azide-sensitive respiration during the early stages of germination. It was found that six other unrelated species had a similar requirement. YENTUR & LEOPOLD (1976) proposed that the SHAM-sensitive pathway provided something essential for the completion of the earliest stages of seed germination. YU, MITCHELL, YENTUR & ROBITAILLE (1979) found that the promotive effect of SHAM on germination of lettuce seeds was particularly noticeable if applied during the first eight hours following R light treatment. On the basis of these results it was suggested that SHAM-sensitive respiration and/or other, as yet unknown, SHAM- sensitive processes are required for light-mediated potentiation of germination of lettuce seeds. Therefore the operation of the SHAM-sensitive pathway during the early stages of germination was again emphasised. A possibility which apparently has not yet received any attention is that although the relative activity of the two pathways results in germination due to the development of a flux balance, the germination trigger is responsible for the change in the activity of the two path-

ways. Therefore the observed changes in respiratory pathways using various compounds are not the primary events causing promotion of germination of seeds in nature. In other words, in nature a primary trigger promotes a particular balance in the relative activity of the two pathways. Therefore although the relative activity of the respiratory pathways provides the block, there is an additional requirement for germination, namely the trigger.

The possible involvement of the SHAM-sensitive respiratory pathway during the early stages of germination (YENTUR & LEOPOLD, 1976) was the motivation for the investigations on *C. odorata* achenes where restricted periods of imbibition on the various compounds were used in an attempt to promote germination in the dark. The results obtained suggest that *C. odorata* achenes have an absolute requirement for the FR light absorbing form of phytochrome (Pfr) whereafter another block might occur which, as has been suggested, is possibly removed by manipulation of the relative fluxes of SHAM- and azide-sensitive respiration.

A second consideration concerning the various hypotheses proposed to explain the block in the germination of dormant seeds is the possibility that a combination of events results in germination. Therefore, with the application of, for example, as was the case in *C. odorata* achenes, both SHAM and azide, not only is a balance in the activity of the SHAM-sensitive and azide-sensitive respiratory pathways established, but in so doing the activity of the PP pathway is increased. As far as can be ascertained, there is no reason why the

application of low concentrations of inhibitors should restrict the activity of the PP pathway.

The relatively low percentage germination obtained for the control achenes experiencing the diurnal light/dark cycle is also suggestive of Pfr involvement in combination with the respiratory pathways in the germination of primarily dormant achenes. This was previously proposed by ERASMUS & VAN STADEN (1983a) for the germination of dormant *Setaria chevalieri* Stapf (syn. *Setaria megaphylla* (Steud.) Dur. and Schinz) seeds. This speculation is based on the assumption that the PP pathway is operative in removal of primary dormancy. As proposed by ERASMUS & VAN STADEN (1983a), insufficient light-promoted production of NAD kinase, the level of which has been shown to be positively correlated with Pfr (TEZUKA & YAMAMOTO, 1972; TAYLORSON & HENDRICKS, 1976) might be stimulated by the Pr to Pfr conversion. The limited levels of NAD kinase consequently do not promote the activity of the PP pathway. However, with the application of compounds suspected of increasing the PP pathway activity (and in regulating the azide-sensitive and/or SHAM-sensitive system) combined with increased levels of light-stimulated Pfr, germination occurs. The Pfr requirement is however, still of primary importance in *C. odorata* achenes because even with combinations of chemicals supposedly promotive for germination, little germination was recorded in the dark, even with non-dormant achenes.

The third consideration which requires attention is that the functional site of SHAM and azide action might include

sites other than those proposed, that is cytochrome oxidase (azide-sensitive) and the SHAM-sensitive oxidase(s). The other possibilities include enzyme systems elsewhere in, for example glycolysis and the Krebs cycle. Or the action of the respiratory inhibitors might merely simulate the endogenous conditions provided by a natural trigger. The nature of this natural trigger can be speculated as being a plant growth regulator (hormone) or a specific threshold balance of a number of hormones since it has been speculated that endogenous plant hormones might be responsible for "perceiving" the environmental conditions. This aspect requires further investigation because the hormonal systems form a common denominator throughout seeds and it might well be that their role is appreciably more important than is thought to be the case by, for example, BEWLEY & BLACK (1982).

2.7 Summary

In the review on *C. odorata* (Chapter 1, section 1.2) mention was made of the importance of the achenes in the life cycle of the weed and this can be briefly summarised as follows:

(i) the pappus-bearing achenes, being small and light, are well adapted to wind dispersal thereby facilitating rapid and widespread dissemination of the reproductive propagules; and

(ii) copious numbers of achenes are produced thus providing this species with a phenomenal reproductive potential.

The results obtained from the studies in the present investigation have provided additional information confirming the importance of the achenes in the life cycle of this weed.

In identifying the optimum conditions for germination of the achenes, it was found that an alternating temperature of 15/30 °C provided for highest germination. In addition, exposure of imbibed achenes to light was necessary to potentiate them. Treatment of imbibed achenes with R and FR light clearly implicated a low-fluence phytochrome-mediated germination response. Thus, although not substantiated in field conditions, it is reasonable to assume that light quality in the natural environment is likely to influence germination. As such, germination will be restricted to those habitats in which achenes are subjected to light comprised of predominantly R wavelengths while under dense canopies germination

will be inhibited. These results are in agreement with field observations which have shown that few or no seedlings emerge under dense vegetation canopies. It is also possible that the HIR might influence germination of achenes where these have been exposed to radiation comprised of predominantly FR irradiation.

In various studies during which achene numbers were investigated, it was confirmed that *C. odorata* has a substantial reproductive potential. This is however, dependent on locality. In areas not particularly well suited to this weed's growth, it was found that although large numbers of achenes were produced, the percentage viable (filled) achenes was comparatively low. However, because germinable achenes were found to be produced in these areas, the threat of further encroachment by this weed is a reality.

In addition to providing a copious propagule source, the achenes are specialised in certain respects since dormancy mechanisms were found to exist. The light-requirement for germination has already been discussed. Further elucidation of the light-requirement showed that removal of the pericarp at least partially substituted for light since germination of "excised" embryos in the dark was appreciably improved. However, merely puncturing the pericarp was inadequate for stimulating germination in the dark. Germination in the dark following a shift of imbibed achenes to elevated temperatures was also low and thus did not substitute for the light-requirement. An additional dormancy mechanism was also found to be operative in achenes harvested at certain times. This dormancy mechanism was removed by dry, dark storage at 25 °C

for seven months, thereby implicating an after-ripening requirement. Elucidation of the after-ripening requirement revealed that an improved germination response could be elicited by the application of a range of chemical compounds. From the results obtained by varying the concentrations of two respiratory inhibitors, azide and SHAM, it is suggested that dormancy during the after-ripening period was probably maintained by an inadequate balance in the flux of the azide-sensitive (cytochrome oxidase) and/or the SHAM-sensitive (alternative) respiratory pathway.

Application of the compounds used to substitute the after-ripening requirement failed to substitute the light-requirement. However, a slight increase in the germination of dark-incubated achenes was obtained with GA_3 and thiourea. This increase was insufficient to warrant further investigation.

Germination tests with achenes harvested at various times showed that achenes which appeared to be obviously immature, were germinable. Germination of these was greatly improved following seven months dry, dark storage at 25 °C. Germination was also improved by the application of azide (and to a lesser extent, GA_3) implicating the involvement of the respiratory pathway(s) in restricting germination during ripening.

Soil-burial studies revealed that achenes remain viable in the soil for at least one year. Although burial resulted in a significant decrease in the percentage germina-

tion, it is suggested that secondary dormancy might have developed in the achenes during burial. Further investigations are however, necessary to clarify this aspect. Burial positively influenced the germination of achenes incubated in the dark. This might have occurred as a result of "erosion" of the pericarp, as discussed previously.

It was also found that large numbers of achenes were present in the soil. The majority of these achene reserves were found on or near the soil surface. Up to 50 per cent of the recovered achenes were found to be germinable. The remainder were either non-viable or secondarily dormant, possibly as a result of the HIR and/or enforced dormancy during burial.

Certain aspects requiring further investigation have been exposed during this germination study. These aspects are outlined briefly below.

(i) As has been mentioned, inhibitors might also play a role in controlling achene germination. Endogenous inhibitors might be involved in prohibiting germination of non-after-ripened achenes and exogenous inhibitors, possibly produced by the parent plants, might contribute to inhibiting germination of achenes on or in the soil in infested areas.

(ii) The effect of Pfr on respiratory substrates also needs further investigation. Information concerning the possible mode of action of Pfr could thus be provided.

(iii) The role of the achene-coat in phytochrome-controlled germination requires greater clarification in order to establish with certainty whether the light-requirement is coat- or embryo-imposed.

(iv) More-detailed studies concerning the effect of burial in the soil on germination and longevity of achenes are necessary. These studies would provide information pertaining to (a) the development of secondary dormancy, which might arise as a result of skotodormancy since germination of imbibed achenes retained in the dark for prolonged periods is reduced when the achenes are subsequently exposed to the light and (b) the potential for re-infestation arising from achenes *in situ*.

(v) Methods of reducing the soil-achene reserves should also be investigated. A reduction in the achene reserves would greatly contribute towards maintaining a *C. odorata*-free situation following the removal of the standing-crop.

CHEMICAL CONTROL

3.1 Introduction

Weed control* originated in conjunction with crop production (KLINGMAN & ASHTON, 1982) and as a result a considerable volume of literature has been published on this topic, the preponderance dealing with agrestal weed control. The original weed control method consisted of hand-pulling undesirable plants occurring together with crop plants. This method progressed to hand-hoeing although hand-pulling of weeds is still extensively used. With the evolution of crop production, the weed control methods gained in efficiency in order to reduce the physical labour involved; this led to the use of animal- and eventually machine-drawn implements. More recently, this physical and mechanical energy input into weed control has and is being replaced by chemical energy in the form of herbicides. In addition, use is also being made of biological control to suppress/prevent the detrimental effect of weeds on desirable plant species.

Although the major categories of weed control vary according to the authority, six methods are generally recognised, each method being comprised of various techniques. The weed control methods and associated techniques are listed in Table 3.1.1.

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* "Control", as used in this dissertation, refers to an acceptable level of weed reduction (mortality) as a result of the implementation of a particular method for combating the weeds.

Table 3.1.1 Weed control methods and techniques used for the control of weeds with special reference to invaders.

METHOD	EXAMPLES OF TECHNIQUES
Manual	Hand-hoeing; hand-pulling; slashing; digging
Mechanical	Brushcutter; chainsaw; ploughing; mowing; mechanical destumping
Chemical	Spraying: coppice, seedlings, mature plants; soil applied, injection, cut-stump and frilling treatments
Biological	Fungi; insects; livestock
Cultural	Land management; fire; preventative (Weeds Act)
Integrated	Combination of two or more methods

All these methods and the respective techniques have been used to combat invader weeds, including *Chromolaena odorata* (L.) R.M. King and H. Robinson, in South Africa. In this study, chemical control of *C. odorata* was investigated.

The use of chemical control apparently started with the application of sea salt (ASHTON & CRAFTS, 1981). The first use of a selective herbicide occurred in France in 1896 (ELLIOTT, HOLMES, LOCKHART & MACKAY, 1977). However, chemical control developed rapidly only after the discovery in 1941 that the salts of the chlorinated phenoxyacetic acids were selectively herbicidal. This discovery took place independently in Britain and in the United States of America (ELLIOTT, HOLMES, LOCKHART & MACKAY, 1977). Understandably, these early developments were directed towards the control of agrestal weeds. However, with the increasing importance of the perennial, ruderal and environmental weeds, chemicals were tested on species comprising these categories and eventually, herbicides were developed specifically for the control of the comparatively large woody plants.

Chemical control of weeds consists of the application of herbicides to the weeds. A herbicide* is a chemical which, when applied in relatively small quantities to plants, results in their death or inhibited growth. Although herbicides can be classified in various ways, use will be made of the classification according to their use.

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* Herbicides are sometimes referred to as weedicides, but the latter term is avoided in this dissertation because it implies that these chemicals are toxic to weeds only.

Two major groups of herbicides are recognised namely those that are selective and those that are non-selective (Figure 3.1.1).

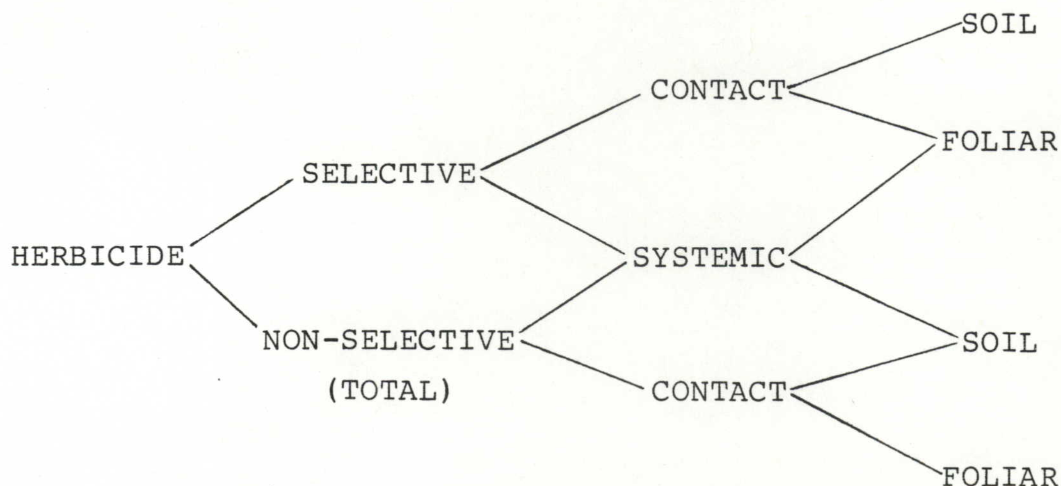

Figure 3.1.1 Classification of herbicides according to use (LOXTON, 1983).

Selective herbicides kill certain groups of plants; for example some herbicides are selectively toxic only to broad-leaved plants when applied according to specific instructions. Non-selective herbicides, which are sometimes referred to as total herbicides, generally kill all plant species. Selectivity of herbicides, which occurs only within certain limits, is dependent on factors such as plant age, growth rate, morphology, root system, physiology, biophysical and biochemical processes of the plant and features of the herbicide itself, such as the molecular configuration, concentration and application technique (KLINGMAN & ASHTON, 1982).

Within the selective and non-selective groups there are a further two categories of herbicides, namely the contact and the systemic herbicides (Figure 3.1.1). The contact

herbicides, as the name implies, result in the death of those parts of the plant with which the herbicide comes into contact; little or no movement of the herbicide occurs in the plant. The systemic herbicides on the other hand, are transported within the plants following penetration. Transport (translocation) of these herbicides within the plants is either in the phloem (symplastic) or in the xylem (apoplastic) (KLINGMAN & ASHTON, 1982). These concepts have been reviewed in detail by HAY (1976). Movement from the symplast or apoplast into the organs and tissues of the plant subsequently occurs where the toxic effects are manifest.

In addition to the two groups and two categories described above, there are those herbicides which are applied to the foliage and those applied to the soil (Figure 3.1.1). Among the foliar applied herbicides, uptake or penetration of systemic herbicides occur predominantly through the leaves. Soil applied herbicides are generally taken-up by the roots although it is difficult to distinguish between effects due to direct contact with the roots and those due to uptake and translocation within the plant (ELLIOTT, HOLMES, LOCKHART & MACKAY, 1977). In certain situations selectivity with soil applied herbicides can be obtained for instance in cultivated lands where these are applied prior to sowing of the crop. In other situations, the use of soil applied herbicides is favoured because persistent herbicide action can be obtained thereby providing long term (years) weed control.

Once inside the plant, systemic herbicides cause mortality through their effect on various vital plant processes.

This is known as the mode of action of the herbicide. This topic has received considerable attention and has been reviewed in detail by AUDUS (1976) and ASHTON & CRAFTS (1981).

For the chemical control of woody perennials, the topic under investigation in this chapter, the herbicides used must of necessity be systemic. The major reason for this is that application of contact herbicides merely results in defoliation. Although defoliation may retard the growth, new leaves are produced thereby ensuring survival of the target weed. Where contact herbicides result in the death of the top-growth, for example of newly produced chlorophyllous shoots, regrowth (coppicing) occurs. The application of contact herbicides to woody perennials at best may therefore have a similar effect as that of mechanical or manual slashing, providing only a temporary suppression of the weed.

As has been mentioned briefly, systemic herbicides are translocated within the plant. In this way it is possible to apply herbicides to parts of the plant remote from the site of herbicide action. For example, in certain herbicides, the site of action is in the roots. The herbicide, if systemic, can be applied to the foliage and following penetration, is translocated, usually along with the photosynthates, to the root region where the mode of action causes death of the plant.

The choice of herbicides for the control of perennials is further dependent on the situation in which the target species is found growing. Where large monospecific infestations occur, non-selective herbicides may be used due to the

lack of desirable species which may be damaged. However, where the weed occurs in a grassland situation, a selective herbicide toxic only to broad-leaved species is generally employed if the grass species are desired.

Selective control of weeds with herbicides which are generally non-selective can also be achieved by employing different application techniques used in the chemical control of woody weeds. These techniques form an important part of invader weed control because although the weed might be susceptible to a selective herbicide, the target weeds often occur amongst desirable indigenous species which are also susceptible to the same selective herbicides. A herbicide which is generally regarded to be selective might therefore be non-selective.

A variety of techniques have been described for herbicide application to woody weeds (KLINGMAN & ASHTON, 1982; LOXTON, 1983); these are listed in Table 3.1.2. There are also variations of the techniques listed in Table 3.1.2. For example, with basal-bark applications, the herbicide mixture may be applied either to the root-collar region, or directly to the stem and surrounding soil or after stripping the bark (cambial layer) around the entire trunk to below ground level.

The selection of a particular technique is dependent on factors related to the target weed. For instance, foliar application to tall shrubs and trees is seldom used. Also, soil applied herbicides are not used where there is a danger

Table 3.1.2 Techniques used for the application of herbicides to woody weeds (After KLINGMAN & ASHTON, 1982).

Technique	Description
Soil	Herbicide as (i) a granular formulation or (ii) water-soluble/miscible formulation; applied directly to the soil.
Foliar	Herbicide water-soluble or miscible; herbicide-carrier (usually H_2O) mixture applied to foliage as a mist, spray or drench.
Cut-stump	Herbicide-carrier (H_2O or diesel oil*) mixture applied as a spray or drench to the cut-surface of the stump.
Basal-bark	Herbicide-carrier (H_2O or diesel oil*) mixture applied as a spray or drench to the basal portion of the main stem.
Frilling	Herbicide-carrier (H_2O or diesel oil*) mixture applied into the frill prepared by overlapping cuts around the main stem.
Stem injection	Herbicide-carrier (H_2O or diesel oil*) mixture applied either by syringe-type injection, axe-injection (hypohatchet) or pouring into cuts made into the bark of the main stem.

* diesel oil is used as the carrier in order to promote penetration of the bark by the herbicide.

of damage to desirable species. Where extensive and dense infestations need to be treated, some of the techniques listed are too labour intensive and laborious to be considered. In these situations overall applications such as foliar sprays and broadcast soil applied herbicides are used.

The decision to embark upon chemical control of *C. odorata* in this investigation was based on a number of factors. Firstly, although there are disadvantages to each of the control methods, the potential of chemical control was adjudged to fulfil the requirements for the development of a control strategy urgently needed to restrict the extent of encroachment and to reduce the current intensity of *C. odorata* infestation. Secondly, the use of chemicals in weed control in conservation areas has been largely avoided due, not only to the danger to desirable species but also, to fears that contamination of the environment could occur. These fears, it seems, can generally be attributed to the lack of knowledge regarding this aspect of invader weed control. It was therefore necessary to demonstrate the advantages of using herbicides as an alternative and effective means of controlling invader weeds. Thirdly, it was felt necessary to demonstrate that additional herbicides are available for the control of *C. odorata*. This would provide an incentive to the herbicide producers and distributors to register their products for the control of *C. odorata*. This factor requires clarification.

Provision has been made in the "Fertilizers, Farm Feeds, Agricultural Remedies and Stock Remedies Act" (Act 36

of 1947) for the registration of herbicides for the control of specific weed species. For registration, a herbicide must be tested on the weed in field trials at a number of localities. The results must clearly demonstrate the suitability and efficacy of the herbicide; that is, it should be shown that an acceptable level of control (measured as mortality of the target weed) can be obtained. In so doing the herbicide is registered at a particular dose and to be applied according to certain specifications. These recommendations are displayed on the herbicide label which is attached to the herbicide container. For the control of agrestals and other weeds occurring in areas of comparatively high economic returns, frequent registration of herbicides takes place since these situations provide a large market for the herbicides. However, the limited use of herbicides in the control of invader weeds has only resulted in a small market. Therefore the input into registration of herbicides for these weeds is small. However, by demonstrating independently the efficacy of herbicides on these weeds, it is hoped that trials and ultimately registration of the herbicides by the producers/ distributors will be encouraged. The greater the number of registered herbicides the greater the range from which chemicals can be selected for specific situations. Act No. 36 of 1947 also provides legislation concerning the recommendation of herbicides since only registered herbicides are allowed to be used; hence the importance of conducting herbicide field trials on *C. odorata* which would eventually provide meaningful results.

For these field trials on *C. odorata* six herbicides were selected (Table 3.1.3) for testing. The common names of these herbicides are used in the text. Each of the herbicides will be briefly reviewed to demonstrate the reasons for their selection for testing on *C. odorata*. A detailed review of each herbicide is not intended since it is not within the context of this study, and in addition, detailed reviews are available for the better known herbicides.

The herbicide (2,4,5-trichlorophenoxy)acetic acid (2,4,5-T) is a well known organic, hormone-type herbicide. Following the discovery during the 1940's that certain chlorinated phenoxyacetic acid derivatives contained herbicidal properties, testing of related compounds resulted in the production of 2,4,5-T (TURNER, 1977). It was found that 2,4,5-T was more active against woody weeds than the other phenoxyacetic acids such as (2,4-dichlorophenoxy)acetic acid (2,4-D) (TAM, 1947). During the early testing of 2,4,5-T it was also found that this herbicide had little effect on grasses, that it was taken-up predominantly by the foliage through the cuticle and stomata (CRAFTS, 1964) and persistence in the soil was limited (TURNER, 1977). By the mid 1950's, 2,4,5-T was widely used in silviculture and in rangelands and along powerlines and other situations in which woody species were a problem in the United States of America (CABLE & TSCHIRLEY, 1961; BOVEY, 1964). In Britain the use of 2,4,5-T for woody weed control was well established by the mid 1960's (BROWN, 1969). The use of 2,4,5-T, together with 2,4-D gained notoriety during the war in Vietnam where mixtures of these

Table 3.1.3 Common names, chemical names, active ingredient concentrations and trade names of herbicides used in screening field trials for *C. odorata*.

Common Name	Chemical Name	Conc. ai in product (g dm^{-3})	Trade Name
2,4,5-T	(2,4,5-trichloro-phenoxy)acetic acid	615	Weedmaster 2,4,5-T
glyphosate	*N*-(phosphonomethyl) glycine	480	Roundup
triclopyr	{(3,5,6-trichloro-2-pyridinyl)oxy} acetic acid	480	Garlon 4E
3,6-dichloro-picolinic acid	3,6-dichloropico-linic acid	300	Lontrel
imazapyr	2-{4,5-dihydro-4-methyl-4-(1-methyl-ethyl)-5-oxo-1*H*-imidazol-2-yl}-3-pyridinecarboxylic acid	250	Arsenal
tebuthiuron	*N*-{5-(1,1-dimethyl-ethyl)-1,3,4-thia-diazol-2-yl}-*N*,*N*'-dimethylurea	200g kg^{-1}	Graslan 20P

herbicides were used to kill or defoliate woody species as described by TURNER (1977). Nonetheless, the use of 2,4,5-T has persisted and the literature abounds in references in which this herbicide has been successfully used. Additional information concerning the history of discovery and development of 2,4,5-T has been supplied by KIRBY (1980).

The chemical structure of 2,4,5-T is shown in Figure 3.1.2. It is a white crystalline solid which is only slightly soluble in water and can form salts or esters. In this investigation the butyl ester of 2,4,5-T was used. The butyl ester is prepared by reacting 2,4,5-T with butyl alcohol (TURNER, 1977).

The most usual route of 2,4,5-T entry is through the foliage, but uptake through branches and twigs can also occur (TURNER, 1977). The foliar absorption and translocation of 2,4,5-T has been reviewed in detail by ROBERTSON & KIRKWOOD (1969) and RICHARDSON (1977). Suffice it to say that environmental factors such as temperature, humidity, moisture stress and growth stage of the target plant influence absorption of the herbicide as do the structure of the leaf and the nature of its components, namely cuticle thickness and number of stomata and trichomes. Chemical factors, including the formulation, pH, concentration and molecular configuration also influence absorption (RICHARDSON, 1977). These factors are also likely to influence the translocation of 2,4,5-T following penetration of the herbicide into the plant. This aspect has been reviewed in detail by ROBERTSON & KIRKWOOD (1970) and RICHARDSON (1977). Translocation of 2,4,5-T is in the symplast

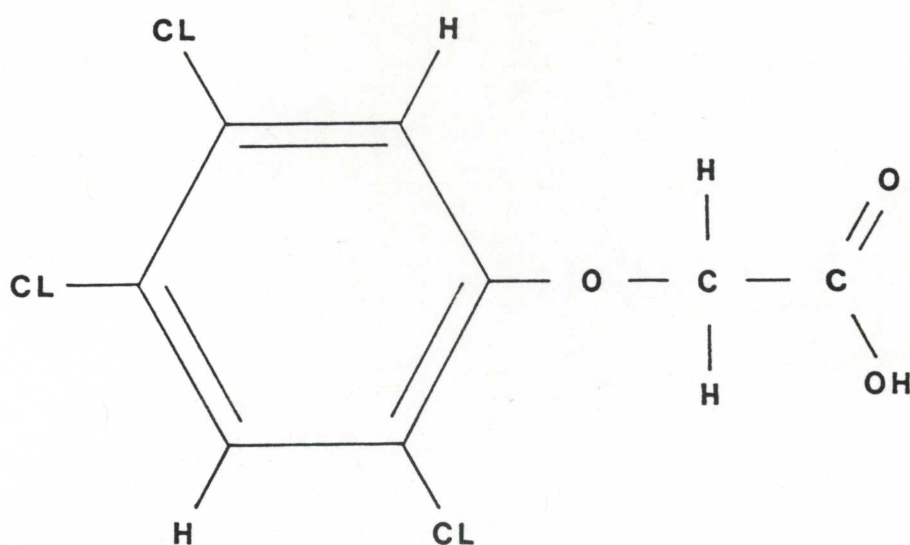

Figure 3.1.2 (2,4,5-trichlorophenoxy)acetic acid (2,4,5-T)

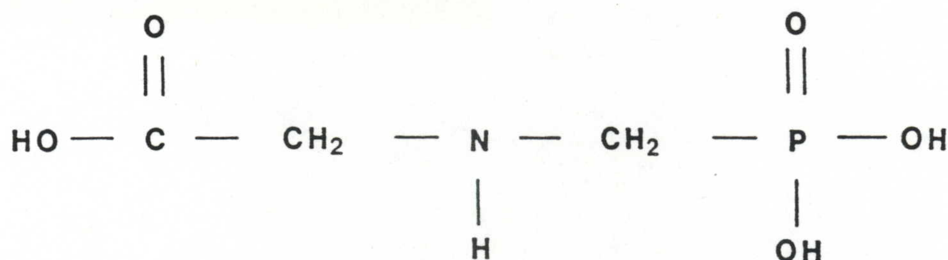

Figure 3.1.3 *N*-(phosphonomethyl)glycine (glyphosate)

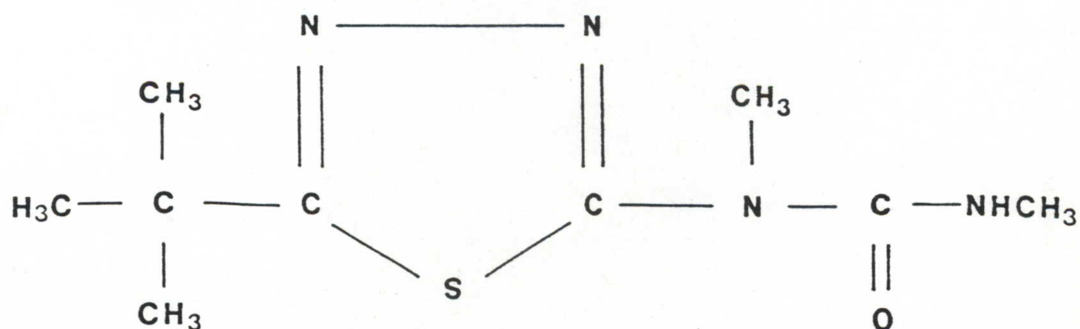

Figure 3.1.4 *N*-{5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl}-*N*,*N'*-dimethylurea (tebuthiuron)

(CRAFTS, 1964), therefore it is a phloem-mobile systemic herbicide. BOVEY & YOUNG (1980) reported that phenoxy herbicides are apparently transported by the assimilation stream within the plants. Most active transport therefore occurs during periods of carbohydrate transfer. Furthermore, the direction of transport is determined by the pattern of food distribution. Thus it is recommended that application of phenoxy herbicides to the foliage should take place when basipetal transport of assimilates is occurring (BOVEY & YOUNG, 1980).

Although many researchers have investigated the mode of action of 2,4,5-T, its precise effect on plant cells is not clear. It seems that the most important effect of 2,4,5-T is upon nuclear function which alters the balance of enzyme systems and consequently has widespread effects on cell division and growth (TURNER, 1977). The visual effect on plants is the characteristic manifestation of epinasty, which is typical of this herbicide's auxin-type mode of action. As a consequence, 2,4,5-T often results in a rapid decline in the carbohydrate reserves of roots, rhizomes and other storage organs (KLINGMAN, 1961).

The use of 2,4,5-T for chemical control of perennial woody weeds has already been mentioned; reference to its use for this purpose has appeared in the large majority of review articles. An indication of the high esteem for this herbicide is the fact that even with the development of many new herbicides as a result of modern technology, 2,4,5-T was recently still extensively used for the control of perennial

and woody weeds (HARVEY, 1982; BOYD, HERNDON & SOSEBEE, 1983; JACOBY & MEADORS, 1984; SCANLAN, 1984; SCANLAN & FOSSETT, 1984a; 1984b).

Based on the data provided by the frequent use of 2,4,5-T for the control of woody weeds and that this herbicide is selectively toxic to broad-leaved species, it was selected for testing on *C. odorata*. An additional motivation was that 2,4,5-T was relatively inexpensive (approximately R9,00 per litre at the time the trials were commenced). In addition, in some silvicultural areas it had been found that in the course of normal weed control measures with 2,4,5-T, *C. odorata* was susceptible to the herbicide. These results were in slight contrast to those of EGBERINK & PICKWORTH (1969) who found that 2,4,5-T was only "moderately effective" for *C. odorata* control. This discrepancy therefore also needed clarification.

Another herbicide which is probably as well, if not better known than 2,4,5-T, is *N*-(phosphonomethyl)glycine (glyphosate). Glyphosate was first marketed in 1971 (HOAGLAND & DUKE , 1981). It is a white solid which is relatively soluble in water. The chemical structure is shown in Figure 3.1.3. The commercial product is manufactured by Monsanto as the isopropylamine salt and is marketed with a surfactant. Glyphosate is a non-selective, broad spectrum post-emergence herbicide and is the only compound of this chemical class which is registered as a herbicide; however an analogue, glyphosine, is a plant growth regulator (HOAGLAND & DUKE, 1982a).

The overall success of glyphosate as a herbicide can also be attributed to several properties in addition to its

phytotoxicity (HOAGLAND & DUKE, 1982a). It has a comparatively low molecular weight (169,1), which together with its high solubility (one to eight per cent in water at 25 to 100 °C) is thought to aid in its rapid absorption and translocation by plant tissues. Glyphosate is readily absorbed by foliage and chlorophyllous tissue and roots (SPRANKLE,MEGGITT & PENNER, 1975; SEGURA, BINGHAM & FOY, 1978). Metabolism of glyphosate within the plant is limited (GOTTRUP, O'SULLIVAN, SCHRAA & VANDEN BORN, 1976; PUTNAM, 1976) and therefore its phytotoxicity is maintained within the plant. According to HOAGLAND & DUKE (1982a) these factors provide this herbicide with the ability to kill deep-rooted perennials and those with vegetative propagules, such as bulbs.

Many studies have been conducted on the absorption and translocation of glyphosate. Certain aspects thought to be of relevance will be mentioned. COUPLAND, TAYLOR & CASELEY (1978) found that glyphosate was most readily absorbed by young but fully expanded leaves. The amount of glyphosate absorbed by leaves has however, been shown to be low. KING & RADOSEVICH (1979) found that only four per cent was absorbed and HADERLIE, SLIFE & BUTLER (1978) reported seven per cent absorption. RICHARD & SLIFE (1979) found that penetration of the leaf by glyphosate could be markedly improved by the combined application of the herbicide and various additives. It was also found that the glyphosate which penetrated the leaves was not tightly bound and it was therefore concluded that the compound was probably freely translocated. KELLS & RIECK (1979) observed that light intensity had no effect on glyphosate

absorption; relative humidity and temperature have however, been found to influence absorption (LUND-HØIE, 1979).

The translocation of glyphosate in plants apparently occurs in the phloem (symplastic), movement following a typical source to sink pattern with accumulation occurring in the meristematic areas (HADERLIE, SLIFE & BUTLER, 1978; SANDBERG, MEGGITT & PENNER, 1978; SCHULTZ & BURNSIDE, 1980; HOAGLAND & DUKE, 1982a). There is however, some evidence of apoplastic movement (SPRANKLE, MEGGITT & PENNER, 1975; CLAUS & BEHRENS, 1976; GOTTRUP, O'SULLIVAN, SCHRAA & VANDEN BORN, 1976). Translocation is influenced by environmental factors. Increase in temperature has been suggested to improve translocation (JORDAN; 1977; LUND-HØIE, 1983a). Light also reportedly affects glyphosate movement; KELLS & RIECK (1979) found that greatest accumulation of glyphosate occurred in the roots of plants exposed to full light. High humidity has been found to greatly increase absorption and translocation of glyphosate (GOTTRUP, O'SULLIVAN, SCHRAA & VANDEN BORN, 1976).

The first visual effect of glyphosate phytotoxicity is generally chlorosis, usually followed by necrosis. No particular plant organ or tissue has however, been conclusively implicated as the primary site of glyphosate action (HOAGLAND & DUKE, 1982a). The mode of action as reviewed by COLE (1982) has been extensively investigated and several plant processes have been found to be affected. These include inhibition of aromatic amino acid synthesis (JAWORSKI, 1972; SHANER & LYON, 1980; HOAGLAND & DUKE, 1982b), retardation of chlorophyll accumulation (KITCHEN, WITT & RIECK, 1981) nitrate reductase

induction (COLE, CASELEY & DODGE, 1983), inhibition of transpiration (SHANER & LYON, 1980), and enhanced destruction of auxins (LEE, 1982a; 1982b).

Phototoxicity of glyphosate has been found to be affected by environmental conditions. This may be as a result of the effect of environmental conditions on absorption and translocation resulting in greater quantities of herbicide reaching the site of action. Plants under moisture stress have been found to be less affected by glyphosate (MOOSAVI-NIA & DORE, 1979a). Increased relative humidity and increased temperature reportedly increased the phytotoxicity of glyphosate (JORDAN, 1977; WILLS, 1978). In contrast to the results obtained by KELLS & RIECK (1979), some researchers have recorded a decreased phytotoxic effect in relation to increased light (MOOSAVI-NIA & DORE, 1979b; LUND-HØIE, 1983b). This may be related either to biochemical changes within the plants or to morphological changes in the nature of the leaves caused by increased shade (MOOSAVI-NIA & DORE, 1979b).

Considerable evidence is provided in the literature (as described above) for the use of glyphosate for perennial weed control. However, there is a notable lack of information concerning the use of this herbicide for the control of woody perennial weeds. It is possible that this has arisen as the result of the cost of glyphosate prohibiting its use for environmental weeds. In South Africa, glyphosate is fairly widely used, especially in silviculture, presumably due to the fact that this herbicide is registered for the control of well known woody perennial weeds such as *Lantana camara* L.,

Solanum mauritianum Scop. and the hard-to-kill perennial *Rubus cuneifolius* Pursh.. It was therefore decided that glyphosate should be included for testing on *C. odorata*, even though it is not selectively phytotoxic. The fact that glyphosate is a systemic herbicide effective against perennials and has a reputation for being a "safe" herbicide (glyphosate is the only herbicide presently approved for use by the Natal Parks Board) was considered sufficient motivation to warrant its inclusion in the trials.

Another herbicide which is well known in the field of chemical control of woody species is *N*-{5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl}-*N*,*N*'-dimethylurea (tebuthiuron). Tebuthiuron is a colourless solid, the chemical structure of which is shown in Figure 3.1.4. This chemical, a substituted urea herbicide, was first marketed in 1974 by Eli Lily (FLETCHER & KIRKWOOD, 1982). There are various forms of the formulated product. The one used in this study was the granular (pellet) formulation consisting of a clay-carrier containing 20 per cent active ingredient and is hereafter referred to as tebuthiuron. This formulation is used for brush and weed control in rangeland situations.

Being a granular formulation, tebuthiuron is soil-applied. During periods of high moisture, that is during and directly after rain, the active ingredient is leached out of the clay-carrier and transported into the soil (CHANG & STRITZKE, 1977). Tebuthiuron can therefore be applied at any time but movement into the soil and therefore subsequent uptake by the plant, only occurs following sufficient rain to solubilise

the tebuthiuron and leach it into the soil.

The fate of tebuthiuron in the soil has been well researched. Microbial degradation of the herbicide occurs in the soil, resulting in low concentrations of metabolites that are either non-herbicidal or such weak herbicides "as to be of no serious concern" (Graslan Technical Manual*). Degradation studies have shown that in humid regions which receive approximately 1000 mm of annual precipitation, the half-life of tebuthiuron is approximately one year (EMMERICH, HELMER, RENARD & LANE, 1983). However, in arid regions the half-life was found to be considerably longer (up to five years). As confirmed by CHANG & STRITZKE (1977), dissipation of tebuthiuron is dependent on soil moisture and temperature. CHANG & STRITZKE (1977) also found that tebuthiuron adsorption is related to the clay and organic matter content. Adsorption is molecular under neutral or alkaline conditions and ionic under acid conditions (WEBER, 1980).

Leaching of tebuthiuron in the soil is dependent on the organic matter and clay content of a soil (CHANG & STRITZKE, 1977) but that of the biologically active herbicide is generally restricted to a depth of 30 cm. Some studies have however, found minor residues at a depth of 60 cm in sandy soils (Graslan Technical Manual).

Tebuthiuron is reported to be readily absorbed by the roots of plants and is then translocated rapidly within the

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* Elanco Products Company (A Division of Eli Lily and Company), P.O. Box 1750, Indianapolis, Indiana 46206, United States of America.

plant (STEINERT & STRITZKE, 1977; McNEIL, STRITZKE & BASLER, 1980). Translocation is thought to be via the apoplastic (xylem) system (KLINGMAN & ASHTON, 1982).

The mode of action of tebuthiuron is thought to occur by way of inhibited RNA and lipid synthesis and electron transport inhibition in photosystem II of photosynthesis (HATZIOS, PENNER & BELL, 1980) thereby inhibiting photo-assimilation which results in reduced levels of non-structural carbohydrates (SHROYER, STRITZKE & CROY, 1979).

As has already been mentioned, the formulation of tebuthiuron used in this investigation is used mainly for brush control, including shrubs and arborescent vegetation. The list of susceptible species emanating from research conducted with tebuthiuron includes 126 genera and almost 250 species of woody perennials (Graslan Technical Manual). Due to its mode of action, tebuthiuron results in the defoliation of these woody perennials, following which refoliation usually occurs. This defoliation-refoliation cycle induced by tebuthiuron is largely dictated by the rainfall pattern, but eventually results in the death of susceptible species (SCIFRES, STUTH & BOVEY, 1981). Usually the defoliation-refoliation cycles are complete within the first growing season following tebuthiuron application (MEYER & BOVEY, 1980) while more tolerant species might only be killed the following growing season, or these might even recover (Graslan Technical Manual).

Considerable use has been made of tebuthiuron for the control of woody perennials in South Africa, especially in bush-

encroachment control in the north Cape Province (JOSEPH, personal communication*). Furthermore, tebuthiuron is also registered for use against numerous woody perennials including such alien invaders as *L. camara*, *S. mauritianum* and several of the exotic *Acacia* species which form a problem in the western Cape. However, the primary reason for the inclusion of tebuthiuron in this study was that at the commencement of these trials, it was the only herbicide registered for *C. odorata* control. Therefore, the application of this herbicide at the recommended dosage was used as a basis for comparison of the efficacy and performance of the other herbicides tested.

The herbicide {(3,5,6-trichloro-2-pyridinyl)oxy}acetic acid,(triclopyr) is manufactured by Dow Chemicals. The chemical structure of this herbicide is shown in Figure 3.1.5. There are three chemical formulations available; the one used in this study consisted of the butoxyethyl ester of triclopyr. Triclopyr was first marketed in the United States of America in 1970 (FLETCHER & KIRKWOOD, 1982).

Triclopyr, as the formulated product, is a liquid concentrate which is usually mixed with water (the carrier) for application to the foliage of plants. Absorption of triclopyr has been found to be greatest in immature leaves, and greater amounts are absorbed by the abaxial than adaxial leaf surfaces (KING & RADOSEVICH, 1979). It was also found that absorption of triclopyr was associated with several characteristics of the foliage, including the amounts of epicuticular wax, stomatal

* JOSEPH, M. Agrihold, P.O. Box 55, Silverton 0127, Republic of South Africa.

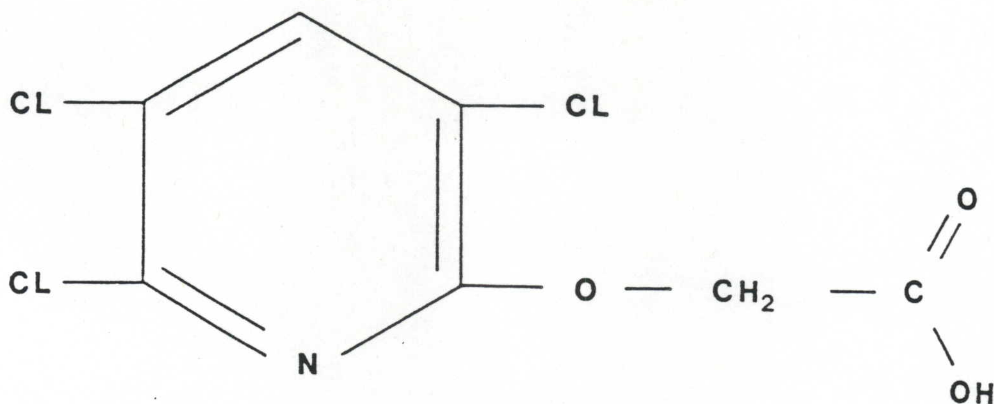

Figure 3.1.5 {(3,5,6-trichloro-2-pyridinyl)oxy}acetic acid (triclopyr)

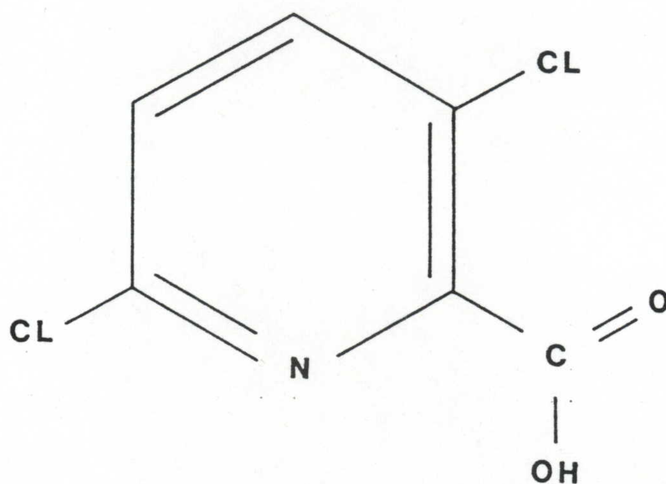

Figure 3.1.6 3,6-dichloropicolinic acid

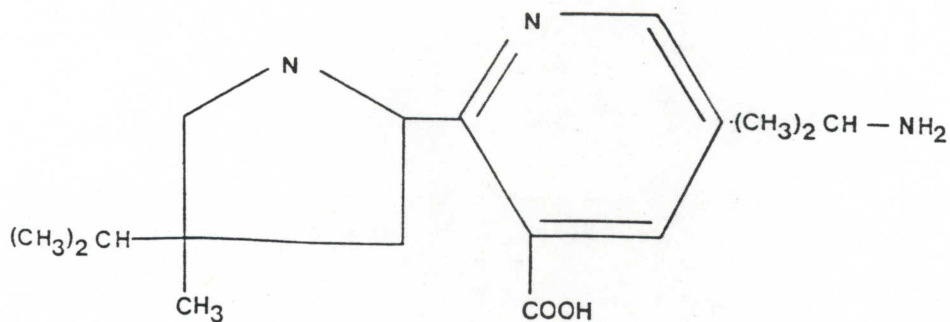

Figure 3.1.7 2-{4,5-dihydro-4-methyl-4-(1-methyl-ethyl)-5-oxo-1*H*-imidazol-2-yl}-3-pyridinecarboxylic acid (imazapyr)

density, cuticular membranes and trichome density. BOVEY, HEIN & MEYER (1983) investigated the absorption of triclopyr. It was found that the herbicide was so rapidly absorbed by the foliage that simulated rainfall within 15 minutes after herbicide application did not reduce the efficacy. BOVEY, KETCHERSID & MERKLE (1979) found that triclopyr was also readily absorbed by the roots of plants and it was suggested that leaves which abscised following herbicide application might serve as an additional source of herbicide to the plant as a result of root uptake of triclopyr washed off these leaves by rain.

The translocation of triclopyr following absorption is also rapid. For instance BOVEY, HEIN & MEYER (1983) found that triclopyr was translocated from the treated leaf to other parts of the plant within four hours of application. RADOSEVICH & BAYER (1979) reported that triclopyr movement was greatest under warm temperatures (29/13 °C alternating) than under cooler temperatures (13/4 °C alternating). In addition, it was found that triclopyr was most mobile under long day conditions (16 hours light).

The mode of action of triclopyr is not known but appears to be similar to that of the phenoxy herbicides (Herbicide Handbook of the Weed Science Society of America*). Triclopyr, like the majority of phenoxy herbicides, is selectively toxic to broad-leaved species including woody perennials. Triclopyr has been found to be effective against many woody peren-

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* Fifth Edition, 1983; Weed Science Society of America, 309 West Clark Street, Champaign, Illinois 61820, United States of America.

nials (BYRD & COLBY, 1978; BOVEY & MAYEUX, 1980; BOVEY & MEYER, 1981; JACOBY & MEADORS, 1984; SCANLAN, 1984; SCANLAN & FOSSETT, 1984a; 1984b). In South Africa the use of triclopyr appears to have increased markedly since the decline in the availability of 2,4,5-T. Consequently, the list of species for which triclopyr is registered is increasing, and currently includes woody species such as *Eucalyptus grandis* W. Hill ex Meiden, several exotic *Acacia* species and *S. mauritianum*. Based on the reputed efficacy of triclopyr on woody species and its selectivity to broad-leaved species, triclopyr was included for testing on *C. odorata*. (At the time the trials were commenced, triclopyr was not registered for use against *C. odorata* but, following application for registration, this product was registered in December 1984).

Not only well known herbicides were tested on *C. odorata*. Two lesser known herbicides were also included. However, due to their newness, limited published scientific literature is available. Consequently, use has largely been made of technical reports.

The herbicide 3,6-dichloropicolinic acid was apparently developed to produce a compound having efficacy similar to that of 4-amino-3,5,6-trichloropyridine-2-carboxylic acid (picloram) but lacking its residual properties (KEYS, 1975). The half-life of 3,6-dichloropicolinic acid in the soil is reportedly about 27 days (HAAGSMA, 1975) which is a great reduction in persistence when compared to that of picloram. The chemical structure is shown in Figure 3.1.6.

Studies on the absorption and translocation of 3,6-dichloropicolinic acid were conducted by O'SULLIVAN & KOSSATZ (1984). Several factors emerged from these experiments on *Cirsium arvense* (L.) Scop.. Absorption of 3,6-dichloropicolinic acid was rapid and continued up to 48 hours after treatment. High relative humidity (95 per cent) approximately doubled absorption compared to that at low relative humidity (40 per cent). The herbicide was also found to be highly mobile since, within 48 hours, over half of the absorbed compound was translocated out of the leaves. Most accumulation of 3,6-dichloropicolinic acid occurred in the apex while approximately eight per cent was recovered from the roots. It was also found that application of the herbicide to the lower leaves decreased the amount of herbicide translocated to the shoot apex; the amount translocated to the roots was increased.

Information regarding 3,6-dichloropicolinic acid was also obtained from the Technical Information Report*. The mode of action of this herbicide is believed to be similar to that of the auxin-type herbicides (phenoxy herbicides). It is also selectively toxic to broad-leaved annual, perennial and woody perennial species, and species from the following plant families, Asteraceae, Polygonaceae, Umbelliferae and Leguminosae, have been found to be particularly susceptible (HAAGSMA, 1975) while grasses are tolerant. For maximum efficacy, it was advised that 3,6-dichloropicolinic acid be

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* Dow Chemical Africa (Pty.) Ltd., P.O.Box 9170, Johannesburg 2000, Republic of South Africa.

applied to the foliage of young, actively growing plants in which case absorption and translocation was reported to be rapid, with little metabolism of the herbicide occurring within the plant.

The published scientific information regarding 3,6-dichloropicolinic acid is scant. It does however, seem that it has been used successfully for the control of perennial weeds (O'SULLIVAN & KIRKLAND, 1984; O'SULLIVAN & KOSSATZ, 1984) and on woody perennials for example *Prosopis juliflora* (Swartz) D.C. (JACOBY, MEADORS & FOSTER, 1981) and *Acacia* species (SCANLAN, 1984) and for the deep-rooted perennial, *Physalis viscosa* L. (DONALDSON, 1984).

Based on the available information (especially the features listed below), 3,6-dichloropicolinic acid was included for testing on *C. odorata*.

- (i) It is a systemic herbicide;
- (ii) it is selective with respect to broad-leaved species including those belonging to the Asteraceae;
- (iii) it is not persistent in the soil; and
- (iv) it has been used for the control of woody perennials.

A herbicide which, until recently, was only known by its code number AC 252,925, has just been released in South Africa. This herbicide is 2-{4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol -2-yl}-3-pyridinecarboxylic acid (imazapyr) and is formulated as the isopropylamine salt (PEOPLES, 1984). Imazapyr was developed at Cyanamid's Agricultural Research Centre, Princeton, New Jersey, United States of America

(ORWICK, MARC, UMEDA, LOS & CIARLANTE, 1983). The chemical structure of imazapyr is presented in Figure 3.1.7.

Imazapyr is readily absorbed by the foliage and roots of plants (HASUI, KADOTA, IKEDA & TANAKA, 1983). Following penetration, imazapyr is reported to be rapidly translocated throughout the target plant, accumulation occurring in meristematic regions (PEOPLES, 1984). According to the Technical Information Report* imazapyr is rapidly translocated into the underground storage organs of perennials resulting in their death and thus preventing regrowth. The efficacy is apparently influenced by soil moisture content, resulting in reduced affect of the herbicide during dry conditions. HASUI, KADOTA, IKEDA & TANAKA (1983) found that, in field studies, the efficacy of imazapyr was greater during warm weather conditions (± 22 °C) than in cooler conditions and LAPADE, MANIMTIM, CALORA & LALAP (1983) found that an increased light intensity enhanced the onset of activity in the target weeds.

Visual observations have shown that the meristematic regions were first affected by imazapyr while complete kill was only observed up to five months after application (HASUI, KADOTA, IKEDA & TANAKA, 1983). SHANER, ANDERSON, REIDER, STIDHAM & ORWICK (1984) reported on the effects of imazapyr on selected physiological processes in *Zea mays* L.. Although various effects were noted, the only physiological process that was greatly inhibited was DNA synthesis. However, it was suggested that imazapyr did not directly inhibit DNA

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* Cyanamid (Pty.) Ltd., Agricultural Research Division, Princeton, New Jersey 08540, United States of America.

synthesis; rather, some other physiological process was thought to be inhibited directly which, in turn, affected DNA synthesis.

The biological activity of imazapyr has been found to persist for three to 12 months in the soil. Under dry conditions, imazapyr may persist for more than 12 months, however, because it is adsorbed to the soil particles, lateral and vertical movement is limited (PEOPLES, 1984).

Although the control of some species in the families Leguminosae, Asteraceae and Rosaceae has been sub-optimal, PEOPLES (1984) reported that imazapyr has demonstrated "excellent activity" against sedges, annual and perennial broad-leaved weeds, and woody species. Although this herbicide is very new in South Africa, several woody weeds have been found to be susceptible to it. This statement is based on the fact that imazapyr has already been registered for use against three perennial woody species namely *E. grandis*, *S. mauritianum* and *L. camara*. Based on the information available, it was decided that this herbicide should be included in the trials. Although imazapyr is non-selective (PEOPLES, 1984), if found to be effective this herbicide would be suitable for use in industrial areas where *C. odorata* occurs and where vegetation-free conditions are desired.

In the control of agrestal weeds, herbicides are applied to eliminate competition from other species, thereby establishing a monospecific population comprised of the crop plants. In the control of environmental weeds however, the situation

is far more complex. This is especially so with alien invaders, since upon removal the objective is to rehabilitate the plant community. However, where dense infestations of invader weeds have become established, their removal might result in the development of an ecological vacuum (HILL, 1977). This vacuum is likely to be filled rapidly by other species. The danger is however, that these species could be comprised predominantly of weed species because the removal of the original infestation by whatever means, constitutes disturbance. It is well known that weeds are opportunistic (HOLZNER, 1982) and therefore rapidly invade disturbed areas. The probability of another invader weed becoming a problem following removal of preceding species is therefore high, especially in South Africa where naturalised exotics are almost automatically regarded as weeds or at least as potential weeds (WELLS & STIRTON, 1982).

Rehabilitation of weed dominated natural communities has received considerable attention in South Africa. This has mainly taken the form of veld-management practices such as grazing and burning cycles. The rehabilitation of indigenous tracts of vegetation from invader weed infestations has also been initiated especially in the western Cape and within bodies committed to conservation such as the National and Provincial Parks Boards and city council Parks Departments. However, it is doubtful that carefully formulated programmes have been drawn-up and adhered to. It is not the objective of this study to fulfil these needs but rather to draw attention to pertinent factors which should form an integral part of invader weed control in South Africa.

A detailed and logical approach to the rehabilitation of weed dominated communities has been proposed by PANETTA (1981), and the following discussion briefly examines aspects of this article.

Firstly, it is essential that the aims of the rehabilitation effort be defined prior to initiating a particular programme. One of the primary objectives should be to determine the desired floristic and structural attributes of the rehabilitated community. Presumably, in the case of *C. odorata*, the rehabilitated community should resemble the original indigenous vegetation prior to the invasion by the weed. It is therefore also necessary to have an understanding of the dynamics of the indigenous community. Clearly aspects which need to be considered include the propagule source of the species composition desired in the rehabilitated community and the interaction of the species within the community. In determining the species composition, it is essential to include those which are likely to provide the establishment of a uniform vegetative cover in order to exclude undesirable species such as the alien invaders.

The second major consideration is the formulation of a rehabilitation strategy which incorporates the method of control to be used in combating the undesired weed species, the introduction of the replacement species and the resources available for the execution of the operation. Regarding the last-mentioned factor, it is crucial that allowance be made for adequate follow-up operations since this is a critical phase for the establishment of the desired species.

The final major consideration involves the selection of the replacement species. Obviously, in the rehabilitation of the indigenous coastal vegetation of Natal, the species composition would be determined by the species likely to have been present prior to invasion by *C. odorata*.

Clearly there are many factors which need to be taken into consideration for the control of alien invader weeds. These include not only the method to be used for the initial control (removal) of the target weed but also actions to be taken following the control. This is especially the case where indigenous, natural communities have been invaded, since removal, which constitutes disturbance, might result in the formation of a habitat vulnerable to infestation by another undesirable species. Investigations were therefore conducted on the possibility of using chemical control to replace or complement the methods presently used for *C. odorata* control. In addition, attention was paid to events occurring following the control of this weed to provide information which could be used in developing a control strategy.

3.2 Materials and Methods: General

Herbicide trials were conducted at two sites; one 10 km south of Melmoth (Vergelegen Estates, Natal Tanning Extract Company) and one five kilometres inland from Clansthal on the Natal south coast (Finningley Estates, Messrs D. & A. Crookes). Only one trial, sprayed in October, 1983, was conducted at Melmoth due to unfavourable climatic conditions during March, 1984 when a second trial was to be sprayed. The majority of trials were carried out at the Clansthal site due mainly to the co-operation of the landowners who ensured protection for the sites and also because of the shorter travelling distances involved.

In the Melmoth region the major land uses are silviculture and sugar cane production. In the Clansthal region the land uses are similar but predominantly sugar cane production. The Melmoth region is classified as "coast hinterland" and Clansthal as"coast lowland" by PHILLIPS (1969). Both these bioclimatic regions are in the summer rainfall area. The mean monthly maximum and minimum temperatures and the mean monthly rainfall for the two regions in which the trial sites were located, are presented in Table 3.2.1.

At both sites extensive and largely homogenous *C. odorata* infestations were present. Mainly for convenience, the trials at both sites were located in timber plantations. At Melmoth, the weed was located in a five-year-old *Pinus taeda* L. plantation growing in a sandy-loam soil (Table 3.2.2) on a northern

Table 3.2.1 Monthly means of daily maximum and minimum temperatures and the mean monthly precipitation recorded for the Melmoth and Clansthal regions during a period of 25 years or more[1].

Month	Climatological Parameters					
	Mean daily max. temperature (°C)[2]		Mean daily min. temperature (°C)[2]		Mean monthly precipitation (mm)	
	Melmoth	Clansthal	Melmoth	Clansthal	Melmoth	Clansthal
January	27	27	16	19	130	120
February					120	110
March	27	27	16	19	100	120
April					60	70
May	23	24	12	15	30	50
June					20	30
July	21	22	10	11	20	30
August					30	40
September	23	23	12	14	50	60
October					90	100
November	25	24	14	17	110	110
December					130	110

[1] Data obtained from SCHULZE (1982)

[2] Only bimonthly data available

Table 3.2.2 Details of soil analyses carried out on soil samples collected from the Melmoth and Clansthal herbicide trial sites[1].

Parameter Analysed	Site	
	Melmoth	Clansthal
Percentage clay	17	13
Percentage silt	5	4
Percentage sand	79	83
Textural class	Sandy loam	Sandy loam
Organic carbon (%)	2,55	1,01
Organic matter (%)	4,39	1,74

[1] Soil analyses conducted by Soil Physics Laboratory, Natal Region, Cedara.

aspect. At Clansthal, the trials were conducted on *C. odorata* infestations growing in a plantation of four-year-old *E. grandis* coppice. Here trials were either on a western or eastern aspect as detailed for each trial. The soil in this region was also a sandy-loam (Table 3.2.2).

The state of the plants and the "history" of the trial sites are described for each of the trials.

All the foliar applied herbicides were mixed with water obtained from the laboratory at the research station located at Cedara, 15 km north of Pietermaritzburg. All mixing was carried out on site immediately prior to spraying. The specifically required volume of herbicide + water mixture was then poured into pump-up Solo sprayers. The sprayers used sprayed to exhaustion, therefore a specific volume could be applied to a particular area. Where high volumes were applied (1000 $dm^3 ha^{-1}$) a solid-cone nozzle (Tee jet-TG1) was used and the mixture sprayed at a pressure of 2,0 to 2,3 kPa cm^{-2}. In the trials where lower mixture volumes were applied (250 to 500 $dm^3 ha^{-1}$) a disc-core type nozzle (disc D1, core (swirl plate) size 35) was used at the same pressure used above to provide a solid-cone spray.

At the time of herbicide application various weather parameters were recorded. These included the temperature, relative humidity, soil moisture content and wind speed. The relative humidity was calculated from readings obtained from a wet and dry bulb thermometer. Soil moisture content was measured as the loss in mass of five soil samples following

drying at 103 °C for two days. The samples were collected at five random points within the trial site and were taken from a depth of five to 10 cm. Wind speed was measured with a portable anemometer. The rainfall following herbicide application was also measured, and the time at which the first rain fell was noted thus providing an indication of the rain-free period.

For the majority of the trials, 10 m^2 plots, arranged where possible, in a randomised block design were used. To provide access for spraying and treatment evaluation, one metre-wide passages were slashed either manually or, where terrain allowed, with an Husqvarna 165R brushcutter.

For assessment of herbicide efficacy, the mortality of *C. odorata* was recorded. The technique used consisted of counting the number of stems at a height of 10 cm above ground level in three separate sample areas (one metre square) within the central portion of each treatment plot before spraying and again at final assessment. The sample areas were marked with pegs so that the same area could again be counted at final assessment. Since three plots (replicates) were used per treatment, the percentage *C. odorata* mortality was calculated from the data obtained from nine sample areas (three replicates per plot). The technique of using stem numbers for calculating mortality was selected because this was preferred to techniques such as visual, subjective estimations of mortality or leaf cover, or those where plant numbers are used. In *C. odorata* infestations it is difficult to define individual plants without excavating the roots and thus this

technique was not used.

The results obtained were all analysed by analysis of variance to enable statistical comparisons to be made. Where necessary, that is, if a binomial distribution was evident, the raw data (presented as the untransformed percentage mortality) was transformed by angular transformation (RAYNER,1967) before executing the analysis of variance.

The herbicides, concentrations and volumes used are presented for each of the trials. It should be mentioned here that the term "rates" will be avoided in describing concentrations and volumes used since use of the former term often results in confusion. Instead use will be made of "volume of herbicide applied" and "volume of mixture applied" (herbicide + water carrier).

3.3 Screening of Herbicides in Field Trials

For the chemical control of *C. odorata*, it was necessary to identify herbicides which would be toxic to this weed. To do this, a number of herbicides were selected, as described in section 3.1, since these were adjudged to be potentially effective against *C. odorata*. The herbicides were applied to the plants in early summer and late summer since considerable controversy generally exists regarding the application time optimum for herbicide efficacy.

Materials and Methods

Sites were prepared as previously described (section 3.2) at Melmoth and Clansthal. At Melmoth, one trial was conducted. In this site the *C. odorata* plants had regrown following slashing which had been implemented two years previously. Apparently this area had been cleared annually in the preceding three years, but due to the size of the plantation trees (*P. taeda*) this was no longer considered to be necessary. At the time of herbicide application the *C. odorata* plants were generally about two metres in height (length), although in some instances the height reached three metres. In this trial, herbicides were applied at the end of October, 1983.

At Clansthal two trials were conducted, the first (trial A) was sprayed in early December , 1983 and the second, (trial B) was sprayed in March, 1984. The *C. odorata* plants

growing in both sites had been subjected to annual slashing. (In this *E. grandis* plantation, manual slashing had been implemented as the weed control method.) Herbicides were therefore applied to regrowth. The plants in trial A were generally approximately 1,5 m in height, having regrown following slashing five to six months previously. In trial B, the *C. odorata* plants were generally about two metres in height. At this time considerable "scrambling" and profuse lateral branching had occurred. The resultant infestation was therefore comprised of an extremely dense thicket.

The herbicide treatments used in the three trials (that is, Melmoth, Clansthal A and Clansthal B) are listed in Table 3.3.1. Included are the concentrations, volume of herbicide applied per hectare and volume of mixture applied per hectare.

In the Melmoth trial, only three foliar applied herbicides and the soil applied tebuthiuron were tested (Table 3.3.1). For each herbicide a range of concentrations was tested. At the concentrations tested and the volume of mixture applied, a realistic value for the volume of product applied per hectare was obtained. This was aimed at because the expense of the herbicides at too high volumes would become prohibitive. The volume of mixture applied was generally 1000 $dm^3 ha^{-1}$. This volume was arrived at by spraying water plus a surfactant (Actipron*) onto the untreated plots; that is, the same area as the treatment plots. It was found that at a volume equivalent

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* Actipron is the wetter recommended for use with triclopyr.

Table 3.3.1 The herbicides and treatment concentrations applied to *C. odorata* in the Melmoth (*) and Clansthal trial A and B.

Herbicide	Product[a] (% of mixture)	Product[a] applied (dm^3 ha^{-1})	Mixture applied[b] (dm^3 ha^{-1})
2,4,5-T*	0,50	5,00	1000
	1,00	10,00	1000
	1,50	15,00	1000
glyphosate*	0,75	3,75	500
	1,50	7,50	500
	3,00	15,00	500
triclopyr*[c]	0,25	2,50	1000
	0,50	5,00	1000
	1,00	10,00	1000
3,6DCPA[d]	0,25	2,50	1000
	0,50	5,00	1000
	1,00	10,00	1000
imazapyr	0,25	2,50	1000
	0,50	5,00	1000
	1,00	10,00	1000
tebuthiuron*	-	20 kg	-

[a] commercial formulation.

[b] herbicide/water mixture

[c] triclopyr mixtures contained 0,5% Actipron, a surfactant recommended for use with this herbicide.

[d] 3,6-dichloropicolinic acid.

N.B. Concentrations used were those recommended by the distributors of the respective herbicides.

to 1000 $dm^3 ha^{-1}$ good overall wetting of the foliage was obtained with little undue run-off occurring from the leaves. For glyphosate, an equivalent of 500 $dm^3 ha^{-1}$ was used because it has been suggested that excessive dilution of the formulated product should be avoided so that the surfactant in the product is not rendered ineffective (FINDLAY, personal communication*).

The trial site plans are presented in Figures 3.3.1 (Melmoth), 3.3.2 (Clansthal A) and 3.3.3 (Clansthal B).

The common names, chemical names and active ingredient concentrations in the formulated products and the trade names were presented earlier (Table 3.1.3, section 3.1).

The environmental parameters measured at the time of herbicide application and the time and amount of rain after treatment are recorded in Table 3.3.2.

The temperature range recorded for each of the three trials was very similar. The humidity during application of herbicides in the Melmoth trial was lower, but not markedly so, than that recorded for the Clansthal trials A and B. The wind speed was low for all three trials. During spraying of the Clansthal trial A, there were times when the wind gusted up to 1,25 m sec^{-1} at which time spraying was halted due to excessive spray drift. The moisture content of the soil in the Melmoth trial was low, in comparison to that of the other two trials, at the time of application. However, 25 mm of

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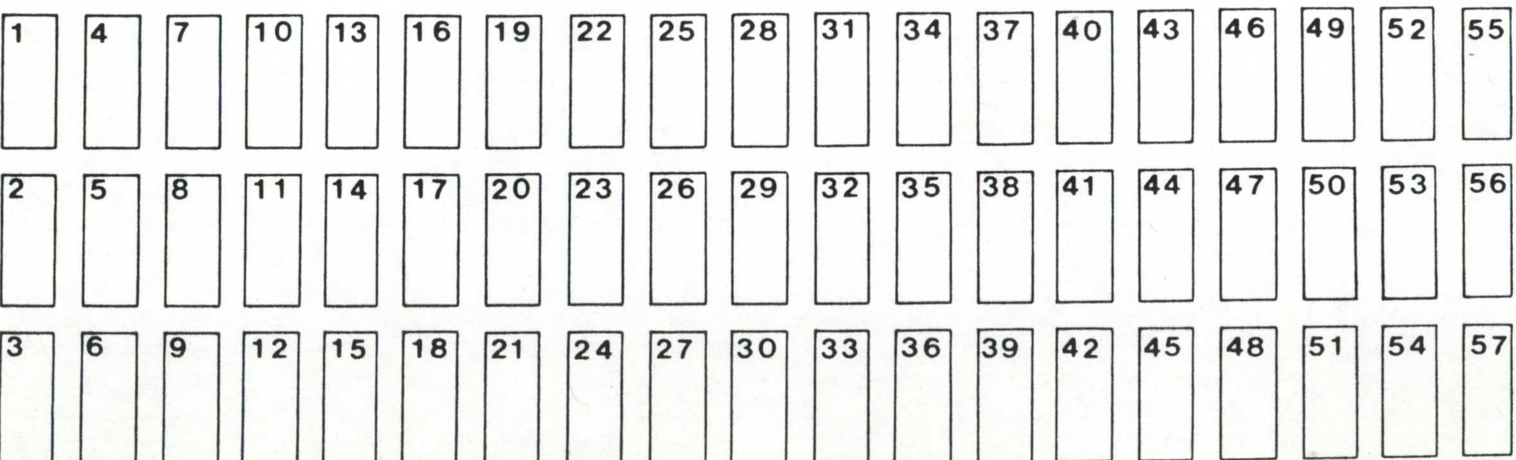
*FINDLAY, J.B.R., Monsanto South Africa (Pty.) Ltd., P.O.Box 78025, Sandton 2146, Republic of South Africa.

17 22
1 4 7 10 13 18 23 27 33 32 31
2 5 8 11 14 19 24 28
3 6 9 12 15 20 25 29
16 21 26 30
N

Treatments	(%)	Plot No.		
2,4,5-T	0,50	5	20	28
"	1,00	2	13	22
"	1,50	7	17	24
glyphosate	0,75	9	18	23
"	1,50	4	16	30
"	3,00	3	12	29

Treatment	(%)	Plot No.		
triclopyr	0,25	6	19	21
"	0,50	8	14	27
"	1,00	10	15	26
tebuthiuron		31	32	33
untreated		1	11	25

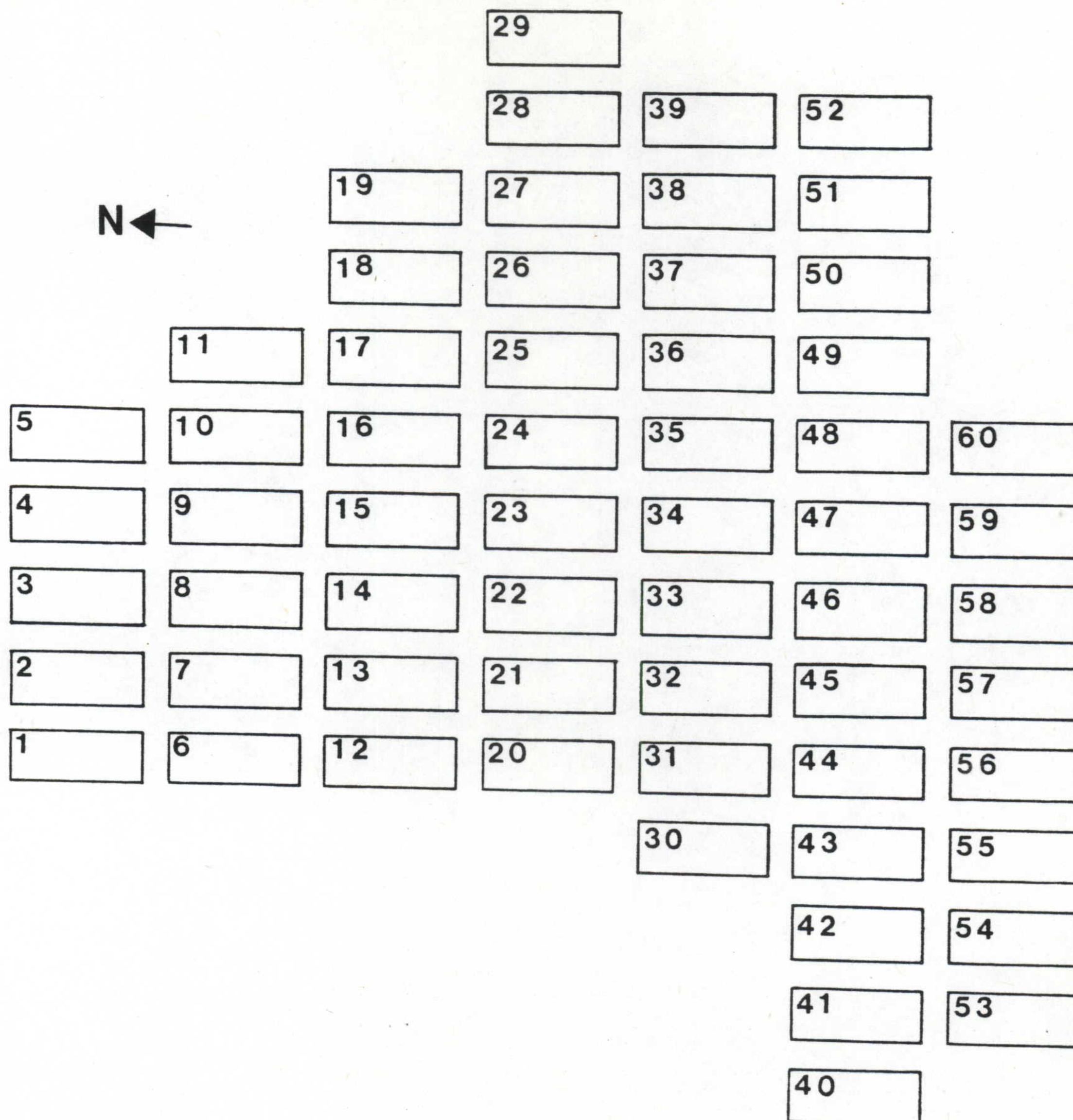
Figure 3.3.1 The Melmoth trial site.



Treatment	(%)	Plot No.		
2,4,5-T	0,50	4	31	44
"	1,00	5	37	42
"	1,50	3	30	54
glyphosate	0,75	6	24	46
"	1,50	14	36	57
"	3,00	9	22	55
tebuthiuron		19	29	51
untreated		2	13	18
"		25	28	38
"		43	50	52

Treatment	(%)	Plot No.		
triclopyr	0,25	11	33	45
"	0,50	8	32	39
"	1,00	16	27	53
3,6DCPA	0,25	7	26	56
"	0,50	1	35	40
"	1,00	12	23	41
imazapyr	0,25	15	20	49
"	0,50	17	34	48
"	1,00	10	21	47

Figure 3.3.2 The Clansthal A trial site.



Treatment	(%)	Plot No.		
2,4,5-T	0,50	5	42	48
"	1,00	4	43	53
"	1,50	16	23	39
glyphosate	0,75	2	36	55
"	1,50	19	33	57
"	3,00	13	20	27
untreated		6	8	9
"		11	14	22
"		24	26	30
"		32	35	37
"		38	58	59

Treatment	(%)	Plot No.		
triclopyr	0,25	18	28	34
"	0,50	10	47	54
"	1,00	17	31	52
3,6-DCPA	0,25	15	49	60
"	0,50	12	41	44
"	1,00	1	25	51
imazapyr	0,25	29	46	50
"	0,50	7	40	45
"	1,00	3	21	56

Figure 3.3.3 The Clansthal B trial site.

Table 3.3.2 Weather conditions and soil moisture content at time of herbicide application and rainfall subsequent to treatment in the Melmoth and the Clansthal A and B trials.

Conditions recorded	Herbicide Trial		
	Melmoth	Clansthal A	Clansthal B
Temp. range (°C)	23,0-27,5	24,0-27,0	23,5-28,0
Relative humidity (%)	41-63	73-83	65-76
Wind speed (m sec^{-1})	0,10-0,75	0,35-1,25	0,30-0,75
Soil moisture (%)[a]	9,5 ±0,9	19,1±0,8	17,1±0,9
Rainfall: (mm) h post application	25 48	5 72	2 144

[a] mean ± S.E. of five samples.

rain fell approximately 48 hours after application. In both the Clansthal trials, the moisture content was high and small amounts of rain fell three and six days after treatment at the Clansthal trial A and B sites respectively. It should be noted that at the time of spraying, the plants were not visibly stressed.

Subsequent to spraying, visual interim assessments were made on each of the trials. These are not reported in detail since these did not make any noteworthy contribution to the end result. However, mention will be made of certain observations should this be deemed necessary. Final assessment of the Melmoth trial was conducted one year after treatment, that of the Clansthal trial A, 10 months after treatment, and the Clansthal trial B, eight months after herbicide application. At final assessment the number of live stems in the sampling areas was recorded, care being taken to positively confirm whether the stems were alive or dead.

Results

For each of the herbicides, three concentrations were tested. To facilitate explanation, the concentrations will be referred to as "low", "medium" and "high". In so doing, continual reference to Table 3.3.1, where details of the treatments are listed, will be avoided.

As described in section 3.2, the percentage mortality was calculated from data collected in nine sample areas (three per replicate plot). In computing the mean percentage morta-

lity per treatment, the standard error for each treatment was also calculated from the data for nine samples. The standard error is included in the tables of results to provide an indication of the variation which occurred within a treatment.

Since the aim of these trials was to identify those herbicides, and the respective concentrations, which provide an acceptable level of control, emphasis will be placed on the treatments which resulted in the highest mortality of *C. odorata*. Only where deemed necessary, will mention be made of the less effective treatments. The treatments were also ranked according to their efficacy; the lower value of the rank, the higher the efficacy.

The percentage mortality, recorded as untransformed (actual mortality) and transformed, for each of the treatments applied in the Melmoth trial is presented in Table 3.3.3. The treatments ranked 1 to 6 provided for a similar (p = 0,05) percentage mortality. These treatments included all three triclopyr concentrations, the medium and high 2,4,5-T concentrations and the tebuthiuron treatment. The actual mortality for these six treatments ranged from 94 to 100 per cent. All the other treatments resulted in a percentage mortality which was lower than 90 per cent. The lowest mortality was predictably obtained in the untreated plots. However, it is pertinent to note that some mortality (two per cent) did occur in these plots (Table 3.3.3).

Within herbicides, a dose related mortality response was observed, especially for the triclopyr and 2,4,5-T concentrations (Table 3.3.3). In the glyphosate treatments, a

Table 3.3.3 *C. odorata* mortality (percentage) following herbicide application in the Melmoth site (± S.E. of nine values).

Herbicide	Mean Percentage Mortality			
	Conc.	Untransformed	Transformed	Rank[1]
untreated	-	2±1	8	11
2,4,5-T	low	48±17	44	10
	medium	94±3	77	6
	high	100	90	1
glyphosate	low	76±7	61	9
	medium	89±3	72	7
	high	82±7	65	8
triclopyr	low	95±2	78	5
	medium	96±3	81	3
	high	98±2	86	2
tebuthiuron	-	96±3	81	4
L.S.D. (p=0,05)			14	

[1] Rank of efficacy

higher percentage mortality occurred with the medium concentration than with the high concentration; however the difference was not significant.

The results obtained for both the Clansthal trials are presented in Table 3.3.4. Included in these results is the mean rank, computed from the efficacy rank obtained for each trial.

In the Clansthal trial A, treatments ranked 1 to 7 resulted in a similar (p = 0,05) percentage mortality. These included the medium and high 2,4,5-T, high glyphosate, medium and high triclopyr, the high 3,6-dichloropicolinic acid and the tebuthiuron treatments. Three treatments resulted in a mean percentage mortality similar to that which occurred in untreated plots. These were the low glyphosate and the low and medium imazapyr treatments.

In trial B the treatments ranked 1 to 7 resulted in a similar (p = 0,05) percentage mortality (Table 3.3.4). These treatments were the medium and high 2,4,5-T, low, medium and high triclopyr and the high glyphosate and imazapyr applications. For this group, the percentage mortality observed in the field ranged from 90 to 100 per cent. Five per cent natural mortality occurred in the untreated plots. This mortality was similar to that obtained for the low 3,6-dichloropicolinic acid and imazapyr treatments (Table 3.3.4). No results are presented for the tebuthiuron treatment because this treatment was not applied in the B trial due to its toxic effect on the plantation trees in the previous two trials.

Table 3.3.4 *C. odorata* mortality (percentage) following herbicide application in both Clansthal trial A and B (± S.E.s of nine values).

		Mean Percentage Mortality						
		Trial A			Trial B			
Herbicide	Conc.	Untransformed	Transformed	Rank[1]	Untransformed	Transformed	Rank[1]	Mean Rank
untreated	-	3±2	6	16	5±3	10	17	16,5
2,4,5-T	low	63±16	54	9	84±5	71	8	8,5
	medium	84±7	68	5	92±5	77	6	5,5
	high	94±2	77	3	99±1	87	3	2,0
glyphosate	low	0	0	17	54±8	47	13	15,0
	medium	27±14	26	13	65±10	54	11	12,0
	high	80±4	64	7	90±8	75	7	7,0
triclopyr	low	62±7	52	10	97±2	84	4	7,0
	medium	90±4	72	4	100	90	1	2,5
	high	93±6	80	1	100	90	1	1,0
3,6DCPA[a]	low	39±10	38	12	19±11	16	15	13,5
	medium	72±4	58	8	67±11	56	10	9,0
	high	84±4	66	6	69±7	56	9	7,5
imazapyr	low	14±9	19	15	19±8	26	14	14,5
	medium	22±14	25	14	61±13	51	12	13,0
	high	49±11	45	11	92±9	80	5	8,0
tebuthiuron[b]	-	95±3	79	2	-	-	-	2,0
L.S.D. (P=0,05)		-	19	-	-	18	-	-

[a] 3,6-dichloropicolinic acid.

[b] tebuthiuron was not applied in trial B because this herbicide killed the plantation trees.

[1] rank of efficacy.

As at Melmoth, a dose related response was obtained for the herbicides in both trials. That is, the higher the herbicide concentration, the higher the percentage mortality recorded at final assessment.

The mean rank can be used to rank the treatments according to consistency of herbicide efficacy. Based on this, the high triclopyr concentration clearly provided the greatest percentage mortality. Other treatments which also consistently resulted in a high percentage mortality (a mean of 90 per cent or more for the A and B trials) were the high 2,4,5-T and medium triclopyr treatments. The tebuthiuron treatment was arbitrarily ranked together with the high 2,4,5-T treatment because, although this herbicide was only used in trial A, it had also provided a very high percentage mortality in the Melmoth trial. The medium 2,4,5-T treatment provided for a mean mortality of 88 per cent and the next most consistently effective treatment was the high glyphosate concentration (85 per cent mean mortality) and the low triclopyr concentration (73 per cent mean mortality). The other treatments used were either inconsistent in their effect, or if consistent, the percentage mortality was comparatively low.

There was a marked difference between the results obtained for the two trials. In all treatments, excluding only the three 3,6-dichloropicolinic acid concentrations, the percentage mortality in trial B was higher than that obtained in trial A. This was especially noticeable with, for example, the glyphosate and imazapyr treatments. However, the ranking order generally followed the same trend in both trials.

Discussion

For evaluation of the trial results obtained in the Melmoth and Clansthal A and B trials, it is firstly necessary to define a level of mortality which can be referred to as acceptable control, although obviously, 100 per cent mortality would be preferred. An acceptable level of control would be an indication of sufficient efficacy of the herbicides on *C. odorata* which would therefore make a particular treatment suitable for use on this weed. Acceptable control of *C. odorata* is however, difficult to define since the parameters requiring consideration are likely to differ due to the situations in which the infestations occur. For example, in silviculture a level of control sufficient to eliminate the detrimental effect of *C. odorata* on timber production would be aimed at. For instance, in a situation where the growth of plantation species seedlings is being suppressed, the *C. odorata* should be reduced to a level where this suppression no longer occurs. This would not necessarily entail complete eradication of *C. odorata*. Similarly, in a rangeland situation complete eradication is not essential since a certain level of control would be adequate to improve grazing production. However, where infestations occur in conservation areas, it would be desirable to eradicate, if possible, all *C. odorata*. Economic considerations therefore play a role in *C. odorata* (and any other weed) control measures. For the purpose of this dissertation, an arbitrary level of 90 per cent mortality, that is, 90 per cent control measured as the decrease in stem number, was selected as "acceptable control". This value was arrived

at by taking into account such factors as the weed's rapid growth and its high reproductive potential as well as visual observations which indicated that a reduction of 90 per cent of dense infestations would appreciably decrease the detrimental effect of *C. odorata* on desirable species and its potential as a fire-hazard.

Based on the assumption that 90 per cent mortality constitutes an acceptable level of *C. odorata* control, those herbicides which provided 90 per cent mortality are considered to be effective in the chemical control of this weed. In the Melmoth trial, these treatments included the three triclopyr concentrations, the medium and high 2,4,5-T concentrations and the tebuthiuron treatment, while the medium glyphosate concentration was a marginal case. In the Clansthal trials, an acceptable level of control was obtained with the high 2,4,5-T, the medium and high triclopyr concentrations and the tebuthiuron treatment where these were applied in early summer. However, where herbicides were applied in late summer, the number of treatments providing acceptable control was higher. These include the medium and low 2,4,5-T, high glyphosate, low, medium and high triclopyr and the high imazapyr treatments.

The most consistently effective herbicide in all three trials was triclopyr. The medium and high concentrations of this herbicide provided similar levels of control of *C. odorata* as did the tebuthiuron treatment, which, at the time these trials were conducted, was the only herbicide registered for use on *C. odorata*. Subsequently, triclopyr was registered

(December, 1984). In the registration trials, conducted by Agrihold, a holding company of Agricura, it was found that a concentration of 0,5 per cent (herbicide in water) provided effective control of *C. odorata* (JOSEPH, personal communication*). Registration was however, obtained for the use of this concentration applied at 400 to 800 $dm^3 ha^{-1}$ mixture volume onto plants 0,5 to 1,5 m in height. These results compare favourably with the results of this study in which herbicide was applied to plants generally ranging from 1,5 to 2,0 m in height.

It is pertinent to compare the suitability of tebuthiuron and triclopyr application for *C. odorata* control. As described in section 3.1, the tebuthiuron formulation used in these trials, Graslan 20 P (registered trade name) is a granular herbicide applied to the soil. This product was formulated in such a manner that although it is a non-selective herbicide, its application generally only results in the death of grasses in small patches in the vicinity of each granule. Toxicity to larger species such as shrubs and trees is however, non-selective. Therefore application of tebuthiuron is likely to result in the death of a variety of species as was the case in the Melmoth and Clansthal A trials and not only the target weed, in this case, *C. odorata*. Consequently, the application of this herbicide is limited to situations where *C. odorata* occurs in a grassland situation or situations where the death of other arborescent and shrub vegetation amongst which the

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weed is growing, is desired.

Triclopyr, which is selectively toxic to broad-leaved species (section 3.1), can also be applied to *C. odorata* growing in a grassland situation with little effect on the desirable grasses. However, where *C. odorata* is growing amongst other vegetation which includes desirable broad-leaved species, death or growth retardation of these will occur if sprayed. The difference between triclopyr and tebuthiuron is however, that triclopyr can be applied selectively, by a directed spray on the target weed, and thus selective control can be obtained which is not generally possible with tebuthiuron. Therefore in a timber plantation where the application to the foliage of timber species can be avoided, triclopyr can be used safely. A similar operation would sometimes be possible where *C. odorata* is found growing below the canopy of desirable, indigenous, arborescent vegetation.

Taking into account the considerations discussed above, triclopyr has a wider application than tebuthiuron for the control of *C. odorata*.

Another factor which should be noted is the cost of the herbicides. (The cost of various application techniques will not be considered however, since this forms a separate issue from the theme of this study.) The costs of the herbicides and the costs for application at the volumes or mass found to provide an acceptable level of *C. odorata* control in this study are presented in Table 3.3.5.

For triclopyr, a figure of 5,0 $dm^3 ha^{-1}$ was used because

Table 3.3.5 The relative costs of triclopyr and tebuthiuron and the cost of the herbicide per hectare which would be required for acceptable control of *C. odorata*[1].

Herbicide	Cost per kg/ dm^3 (Rands)	Mass or vol. required ha^{-1}	Total cost of herbicide ha^{-1} (Rands)
Tebuthiuron	17,09	20 kg	341,80
Triclopyr	52,00	5 ℓ	260,00

[1] Costs provided by Agricura, Pietermaritzburg, Republic of South Africa (April, 1985)

the equivalent of this was found to consistently provide an acceptable level of *C. odorata* control, similar to that of the 1,0 per cent concentration which is equivalent to 10,0 $dm^3 ha^{-1}$.

The figures provided refer to a situation where dense, homogenous infestations of *C. odorata* 1,5 to 2,0 m in height are present. The cost of the triclopyr per hectare is less than that of tebuthiuron; the cost is therefore an additional recommendation for the use of triclopyr as opposed to tebuthiuron. However, an additional amount should also be allowed for the price of the surfactant (Actipron, registered trade name) which is recommended for use with the application of triclopyr.

These costs, even that of triclopyr, are high; therefore additional studies were conducted (section 3.5(i) and (ii)) in which attempts were made to reduce the volume of herbicide required for an acceptable level of *C. odorata* control.

Of the other herbicides tested, 2,4,5-T at a high concentration and to a lesser degree, the medium concentration, provided an acceptable level of control. However, the availability of this herbicide, which has similar advantages in application to those of triclopyr, and is also selectively toxic to broad-leaved species, is limited in South Africa. Furthermore, it is reported that 2,4,5-T is to be removed from the market due to unacceptable levels of dioxin (a carcinogenic by-product of 2,4,5-T production) in 2,4,5-T formulations.

A high concentration of glyphosate (three per cent

in water) was also found to provide an acceptable level of control. At this concentration, the equivalent of 15 $dm^3 ha^{-1}$ product (that is, 7,2 kg ha^{-1} active ingredient) was applied. In the Ivory Coast, DUFOUR, QUENCEZ & BOUTIN (1979) found that *C. odorata* could be controlled with three kilogrammes per hectare active ingredient glyphosate. Therefore, the results obtained in this study do not compare with those found in the Ivory Coast. The discrepancy might have been due to poor spray coverage of the plants since the equivalent of only 500 $dm^3 ha^{-1}$ mixture was applied. This possibility was investigated in subsequent trials (section 3.5).

During the interim visual assessments, it appeared as though imazapyr would result in acceptable levels of *C. odorata* control. Although intially slow, the effect of this herbicide resulted in complete defoliation, even at the lowest concentration. In addition, apical necrosis occurred and, with the high concentrations die-back to ground level occurred. However, by the time final assessment was made considerable regrowth had occurred. Subsequent monitoring of these plots has shown that dense, apparently healthy thickets of *C. odorata* have become established. It is also notable that in the Clansthal B trial, *E. grandis* trees in the imazapyr treatment plots, had died, presumably due to root-uptake of the herbicide washed into the soil, confirming that imazapyr is readily absorbed by roots (CIARLANTE, FINE & PEOPLES, 1983).

The other herbicide tested in the Clansthal trials, 3,6-dichloropicolinic acid, did not provide acceptably high mortality of *C. odorata* even though this herbicide is re-

portedly highly toxic to the Asteraceae (HAAGSMA, 1975). Based on these results therefore, this herbicide cannot be recommended for *C. odorata* control.

As can be seen in the results presented in Table 3.3.4, and mentioned earlier, the percentage mortality for the majority of treatments was higher in the Clansthal trial B than in the trial A. This could possibly be attributed to the time of application, that is, late summer versus early summer. The assumption would be supported by reports in which it has been found that translocation of assimilates, and therefore presumably phloem-transported herbicides, is predominantly basipetal during late summer (ERASMUS & VAN STADEN, 1983b). However, caution is required in the interpretation of these results obtained for *C. odorata* since it is possible that the differences in percentage mortality were due to other factors.

In section 3.1 where certain aspects pertaining to the herbicides used in this investigation were reviewed, reference was made to factors influencing the absorption, translocation and mode of action of the herbicides. There are several factors which are common to all the herbicides used, especially those applied to the foliage. For instance, temperature, humidity and moisture stress have been found to influence the absorption and translocation of 2,4,5-T (RICHARDSON, 1977), glyphosate (GOTTRUP, O'SULLIVAN, SCHRAA & VANDEN BORN, 1976; JORDAN, 1977), triclopyr (RADOSEVICH & BAYER, 1979), 3,6-dichloropicolinic acid (O'SULLIVAN & KOSSATZ, 1984) and imazapyr (HASUI, KADOTA, IKEDA & TANAKA, 1983). In addition, the light intensity has been implicated in influencing absorption, trans-

location and mode of action of 2,4,5-T (BRADY, 1969), glyphosate (KELLS & RIECK, 1979; LUND-HØIE, 1979; MOOSAVI-NIA & DORE, 1979b) and imazapyr (LAPADE, MANIMTIM, CALORA & LALAP, 1983). Any one or more of these factors may have been responsible for the differences observed in the Clansthal A and B trials.

Although the trial sites were adjacent, the density of canopy formed by the *E. grandis* trees in the region of trial B was greater. Being on a hot western aspect, the denser canopy might have provided for a more favourable micro-climate than was the case in trial A. It is therefore possible that the difference in mortality might have occurred as a result of differences in the micro-climate of the two sites and their influence on absorption, translocation and mode of action of the herbicides. The more "favourable" micro-climate for herbicide action in the trial B site might thus have resulted in enhanced herbicide efficacy. The assumption that the greater efficacy in trial B than in trial A might be due to factors other than, or in combination with a seasonal effect, is supported by the results obtained in the Melmoth trial which resemble those of the Clansthal B trial, even though the former trial was conducted earlier in the summer than the Clansthal trial A.

Other factors than those already mentioned might also have contributed to the enhanced efficacy observed in the Clansthal trial B. As reviewed by HOLLY (1976), the morphological features of the absorption surfaces and absorption area, predominantly the leaves, also influence herbicide ab-

sorption. Therefore the differences in the light intensities between trial A and B might have provided for not only a difference in the morphology of the *C. odorata* leaves, but also an increased area for absorption of herbicide as has been suggested for the increased activity of glyphosate recorded for *Imperata cylindrica* (L.) Beauv. and for *Cyperus rotundus* L. growing in shaded areas (MOOSAVI-NIA & DORE, 1979b).

Two other considerations regarding the chemical control of *C. odorata* were investigated. Firstly, the persistence of the herbicides in the soil and secondly, the colonisation of the treatment plots following control. These are reported in the following section (3.4).

3.4 Post-spray Colonisation of Herbicide Treatment Plots

A possible consequence of the control of *C. odorata* infestations is the creation of the classical "ecological vacuum" (HILL, 1977) situation, thus providing a disturbed area suitable for colonisation by species which are opportunistic, such as environmental (invader) weeds (HOLZNER, 1982). It was therefore decided that a pre-and post-spray species check-list should be prepared in order to determine the short-term course of events following chemical control of *C. odorata*. Additionally, this would provide information pertaining to the persistence in the soil of herbicides tested in the screening trials described in the previous section (3.3).

Materials and Methods

This study was conducted concurrently with stem counts performed before spraying and at final assessment of the three herbicide trials, namely in the Melmoth and Clansthal A and B sites, conducted to screen herbicides for efficacy against *C. odorata*. The details of the sites and the site plans were presented in section 3.3.

During stem counting in each of the treatment plots prior to herbicide application a survey of species present, other than *C. odorata* and the timber species, was made. These are referred to as the pre-spray species and constitute the species present in the trial site at the time herbicides were applied. The height of these species relative to the height

of the *C. odorata* canopy was also noted. That is, species present were recorded either as being low-growing (below the *C. odorata* canopy) or tall-growing (same height as the *C .odorata* canopy). In no cases were there any species recorded taller than the *C. odorata*. At final assessment of the treatment effects, the species survey was repeated; these are referred to as the post-spray species and constitute the species present in the trial sites at the time of final assessment. Where species could not be positively identified *in situ*, samples were collected for later identification.

The data obtained for the pre- and post-spray species checklist were used to calculate the frequency of occurrence of the various species in each treatment (each treatment being comprised of three plots). At final assessment, an estimate of the percentage cover formed by the combined presence of the species in each plot (excluding *C. odorata* and timber species), was made. The estimates of percentage cover were scored on a scale of one to five where one represented one to 20 per cent, two 21 to 40 per cent, three 41 to 60 per cent, four 61 to 80 per cent and five 81 to 100 per cent cover. These data were used to calculate a weighted mean percentage cover for each herbicide treatment. At final assessment, the presence or absence of *C. odorata* seedlings was also recorded.

The results obtained in this investigation are discussed in association with those obtained for the herbicide trials in section 3.3. Nomenclature is according to GIBBS RUSSELL (1984).

Results

The species recorded pre- and post-spray represent a diverse collection of species ranging from annuals to woody perennials. However, special attention will only be paid to those species which are considered to be a threat as invader weeds. Therefore, details presented in the text are predominantly those of the woody perennials.

Four pre-spray woody perennial species and a grass species were present in the treatment plots of the Melmoth trial (Table 3.4.1). These species were *Lantana camara* L., *Psidium guajava* L. (guava) and *Trema orientalis* (L.) Blume and *Solanum mauritianum* Scop. (bugweed) and the grass, *Setaria megaphylla* (Steud.) Dur. & Schinz.. Tall-growing *L. camara*, guava and *T. orientalis* survived triclopyr application and tall-growing guava also survived glyphosate application. All the bugweed plants which survived were low-growing. *S. megaphylla* survived the tebuthiuron treatment.

One year after herbicide application, at final assessment, 15 species were present in the Melmoth trial plots (Table 3.4.1). The most frequently occurring of these post-spray species were the broad-leaved annual, *Ageratum houstonianum* Mill. and the grass, *Panicum maximum* Jacq. both of which were present in 10 treatments. Bugweed was a frequently occurring species, being present in the plots of nine treatments. Of the 15 species, six occurred infrequently and were only present in a single treatment (Table 3.4.1). The remaining species were not considered to be of relevance in the

Table 3.4.1 The pre- (•) and post-spray (x) species occurring in the Melmoth trial.

Herbicide Applied: Common name		2,4,5-T		glyphosate			triclopyr		tebuthiuron	untreated	
Herbicide Applied: Product (%)	0,50	1,00	1,50	0,75	1,50	3,00	0,25	0,50	1,00	2 mg-²	-	
C. odorata: Mortality (%)	48	94	100	76	89	82	95	96	98	96	2	
Other species: Mean cover (%)	21-40	21-40	41-60	21-40	21-40	21-40	41-60	21-40	41-60	1-20	21-40	
Other species: No. per treatment	5	4	8	6	5	5	9	5	6	1	4	Frequency of Occurrence
Species												
Ageratum houstonianum Mill.	x	x	x	x	x	x	x	x	x		x	10
Bidens pilosa L.			x				x					2
Berkheya rhapontica (DC.)Hutch.& Burtt Davy			x									1
Commelina livingstonii C.B. Cl.			x		x		x		x			4
Dioscorea sylvatica (Kunth) Eckl.				x								1
Ipomea L., sp.									x			1
Lantana camara L.						x	•x	x				3
Mikania natalensis DC.	x	x			x	x	x				x	6
Panicum maximum Jacq.	x	x	x	x	x	x	x	x	x		x	10
Psidium guajava L.		x	x	•x			•x			•		4
Setaria megaphylla (Steud.)Dur. & Schinz.										•x		1
Solanum mauritianum Scop.	x		x	x	x	•x	•x	•x	x		x	9
Senecio L., sp.	x											1
Trema orientalis (L.) Blume			x	x	•			•x	x			4
Vernonia natalensis Sch. Bip.							x					1

context of this study.

The mean percentage cover formed by the post-spray species in the treatments was generally not higher than 21 to 40 per cent (Table 3.4.1). In three treatments (high 2,4,5-T and the low and high triclopyr) however, the cover was 41 to 60 per cent. With each of these treatments, a high percentage of *C. odorata* mortality had been achieved.

As regards the number of species occurring in the plots of each treatment, the lowest number (one, *S. megaphylla*) was present in the tebuthiuron treatment (Table 3.4.1); this co-incided with the lowest percentage cover recorded in the Melmoth trial. There was no apparent trend between the number of species and herbicide applied. However, it is notable that the number of species in the untreated plots was lower than the mean number of species per treatment.

In the Clansthal trial A, six pre-spray species were recorded (Table 3.4.2). These consisted of three grass species, low-growing *P. maximum* and *Rhynchelytrum repens* (Willd.) C.E. Hubb and tall-growing *Sorghum halepense* (L.) Pers., two woody perennials, *Dichrostachys cinerea* (L.) Wight and Arn. (tall-growing) and *L. camara* (low- and tall-growing) was also present. A broad-leaved creeper, *Mikania natalensis* D.C. was found growing on the *C. odorata* canopy.

Both *P. maximum* and *R. repens* were again present in the post-spray species checklist, having survived glyphosate and triclopyr treatments. However, while *S. halepense* survived 2,4,5-T application it was killed by imazapyr. The

Table 3.4.2 The pre- (•) and post-spray (x) species occurring in the Clansthal trial A site.

Species																		Frequency of occurrence
Herbicide Applied: Common name	2,4,5-T			glyphosate			triclopyr			3,6-DCP[a]			imazapyr			tebuthiuron	untreated	
Herbicide Applied: Product (%)	0,50	1,00	1,50	0,75	1,50	3,00	0,25	0,50	1,00	0,25	0,50	1,00	0,25	0,50	1,00	2 gm^{-2}	-	
C. odorata: Mortality (%)	63	84	94	0	27	80	62	89	93	39	72	84	14	22	49	95	3	
Other Species: Mean cover (%)	41-60	41-60	61-80	21-40	21-40	21-40	41-60	41-60	61-80	21-40	41-60	41-60	21-40	21-40	21-40	41-60	21-40	
Other Species: No. per treatment	12	17	16	12	11	10	10	14	15	7	11	14	6	14	11	8	6	
Abutilon grantii A. Meeuse									x									1
Abutilon mauritianum (Jacq.) Medic.		x	x	x	x	x		x	x		x	x	x	x	x			12
Abutilon sonneratianum (Cav.) Sweet	x	x		x	x	x		x	x	x	x	x		x	x			12
Ageratum houstonianum Mill.		x	x	x			x	x			x				x			7
Anomatheca laxa (Thunb.) Goldbl.	x																	1
Asclepias physocarpa (E. Mey.) Schltr.	x	x	x		x	x			x			x		x				8
Asparagus saundersiae Bak.				x														1
Berkheya rhapontica (DC.) Hutch. & Burtt Davy	x	x	x	x	x	x	x	x	x	x		x	x	x	x	x	x	16
Canthium c.f. *spinosum* (Klotzsch) Kuntze															x			1
Cnestis natalensis (Hochst.) Planch. & Sond.			x															1
Commelina livingstonii C.B. Cl.	x																	1
Conyza bonariensis (L.) Cronq.				x														1
Crotalaria pallida Ait.				x		x												2
Croton L., sp.			x															1
Cyperus rotundus L.		x	x					x	x		x	x				x		7
Dalbergia obovata E. Mey.										x				x				2
Desmodium canum (J.F.Gmel.) Schinz & Thell.				x	x													2
Dichrostachys cinerea (L.) Wight & Arn.												•x						1
Erigeron karvinskianus DC.	x								x					x				3
Hewittia sublobata (L.f.) Kuntze	x	x	x			x	x	x			x	x		x		x		10
Hibiscus aethiopicus L.										x								1
Hibiscus cannabinus L.		x	x			x	x		x		x					x		7
Hibiscus fuscus Garcke		x										x					x	3
Indigofera eriocarpa E. Mey.														x				1
Lantana camara L.			x					•x										2
Melhania prostrata DC.												x					x	2
Mikania natalensis DC.		x			•		•					•			•			1
Oxalis corniculata L.		x									x			x				3
Panicum maximum Jacq.	x	x	•x	x	x	x	x	x	•x	x	x	x	•x	x	x	x	x	17
Passiflora suberosa L.			x	x														2
Phyllanthus burchelli Muell. Arg.									x									1
Psidium guajava L.					x													1
Rhus chirindensis Bak. f.					x													1
Rhynchelytrum repens (Willd.) C.E. Hubb.		x	x		x	•	x	•x	x		x	x	x	x	x			11
Senecio madagascariensis Poir.	x						x		x									3
Senecio c.f. *polyanthemoides* Sch. Bip.								x	x						x			3
Setaria megaphylla (Steud.) Dur. & Schinz		x		x				x				x						4
Sida rhombofolia L.	x						x	x				x		x		x		6
Solanum incanum L.								x										1
Solanum mauritianum Scop.															x			1
Sorghum halepense (L.) Pers.	•x	x	x	x	x	x	x	x	x	x	x	x	•	x	x		x	15
Sporobolus R. Br., sp.													x					1
Tagetes minuta L.															x			1
Trimeria grandifolia (Hochst.) Warb.																x		1
Vernonia natalensis Sch. Bip.	x	x	x		x	x	x			x	x		x	x		x	x	12
Vigna vexillata (L.) A Rich. sens. lat.									x									1
Zanthoxylum capense (Thunb.) Harv.		x	x															2

[a] 3,6-dichloropicolinic acid

low-growing *L. camara* was still present at final assessment, while the tall-growing *L. camara* survived triclopyr application and tall *D. cinerea* survived 3,6-dichloropicolinic acid application. *M. natalensis* was killed by all herbicides applied to the plots in which it had been present.

Ten months after herbicide application, the 47 species listed in Table 3.4.2 were recorded in the Clansthal trial A. The two grasses, *P. maximum* and *S. halepense*, and the herbaceous *Berkheya rhapontica* (D.C.) Hutch. and Burtt Davy were the most recurrent species. The other frequently occurring species are of little importance as invaders. No appreciable increase in perennial woody species frequency was recorded.

Although not distinctive, certain trends appear to exist in the data presented in Table 3.4.2. For instance, increased mortality of *C. odorata* generally co-incided with a higher percentage cover. This was especially true with those herbicides which are selectively toxic to broad-leaved species, for example in the triclopyr treatments. With the low concentration of triclopyr, where 62 per cent *C. odorata* mortality was obtained, a cover of 21 to 40 per cent was recorded while with the high triclopyr concentration, resulting in 93 per cent *C. odorata* mortality, the cover was 61 to 80 per cent (Table 3.4.2). With the non-selective herbicides (glyphosate and imazapyr) the percentage cover was low, similar to the untreated plots, regardless of *C. odorata* mortality. Percentage mortality also appeared to be associated with a higher number of post-spray species, for example in the triclopyr treatments. The lowest number of species was recorded

for the low imazapyr treatment and the untreated plots, both of which contained a high density of *C. odorata*.

In the Clansthal trial B, the only notable pre-spray woody species was tall-growing guava; this survived the high 3,6-dichloropicolinic acid treatment (Table 3.4.3). The four other pre-spray species were the indigenous perennial *Cnestis natalensis* (Hochst.) Planch and Sond., the geophyte *Hypoxis rooperi* S. Moore, the grass *P. maximum* and *Phoenix reclinata* Jacq., a palm species. These four, which were all low-growing, survived herbicide application.

At final assessment, 40 species were found in the Clansthal trial B. The most recurrent species was *P. maximum*. Other frequently occurring species included the annuals *A. houstonianum* and *Erigeron karvinskianus* D.C. and the sedge *Cyperus rotundus* L.. More than half the species (22) were present in a single treatment only (Table 3.4.3).

As with the Clansthal trial A, no definite trends were apparent. However, certain inclinations were observed. With the application of the selective herbicides (2,4,5-T, triclopyr and 3,6-dichloropicolinic acid) the percentage cover was high in the treatments where high *C. odorata* mortality had occurred (Table 3.4.3). In the non-selective herbicide treatments, glyphosate and especially imazapyr, the percentage cover was generally lower than that of the selective herbicides, although the percentage cover recorded was relatively high in those treatments which had provided for a high percentage mortality of *C. odorata*. With the exception of the

Table 3.4.3 The pre- (•)and post-spray (x)species occurring in the Clansthal trial B site.

Herbicide Applied: Common Name	2,4,5-T	2,4,5-T	2,4,5-T	glyphosate	glyphosate	glyphosate	triclopyr	triclopyr	triclopyr	3,6-DCPA[a]	3,6-DCPA[a]	3,6-DCPA[a]	imazapyr	imazapyr	imazapyr	untreated	Frequency of occurrence
Product (%)	0,50	1,00	1,50	0,75	1,50	3,00	0,25	0,50	1,00	0,25	0,50	1,00	0,25	0,50	1,00	-	
Species																	
Ageratum houstonianum Mill.	x		x	x	x	x	x	x	x		x			x		x	11
Anomotheca laxa (Thunb.) Goldbl.	x														x		2
Asclepias physocarpa (E. Mey.) Schlt.	x	x	x									x					4
Berkheya rhapontica (DC.) Hutch. & Burtt Davy		x															1
Canthium c.f. *spinosum* (Klotzsch) Kuntze														x			1
Cirsium vulgare (Savi) Ten.						x											1
Cnestis natalensis (Hochst.) Planch & Sond.			•x														1
Commelina livingstonii C.B. Cl.						x									x		2
Cyperus rotundus L.	x		x		x		x	x	x	x	x	x		x		x	11
Desmodium canum (J.F. Gmel.)Schinz & Thell.														x			1
Ekebergia capensis Sparrm.				x													1
Eragrostis curvula (Shrad.) Nees							x										1
Erigeron karvinskianus DC.	x	x	x	x		x	x		x			x	x	x	x		11
Eriosema (DC.) Desv., sp.		x															1
Helichrysum Mill., sp.												x					1
Hewitta sublobata (L.f.) Kuntze						x											1
Hibiscus cannabinus L.				x							x						2
Hibiscus fuscus Garcke	x											x					2
Hydrocotyle americana L.								x									1
Hypoxis rooperi S. Moore.	x	•x															2
Lactuca capensis Thunb.				x													1
Oxalis corniculata L.			x		x		x					x					4
Panicum maximum Jacq.	x	x	x		•x		x	x	•x	x	x	x		x	x	x	13
Phoenix reclinata Jacq.								•x									1
Phyllanthus burchelli Muell. Arg.		x								x							2
Psidium guajava L.												•x					1
Rhynchelytrum repens (Willd.) C.E. Hubb	x	x									x						3
Sarcostemma viminale (L.) R. Br.												x					1
Senecio madagascariensis Poir.			x	x				x	x					x	x		6
Senecio c.f. *polyanthemoides* Sch. Bip.							x										1
Sida cordifolia L.								x									1
Sida rhombifolia L.	x			x													2
Solanum incanum L.														x			1
Solanum mauritianum Scop.			x					x				x			x		4
Sorghum halepense (L.) Pers.	x		x												x		3
Sporobolus R. Br., sp.							x										1
Tragia meyeriana Muell. Arg.										x							1
Trimeria grandifolia (Hochst.) Warb.				x													1
Vernonia natalensis Sch. Bip.									x								1
Vigna vexillata (L.) A. Rich. sens. lat.								x			x						2
C. odorata: Mortality (%)	84	92	99	54	65	90	97	100	100	19	67	69	19	61	92	2	
Other species: Mean cover (%)	21-40	21-40	41-60	1-20	21-40	21-40	41-60	41-60	41-60	1-20	21-40	21-40	1-20	1-20	21-40	1-20	
Other species: No. per treatment	11	8	10	8	4	5	8	9	6	4	6	10	1	8	7	3	

[a] 3,6-dichloropicolinic acid

low imazapyr concentration, the number of species per treatment was higher than that of the untreated plots (Table 3.4.3). As mentioned earlier, the presence and absence of *C. odorata* seedlings in the treated plots was recorded. It was found that in the Melmoth and Clansthal A trial, few plots contained seedlings. In the Clansthal B trial however, 80 per cent of the plots contained seedlings. Large numbers were generally observed in plots where high *C. odorata* mortality had been obtained. This was especially the case where a sparse cover had become established. However, seedlings were even found in the untreated plots.

Discussion

As mentioned previously, invader weeds are generally comprised of woody perennials. In addition this study was primarily concerned with alien invaders. Therefore, only those species which are recognised alien invaders and occurred in the trial sites will be discussed in this section.

The subject of rehabilitation of weed dominated communities is not new and has previously been investigated (PANETTA, 1981). In South Africa this topic is currently also receiving attention, especially in the eastern Cape Province where research has been aimed at replacing undesirable invader weeds with useful species. There are numerous instances in South Africa where areas have been invaded and subsequently dominated by exotic species. The encroachment by *C. odorata* in large parts of Natal is one such example.

It was therefore decided to investigate, by means of species checklists and the cover established by colonising species, the events occurring after chemical control of *C. odorata*.

Before discussing the results obtained, it is necessary to firstly mention certain pertinent considerations associated with this study. The trials were conducted in exotic timber plantations, due mainly to the long-term protection of the trial sites against fire and other disturbances. It might therefore be an unnatural situation for this type of study. However, it was decided that the beneficial aspects sufficiently outweighed the negative aspects. In addition, the results would provide an indication of whether other invader weeds are likely to replace *C. odorata* as the major problem. If this was found to be the case, the results would still provide a forewarning and therefore appropriate measures could be instigated.

The results of this study showed that very few species were present within the *C. odorata* infestations at the time of spraying. This confirms that *C. odorata* suppresses and excludes the establishment of other species (both desirable and undesirable) and therefore occurs in predominantly monospecific thickets. Observations have shown this is also often the case in infestations in conservation areas.

It was also found that following chemical control of *C. odorata* there was rapid colonisation of the cleared areas. The extent of colonisation appeared to be determined by the degree of *C. odorata* control. This indicates that either the reduction in the density of *C. odorata* resulted in a

decreased allelopathic effect on other species as proposed by AMBIKA (1980), or merely that the competitive advantage over other species was reduced sufficiently to promote their establishment. It would seem that the latter is of greater importance since the number of species in the untreated plots also increased and is thought to have resulted from increased light penetration following the slashing of access paths around each of the plots. Results obtained in pot trials using *Chrysanthomoides monilifera* (L.) Nordlindh. as the test plant, indicated that the addition of freshly harvested *C. odorata* material actually promoted the growth of the test plants (results not presented). Therefore, if an allelopathic effect is exhibited by *C. odorata*, then it is not present in "dead" material. The use of *C. odorata* as a "green-manure" has previously also been reported (MOHAN LAL, 1960).

The smallest increase in species number was recorded for the Melmoth trial. The probable reason for this is that this trial was located in a *P. taeda* plantation. The thick litter layer consisted predominantly of pine needles. Pine litter is reported to have a pH of 3,5 to 4,2 (MITCHELL & MILLAR, 1978) and leachates, which inhibit the growth of plants, have been extracted from coniferous leaf litter (BLANSCHKE, 1977). Both these factors are likely to restrict and suppress growth of seedlings. In addition, light attenuation by the litter layer, combined with the restricted incident radiation by the *P. taeda* canopy, is likely to reduce germination of seeds of species in which light is required for germination.

In plant succession, the species which determine the

character of the community are referred to as the dominants (DAUBENMIRE, 1968; COLINVAUX, 1973). Although the dominants of a plant community are not necessarily the most frequently occurring, the frequency of occurrence recorded in these trials was an indication of the species which were the post-spray dominants by virtue of their preponderance. It is realised however, that the post-spray species composition is likely to change, as a natural progression of succession, with time. This is an additional reason for the particular attention paid to woody perennials since these might ultimately become the dominants.

The post-spray dominants, in the context of this discussion, in the Melmoth trial were *A. houstonianum*, *P. maximum* and bugweed. Although *A. houstonianum* is a weed in many countries (HOLM, PLUCKNETT, PANCHO & HERBERGER, 1977), it is an annual and therefore of little consequence as an invader weed. *P. maximum*, which is widespread in Natal (ROSS, 1972), is a palatable and nutritious grass for livestock grazing (TAINTON, BRANSBY & DEVILLIERS BOOYSEN, 1976). This species can therefore be considered to be a desirable dominant. The third dominant, bugweed, is an exotic woody perennial with a widespread distribution in South Africa (LE ROUX, 1979) and a high viable-seed production (CAMPBELL & VAN STADEN, 1983). This species is a recognised invader weed. Its frequent occurrence in the Melmoth trial is therefore an indication that it could become a dominant and could thus replace *C. odorata* as the problem species in this region. This is supported by results obtained in soil-seed reserve studies

conducted in this region in which high numbers of bugweed seeds were found (results not presented).

Two other potentially serious invader weeds were recorded in the Melmoth trial, namely *L. camara* and guava. Although the frequency of occurrence of these two species was not high, it is pertinent to note that their frequencies had increased during the study period. It is therefore possible that these could also present a weed problem subsequent to *C. odorata* control.

The majority of the dominants found in the Clansthal trials are not likely to form serious invader weed problems following *C. odorata* control. These consisted of either annual and herbaceous weeds of disturbed areas (*A. houstonianum* and *B. rhapontica*), sedges (*C. rotundus*) or grasses (*P. maximum* and *R. repens*). *S. halepense*, a dominant in the Clansthal trial A, is reported to be a widespread weed in Natal (ROSS, 1972) and is rated the sixth worst weed in the world by HOLM, PLUCKNETT, PANCHO & HERBERGER (1977). However, it is principally a weed-grass in crops and although its presence might initially suppress the growth of desirable species in silviculture and natural areas, it is not rated as an invader weed.

Based on the information recorded for the percentage cover and number of species per treatment in the Melmoth and Clansthal A and B trials, certain deductions can be made.

The degree of *C. odorata* control, measured as percentage mortality, appeared to positively influence the cover formed by the post-spray species since generally, the greater

the control of *C. odorata* the higher was the resultant percentage cover. Thus, chemical control of *C. odorata* resulted in rapid colonisation and establishment of a favourable cover. This has two major implications. Firstly, the rapid and sometimes substantial cover established is an indication that little biologically active herbicide persistence occurred. Even with the application of tebuthiuron, which is reported to be persistent in the soil for 12 to 15 months (CHANG & STRITZKE, 1977), colonisation was not prevented in the Clansthal trial A. Imazapyr, which is reported to persist in the soil for three to five months under tropical conditions (PEOPLES, 1984) also did not appear to restrict re-establishment of species in plots where this herbicide was applied. Rather, it seems that colonisation was suppressed by high density *C. odorata* presence as already discussed. The apparent non-persistence even of herbicides known to be persistent, could be due to various factors. The soils occurring in the trial sites are sandy and this, together with the comparatively high rainfall occurring in these areas, could have resulted in rapid leaching of the herbicides out of the root zone of the colonising species. In addition, frequent reference is made to rapid degradation of herbicides in hot, humid conditions. This might also have contributed to the lack of persistence of the herbicides.

A second major implication of a rapid cover establishment indicates that areas which are cleared of *C. odorata* by chemical control are not generally susceptible to erosion. This is an important consideration since sandy soils are prone to erosion and in addition, *C. odorata* often occurs on

steep slopes which are more likely to be eroded if denuded of vegetation. It is also suspected that the likelihood of erosion is reduced by chemical control of *C. odorata* as opposed to manual/mechanical digging-up of the weed. This is because the *C. odorata* plants remain *in situ* and thus their roots, although dead, assist in binding the soil.

Re-infestation of the treatment plots was observed to occur especially in the Clansthal trial B site. The source of re-infestation was either by way of wind-dispersed achenes emanating from an infestation adjacent to the trial sites where achene production occurred, or from achenes present in the soil. As discussed in detail in Chapter 2, the achenes of *C. odorata* are positively photoblastic and therefore a reduction in the leaf-canopy formed by *C. odorata* might have resulted in sufficient light for the promotion of germination as shown by the preponderance of seedlings in plots where high *C. odorata* mortality had occurred. This could also explain the increased colonisation of the treatment plots by other species. The observation that *C. odorata* seedlings were also present in the untreated plots may be explained by the increased light-penetration resulting from the access paths around each plot. The low occurrence of *C. odorata* seedlings in the Melmoth plot was probably due to the pine litter as already discussed. In the Clansthal trial A, where few *C. odorata* seedlings were observed, the establishment of a high percentage cover was possibly the cause of reduced achene germination and seedling establishment, suggesting that a shift in the balance of species diversity provided a competitive advantage to other species.

Additional information concerning the effect of herbicides on other species was also obtained in this study. Two recognised woody invader species were found to be resistant to some of the herbicides. Guava, growing at a similar height as the *C. odorata* survived glyphosate and triclopyr application in the Melmoth trial and 3,6-dichloropicolinic acid in the Clansthal trial B. Although it is possible that control of this species could be obtained with higher concentrations, the indications are that guava is likely to be a "hard-to-kill" species. This has been confirmed by preliminary investigations in Ndumu Game Reserve, Natal, where this species is a serious alien invader weed (JACKSON, personal communication*). Another exotic woody perennial, *L. camara* survived triclopyr application in the Melmoth and Clansthal A trials. Herbicide trials on this species have also shown it to be resistant to triclopyr (GRAAFF, personal communication**). Triclopyr has also been found to be ineffective on another woody weed, *Quercus virginiana* Mill. (MEYER, BOVEY, BOUSE & CARLTON, 1983).

Low-growing pre-spray species were also found to survive the herbicide treatments. This is probably due to their being sheltered from direct herbicide spray by the dense *C. odorata* canopy. Therefore, desirable species within a *C. odorata* infestation are unlikely to be damaged by herbicides applied to the target weed.

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The results of this study and the factors which have emerged in the discussion have shown the importance of studies of this nature. However, certain limitations have also been exposed and it is suggested that to obtain a clearer picture of the events occurring subsequent to chemical control of *C. odorata*, a similar study should be undertaken in natural areas infested with this weed. In addition, the possibility of replacing *C. odorata* with desirable species should be considered. Here the primary objective would be firstly to determine the desired floristic and structural attributes of the rehabilitated community required. Presumably the rehabilitated community should resemble the original community as closely as possible. To this end, a preliminary study has been conducted in which cuttings of *C. monilifera*, an indigenous shrub, have been planted in plots where *C. odorata* has been removed. The choice of *C. monilifera* in this investigation (and in the allelopathy studies briefly mentioned earlier) was based on the observation that in some situations it occupies the same ecological niche invaded by *C. odorata*. Additionally, *C. monilifera* is easily propagated from cuttings thereby providing a readily available source of plants. The results to date have indicated that with management of these plots (that is, continued removal of *C. odorata*) a dense stand of *C. monilifera* has become established. Obviously, more information is required, but it does seem as though active replacement of *C. odorata* with a particular species, or a number of species, could play a major role in the rehabilitation of infested areas.

3.5 Herbicide and Mixture Volume Reduction Trials

In section 3.3 the screening of herbicide efficacy on *C. odorata* was described. But, as discussed in that section, the cost of chemical control using those particular procedures and doses might prove to be prohibitively expensive. Therefore additional research on the chemical control of this weed was initiated; this was aimed at (i) reducing the volume of herbicide required for control and (ii) selective application of herbicides on to *C. odorata*.

In attempting to fulfil the first aim, a site was prepared on *C. odorata* re-growth (coppice) to which all the herbicides and concentrations used in the Clansthal A and B trials described in section 3.3 were applied at 250 and 500 $dm^3 ha^{-1}$. Therefore the volume of herbicide per hectare was effectively reduced to one half and one quarter of that applied in the screening trials. Unfortunately however, this entire trial came to nought since rain fell within 3,5 hours of the first applied treatments (glyphosate) and within 30 minutes of the last treatments (triclopyr). Subsequent visual assessments showed that the results were extremely variable, probably as a consequence of the rain. Although this trial was abandoned, it is pertinent to note that all the herbicides, with the exception of imazapyr, showed negligible phytotoxic symptoms indicating that herbicides applied shortly before rain (within 30 minutes to 3,5 hours of rain) are likely to be ineffective. The comparatively more noticeable effect of imazapyr is consistent with the herbicide's foliar- and root-absorption

characteristics as described in section 3.1.

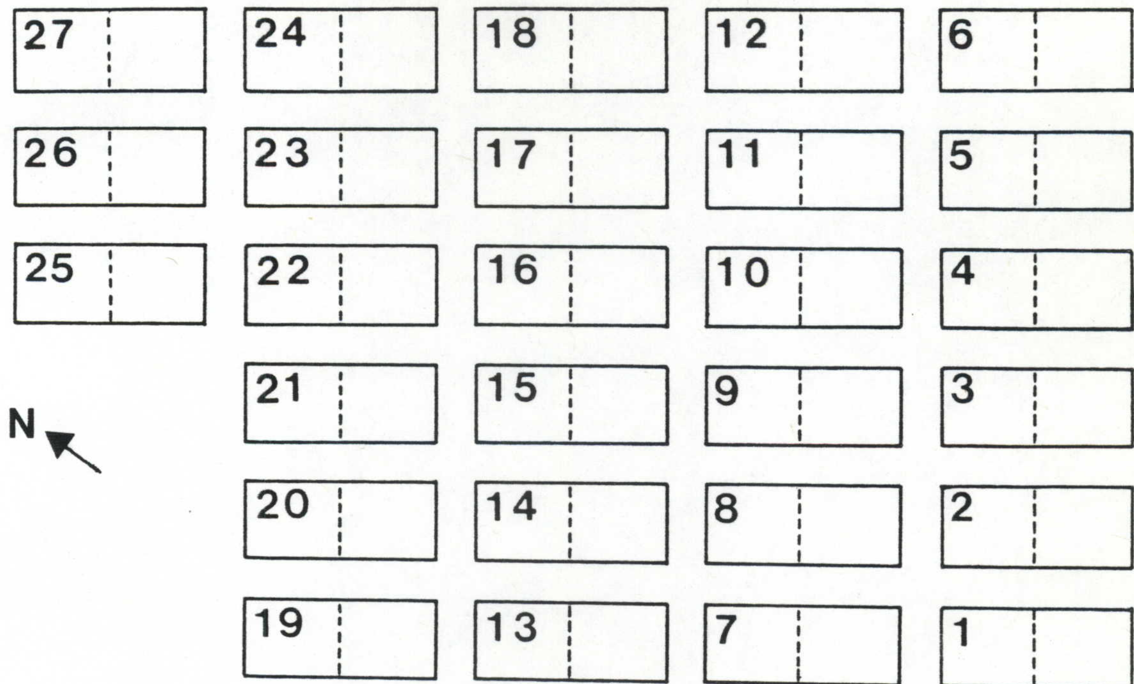
Following this trial, only two herbicides were used in further trials. These were triclopyr, which had shown high efficacy, and glyphosate which, although it had not resulted in an acceptable level of control, was suspected of being ineffectual due to insufficient spray-coverage of the plants. These two herbicides were tested on *C. odorata* coppice by both directed-spray application involving the use of a shield, and overall spray as described in the screening trials. The use of the directed-spray application technique was tested because it was thought to be a potentially useful tool in the control of *C. odorata* occurring in close association with desirable species.

(i) Directed-spray trial

Materials and Methods

This trial was also conducted at Finningley Estates, Clansthal. The site was located on a south-western aspect in an *E. grandis* coppice plantation. The soil was similar to that described previously for the Clansthal site (Table 3.2.2).

The trial site plan is shown in Figure 3.5.1. The plots measured two metres by five metres (10 m^2) and each plot was halved to produce two sub-plots measuring 2,0 m by 2,5 m as demonstrated in Figure 3.5.1. Within the central portion of each sub-plot, a sampling area (one metre by one metre) was staked-out in which the number of stems at 10 cm above ground level was counted before herbicide application and again



Treatment	(%)	Plot No.		
glyphosate	0,25	2	14	24
"	0,50	4	17	27
"	1,00	5	11	20
"	2,00	8	13	23
untreated		7	12	26

Treatment	(%)	Plot No.		
triclopyr	0,25	3	16	21
"	0,50	1	10	19
"	1,00	6	15	22
"	2,00	9	18	25

Figure 3.5.1 The direct-spray trial site. The north-western half was sprayed with the equivalent of 500 dm^{3} ha^{-1} and the south-eastern half with 250 dm^{3} ha^{-1} .

at final assessment.

Herbicide concentrations applied were 0,25; 0,50; 1,00 and 2,00 per cent of each herbicide (triclopyr and glyphosate) mixed in water. As was previously the case, the surfactant Actipron (0,5 per cent of the final mixture volume) was added to the triclopyr mixtures. The herbicides were applied as a foliar spray. Application was effected by means of a knapsack fitted with a plunge dispenser which could be calibrated to deliver a specific volume. Instead of the spray nozzle being attached to the end of a lance, two nozzles were fixed onto the inside of a fibreglass shield as illustrated in Plate 6 and Figure 3.5.2 . (Originally a single nozzle was fixed to the inside of the shield at its apex, but in preliminary trials, it was found that the nozzle became blocked by the foliage of the target plant; consequently poor spray coverage was obtained.) Each of the two nozzles was fitted with pressure shut-off springs; these ensured that the herbicide mixture pumped by the plunge dispenser was delivered as a spray. Previously it had been found that where the pressure shut-off springs were excluded, a dribble of herbicide mixture occurred as the pressure subsided.

For actual application the shield was placed over individual plants or groups of plants (Figure 3.5.2). The volumes sprayed were pre-calibrated by the adjustment on the dispenser. A mixture volume equivalent to 250 $dm^3 ha^{-1}$ was applied to the north-western half of each plot, and 500 $dm^3 ha^{-1}$ to the south-eastern half. At the time of application, 26th July, 1984, the *C. odorata* plants consisted of coppice 0,5 to

Plate 6A The knapsack and shield used in the directed-spray trial. X 0,4.

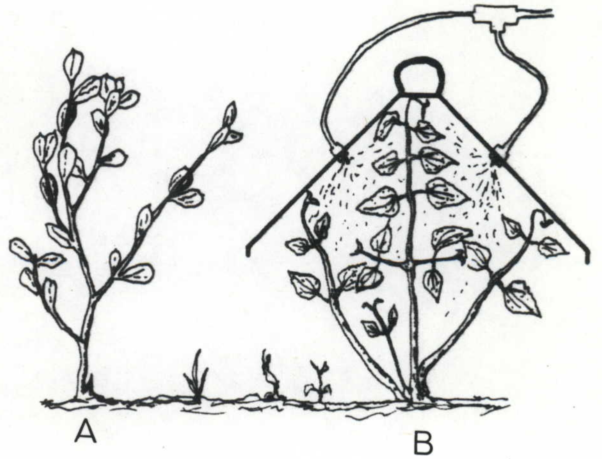


Figure 3.5.2 An illustration of directed-spray application to the target weed (B) whilst avoiding a desirable plant (A). X 0,4.

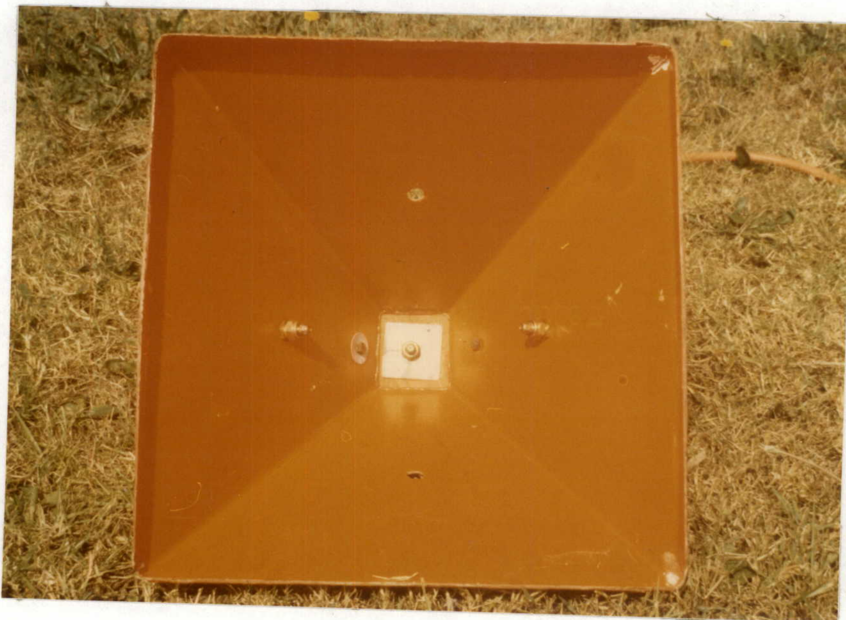


Plate 6B The shield, showing positioning of the nozzles. X 0,15.

0,7 m tall, the infestation having been slashed two months previously.

The environmental parameters measured at the time of herbicide application are recorded in Table 3.5.1.

Results

The results obtained for the directed-spray trial are presented in Table 3.5.2 as the untransformed and transformed percentage mortality for each of the treatments. Included are the actual volumes of product applied (calculated from the number of squirts applied) and the volumes of product which would have been applied if application had been by means of an overall spray; both are expressed as the equivalent volume per hectare.

At an application equivalent to 250 $dm^3 ha^{-1}$ mixture, the 1,0 and 2,0 per cent triclopyr and the 2,0 per cent glyphosate resulted in the highest ($p = 0,05$) percentage mortality of *C. odorata*. The 0,25 per cent glyphosate was particularly ineffectual and resulted in the lowest percentage mortality. As indicated by the actual volume applied, the volumes were similar for the two herbicides; these were consistently lower than the volume which would have been applied with an overall spray application. The actual volume applied ranged from 64 to 80 per cent of that which would have been applied, or a mean of approximately 73 per cent of that which would have been applied by overall spraying.

Where the equivalent of 500 $dm^3 ha^{-1}$ mixture was applied

Table 3.5.1 Environmental conditions recorded at the time of herbicide application in the directed-spray trial (±S.E.)

Parameter measured[1]	Range
Temperature (°C)	17 - 23
Relative Humidity (%)	63 - 73
Soil Moisture (%)	15,4±0,1

[1] Wind speed was not measured as this parameter had no influence on the spraying technique.

Table 3.5.2 Percentage mortality of *C. odorata* treated with triclopyr or glyphosate. Herbicides were applied to coppice by the directed-spray technique (± S.E.)

Herbicide Conc. (%)	Percentage Mortality							
	250 $dm^3 ha^{-1}$				500 $dm^3 ha^{-1}$			
	UT[1]	T[2]	Actual[3] ($dm^3 ha^{-1}$)	Overall[4] ($dm^3 ha^{-1}$)	UT[1]	T[2]	Actual[3] ($dm^3 ha^{-1}$)	Overall[4] ($dm^3 ha^{-1}$)
Triclopyr								
0,25	62±26	53	0,4	0,625	96±6	81	0,9	1,250
0,50	74± 9	59	0,9	1,250	93±9	79	1,8	2,500
1,00	100± 0	90	1,9	2,500	100±0	90	3,4	5,000
2,00	100± 0	90	3,8	5,000	100±0	90	5,3	10,000
Glyphosate								
0,25	30± 7	33	0,5	0,625	38±4	38	1,0	1,250
0,50	65± 7	54	0,8	1,250	72±6	58	1,4	2,500
1,00	72± 8	58	2,0	2,500	97±5	84	4,3	5,000
2,00	94±10	82	3,5	5,000	94±5	79	5,7	10,000
LSD (p=0,05)		16				16		

[1] Untransformed data

[2] Transformed data

[3] Actual volume applied per hectare

[4] Volume per hectare if application had been made by overall spraying

all four triclopyr concentrations provided a high percentage mortality of *C. odorata* while only the 1,0 and 2,0 per cent glyphosate treatments resulted in high *C. odorata* mortality. The 0,25 per cent glyphosate treatment again resulted in the lowest percentage (p = 0,05) mortality. In this trial the actual volume of herbicide applied ranged from 53 to 86 per cent of that which would have been applied by overall spraying, or, for the eight treatments, a mean of approximately 57 per cent.

Discussion

The volume of herbicide actually applied to the treatment plots (expressed as a volume per hectare) was compared to that which would have been applied were the herbicides to be applied as an overall spray. This provided an indication of whether this application technique provided for a reduction in the volume of herbicide used. Clearly, this was achieved as a result of restricted application in the form of a spot-spray. The only difference to a normal spot-spray application is that a shield was also used.

In addition to the reduced volume of product used, which not only restricts the volume released into the environment but also reduces the cost of the herbicide, it was found that an acceptable level of control was achieved with certain treatments. Two levels of herbicide mixture volume were applied. With the low volume (250 $dm^3 ha^{-1}$) an acceptable level of *C. odorata* control was obtained with the

1,0 and 2,0 per cent triclopyr and the 2,0 per cent glyphosate. To obtain this acceptable level of control, an appreciably lower volume of herbicide was required than that found necessary in the trials discussed in section 3.3. Where the equivalent of 500 $dm^3 ha^{-1}$ mixture was applied an acceptable level of control was achieved with all four triclopyr treatments and the 1,0 and 2,0 per cent glyphosate treatments. Again a lower volume was necessary for acceptable control than was previously found.

Two aspects of these results require elaboration. Firstly, the results indicate that the design of the shield used was suitable for practical use in *C. odorata* control since an acceptable level of control was obtained. It is therefore proposed that this type of herbicide application might be useful in combating *C. odorata* (and other weed) infestations in indigenous vegetation. The advantages of this application technique are:

(i) reduced herbicide volumes used therefore reduced costs and volume of herbicide released into the environment;

(ii) no spray drift due to the shield, therefore longer spraying time even during windy conditions; and

(iii) little danger to desirable species growing in close proximity to the target weed.

There are two disadvantages namely:

(i) site preparation (slashing and time for regrowth) is necessary before spraying; and

(ii) the shield could be cumbersome in harsh terrain and dense vegetation.

The second aspect of note emanating from this trial, is that *C. odorata* in the form of "immature coppice" is more susceptible to herbicides (triclopyr and glyphosate) than the "mature coppice" sprayed in the trials described in section 3.3. This is obvious from the lower mixture volumes and, therefore, product volumes used for attaining a high percentage mortality. Even some of the glyphosate treatments provided an acceptable level of control. The possible reasons for the increased herbicide efficacy are four-fold.

In the first instance the size of the above-ground material was greatly reduced and therefore, although the leaf-surface area for herbicide absorption was greatly reduced, the distance for herbicide translocation to the root system was markedly decreased. The translocation distance might therefore have played a major or contributary role in the increased efficacy.

Secondly, the physiological status of the plants (immature coppice) was likely to have been markedly different to the mature coppice. Following slashing, the plants rapidly regrew and multiple stems were produced by each plant, probably as a result of the removal of apical dominance. Since the primary factor controlling the transport and utilisation of assimilates in plants is the existence of nutrient sink regions (ESCHRICH, 1975; GIAQUINTA, 1980; HEROLD, 1980) the nutrient reserves in the roots were probably depleted by the vigorously growing coppice. This might have served to lower the tolerance of the plant to herbicides. Although identification of the precise mechanism governing

the susceptibility in immature and mature coppice plants would require detailed investigations not within the objectives of this study, a possible role of "weak" versus "healthy" plants in reaction to glyphosate will be ventured. The primary mode of action of glyphosate has been, almost positively, determined; namely the inhibition of amino acid synthesis. In a healthy plant, such as the mature coppice, there is likely to be a pool of amino acid and protein reserves. Therefore, upon application of glyphosate, sub-optimal levels of glyphosate in the roots, for example, are likely to halt temporarily, amino acid synthesis, during which time reserves are utilised. The plant is therefore able to withstand a transient inhibition by glyphosate albeit weakened by reserve utilisation. However, in the immature coppice, even sub-optimal levels of glyphosate are likely to kill plants because no reserve pool of amino acids exists and, even what would normally be a temporary inhibition of the amino acid synthetic pathway, results in death.

The third aspect which might have resulted in the enhanced efficacy of the herbicides applied to immature coppice is that environmental factors such as temperature, humidity, soil moisture and light were optimal for absorption and translocation of the herbicides. These factors were discussed in section 3.3 and have been reviewed by BUKOVAC (1976) and HAY (1976) and will not be repeated. Suffice to say that any one or a combination of these factors could have led to an increase in the amount of herbicide within the plant and therefore enhanced efficacy.

The final aspect relates to the morphological differences between immature and mature coppice since, as reviewed by BUKOVAC (1976) herbicide entry into the plant is determined by the nature of the absorption surface. In the immature coppice the leaves were mostly fully expanded but in contrast to mature coppice leaves, these were young. It is commonly agreed that penetration of organic and inorganic chemicals into young leaves is more rapid than that of older leaves (CURRIER & DYBING, 1959; HULL, 1970). For example COUPLAND, TAYLOR & CASELEY (1978) found that glyphosate was most readily absorbed by young but fully expanded leaves, and since glyphosate absorption appears to be a primary factor in its action due to limited uptake (HADERLIE, SLIFE & BUTLER, 1978; KING & RADOSEVICH, 1979), increased absorption by the immature coppice is likely to have resulted in the enhanced efficacy observed. Absorption of triclopyr has been shown to be retarded by epicuticular wax (KING & RADOSEVICH, 1979) and therefore increased absorption by immature *C. odorata* coppice might have increased the efficacy of this already effective herbicide.

This study has clearly demonstrated two important aspects in the control of *C. odorata*, namely that lower volumes of herbicide than those proposed for mature coppice control, can be used to obtained an acceptable level of control if applied to immature coppice, and secondly, the application technique provides an effective means of applying the herbicide target specifically and might thus provide a useful tool in combating *C. odorata* where it occurs in close proximity to desirable species.

(ii) Overall-spray trial

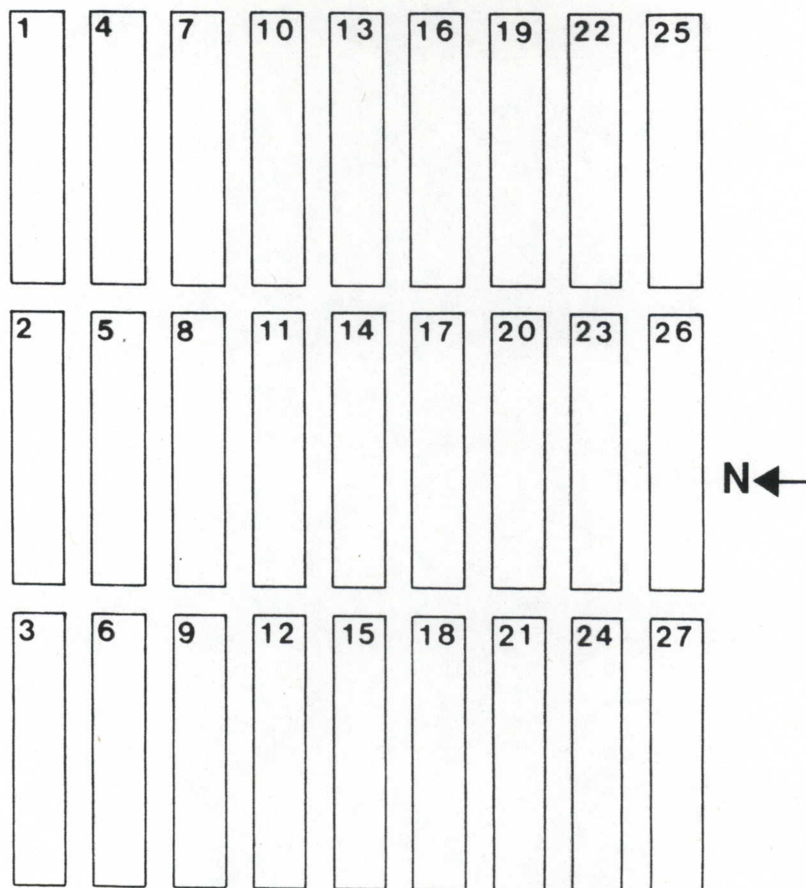
Materials and Methods

This trial was also conducted on Finningley Estates, Clansthal in an *E. grandis* coppice plantation. The site was on an eastern aspect and had similar soils to the other sites on this estate, as described previously.

The trial site plan is shown in Figure 3.5.3. Each plot measured 2,0 by 10,0 m (20 m^2). Within the central portion of each plot, four sample areas measuring one metre by one metre were staked. In these, the number of stems of *C. odorata* 10 cm above ground level were counted. At final assessment, these were re-counted; these data were then used to calculate the percentage mortality obtained for each of the treatments. Each treatment was triplicated and thus 12 samples were counted (four per plot). These data were used to calculate the standard errors for each treatment in order to provide a measure of the variation. The mean percentage mortality per plot was used in the analysis of variance. For this particular trial, the means were not transformed since the data did not fulfil the requirements for transformation of percentages since there was no binomial distribution of the standard errors.

The *C. odorata* plants growing in this site had been slashed four months prior to treatment. At application time the coppice ranged from 0,7 to 1,0 m in height.

The herbicide treatments applied were 0,25; 0,5 and 1,00 per cent herbicide in water of both herbicides applied



Treatment	(%)	Plot No.		
triclopyr	0,25	3	16	21
"	0,50	6	15	22
"	1,00	9	18	25
($250 dm^3 ha^-$)	1,00	1	10	19
untreated		7	12	26

Treatment	(%)	Plot No.		
glyphosate	0,25	2	14	24
"	0,50	4	17	27
"	1,00	5	11	20
($250 dm^3 ha^-$)	1,00	8	13	23

Figure 3.5.3 The site plan for the overall-spray trial in which triclopyr and glyphosate were applied to *C. odorata* coppice.

at an equivalent of 500 $dm^3 ha^{-1}$. Two additional treatments were included. These were a 1,00 per cent solution of triclopyr and glyphosate, but applied at an equivalent of 250 dm^3 ha^{-1}. Again Actipron (0,5 per cent of final mixture) was added to the triclopyr mixtures. Three plots were not treated; these provided a basis for comparison.

The herbicides were applied on 25th October, 1984 and final assessment was made on 9th March, 1985.

Results

The results obtained for the overall spray applied to *C. odorata* coppice are presented in Table 3.5.3. All triclopyr concentrations provided for a high percentage mortality. The 0,50 and 1,00 per cent concentrations (500 $dm^3 ha^{-1}$) and the 1,00 per cent concentration applied at the equivalent of 250 $dm^3 ha^{-1}$ were especially effective but not significantly ($p = 0,05$) better than the 0,25 per cent concentration which, in one plot, was variable. The glyphosate treatments were however, generally ineffective with the exception of the 1,00 per cent concentration (applied at 500 $dm^3 ha^{-1}$) which was not significantly different from the triclopyr treatments (Table 3.5.3). With the lower concentrations of glyphosate, the results were very variable (as shown by the large standard errors which were largely responsible for the high value of the L.S.D.) and in some of the sample areas in the plots, an increased number of stems were recorded at final assessment.

Table 3.5.3 Percentage mortality of *C. odorata* following the application of various concentrations of triclopyr and glyphosate to coppice (±S.E.)

Herbicide Conc. (%)	Percentage Mortality		Volume product $(dm^3 ha^{-1})$[1]
	Triclopyr	Glyphosate	
0,25	89±7	5±15	1,25
0,50	99±1	0± 9	2,50
1,00	100±0	73± 3	5,00
1,00*	99±1	33± 9	2,50
LSD (p=0,05)	27	27	

[1] Equivalent volume of herbicide per hectare

* Applied at the equivalent of 250 $dm^3 ha^{-1}$ mixture

Discussion

Two factors discussed in section 3.3 need to be reiterated. Firstly, the volume of triclopyr required to obtain an acceptable level of control of mature *C. odorata* was found to be the equivalent of 5,0 $dm^3 ha^{-1}$. Secondly, the low efficacy obtained with glyphosate was thought to have resulted from inadequate spray coverage. Both these factors have been clarified in this trial. However, before discussing these it is necessary to accentuate certain similarities between the *C. odorata* plants in this trial and those in the Clansthal A and B trials described in section 3.3.

Although the sites were on different aspects, the density of the *C. odorata* stand prior to slashing (to provide a coppice site) was similar to that of the previous trials. In addition the height of the plants prior to slashing was the same. In general appearance the sites were identical although the canopy formed by *E. grandis* was more dense in the Clansthal trial B site. The sites were adjacent (but on different aspects) and had received the same weed control measures, namely annual slashing. However, direct comparison between this trial and the Clansthal A and B trials would be unjustified because the herbicides were applied a year later than in the Clansthal trial A and in a different season to trial B. Nevertheless, in the context of herbicide efficacy on *C. odorata*, certain comparisons can be made in determining the most effective control treatment.

In this coppice trial it was found that high mortality

could be obtained with the equivalent of 2,5 $dm^3 ha^{-1}$ triclopyr applied either as a 0,50 per cent mixture at the equivalent of 500 $dm^3 ha^{-1}$ or as a 1,00 per cent mixture at the equivalent of 250 $dm^3 ha^{-1}$. These results therefore illustrate that *C. odorata* can be controlled effectively with half the volume of herbicide suggested for application on taller, mature coppice, plants (section 3.3). Furthermore, the percentage mortality obtained with the 0,25 per cent concentration which was not significantly different to the other triclopyr treatments, can be considered to have provided an acceptable level of control, especially since high percentage mortality was also obtained using this concentration at 500 $dm^3 ha^{-1}$ in the directed-spray trial. As such, the previously suggested dosage of 5,00 $dm^3 ha^{-1}$ is quartered, thereby further reducing the cost of the herbicide. The indications are however, that to obtain uniform control of *C. odorata* a mixture percentage of between 0,25 and 0,50 applied at 500 $dm^3 ha^{-1}$, or in the region of a 0,75 per cent mixture concentration applied at 250 $dm^3 ha^{-1}$ is necessary. Further trials are necessary to affirm these projections.

As discussed above, the cost of the herbicide can be appreciably reduced by applying triclopyr to immature coppice However, it should be mentioned that when taking into account the actual cost of control, an additional cost will be incurred for the slashing required in site preparation.

In comparison to triclopyr, glyphosate was again inferior for *C. odorata* control. At the volumes applied in these trials, which were the same as those for the triclopyr

treatments, good overall wetting of the plants was obtained. This however, did not markedly improve the level of control obtained, although the percentage mortality obtained with the 1,0 per cent concentration applied at 500 $dm^3 ha^{-1}$ was not significantly different from the triclopyr treatments. But, as with the triclopyr, it would appear that immature *C. odorata* coppice is more susceptible to herbicides than the mature regrowth.

In considering chemical control of *C. odorata* there are several major differences between immature and mature coppice as have already been discussed in the directed-spray trial. These features might all have contributed to varying degrees, to the enhanced efficacy obtained in the overall-coppice-spray trial. Since these have already been discussed it is suffice to say that this trial has again shown that herbicide efficacy is greater on immature coppice than on mature coppice.

As with the directed-spray application described earlier, there are advantages and disadvantages to overall application. The advantages of overall-spray include:

(i) application is likely to be more rapid than directed-spray application using a shield; and

(ii) the spraying operation will not be hindered by terrain and dense vegetation to the same extent as the directed-spray technique.

The disadvantages include:

(i) more herbicide is used by overall spraying therefore costs are increased and more herbicide is being

released into the environment, however, spray (mixture) volume could be decreased by merely spot-spraying without a shield;

(ii) spray-drift could occur during windy conditions, therefore there is a greater likelihood of damage to desirable species and a reduced time for spraying; and

(iii) no shielding of desirable species takes place, therefore the risk of damage to these species growing in close proximity to the target weed is increased.

3.6 Summary

Three field trials were conducted in which herbicides were screened for efficacy on mature *C. odorata* coppice. In the Melmoth trial three herbicides, namely 2,4,5-T, glyphosate and triclopyr were tested and compared with tebuthiuron, the only herbicide registered at that time for use on *C. odorata*. Here the herbicides were applied in early summer. Two trials were conducted at Clansthal. In both these trials, five herbicides, 2,4,5-T, glyphosate, triclopyr, 3,6-dichloropicolinic acid and imazapyr were compared to tebuthiuron. In the first trial (Clansthal trial A) the herbicides were applied in early summer and in the second (Clansthal trial B) in late summer. The results showed that tebuthiuron, applied at the registered dosage, provided an acceptable level of control (>90 per cent mortality). Triclopyr, applied as a 0,5 and 1,0 per cent mixture (formulated product in water) at 1000 $dm^3 ha^{-1}$ consistently provided for an acceptable level of control of *C. odorata*. Another herbicide, 2,4,5-T, applied either as a 1,0 or 1,5 per cent mixture (formulated product in water) at 1000 $dm^3 ha^{-1}$, also consistently provided for an acceptable level of control. The other herbicides, glyphosate, 3,6-dichloropicolinic acid and imazapyr, were found to be relatively ineffective at the doses applied.

Differences in the percentage mortality of *C. odorata* were noted for the Clansthal A and B trials. Although these differences may be attributed to enhanced efficacy of herbicides applied at a time when assimilate transport was likely

to have been predominantly basipetal (that is, late summer) it was suggested that caution in the interpretation of the results was necessary since other factors related to site differences, might have contributed to the results obtained.

Based on the results of the trials discussed above, triclopyr and glyphosate were selected for further testing; triclopyr because of its high efficacy and glyphosate because it was suspected that the comparatively low efficacy obtained previously was due to insufficient spray coverage since this herbicide was applied at only 500 $dm^3 ha^{-1}$. (2,4,5-T was excluded due to its unavailability). A range of concentrations of triclopyr and glyphosate was applied to immature coppice in an attempt to reduce the volume of herbicide required to elicit an acceptable level of control. Two trials were conducted in which two application techniques were tested. These were a directed-spray and an overall-spray technique.

In the directed-spray trial, herbicides were applied using a shield. It was found that the dosage of triclopyr required to provide an acceptable level of control could be reduced in comparison to that found to be effective on mature coppice. Although the dosage of glyphosate required for an acceptable level of control was also reduced, this herbicide was inferior to triclopyr. Similar results were obtained for the overall-spray trial. The major difference between the two techniques was that a lower volume of spray mixture was applied with the directed-spray technique.

In a broad context, the control of alien invader weeds in natural habitats is comprised of two distinct operations. The first consists of killing of the target weed and the second, the rehabilitation of the infested natural plant community. The first operation was the control of *C. odorata* by the chemical control method, as already discussed. For the second operation, a preliminary investigation into the events occurring following *C. odorata* mortality, namely the species and extent of colonisation of the treatment plots, was initiated in the Melmoth and Clansthal A and B trials. Particular attention was paid to those species recognised as alien invaders. In the Melmoth trial, it was found that there was a noticeable increase in the occurrence of *S. mauritianum*, *P. guajava* and *L. camara* following the control of *C. odorata*. In the Clansthal A and B trials, no obvious increase of alien invader weed species was noted. However, in the Clansthal trial B especially, re-infestation by *C. odorata* in the form of seedlings was observed. Further observations were also made which have important implications.

Firstly, rapid colonisation of the treatment plots by species other than *C. odorata* and those previously mentioned, occurred. The extent of the colonisation, measured as number and estimated ground cover establishment, was directly related to the percentage *C. odorata* mortality. Additionally, this indicates that little or no biologically-active herbicide persistence occurred. Secondly, the few species occurring below the *C. odorata* canopy generally were not visably damaged by the herbicide spray. Thirdly, *P. guajava* growing at the

same height as the *C. odorata* canopy (and therefore presumably sprayed directly) was resistant to several of the herbicides. *L. camara*, in the same situation, was found to be resistant to triclopyr applications.

This study on the chemical control of *C. odorata* has shown that this species is susceptible to certain herbicides. In addition, by incorporating preparative procedures, the dosage of herbicide required to obtain an acceptable level of control can be reduced. However, *C. odorata* seedlings were observed in the treatment plots following mortality of the standing crop, and thus follow-up operations are essential.

GENERAL DISCUSSION AND CONCLUSION

The results obtained in this study have been summarised in sections 2.7 and 3.6, thus no further summary will be included here. However, it is necessary to clarify the aspects investigated since these, germination of the achenes and the chemical control, are apparently two unrelated topics.

Generally, "control" of a weed refers to the actual killing of the target species. In solving the problem of invader weeds, and especially alien species, control only forms the first of three major stages in the control strategy; the second is the maintenance of a weed-free situation and the third the rehabilitation of the natural (indigenous) community. Besides contributing to the presently small pool of knowledge on *C. odorata* in South Africa, the results obtained in this study can be used as a basis for formulating guidelines for reducing the impact currently exerted by *C. odorata*. Each of the three stages mentioned above will be discussed briefly in association with the results obtained.

In South Africa, very few known cases of chemical control of *C. odorata* are recorded, even though this species has been recognised as a serious problem since the early 1960's; the work of EGBERINK & PICKWORTH (1969) in which it was found that *C. odorata* was susceptible to certain herbicides, also seems to have been overlooked. LIGGITT (1983) provided a substantial list of herbicides to which *C. odorata* is apparently susceptible, but unfortunately many of these herbicides are not available in South Africa. In addition, as was previously

discussed, herbicides must be registered for specific species before they can be legally used. Nonetheless, a number of candidate herbicides were screened in this study and the results obtained have been used to support the registration application of triclopyr which was found to be particularly effective on *C. odorata*. Consequently, two herbicides are now registered; the registration of triclopyr greatly improving the selection of herbicides which can be recommended for use. This is especially so since tebuthiuron, the other registered herbicide, has limited application in that, being a soil-applied herbicide, it is not selective and cannot generally be applied target specifically; certainly not for the control of *C. odorata* occurring in indigenous arborescent vegetation or in timber plantations.

The use of triclopyr is recommended for use in the first stage of the control strategy aimed at reducing the *C. odorata* problem. Preferably, triclopyr should be applied to immature coppice (< one metre tall) since this growth stage was found to be particularly susceptible to triclopyr. In addition, application to immature coppice is desirable where *C. odorata* occurs amongst desirable arborescent species (indigenous or exotic timber species). In these situations, triclopyr can generally be applied target specifically. Should *C. odorata* be found growing in close proximity to desirable species which could be damaged, alternative herbicide application techniques may be used. In this study, a shield was used to confine the spray. Although fairly cumbersome, a similar type of shield might prove to be a useful tool in chemical control of *C. odorata* and other alien invader species because

not only is the spray confined but also reduced volumes of spray mixture are applied. Thus control costs and the volume of herbicide released into the environment are reduced.

The above discussion clearly indicates that chemical control of *C. odorata* is a viable alternative to the methods currently being used to control this weed. Not only is chemical control likely to improve the rate of control but certain other advantages might also be gained by using this method. For example, manual control methods result in soil disturbance during uprooting of plants, consequently germination of light-requiring seeds, including *C. odorata* achenes, is promoted. With chemical control, no soil disturbance occurs. Futhermore, erosion is less likely to occur in areas where chemical control has been practised since the dead plants remain *in situ* and thus their roots continue to bind the soil. Chemical control also provides for greater permanency of control than does slashing and therefore the return time (time between initial and follow-up control measures) is greatly increased.

The second major stage in reducing the problems caused by alien invaders, is the prevention of re-infestation. In this context, valuable information was obtained from the germination studies. Clearly *C. odorata* has a massive reproductive potential in the form of the achenes. The achenes are the reproductive propagules and are thus the source of re-infestation and further encroachment. Several aspects emanating from the germination studies showed how the achenes pro-

vide *C. odorata* with a characteristic common to the majority of problem weeds. Besides the large numbers produced, the achenes are well adapted to wind-dispersal. In addition, germination of the achenes is controlled by at least two dormancy mechanisms, the phytochrome-mediated dormancy mechanism and the after-ripening requirement. Both these mechanisms ensure a viable propagule source, the former through its prevention of germination of achenes present in dense vegetation and below the soil surface and the second in preventing germination of achenes while still on the parent plant or in the capitula. The phytochrome-mediated dormancy mechanism is of primary importance and was probably responsible for the large reserves of achenes found to be present on and in the soil under a dense vegetation canopy. This mechanism might also be responsible for the unsubstantiated secondary dormancy system which might be operative. Together, the dormancy mechanisms ensure that achenes remain viable for at least one year of burial.

The identification of the phytochrome-mediated dormancy mechanism is an important discovery in another context; namely this mechanism can be exploited in preventing or at least restricting re-infestation and further encroachment by this invader. The results obtained in laboratory studies using different qualities of light, implied that germination is likely to be restricted to disturbed areas denuded of a vegetation canopy. Therefore, the establishment of dense vegetation cover, preferably comprised of desirable species will theoretically restrict germination and consequently also re-

infestation and further encroachment. The aim is therefore to prevent, as far as possible, germination not only of achenes blown-in from neighbouring areas, but also those *in situ*, by creating a habitat unfavourable for germination. (Although methods of reducing the soil-achene reserves, such as the use of fire and cultivation were considered, these were excluded from this study because of the extremely large areas involved and, in addition, wind-dispersed achenes present a propagule source which will be unaffected by such measures.)

The maintenance of a *C. odorata*-free habitat is closely associated with the third stage of the control strategy; this is the rehabilitation of the natural community. (This third stage is of relatively little importance in timber plantations.) In natural communities, the initial phase of the rehabilitation should incorporate the creation of a situation unfavourable for the re-infestation of *C. odorata*. As such, the colonisation of the treated area must be encouraged. The pre- and post-spray species investigation showed that even in a timber plantation, colonisation is extremely rapid and often results in the establishment of a dense vegetation cover which not only reduces the likelihood of erosion, but also discourages re-infestation by *C. odorata* from achenes *in situ* and those being dispersed by the wind. The post-spray species checklist indicated that in some areas, other invader weeds might fill the ecological vacuum created by the disturbance caused in killing of *C. odorata*. It is thus proposed that an attempt should be made to manipulate the species composition of areas where *C. odorata* has been killed. Obviously, the species

selected should be easily propagated, have an abundant propagule source, be ecologically suitable and be fast-growing. Further details required for the successful implementation of this phase of the control strategy urgently require investigation.

Another aspect of paramount importance is the necessity for follow-up measures to ensure the exclusion of *C. odorata* from areas previously infested. Motivation for this is that seedlings were recorded in some of the trials at final assessment. Observations have shown that other methods of control appear to stimulate an even greater response in as much as carpets of seedlings sometimes result. Removal/killing of the *C. odorata* seedlings either chemically or merely by hand-pulling is therefore an essential part of the control strategy. It is suggested that the extent of the area treated in the initial phases of the control strategy be limited to a size which can be thoroughly treated by the follow-up measures. In addition, the follow-up measures should be implemented prior to initiating further initial treatments. Incidentally, a follow-up type of treatment is also recommended for all areas in which this weed may become established.

This study has also revealed certain aspects requiring further investigation and which are thought to be necessary for success in combating this weed. As far as achene biology is concerned mention has already been made of these in section 2.7. In the chemical control context, there are also certain techniques which require investigation in order to improve the control effort. Two of these are, application of herbicides

to cut-stems and secondly, the use of herbicide mixtures (cocktails). In considering the former, it has been observed that during manual control of well-established infestations of *C. odorata* in natural habitats, the possibility exists that this process might be improved upon by incorporating the use of herbicides. Further trials are therefore envisaged in which the efficacy of herbicides applied to cut-stems would be investigated. Should this technique result in high mortality of *C. odorata*, the uprooting stage would be obviated. Additionally, awaiting regrowth suitable for spraying would not be necessary and thus the control treatment would be effected in a single operation. The use of herbicide cocktails in the control of *C. odorata* is also thought to be of high priority since the herbicide found to be particularly effective on this weed was found to be relatively ineffective on another problem alien invader weed, *Lantana camara* L.. Consequently, two spraying operations are currently necessary in situations where these two species occur in close proximity, as is often the case along the Natal coastal area.

It is hoped that with the implementation of the recommendations made in this dissertation, together with the general knowledge gathered on this weed, the problem of *C. odorata* will be diminished. In so doing, the aesthetically valuable indigenous vegetation of Natal and the eastern Transvaal will be saved from further degradation and possible extinction. However, solving of the *C. odorata* problem will require a national effort and hopefully this study will contribute to and accelerate the momentum of the effort being

put into the fight against widespread encroachment by un-
desirable alien weed species.

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