The Reproductive Biology, Natural Enemies
and Biological Control of
Delairea odorata Lem.

by

Carol Ann Rolando

Submitted in fulfilment of the requirements for the degree of

Master of Science

in the

School of Botany and Zoology,
University of Natal, Pietermaritzburg,

March 2000

ABSTRACT

Delairea odorata Lem., an asteraceous perennial vine indigenous to southern Africa, has become naturalised and invasive in many subtropical regions including California, South Australia and Hawaii. Biological control offers a potential long term solution to the management of this species in exotic locations. This study analysed aspects of the biology of *D. odorata* in its native environment to determine its suitability to classical biological control. To this end an examination of the reproductive biology and natural enemies of *D. odorata* was made. A study of the pyrrolizidine alkaloid profile was also conducted.

Reproductive biology: *Delairea odorata* reproduces both sexually by seeds and asexually by stolons. The flowering season occurs over the autumn months from April to June. Results of the pollination trials indicate that *D. odorata* is a cross compatible species and an obligate outbreeder. There is no specialised pollination system and the predominant pollinators belong to the families Apidae, Syrphidae and Calliphoridae. Following pollination, numerous small achenes are produced. Laboratory trials indicate that these achenes germinate readily between 10 and 25 °C and, although germination occurs in both the light and dark, light clearly stimulates seed germination. Greenhouse trials conducted to determine the effect of light on growth and reproduction indicate that *D. odorata* is a shade tolerant species which shows plasticity in terms of growth form and deployment of biomass in response to changes in light intensity. Growth rate and allocation of biomass to vegetative and sexual reproduction are highest at an intermediate light level. However, greatest allocation of biomass is to stem growth regardless of light level.

Natural enemies: Surveys for potential biological control agents against *Delairea odorata* were conducted in KwaZulu-Natal and several phytophagous species were associated with the plant. However, only one potentially suitable control agent was identified, a stem galling tephritid fly, *Parafreutreta regalis* Munro. Preliminary studies indicate this species to be fairly host-specific, a valuable asset if it is to be considered as a control agent. Furthermore, as *D. odorata* proliferates extensively by means of stem regeneration and elongation, galling of these growing points by *P. regalis* may limit stolon spread in exotic locations. Two species of parasitic wasp (Braconidae) were found to parasitise *P. regalis* pupae. If *P. regalis* is to be used as a control agent the likelihood of parasitisation in the new environment must be determined.

Pyrrolizidine alkaloids: Host-specificity in insects is often dependent on host-plant chemistry (e.g. alkaloids or essential oils). Thus prior to any biological control programme it is important to determine if there are ecotypes of the host plant present. An investigation to determine the specificity of the pyrrolizidine alkaloid profile of *D. odorata*, occurring across KwaZulu-Natal, was made. The results indicate the presence of nine retronecine based pyrrolizidine alkaloids which occur in similar proportions in locally distributed plants. However, these alkaloid profiles differ considerably from those published for *D. odorata* occurring in California. This is an interesting and important result which indicates that chemotypes of *D. odorata* may exist, a factor which must be considered in the initiation of any biocontrol. If chemotypes of *D. odorata* are present this may affect the behaviour of natural control agents on the exotic plant populations.

Preface

The experimental work in this thesis was carried out under the supervision of Dr T. Edwards, at the School of Zoology and Botany, University of Natal, Pietermaritzburg from February 1998 to August 1999.

The results of this study have not been submitted in any form to another university and, except where acknowledged, are the results of my own work.

Carol Ann Rolando

Contents

Acknowledgements

I would like to thank my supervisor, **Dr T. Edwards**, for the advice and encouragement which he gave me during the course of this investigation.

Special thanks must also go to:

Prof. J. van Staden for both his academic and financial support for which I am extremely grateful.

Dr R. M. Miller for his advice and help with identification of invertebrates.

Dr M. Smith for his expertise, assistance and patience concerning the pyrrolizidine alkaloid analysis.

Credit is also due to the following persons:

Dr E. Grobbelaar (PPRI) and Dr R. Muller (Transvaal Museum) for their help with identification of beetles.

Dr M. Kruger (Transvaal Museum) for his assistance with the identification of moths and butterflies.

The staff of the Electron Microscope Unit for their help with scanning electron microscopy.

I would also like to thank my parents for their continued support and encouragement.

Finally, I would like to thank Hallam who provided the confidence, inspiration, motivation and support when I needed it - which was most of the time!

List of Figures

List of Tables

Chapter 1

General Introduction

1.1   Project description

Delairea odorata Lem., (syn. *Senecio mikanioides* Otto ex Walp.) commonly known as the Cape Ivy or German Ivy is a perennial semi-climbing plant that is indigenous to southern Africa. Here it occurs sporadically along the mist-belt region stretching from Mpumalanga to the Eastern Cape, typically growing along forest margins or in the forest between 800 - 1900m above sea level. During the early 20th century *D. odorata* was introduced into several countries as a Victorian-era ground cover and house plant (Hilliard, 1977; Jacobi and Warshauer, 1992; Archbald, 1995; Alvarez, 1997). Today, *D. odorata* is naturalised in many subtropical countries including the USA (California and Hawaii), Argentina (Buenos Aires), Chile, South Australia (New South Wales and Victoria), Tasmania, Italy and northern Spain (Hilliard, 1977; Fagg, 1989; Montenego *et al.*, 1991; Jacobi and Warshauer, 1992). In most of these areas *Delairea* is listed as an invasive species and a serious environmental weed warranting evaluation for biological control (Scott and Delfosse, 1992; Elliot, 1994; Archbald, 1995).

In exotic locations *Delairea odorata* is presently controlled by mechanical and chemical means (Cudney and Hodel, 1986; Archbald, 1995; Bossard and Benefield, 1995; Moore, 1997; Forbert, 1998). However, due to the high costs of chemical and mechanical control coupled with the need for regular monitoring and follow-up treatments after removal, thousands of dollars are currently spent on the control and eradication of *D. odorata* (Robison, 1999). The only long term solution to the management of *D. odorata* that seems practical is the introduction of organisms that feed on it, *i.e.* biological control (Elliot, 1994; Balciunas pers. comm.). If successful, biological control provides a low cost, long-lasting, alternative to mechanical and chemical control methods. Elliot (1994) suggested the release of two insects introduced to control *Senecio jacobaeae* L. onto *D. odorata*. During 1994, studies were conducted to test if these insects could reduce the vigour of *D. odorata* in the laboratory. No follow up report on these trials could be found. The alternative to using existing biocontrol agents is to search for the natural enemies of *D. odorata* in southern Africa. This involves the development of a classical biological control programme. Researchers from the USA are of the opinion that, in southern Africa, the growth of *D. odorata* is stunted due to the action of herbivores (Balciunas, pers. comm.).

This project was aimed at investigating aspects of the biology of *Delairea odorata* in southern Africa. For a biological control programme to be effective knowledge of the distribution, biology and ecology of the plant species in its native environment is essential. It is hoped that this research will provide a series of baseline studies which will assist land managers and biocontrol specialists in developing control strategies for *D. odorata*.

1.2   Weeds: their definition, attributes and control

One of the main threats to the biodiversity of our environment is the direct destruction of habitats by the activities of man. Another serious, but largely underestimated problem, is the threat to natural and semi-natural habitats by the invasion of alien organisms (Rejmánek, 1995). Plant invaders pose a potentially lasting and pervasive threat as they may physically displace native plants and animals and in some cases change the soil composition enough to make native plant re-establishment impossible (Cronk and Fuller, 1995; Rejmánek, 1995). Conservationists are becoming increasingly aware of the problems posed by invasive plants as attempts to conserve native vegetation fail due to the disturbance effects created by vigorous aliens.

There is much general agreement as to which plants are weeds. However, definitions of what constitutes a "weed" or an "invader" and the terminology used in the study of plant invasions and weed biology can be confusing (Pysek, 1995). Plants occurring in regions where they are not wanted have been termed weeds, aliens, invaders, exotics, immigrants, introduced, naturalised and colonisers (Baker, 1974; Heywood, 1989; Perrins *et al.*, 1992; Pysek, 1995; Mack, 1996). In the literature a distinction between these terms is rarely made yet, technically, they do not reflect identical concepts.

The term weed is traditionally associated with plants invading highly disturbed man-made or agricultural habitats and thus often reflects an anthropocentric viewpoint (weeds are plants growing where they are not desired) (Baker, 1974; Cronk and Fuller, 1995; Rejmánek, 1995). Baker (1974) designated a plant a weed "if, in any specified geographical area, its populations grow entirely or predominately in situations markedly disturbed by man (without being deliberately cultivated plants)". This definition does not include those plants which invade undisturbed and successionally advanced communities or natural/semi-natural habitats - the plants most frequently termed colonisers, invaders and aliens (plants spreading into areas where they are

not native) (Rejmánek, 1989; Pysek, 1995; Rejmánek, 1995). Furthermore, many non-native plants in protected areas are often classified as weeds ("ecological" or "environmental") because in national parks and similar areas non-native species interfere with the major management goals, *i.e.* protection of the native biota (Rejmánek, 1995). Since the term weed is so widely used, a more acceptable definition of this term might be: " A weed is a plant that is growing where it is not wanted" (Mortimer, 1990). Thus alien invaders may frequently be referred to as weeds.

An alien species has been defined as one which has reached an area as a consequence of the activities of man or his domestic animals but is not necessarily a weed (Pysek, 1995). It has also been termed an introduced species (Williamson and Fitter, 1996). An invader is an alien with increasing distribution in the wild, regardless of habitat (Pysek, 1995). Theoretically, an alien species can be considered invasive when it enters the exponential phase of spread (Rejmánek, 1995). An invasive species may also be referred to as a naturalised species (Williamson and Fitter, 1996). A weed may also be an indigenous species increasing in abundance and range. Prach and Wade (1992) termed a native species exhibiting such behaviour as expanding rather than invasive.

Over the past 30 years, numerous attempts to describe and define attributes common to plant invaders or weeds have been made. Contemporary scientists are asking the following questions: What makes a species invasive ? Why are some plants more likely to become weeds than others? (Baker, 1974; Lawrence, 1980; Rejmánek, 1989; Rejmánek *et al.*, 1991; Perrins *et al.*, 1992; Vogt Anderson, 1995; Pysek, *et al.*, 1995; Rejmánek, 1995; Mack, 1996; Williamson and Fitter, 1996; Vermeij, 1996). One of the first, and best known, attempts to describe attributes common to weedy species is Baker's (1965) list of "ideal weed" characters. In summary, he described an "ideal weed" as one which produces many widely dispersed, long-lived seeds which lack special requirements for germination, grows quickly, reproduces both vegetatively and sexually, flowers early, is self compatible and is a good competitor. Baker (1965) proposed a continuum of "weediness" where species with few "ideal characters" were unlikely to be weedy while those possessing many were likely to be invasive.

A review of contemporary literature indicates that many of Baker's "ideal weed" characters are now being rejected. The two major reasons for this are: (1) many invaders have remarkably few of the Baker characters while other species, that have not become extensively naturalised, possess

many, e.g. *Pinus pinaster* Loud in South Africa (Kruger, 1977) (2) Baker's list is compiled for weeds which grow in areas markedly disturbed by man and while these characters may be common to agricultural weeds they do appear to hold for invasive plants of natural habitats (Rejmánek, 1989; Cronk and Fuller, 1995; Mack, 1996). However, despite a multitude of more recent attempts to define attributes common to environmental weeds (Rejmánek, 1989; Panetta and Mitchell, 1991; Perrins *et al.*, 1992; Vogt Anderson, 1995; Rejmánek, 1995; Williamson and Fitter, 1996; Rejmánek and Richardson, 1996; Vermeij, 1996) as yet, few shared attributes have been found to be associated with invasive potential. The general conclusions from these studies are that there is no one suite of characteristics which make a plant invasive therefore one species with a given set of characters will be a weed in one place but not in another. Several predisposing factors have been suggested to increase the chance of a plant becoming invasive. Some of these factors include taxonomic position, homoclines (climate), habitat, ecological status, dispersal characteristics, seed biology, genetic systems and mode of reproduction (Groves, 1986). In other words, the potential for a plant to become a weed depends on: (1) the attributes of the species (life history, growth form and reproductive behaviour) (2) the environment in which it is growing (habitat and climate), and (3) the interaction between these two.

Weeds may be controlled by biological, chemical, mechanical or cultural means. Today, mechanical and cultural weed control are important only in the underdeveloped nations. These methods are labour intensive and frequently require rigorous monitoring and follow up treatments (Stephens, 1982). Following the discovery of potent herbicides during the 1940s, chemical control was seen as a permanent solution to weed problems. Soon, however, the ecological implications of these herbicides became apparent. This promoted the development of integrated control where pest infestations are reduced through a combination of methods - chemical, biological, mechanical and/or cultural. Only recently, however, has the concept of integrated control been applied with conviction to the control of weeds. The development of herbicide-resistant floras and their interaction with pests and disease organisms has made an integrated approach to weed control essential (Altmann and Campbell, 1977; Stephens, 1982).

While an integrated approach to weed control in agriculture is very effective, in the case of environmental weeds, which infest larger areas and where access is more difficult, biological control is frequently a better option (Bennett, 1986). Biological control is more convenient as

once an agent has established the control programme is self-perpetuating. Moreover, economic and social pressures to reduce reliance on pesticides is encouraging biologists to seek biological solutions to pest control (Stephens, 1982).

1.3   Biological control

Biological control began in the thirteenth century when the ancient Chinese manipulated the predacious ant, *Oecophylla smaragdina* F. in mandarin orange trees to reduce the number of foliage feeding insects (DeBach, 1964). It was not until 1836, however, that the biological control of weeds began. In 1836 the cochineal insect, *Dactylopius ceylonicus* Green, was taken from northern to southern India to control the cactus *Opuntia vulgaris* Miller (Goeden, 1988; Julien, 1989). Since then research in biological control of weeds has dramatically increased and progress towards a greater understanding of the ecological principles underlying this practice has been made. Today, biocontrol is recognised as a multidisciplinary and applied science (Julien, 1989; Harris, 1991; Shepherd, 1993; van Driesche and Bellows, 1996).

What is biological control? A review of the literature reveals that the term "biological control" has no single definition. As argued by DeBach (1964) and Harris (1991), no single definition appears to be satisfactory because the term "biological control" is used with different meanings. Traditionally, biological control refers to the process whereby the natural enemies of a plant (*i.e.* those that exist in nature), are used to control populations of the plant (DeBach, 1964; Anon, 1971; Dennil and Hokkanen, 1990; Shepherd, 1993; van Driesche and Bellows, 1996). Thus the term "classical biological control" refers to the process whereby the natural enemies of a weed are collected in the country of origin of the weed and released in the country of introduction in order to control the weed. For the past 30 years the traditional approach to biocontrol has been much disputed and alternative methods to the " classical biocontrol" approach have been used (Dennil and Moran, 1989; Dennil and Hokkanen, 1990). A contemporary and suitable definition of biological control is thus "the study and utilisation of parasites, predators and pathogens to regulate populations of pests" (Harris, 1991).

Since the classical biological control approach is the method to be adopted for this investigation, the following section will focus on the principles and methods underlying the traditional approach.

Classical biological control

Biological control of weeds is based upon a mutual dependance in the status of a weed and a phytophagous predator or fungal pathogen capable of controlling it. It has usually been applied against alien weeds, the abundance of which is often due to their having escaped the natural enemies common to them in their native lands (Huffaker, 1964; van Driesche and Bellows, 1996). The main assumptions of the classical biological control approach are: (1) that the natural enemies (mono-or oligophages) are most likely to be host-specific and therefore safe for introduction into other countries and (2) that irrespective of the evolutionary age of the relationship between plant and natural enemy, and thus any possible homeostasis that may have developed between the two, it is the release of the biocontrol agents from their natural enemies that is the crucial factor in the achievement of control (Bartlett and van den Bosch, 1971; Dennil and Moran, 1989; van Driesche and Bellows, 1996).

A number of scientists have challenged the basic assumptions of classical control (see review by Dennil and Moran, 1989) and today the search for effective biocontrol agents is less restrictive. The search for biological control agents can be extended to (1) areas other than the native home of a weed (2) to natural enemies which normally feed on a relative of the weed, and (3) to existing control agents (Bartlett and van den Bosch, 1971; Dennil and Moran, 1989; Dennil and Hokkanen, 1990). Despite the development of a number supplementary techniques many researchers still approach biological control in the traditional way (Dodd, 1961; Marohasy, 1989; Muller, 1989; Scott and Way, 1990; Gillet *et al.*, 1991; McClay and Palmer, 1995).

Methods used in biocontrol studies

Biological control is dependent upon the availability of host-specific control agents (Huffaker, 1964; Anon, 1971; Julien, 1989; Harris, 1991; Shepherd, 1993). Furthermore, the objective of biological control is not eradication, it is the gradual reduction of a weed's density and impact (Huffaker, 1964; Julien, 1989). Agriculturalists have been reluctant to use biological control methods as the risks, pertaining to the introduction of new organisms into an already disturbed environment, are too great when compared with the chances of obtaining an adequate degree of control. Added to this there is frequently conflict in the acceptance of a given plant as a weed (Bartlett and van den Bosch, 1971). To reduce conflict and to increase confidence in biological weed control, extensive codes of practice and legislation are now in place to set out the required

procedures for the development of a biological control programme (DeBach, 1964; Zwolfer and Harris, 1971; Harris, 1973; Wapshere, 1974; Lawton, 1984; Wapshere, 1989; Muller-Scharer *et al.*, 1991; Shepherd, 1993; Cronk and Fuller, 1995; van Driesche and Bellows, 1996; FAO, 1997).

Today, classical biological control programmes proceed according to a general protocol designed to minimise the associated risks. This protocol was originally described by Zwolfer and Harris (1971). Generally, the procedure is to:

1. Determine the weed's suitability to biological control.
2. Conduct surveys of the weed's natural enemies.
3. Evaluate and select potential biological control agents from a range of natural enemies.
4. Conduct host-specificity studies of potential agents to ascertain the safety of their introduction into the new area.
5. Introduce the biocontrol agents into the control area.
6. Conduct evaluation studies.

The following section will expand on the biological and ecological theories upon which the above procedure is based.

Determine the weed's suitability for biological control

It is important to determine the suitability of a weed for biological control before embarking on an extensive research programme. Thus, prior to the beginning of the actual search for biological control agents, the following basic information on a weed should be established: taxonomic position, biology, ecology, native geographic distribution, total present distribution, probable centre of origin and that of its close relatives and its economic importance (DeBach, 1964; Anon, 1971; Scott and Delfosse, 1992; Shepherd, 1993; van Driesche and Bellows, 1996).

A basic requirement of all projects is a thorough understanding of the taxonomy of the target weed (Huffaker, 1964; Schroeder and Goeden, 1986; Harris, 1991; van Driesche and Bellows, 1996). A study of the taxonomy of the plant in the country of introduction as well as in the country of origin ensures that agents are collected from the correct plant species. Projects have failed because of a lack of knowledge of the host plant; for example, early projects against *Salvinia molesta* D.S. Mitchell failed as this species had not been taxonomically separated from

S. auriculata D.S. Mitchell. In absence of knowledge of *S. molesta*'s native range, agents had been collected from *S. auriculata* and were thus unable to establish on *S. molesta* (Schroeder, 1985).

The distribution, pest status and economic impact of the weed in the countries of introduction should be ascertained through literature surveys to enable the researcher to find out what is known about the plant (Julien, 1989; McClay, 1989; Shepherd, 1993; van Driesche and Bellows, 1996). Given present economic conditions, effort should be focused on weeds which are serious economic problems and which offer a realistic chance of success. However, there may be little or no information on the economic impact of a weed species, particularly if the weed in question is an environmental as opposed to agricultural weed, and in such cases the study may be limited to determining the biological suitability of a weed as a target species (McClay, 1989).

A study of the biology and ecology of the plant should be made in both the country of origin and introduction. This will help to determine why the plant is a weed, the vulnerable stages of the weed to natural enemies and whether there are any major differences in the life forms and/or habits of the plant. The study should determine if there are different ecotypes or biotypes of the plant present and should take into account seasonal variations in life history characters with respect to climate, temperature and rainfall (Andres *et al.*, 1976; Shepherd, 1993; van Driesche and Bellows, 1996). Extensive intraspecific variation in the target weed can make biological control difficult as the ecotypes may differ in their susceptibility to the biocontrol agent (Andres *et al.*, 1976; McClay, 1989; Shepherd, 1993). Furthermore, it may be difficult to identify effective biocontrol agents for a particular ecotype, and if several forms are present, several biotypes of the control agent may have to be introduced (McClay, 1989; Dennil and Hokkanen, 1990; van Driesche and Bellows, 1996). Critical to a biological control programme is determining the vulnerable stages in the plants' life history. Any agent which is to be introduced must attack the plant so as to limit its reproductive output, be that sexual or vegetative (McClay, 1989; Shepherd, 1993; van Driesche and Bellows, 1996).

Natural enemies

Many types of herbivorous insects and pathogenic fungi have been considered as biocontrol agents (Crawley, 1989; Julien, 1989). The most severe constraint on the choice of agents is their

host-specificity. Controversy exists over whether it is possible to empirically analyse the qualities of candidate enemies and introduce only the best species (Huffaker *et al.*, 1971; Harris, 1991; McEnvoy *et al.*, 1993; van Driesche and Bellows, 1996). An introduced enemy's efficiency in controlling a given weed is inherently related to other stresses the weed faces from its direct competitors (Anon, 1971; Harris, 1991). Judgement of the "best" enemy in one vegetation complex will, therefore, have only limited meaning in another vegetation complex. However, ecological information and analysis of previous control attempts have been used to indicate which species should be tried first (Anon, 1971; Huffaker *et al.*, 1971; Andres *et al.*, 1976; van Driesche and Bellows, 1996). Effective biocontrol agents should possess the following attributes:

1.   Adaptability to varying physical conditions.

2.   Searching capacity, including general mobility (or capacity for dispersal).

3.   Power of increase (in population size) relative to that of its host.

4.   Power of host consumption (i.e. negative impact on host growth capacity).

5.   Other intrinsic properties such as synchronisation with the hosts' life history, host specificity and ability to survive host-free periods.

Evaluation of natural enemies should comprise the following stages (Anon, 1971):

1.   Broad survey of the literature and regular sampling at local populations to determine the natural enemies.

2.   Concentrated studies on single control agents that cover life history, distribution and parasitoids of the agent.

3.   Host-specificity studies.

Harris (1973) outlined some useful parameters for the selection of effective agents for the biological control of weeds.

Host-specificity studies

One of the most important considerations in biological control is the host-specificity of the control agent as it is important to demonstrate that on introduction to a new area it will not damage plants of economic and conservation importance (Huffaker, 1964; Zwolfer and Harris, 1971; Wapshere, 1974; Andres *et al.*, 1976; Wapshere, 1989; Blossey *et al.*, 1994; van Driesche and Bellows, 1996). The absence of insects from plants related to the weed is a good indication of host-specificity as the propensity for feeding on other plants is usually greatest for those having

taxonomic affinity with the host (Zwolfer and Harris, 1971; Shepherd, 1993). However, host recognition may depend on a single chemical or physical stimulus which has arisen independently in several plant taxa. Therefore, once suitable agents have been selected, scientific tests defining their host range need to be conducted (Anon, 1971; Wapshere, 1974; van Driesche and Bellows, 1996). These are in the form of laboratory and field tests.

Laboratory tests

Tests to determine the specificity of herbivorous arthropods have been conducted as either "choice" or "no choice" tests (Syrett, 1985; Adair and Scott, 1991; Dunn and Campobasso, 1993; Woodburn, 1993; Blossey *et al.*, 1994; Wan *et al.*, 1996). However, there has been considerable variation in the experimental techniques used and critical plants tested (Harris and Zwolfer, 1968; Zwolfer and Harris, 1971; Wapshere 1974; Wapshere, 1989; Clement and Cristofaro, 1995). "No choice" tests are conducted by isolating the insect in a cage with a plant species to determine whether any feeding or oviposition can occur. In "choice" tests the agent is given a choice, in a single cage, of two or more plant species and feeding and reproduction on each is recorded (Zwolfer and Harris, 1971).

The criteria for selection of plant species to test has been the subject of much debate. Typically, the species selected are close taxonomic allies (same genus, tribe or family) (Harris and Zwolfer, 1968; Harris, 1973; Wapshere, 1974; Wapshere, 1989; Shepherd, 1993). The testing of plants by phylogenetic methods is based on the assumption that related plants are morphologically and biochemically more similar than unrelated plants (Wapshere, 1974; Wapshere, 1989; van Driesche and Bellows, 1996). That closely related plants are morphologically and biochemically similar is a *sine qua non* since plant taxonomy and phylogenetic relationships have been based on these aspects. Tests should also be conducted on both cultivated plants botanically related to the weed and cultivated plants known to be attacked by organisms closely related to the biocontrol agent under investigation (Woodburn, 1993).

Field tests

Several authors have questioned the applicability of results obtained under laboratory conditions to the behaviour of the potential biocontrol agent in natural plant settings (Zwolfer and Harris, 1971; Lawton 1984; Wapshere, 1989). Tests confining insects often give unnatural results because

dispersal is eliminated and this is an important part of the host selection process. Thus an organism may feed/oviposit on plants in the laboratory which it would not normally select in the field. The difficulties arising from laboratory testing can be avoided by testing for host-specificity under field conditions. In open field tests the selected arthropods can have "free choice" of plants without the constraints on their host selection behaviour (Maddox and Sobhian, 1987; Clement and Cristofaro, 1995). Clement and Cristofaro (1995) reviewed the development and use of open field tests in host-specificity determination. They proposed that during insect screening programmes both laboratory and field methods should be used (Dunn and Campobasso, 1993; Blossey *et al.*, 1994).

Introduction and evaluation of biocontrol agents

Once tests regarding the host-specificity of a natural enemy are complete, decisions to introduce an agent into a weed-infested area can be made (Anon, 1971; van Driesche and Bellows, 1996). Such decisions are usually made at higher official levels than the research unit conducting the tests and regulations regarding the mode of introduction will vary depending on the organisation in charge of biological weed control for that specific area (country/state) (Shepherd, 1993). Once an enemy has been introduced, close descriptions of the destructive action (if any) and patterns of destruction caused by the agent should be made if the results of the introductions are to be evaluated accurately (Shepherd, 1993; Cronk and Fuller, 1995; FAO, 1997). A principle tool in the evaluation of the impact of introduced agents is close studies of the changes in the general vegetation, in association with studies on the specific impact of the enemy on the weed in question (Anon, 1971; van Driesche and Bellows, 1996).

1.4   General background to the present study

Presently chemical and mechanical methods are proving inadequate, and expensive, for the control of *D. odorata*. Consequently, biological control offers a seemingly attractive long term, economically viable alternative to the management of this plant. Despite the status of *D. odorata* as an highly invasive species, very little is known about the biology and ecology of this plant in both its native and exotic environments. If a biological control programme for *D. odorata* is to be developed it is vital that aspects of its biology be investigated.

Little has been published about *D. odorata* in southern Africa, South America, Europe and Australia. Some work has been conducted into the status of *D. odorata* in California and Hawaii (Cudney and Hodel, 1986; Jacobi and Warshauer, 1992; Chipping, 1993; Elliot, 1994; Archbald, 1995; Alvarez and Cushman, 1997; Archbald, 1997; Forbert, 1998). However, most of this is unpublished research obtained largely from files of interested individuals and biological newsletters. Increasing concern about the rate of spread of *D. odorata* in California has recently motivated the development of several research initiatives aimed at investigating its biology and developing effective methods of control (Balciunas, pers. comm.; Robison, pers. comm.).

The following section is an account of the current status of knowledge on the taxonomy, distribution, biology and ecology of *D. odorata*.

Taxonomic position

Delairea odorata is a member of the Asteraceae tribe Senecioneae, subtribe Senecioninae (Jeffrey and Chen, 1984). The genus *Delairea* Lem. (syn: *Senecio* sect. *scandentes* DC.) consisting of the single South African species *D. odorata* is allied to the genera *Mikaniopsis, Cissampelopsis* and *Austrosynotis* (Jeffrey, 1986). All three genera are grouped into the Synotoid complex which is represented in Africa and Madagascar by scandent species. They are characterised by the presence of tailed anthers with sterile basal auricles (Jeffrey, 1986). Closely related species occurring in southern Africa include: *Mikaniopsis cissampelina* DC., *Senecio cinarescens* DC., *Senecio quinquelobus* DC. and *Senecio tamoides* DC. (Arnold and de Wet, 1993; Jeffrey, 1986).

Delairea odorata is a bright green, slender, herbaceous twiner, stems and leaves slightly succulent, glabrous to glabrescent. *Leaves* alternate, deltoid-ovate, up to 70-80 mm in diameter, margin 6-10 lobed, base cordate. *Petioles* 70-80 mm, auriculate, often twisted. *Heads* discoid, many in congested compound corymbose panicles, terminating short lateral branchlets which are nude or with few reduced leaves. *Involucre* narrowly campanulate, bracts 8, much shorter than the disc and keeled at the base. *Flowers* bright yellow, ovary 1.5 mm long, sparsely hispid (Hilliard, 1977).

Distribution, status and community associations

Delairea odorata is uncommon in South Africa. It occurs sporadically along forest margins in mistbelt vegetation stretching from the southern and Eastern Cape to Lesotho and grows as far north as Nkandla, Nhlazatshe Mt. and the Biggarsberg north of Ladysmith (Fig. 1.1). In South Africa it flowers in the late autumn and early winter months of May, June and July. Only one South African population seems to grow in an invasive smothering manner similar to plants where it has become an invasive weed. At Ingele Forest, Harding, *D. odorata* grows vigorously along the road and forest edges (pers. observation, Robison, pers. comm.).

In Australia *D. odorata* occurs in South Australia, Tasmania, New South Wales and Victoria. In Victoria it is now widespread in the southern districts from Portland and Warrnambool on the Western coast, to Wilsons Promonotory, and to Mallacoota in far east Gippsland (Fagg, 1989). *D. odorata* has been listed as an important weed of Australian conservation areas (Scott and Delfosse, 1992). It spreads most vigorously in moist semi-shaded environments such as damp gullies with tree cover and frequently occurs along roadsides (Fagg, 1989). It seems less successful in dry, exposed sunny conditions (Cox, 1998). Flowering occurs during the late winter early spring months of July, August and September (Cox, 1998).

A map published by HEAR (1986) indicates that in Hawaii *D. odorata* is present on the islands of Hawaii, Maui and Oahu (Fig. 1.2). However, this reference gives no information on the habitat types in which *D. odorata* occurs. A study of the distribution of *D. odorata* on the island of Hawaii was completed in conjunction with bird counts from 1976 to 1981 (Jacobi and Warshauer, 1992). This study found that on the island of Hawaii *D. odorata* occurs between 500 and 2500 m above sea level in areas with 1250 to 2500 mm annual rainfall. Major habitat types include xeric to mesic scrub and forest and hydric forest. Jacobi and Warshauer (1992) found most populations to be concentrated in the North Kona and Mauna Kea -Mauna Los saddle areas. In Hawaii *D. odorata* flowers in December and January.

Delairea odorata is also present in Europe. Almost nothing is known about the nature of the infestations in this region. Catalano *et al.*, (1996) report its naturalisation in south and west Europe, including Italy. Researchers in northern Spain have also recently begun herbicide control tests (Robison, pers. comm.). The reported range of *D. odorata* in North America extends from

2223	2224	2225	2226	2227	2228	2229	2230	2231	2232	2233	2234	2235	**22**
2323	2324	2325	2326	2327	2328	2329	2330	2331	2332	2333	2334	2335	
2423	2424	2425	2426	2427	2428	2429	2430	2431	2432	2433	2434	2435	**24**
2523	2524	2525	2526	2527	2528	2529	2530	2531	2532				
2623	2624	2625	2626	2627	2628	2629	2630	2631	2632				**26**
2723	2724	2725	2726	2727	2728	2729	2730	2731	2732				
2823	2824	2825	2826	2827	2828	2829	2830	2831	2832				**28**
2923	2924	2925	2926	2927	2928	2929	2930	2931					
3023	3024	3025	3026	3027	3028	3029	3030						**30**
3123	3124	3125	3126	3127	3128	3129	3130						
3223	3224	3225	3226	3227	3228	3229							**32**
3323	3324	3325	3326	3327									
3423	3424	3425											**34**

24 26 28 30 32 34 36

NBI 1994

Fig. 1.1. Distribution of *Delairea odorata* in South Africa. (Distribution map is based on specimens lodged at herbaria: PRE and NU)

Present

May be present (search needed)
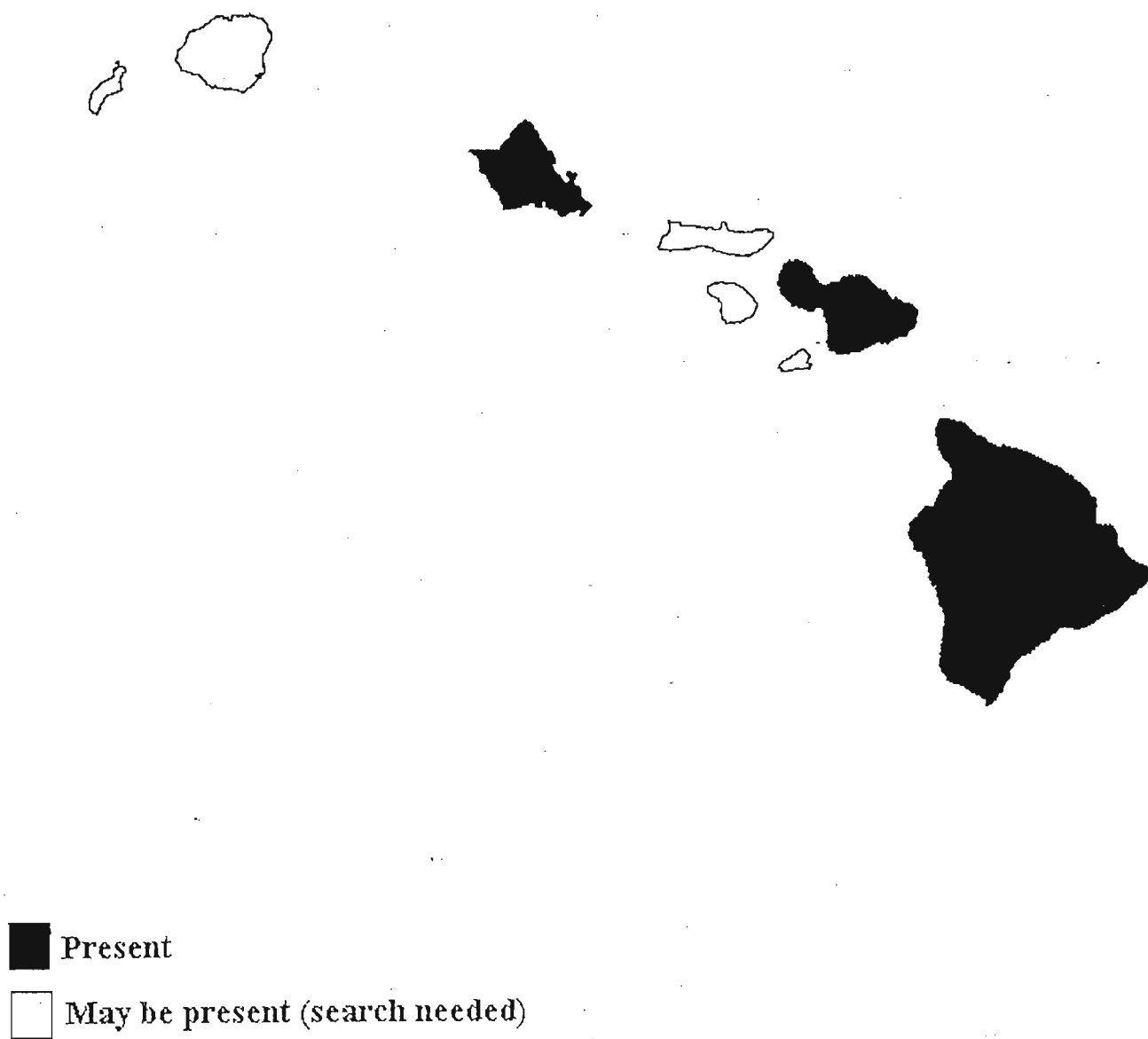
Fig. 1.2. Distribution of *Delairea odorata* on the main Hawaiian islands as of 1986 (HEAR, 1986).

Oregon South through California into Baja California, though no comprehensive mapping efforts have yet been completed (Balciunas pers. comm.). In California *D. odorata* has invaded the South Coast, the Central Coast and the North Coast, the San Francisco Bay area and many of the state's national parks (Anon, 1996; Alvarez, 1997). In the Golden Gate National Recreation Area (GGNRA) *D. odorata* is spreading more rapidly than any other non-native invasive plant species (Alvarez, 1997). Monitoring has shown that from 1987 to 1996 populations of *D. odorata* moved from infesting less than 30 acres to more than 200 acres within the GGNRA. This represents an average population size increase of 25 % per year (Fisher, 1997). The species has been rated as "highly invasive" by the new Jepson Manual and is also included in the California Exotic Pest Plant Councils' (CalEPPC) A-1 list of "Most Invasive Wildland Pest Plants: Widespread". In California, *D. odorata* appears to prefer moist, shady environments along the coast. However, it is also spreading in riparian forest, coastal shrubland, grassland, Monterey pine forest, coastal bluff communities and seasonal wetlands. It has even been noted on serpentine soils (Archbald, 1995). Flowering occurs during the winter months of December and January.

Basic biology

In its exotic locations, *D. odorata* forms impenetrable mats in both the shade and the sun, climbs native shrubs and trees and often forms a dark canopy layer up to nine metres high, smothering native vegetation in the immediate vicinity (Fagg, 1989; Chipping, 1993; Alvarez, 1997; Fisher, 1997). *D. odorata* expands vegetatively as a vine through spreading stolons and frequently sends out runners in response to poor light conditions. These runners have leaves at each node and each of these nodes has the potential to become a new plant (Chipping, 1993). Growth rates of individual plants and populations measured at the GGNRA have shown that increase in growth may be up to 30 cm per month per individual stem (Alvarez, 1997).

Delairea odorata has the ability to regenerate from small pieces of above ground stem material (Archbald, 1995). Stem fragments as short as two cm carried by runoff or landscape machinery can take root and colonize new areas (Chipping, 1993; Archbald, 1995; Alvarez, 1997). In addition, it has thick rhizomes that support regrowth of above ground portions when shoots are damaged or removed. The system of rhizomes was aptly described by Chipping (1993):" I could not understand how resprouting shoots kept showing up in areas unfit for growth.......down under the grass was a mass of purple, leafless vines, not particularly woody, and numerous enough to

look like the control panel wiring for a B-1 bomber". This strong predisposition for vegetative reproduction makes mechanical control of this species very difficult.

A series of experiments conducted by a student group in California have shown *D. odorata* to be drought tolerant. In a 10 week drought tolerance test they found that not a single well established plant was killed by moisture regimes ranging from 0-100 % for the full 10 weeks. Even cut stems left to dry on the laboratory table for the 10 week period were alive at the end of the test. Furthermore, when the drought stressed individuals were watered at the end of 10 weeks they recovered normal water potential in their tissues after two days, even though the drought stressed plants were observed to have air embolisms in their tissues (Archbald, 1995).

Very little research has been conducted into the breeding system and seed biology of *D. odorata*. In California, no viable seed has been collected and the plant appears to be spreading only by vegetative means (Balciunas, pers. comm.; Alvarez, 1997). In Australia and Hawaii *D. odorata* apparently spreads both vegetatively and by wind blown fruits (Jacobi and Warshauer, 1992; Cox, 1998). However, no data or research has been published in support of the above statement. Prior to this study no research had been conducted into the reproductive biology of *D. odorata* in southern Africa. The nature of the contrasting modes of reproduction in exotic locations raises some interesting questions as to the genetic attributes and the nature of the spread of populations of *D. odorata* in each of these areas.

Ecological effects of Delairea odorata

At the GGNRA, California, research on the impact of *D. odorata* on coastal plant communities has shown that it is associated with significant reductions in species richness (Alvarez and Cushman, 1997). Grasses and annual species were found to be consistently missing from *Delairea*-infested plant communities. In addition, the abundance and richness of seedling plants is significantly reduced indicating that after weed control there may be little re-establishment of native plants in the infested sites (Alvarez, 1997). There is also evidence that infestation is associated with a reduction of two insect orders (Coleoptera and Diptera) in two riparian plant communities which could affect other species densities dependent on these insects (Fisher, 1997). Furthermore, *D. odorata* is a threat at the species level, with four federally listed species and nine state listed species threatened by habitat modification, displacement and shading from this plant.

Host and nectar plant populations for two endangered butterflies, the Mission Blue (*Plebejus (Icaria) icarioides* Boisduval (Lepidoptera: Lycaenidae)) and the San Bruno Elfin (*Callophyrs mossii* Edwards (Lepidoptera: Lyacaenidae)) are also threatened by the spread of *D. odorata* (Alvarez, 1997).

Delairea odorata contains pyrrolizidine alkaloids which are a group of 'highly toxic' secondary plant compounds. Pyrrolizidine alkaloids of a related species, *Senecio jacobaeae*, have been associated with growth depression, mortality and development of hepatic lesions in rainbow trout (Hendricks *et al.*, 1981). *D. odorata* has been associated with a toxic effect on aquatic organisms (Archbald, 1997). Preliminary studies, to determine the toxicity of *D. odorata*, have shown that liquid extracted from blended plant material or small stem fragments has a significant toxic effect on the golden shiner (*Notemigonus crysoleucus* Mitchell), a common toxicological subject (Archbald, 1995). Further studies are being conducted with rainbow trout (*Salmo gairdneri* Richardson) and three-spine stickleback (*Gasterosteus aculeatus* L.), fish more characteristic of the coastal riparian habitats which *D. odorata* could invade. These results will indicate whether the threatened coho salmon *(Oncorynchus kusutch* Walbaum*)* and the California freshwater shrimp (*Syncaria pacifica*) will be directly affected by *D. odorata* infestations.

Mechanical and chemical control

The environments in which *D. odorata* grows frequently makes access to the plant difficult and this hinders mechanical and chemical control (Moore, 1997). Effective mechanical control requires the removal of all vegetation, native and exotic, from the infested area in order to gain visual and physical access to locations where stems emerge from the ground. Above-ground shoots are then pulled or cut away and the root mass dug out with hand tools (Archbald, 1995; Moore, 1997). At sites where the plant is growing mat-like on the ground it is sometimes possible to role up the entire infestation like a carpet (Archbald 1995). As *D. odorata* reproduces vegetatively it is difficult to dispose of the plant material. Usually, after clearing, the plant is removed from the site and laid out in the sun to dry to ensure complete desiccation. Continued monitoring and repulling at the cleared site is needed as resprouting occurs from remaining fragments (Moore, 1997).

Preliminary herbicide trials, carried out by the Presidio National Park in San Francisco, have shown that spraying with Triclopyr®, Glyphosate®and Sylgard® reduces resprouting in *D. odorata*. Furthermore, weed whipping and application of a low volume of Triclopyr® successfully reduces above and below ground biomass (Archbald, 1995). Archbald (1995) observed that where plants were not weed-whipped prior to application of the herbicide, translocation of the herbicide along the rhizome was blocked by much thickened nodes or storage organs. Thus the rhizome on one side of the storage organ was killed, but the rhizome continuing from the other side was not visibly damaged. Other herbicides effective against *D. odorata* include Roundup® (active constituent: glyphosate) and Rodeo® (Archbald, 1995; Forbert, 1998). After spraying an infestation, regular follow-up treatments are essential.

1.5 Overall aims of the present study

The aim of the present study was to survey the natural enemies associated with *D. odorata* and to determine their suitability to biological control. However, as indicated previously, prior to any extensive investigations on natural enemies and their effect on plant populations of a pest, studies on the suitability of a weed to biocontrol should be conducted. Critical to this is an understanding of a weed's reproductive biology in both the native and exotic locations.

For this investigation emphasis was placed on:

i.   Investigating the reproductive biology of *D. odorata* in southern Africa.

ii.   Identifying natural enemies which may be useful in the control of *D. odorata*.

Finally, a study was conducted into the pyrrolizidine alkaloids of *D. odorata*. The toxicity of *D. odorata* to aquatic organisms has been associated with the presence of pyrrolizidine alkaloids. Furthermore, several studies have already been conducted into the pyrrolizidine alkaloids of *D. odorata* in the USA (Stelljes and Seiber, 1990; Stelljes *et al.*, 1991). A study of the profile of these compounds for *D. odorata* across its native range will indicate to what extent the alkaloid profiles in native and exotic locations differ. Perhaps the breeding behaviour in exotic locations favours the formation of genetically isolated populations which may preserve their specific chemical characters. If so, this could have important implications for a biological control programme.

Due to the diversity of this project, the theory, specific objectives, methods and results of each aspect of the research will be introduced and dealt with separately in the relevant chapters.

Chapter 1 introduces this study and also reviews the literature pertinent to biological control.

Chapter 2 is a review on the biological control programmes targeted for weeds of the Asteraceae.

Chapters 3, 4 and 5 are investigations into sexual and vegetative reproduction in *D. odorata*.

Chapter 6 details the natural enemies of *D. odorata* and their potential as control agents.

Chapter 7 is a study on the pyrrolizidine alkaloids of *D. odorata*, and

Chapter 8 is a summary of this study and outlines directions for future research.

Chapter 2

Weeds of the Asteraceae and their biological control: a review

2.1 Introduction

The Asteraceae as weeds

If the evolutionary success of an organism is to be measured in terms of the numbers of individuals in existence, the extent of their reproductive output, the area of the world's surface they occupy, the range of habitats they can enter and their potentiality for putting their descendants in a position to continue the genetic line through time (Baker, 1974), then most members of the Asteraceae rate as highly successful. From an evolutionary point of view the Asteraceae are regarded as one of the most advanced families and there are few families with such an abundance of weedy members particularly in the temperate areas of the world (Heywood *et al*, 1977; Heywood, 1989).

The Asteraceae is one of the largest families of flowering plants with 1100 currently accepted genera and 25 000 species. The diversity of this family is greatest in montane subtropical or tropical latitudes particularly in areas that border arid regions. Among the members of the Asteraceae are evergreen shrubs; annual, biennial and perennial herbs; tap-rooted or tuberous rooted perennials; trees, scramblers, climbers and succulents. The most characteristic feature of this group is the head-like inflorescence, known as the capitulum, made up of numerous small individual flowers, the florets, surrounded by an involucre of protective bracts. This type of inflorescence is constant throughout the family and is sometimes accompanied by various modifications (Heywood, 1993).

Heywood (1989) analysed "A geographical atlas of world weeds" by Holm *et al.* (1979) and found that the families with the highest number of weeds worldwide were the Asteraceae, with 224 genera and 830 species (13 %), and the Poaceae, with 166 genera and 753 species (12 %), considered as weeds. Similar high percentages of these families have been recorded elsewhere in the literature, for example, in the alien flora of individual regions such as California, where 14 % belong to the Asteraceae and 15 % to the Poaceae (Rejmánek *et al.*, 1991). The plant families containing the most weed species in South Africa, are the Asteraceae, Poaceae, Fabaceae and

Solanaceae (in order of decreasing magnitude) (Wells and Stirton, 1982). Among the families over represented in the Czech alien flora composition, the Asteraceae rate the highest, with 20.5 % present in the alien flora and only 10 % in the native flora (Pysek *et al.*, 1995). A review of "World Weeds" by Holm *et al.* (1997) again showed the same pattern, with the leading families being Poaceae (15 %) and Asteraceae (14 %) . The Poaceae and Asteraceae rank amongst the largest angiosperm families, perhaps it is the very features that have been responsible for their evolutionary success and adaptive diversity that are responsible for their successful spread and establishment as aliens worldwide (Heywood, 1989).

Attributes associated with the success of the Asteraceae

The success of the Asteraceae has been attributed to their ability to tolerate a wide range of habitats, their high reproductive rate and their unique chemical compounds. The extreme plasticity of this family is reflected in their habitat accommodation where extreme xerophytes or mesophytes often occur in the same genus *viz. Senecio.* This ecological plasticity has been made possible through diversification of growth habit, which includes everything from slow growing, cold enduring, hard woody shrubs to rapidly growing, cold-sensitive herbaceous perennials (Heywood *et al*, 1977). Their diversity of growth habit has also contributed to their success as weeds and this is reflected in those species that are invasive - where annual, biennial and perennial herbs, shrubs and trees are all represented in the weedy flora of invaded regions (Heywood, 1989).

According to Heywood (1989) the factors which have contributed to the reproductive success of the Asteraceae are associated with the development of the capitulum, this includes: a geitonogamous breeding system (often superimposed with agamospermy), increased pollinator attraction (associated with the aggregation of flowers) and the development of complicated dispersal mechanisms. The one-seeded fruit often has a pappus or wings to aid in wind dispersal. Hooks and spines frequently aid dispersal of seeds by animals and man (Heywood *et al*, 1977; Heywood,1989).

The family is very distinctive in its chemical attributes. Although no single compound is unique to the family, the Asteraceae are remarkable in the range of secondary compounds present and also in the number of complex structures known in any one class (Heywood *et al*, 1977). Many

of the substances produced by the family are toxic or show other physiological activity and this provides a great deal of protection from phytophagous insects, overgrazing by livestock and use by man. It is for this reason that the chemical composition of the Asteraceae is believed to have played a part in their success as weeds.

Several members of the Asteraceae are serious noxious agricultural and/or environmental weeds in areas far removed from their indigenous ranges. They include: *Chondrilla juncea* L. (skeleton weed), *Chromolaena odorata* (L.) R. King & H. Robinson, *Ageratina riparia* (Regel) R. King and H. Robinson, *Taraxacum* species (dandelions), several *Cirsium* and *Carduus* species (thistles), and many *Senecio*'s (Heywood, 1989). Since there are few families with such an abundance of weedy members as the Asteraceae it is not surprising that a number of species have been the targets of biological control programmes (Julien, 1989).

Biological control of the Asteraceae

The following section is a short review on the biological control programmes aimed at weeds of the Asteraceae. As the history of the biological control of weeds is characterized by both successes and failures (Crawley, 1989), many researchers are now analysing previous biological control programmes to determine whether patterns which emerge from such analyses will be useful in guiding future control attempts (Crawley, 1989; Harris, 1989; Julien, 1989; McEnvoy *et al.*, 1993). Since this study is aimed at the biological control of a species of the Asteraceae a review of the literature on biological programmes, of weeds in this family, was carried out to determine any trends which may be of assistance in the development of a control programme for *Delairea odorata*.

2.2   The database and methods used for this review

The following analysis has been based largely on Julien's (1992) catalogue of weed biocontrol cases, specifically those of the Asteraceae. Use of this catalogue as a database limits the scope of this study. However, the catalogue is sufficiently comprehensive to reveal the general trends in biological control of this group. Where necessary data from other sources have been used. In an attempt to understand the functional causes of the successful programmes particular attention has been paid to biocontrol agents which have controlled their respective hosts.

There is no absolute measure of "success" of a weed biocontrol project as each programme is designed for a specific weed problem. Therefore, whether a project is a success or not will depend on the aims of the weed controlling body (Crawley, 1989). For the purpose of this review, to categorise "success", biocontrol programmes have been divided into three categories: (1) those where the agent released did not establish or established but has had no effect on the weed population (2) those where release of an agent has achieved partial control in at least one area of infestation, and (3) those where release of an agent has lead to marked to complete control of the weed in at least one infested area. As the study is based largely on Julien's (1992) catalogue of biocontrol programmes, placement of projects into each of the above categories is partly limited by the information supplied in the catalogue.

2.3   Biocontrol agents used in the control of the Asteraceae

Julien (1992) lists a total of 117 weedy species for which exotic invertebrates and fungi have been released, of these, 32 species (27 %) belong to 18 genera within the Asteraceae. According to Julien (1992), the Asteraceae represent the largest group of terrestrial weeds which have been targeted by biological control programmes. Second to the Asteraceae are the Cactaceae where 22 species (18 *Opuntia* spp.) belonging to five genera have been evaluated and subjected to biological control. Clearly the Asteraceae represent the largest and most diverse group of weeds subjected to evaluation for biocontrol.

A total of 81 species (77 invertebrates and 4 fungi) have been introduced into exotic locations to control weeds of the Asteraceae (Table 2.1). Invertebrates belonging to the orders Coleoptera, Diptera and Lepidoptera have been the most popular control agents followed by the pathogenic fungi (Table 2.1). The minor agents include Hemiptera, thrips, nematodes and acarines. Despite the number of agents which have been used, only 12 weed species have been partially controlled by their agent in at least one region of infestation. Programmes against only four weed species have achieved good to complete control in at least one infested area (Tables 2.2 & 2.3). For 54 % of weeds targeted for biological control, no control was achieved with the release of an evaluated biocontrol agent.

Table 2.4 illustrates the success of the invertebrate and fungi families which have been used in the biocontrol of weeds of the Asteraceae. Most of the exotic invertebrates which have been

Table 2.1. Taxa of exotic invertebrates and fungi released to control weeds of the Asteraceae. Taxa are ranked in decreasing magnitude (based on the number of species released within the taxon).

Order	No. spp (/ 81)	% Total
Coleoptera	31	38
Diptera	23	28
Lepidoptera	19	23
Fungi	4	5
Thysanoptera	1	1
Hemiptera	1	1
Acarina	1	1
Nematoda	1	1

Table 2.2. Weed species (Asteraceae) where good control has been achieved in at least one area of infestation and the agent/s which achieved control (Julien, 1992).

Weed species	Agent species
Ageratina riparia (Regel) R. King & H. Robinson	*Entyloma ageritinae* Barreto and Evans (fungus: Ustaligenales) *Procecidochares alani* Steyskal (Diptera: Tephritidae) *Oidaematophorus beneficus* Yano & Heppner (Lepidoptera: Pterophoridae)
Carduus nutans L.	*Rhinocyllus conicus* Frölich (Coleoptera: Curculionidae)
Carduus thoermeri Weinmann	*Rhinocyllus conicus* (Coleoptera: Curculionidae) *Trichosirocalus horridus* Panzer (Coleoptera: Curculionidae)
Senecio jacobaeae L.	*Longitarsus flavicornis* Stephens; *S. jacobaeae* Waterhouse & *Longitarsus* sp. (Coleoptera: Chrysomelidae)

Table 2.3. Weed species (Asteraceae) partially controlled in at least one area of infestation and the agent/s which achieved this level of control (Julien, 1992).

Weed species	Agent species
Ageratina adenophora (Sprengel) R. King & H. Robinson	*Procecidochares utilis* Stone (Diptera: Tephritidae)
Ambrosa artemisifolia L.	*Zygogramma suturalis* Fabricius (Coleoptera: Chrysomelidae)
Baccharris halimifolia L.	*Trirhabda baccharidis* Weber (Coleoptera: Chrysomelidae) *Megacyllene mellyi* Chevrolat (Coleoptera: Chrysomelidae), *Oidaematophorus balanotes* Meyrick (Lepidoptera: Pterophoridae)
Carduus acanthoides L.	*Rhinocyllus conicus* (Coleoptera: Curculionidae) *Trichosirocalus horridus* (Coleoptera: Curculionidae)
Carduus pycnocephalus L.	*Rhinocyllus conicus* (Coleoptera: Curculionidae)
Carduus tenuiflorus Curtis	*Puccinia carduorum* Jacky (fungus: Uredinales) *Rhinocyllus conicus* (Coleoptera: Curculionidae)
Centaurea maculosa Lam.	*Urophora affinis* Frauenfield (Diptera: Tephritidae) *Urophora quadrifasciata* Meigen (Diptera: Tephritidae)
Chondrilla juncea L.	*Puccinia chondrillina* Bubak & Sydenham (fungus:Uredinales) *Eiophyes chondrillae* Canestrini (Acarina: Eriophyidae)
Chromolaena odorata L.	*Pareuchaetes pseudoinsulata* Rego & Barros (Lepidoptera: Arctiidae)
Parthenium hystophorus L.	*Buccaltrix parthenica* Bradley (Lepidoptera: Lyonetidae) *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae)
Senecio jacobaeae L.	*Tyria jacobaeae* L. (Lepidoptera: Arctiidae)
Xanthium strumarium L.	*Epiblema strenuana* Walker (Lepidoptera: Tortrichidae)

Table 2.4. Taxa of exotic invertebrates and fungi used in the biocontrol of weeds of the Asteraceae. Number of species released within each order/family and their effect on weed populations is indicated. Families with agents which have achieved good control are highlighted in bold.

Taxon	Order/Family	No effect	Partial control	Good control
Coleoptera	Anthribidae	1		
	Cerambycidae	3		
	Chrysomelidae	5	4	3
	Curculionidae	7	2*	
	Buprestidae	1		
	Apionidae	1		
Diptera	Agromyzidae	2		
	Anthomyiidae	2		
	Cecidomyiidae	3		
	Syrphidae	1		
	Tephritidae	13	3	1
Lepidoptera	Arctiidae		2	
	Cochylidae	3		
	Gelechiidae	2		
	Geometridae	2		
	Lyonetiidae	1	1	
	Noctuidae	1		
	Pterolonchidae	1		
	Pyralidae	1	1	
	Pterophoridae		1	1
	Tortrichidae	1	1	
Fungi	Hyphomycetes	1		
	Ustilaginales			1
	Uredinales	1	1	
Hemiptera	Delphacidae	1		
Acarina	Eriophyidae		1	
Nematoda	Tylenchidae	1		
Thysanoptera	Phlaoethripidae	1		

* The same two species have achieved partial or good control of a weed in at least one locality.

released belong to the order Coleoptera with high success rates occurring in the Chrysomelidae and Curculionidae. Agents from these two families have achieved a measure of control against nine weed species (Tables 2.2 & 2.3). Amongst the dipterans, only the Tephritidae have been partly successful. The tephritid flies are favoured for their host-specificity and are frequently used as agents for control of weeds of the Asteraceae. However, their success as control agents is debatable. Only four species (23 %) have achieved a measure of success against their targets. Within the Lepidoptera several have lead to some degree of control of their host, no particular family has been favoured. Out of the less frequently used groups (fungi, Hemiptera, Thysanoptera, Acarina and Nematoda), the fungi represent the most promising agents.

The following section will focus on each of the more successful groups, reference to previous effective biological control programmes is made.

Beetles

In support of the results obtained in this review, Crawley (1989) found that the most successful weed biocontrol agents were beetles; particularly, those belonging to the families Curculionidae and Chrysomelidae. This is contrary to expectation as, generally, the endophagous herbivores, like the dipterans, would be the agents most likely to be successful; especially for the Asteraceae, where many tephritid flies are specific to genera within this group (Freidberg, 1984; Crawley, 1989; Harris, 1989). The biological attributes responsible for the success of the coleopterans as agents are not fully understood. Factors associated with high establishment probability may be involved, this includes good host seeking mechanisms, high rates of population increase relative to the host, long lived adults and high voltinism (polymorphism in invertebrates where some individuals enter diapause and some do not). Crawley's (1989) data suggested that size is an important factor for establishment. According to Crawley (1989) smaller insects are more likely to become established following introduction as biocontrol agents.

The Curculionidae are recognised as "premier biocontrol agents" (O' Brien, 1995; Syrett *et al.*, 1996). The most frequently released and successful biocontrol agent for the Asteraceae is *Rhinocyllus conicus* Frölich (Coleoptera: Curculionidae) (Table 2.5) (Julien, 1992). This thistle-head weevil has been released against 10 weed species in eight countries (Argentina; Canada; New Zealand; USA; Australia; New Zealand and South Africa) on at least 21 different occasions

Table 2.5. A list of the six most successful control agents (species which have achieved good to complete control on at least one weed in one area of infestation). The number of times these control agents have been released and the weed species they have controlled is indicated (Julien, 1992).

Species	No. of major releases	Weed species
Rhynocyllus conicus (Coleoptera: Curculionidae)	21	*Carduus acanthoides*; C. nutans**; C. pycnocephalus *; C. tenuiflorus*; C. thoermeri**; Cirsium arvense; C. vulgare; Silybum marianum; Ageratina riparia*
Trichosirocalus horridus (Coleoptera: Curculionidae)	10	*Carduus acanthoides*; C. nutans, C. pycnocephalus; C. thoermeri**; Cirsium palustre; Cirsium vulgare*
Longitarsus jacobaeae (Coleoptera: Chrysomelidae)	2	*S. jacobaeae***
Procecidochares alani (Diptera: Tephritidae)	2	*Ageratina riparia***
Oidaematophorus beneficus (Lepidoptera: Pterophoridae)	1	*Ageratina riparia***
Entyloma ageratinae (Fungus: Ustilaginales)	2	*Ageratina riparia***

* Indicates partial control attained in at least one area of weed infestation.
** Indicates good/complete control attained in at least one area of weed infestation.

(Julien, 1992). The weevil has achieved some measure of control on five species of thistles belonging to the genus *Carduus*. The adults feed on stems, leaves and bracts of flower heads and also oviposit on these bracts. Most control resides with the larvae which feed within the receptacles inhibiting seed production (Surles and Kok, 1978; Dunn and Campobasso, 1993). *Carduus nutans* L., *Carduus pycnocephalus* L., *Carduus tenuiflorus* Curtis and *Carduus thoermeri* Weinmann are all annual or biennial herbs propagated principally by wind dispersed seeds. Destruction of the thistle heads by the larvae thus reduces seed output and hence achieves a measure of control (Holm *et al.*, 1997). *Trichosirocalus horridus* Panzer (Coleoptera: Curculionidae), a curculionod, has also achieved a measure of control on several thistle species (Table 2.5). The larvae of this beetle damage thistle rosettes by feeding on meristem tissue. In some instances the most effective control has been achieved when both agents have been targeted against the same weed (thistle species) (Dunn and Campobasso, 1993).

More species of Chrysomelidae have been used as weed biocontrol agents than any other family of insects (Syrett *et al.*, 1996). All species are phytophagous, they are generally host-specific and often cause complete defoliation of their host. Furthermore, they are usually easy to rear, fecund, disperse well and reach high densities in field populations (Syrett *et al.*, 1996). *Longitarsus jacobaeae* Waterhouse, a chrysomelid, has achieved successful control of *Senecio jacobaeae* L., a serious weed, in a number of locations (Table 2.5) (McEnvoy, 1984; James *et al.*, 1992; Julien, 1992; McEnvoy *et al.*, 1993). This plant is a biennial or short-lived perennial reproducing principally by wind dispersed seeds but is also able to reproduce vegetatively by root and crown buds (James *et al.*, 1992; McEnvoy *et al.*, 1993). The first agent to be released against *S. jacobaeae* (tansy ragwort) was *Tyria jacobaeae* L. (Lepidoptera: Arctiidae). The larvae of this moth feed on the leaves of the ragwort, and although they may completely defoliate the plants, their activity has been shown to increase plant density (Dempster, 1971). Later the ragwort flea beetle, *Longitarsus jacobaeae* (Coleoptera: Chrysomelidae), was introduced. It is by far a superior control agent. The adults of the flea beetles feed on leaves. Larvae damage the plant by boring into the roots and petioles where they feed throughout the winter (James *et al.*, 1992). Experiments have shown that the two insects together have a greater impact on the plant than either insect acting alone (James *et al.*, 1992). However, high mortality of young plants caused by the boring larvae of the flea beetle indicate that the beetle alone can be an effective agent (James *et al.*, 1992; McEnvoy *et al.*, 1993). The ragwort flea beetle is said to epitomise the

"search and destroy" strategy, in which the agent is monophagous on the host and highly capable of finding and destroying it.

Flies

The family from which the most species have been released, for the control of the Asteraceae, is the Tephritidae (Table 2.4). These flies have been only partly successful control agents (Syrett *et al.*, 1996). Seventeen species of tephritids are listed in Julien's (1992) catalogue of weed biocontrol agents for the Asteraceae. Only three out of the 17 species have partially controlled their target weed and only one species has inflicted substantial damage to its host (Tables 2.3, 2.4 & 2.5). The tephritids have been selected as biocontrol agents largely for their host- specificity. A tephritid genus is usually restricted to a specific group of plants, usually at the level of tribe or below (Edwards *et al.*, 1996).

Procecidochares alani Steyskal (Diptera: Tephritidae) and *Procecidochares utilis* Stone (Diptera: Tephritidae), both stem gallers, are credited with the control of *Ageratina riparia* (syn: *Eupatorium riparium*) and *Ageratina adenophora* (Sprengel) R. King and H. Robinson (syn: *Eupatorium adenophorum)* respectively and this represents the best result to date (Tables 2.2, 2.3 & 2.5) (Bess and Haramoto, 1959; Dodd, 1961; Harris, 1989; Julien,1992; Morin *et al.*, 1997). Both of these plants are serious economic weeds in many countries. *Ageratina adenophora* is a perennial which depends on both sexual and vegetative reproduction for population increase. *Procecidochares utilis* eggs are deposited between the young paired leaves before they unfold, where they hatch and feed, producing a gall. Control is manifested in shortened stems and reduced foliage production (Bess and Haramoto, 1959). During a dry season fewer young leaves are produced by the weed and hence there are fewer ovipositional sites. Continued heavy attack by the fly causes the growing tips to die, reducing population spread.

Several tephritid species which form flower head galls have also been employed for biological control of the Asteraceae (Freidberg, 1984; Harris, 1989; Julien, 1992). Seven species of *Urophora*, a flower-head galler, have been released against thistles and knapweeds (Julien, 1992). As most of these weeds are herbaceous annuals, biennials or short lived perennials propagated mainly by wind dispersed seeds, destruction of the seed head has a bearing on the reproductive capacity of these plants. The flower-head gall acts as a powerfull metabolic sink and therefore

accounts for much energy loss from the capitulae of the host (Harris, 1989). Although many species of *Urophora* have established on their respective hosts, *Urophora affinis* Frauenfeld and *Urophora quadrifasciata* Meigen are the only two species to have partly controlled their target weed, *Centaurea maculosa* Lam. (Table 2.3) (Julien, 1992).

The high rate of failure of many tephritid species to achieve control of their hosts is partly attributed to parasitism of tephritids in the galls (Edwards *et al.*, 1996). Parasitism of tephritid galls by parasitic wasps is not uncommon and may effectively reduce agent population numbers and hence their control potential (Crawley, 1989). Unsuitability of the target weed as a host may also account for establishment failure, *i.e.* the chosen biocontrol agent cannot breed successfully on the target weed (Crawley, 1989; Harris, 1989). Furthermore, an agent can only be successful if it targets the life strategy of the plant. A tephritid fly which reduces seed production will have no effect on the plant population if reproduction is not limited by seed.

Moths

Out of the 19 species of Lepidoptera released onto the Asteraceae, only one has achieved good control of its host (Table 2.4) (Julien, 1992). *Oidaematophorus beneficus* Yano and Heppner (Lepidoptera: Pterophoridae), a plume moth, has achieved good control of *A. riparia*, commonly known as the mist flower, in Hawaii (Morin *et al.*, 1997). On hatching, the larvae of *O. beneficus* feed *in situ* on the underside of the leaves. Later, they move to the new leaves at the terminals of shoots where they consume all parts of the young leaves. In Hawaii, the plume moth established rapidly on *A. riparia*. It is most active in August which coincides with the period of maximum growth and can completely defoliate the plants (Morin *et al.*, 1997). However, at low altitudes and in the warmer regions, egg parasitism by braconid wasps reduce the impact of this species (Yano and Heppner, 1983; Morin *et al.*, 1997).

Pareuchaetes pseudoinsulata Rego Barros (Lepidoptera: Arctiidae) has partly controlled *C. odorata* in Ghana, Guam and parts of Asia (Julien, 1992; Timbilla and Braimha, 1996). The damage caused to the terminal and axillary buds of *C. odorata* by the larvae of *P. pseudoinsulata* reduces seed set and consequently has the potential to reduce populations of this weed. Various degrees of control of *C. odorata* have been achieved using this agent (Julien, 1992). Timbilla and Braimha (1996) are of the opinion that effective control of this weed in most locations will require more than just defoliation by *P. pseudoinsulata*.

Crawley (1989), found that the Lepidoptera rated highly amongst the "poor establishers" for weed biocontrol agents and attributed this to high levels of predation. Despite the above examples, the Lepidoptera are not outstanding as control agents for weeds of the Asteraceae (Table 2.4). Crawley (1989) suggested that as the Lepidoptera are generally external feeders, their eggs and larvae are extremely vulnerable to generalist predators and, coupled with this, they are especially prone to viral and fungal diseases (Entwistle 1983; Crawley, 1989). No doubt these factors reduce the probability that an introduced agent will establish.

Pathogens

The use of plant pathogens as biological control agents has increased dramatically since the 1970s (Freeman and Charudattan, 1984; Julien, 1989). Such research holds much potential as fungal pathogens generally have host ranges limited to one or a few species and are therefore ideal biocontrol agents (Watson, 1984). Two of the four species listed as control agents in Julien's (1992) catalogue have achieved a measure of success against their target (Table 2.4). *Entyloma ageratinae* Barreto and Evans (syn. *Cercosporella ageritinae, Entyloma compositarum*) causes leaf blight on *A. riparia* and has achieved striking control of this weed in Hawaii (Trujillo, 1984; Julien, 1992; Morin *et al.*, 1997). In 1975, inoculation of the plants with the fungus caused population decreases of up 80 %, moreover, at several sites this was achieved in just nine months (Trujillo, 1984). The rust fungus *Puccinia chondrillina* Bubak and Sydenham kills the leaves and stems of *Chondrilla juncea* and subsequently reduces flowering. The specificity of this pathogen is so strict that certain strains are virulent against only one form of *C. juncea.*

2.4   Summary and conclusions

Two important points emerge form this review: firstly, regardless of type, a good agent is one which targets the life strategy of the host plant; secondly, good control of a weed is often achieved when multiple species introductions are made. Even if an agent is host-specific and has the ability to defoliate its host, if plant reproduction (vegetative or sexual) is not limited by herbivory then control is not initiated. Successful agents are also those not prone to attack by parasitoids in the new environment. Tephritids are potentially ideal candidates for biocontrol of the Asteraceae - they are host-specific (largely on the Asteraceae) and also endophagous herbivores. However, parasitisation of the flies by native parasitoids of the new environment often limits their establishment on their host plant. This analysis has also shown that trends in the

biological control of the Asteraceae echo that of weed biocontrol in general (Crawley, 1989).
Coleopterans are usually the most successful agents, and this is attributed to their host-specificity,
ease of dispersal and powers of consumption (Crawley, 1989).

Although this analysis is useful in highlighting groups which may contain potential control agents
for *D. odorata*, it would be a mistake to read too much into the results. Reasons for failure of a
biocontrol project are often many, complex and interacting; for example, genetic variation of the
plant population often means only a fraction of the weed is susceptible to control by an
introduced agent. Thus potential agents should not be rejected merely because they have a poor
"track record". Rather the results of this review should be used to highlight the weaknesses of
a potential agent, thorough investigation into these aspects prior to release may help to determine
whether successful establishment and control are feasable.

Julien (1989) highlighted the use of pathogens as control agents. As of 1989, 66.6 % of the
pathogens released had achieved control of their target weeds. Use of pathogens has generally
been restricted due to concerns about their stability and specificity (Julien, 1989). However, these
problems are being overcome by the development of guidelines for testing (Freeman and
Charudatten, 1984, Julien, 1989). Perhaps the best option for control of *D. odorata* is by the
introduction of a host-specific microorganism or the development of a bioherbicide.

Chapter 3

Floral biology, breeding system and pollination

3.1   Introduction

The search for correlations between colonising ability and breeding system has been a recurring theme in invasion biology (Baker, 1955; Baker, 1965; Price and Jain, 1981; Carr and Powell, 1986; Abbot and Forbes, 1993; Rejmánek and Richardson, 1996; Williamson and Fitter, 1996). It was little more than 40 years ago that Baker (1955) pointed out the correlation between the breeding system of an organism and its ability to establish sexual populations after a long distance dispersal event. Baker (1965) suggested that for the "ideal weed" the most suitable breeding system for colonising new areas is "self compatible", but not completely autogamous or apomictic. Self-compatibility allows seed set at low population density in the absence of suitable pollinators. However, obligate self-pollination can lead to inbreeding and associated lack of variation and vigour in the population. For many years it was generally assumed that only self-compatible species have weedy potential and although there is evidence in support of this, more recent studies have shown that the type of breeding system may not always be an important factor in determining invasiveness (Brown and Marshall, 1980; Price and Jain, 1981; Carr and Powell, 1986; Noble, 1989; Perrins et al., 1992; Cronk and Fuller, 1995; Mack, 1996; Rejmánek and Richardson, 1996; Williamson and Fitter, 1996).

Although the breeding system of a plant may not be a determinant of its invasive potential, it is an important factor to consider in the development of a biological control programme. This is because the genetic structure of populations of a weed influence the ability of introduced pests or pathogens to substantially reduce host stand density. In their analysis on biological control and the reproductive mode of weeds, Burdon and Marshall (1981) found a preponderance of apomictic species selected as targets in biological control programmes. Furthermore, the degree of success achieved in weed control was significantly affected by the reproductive mode of the target species - those reproducing asexually being more successful. The greater efficacy of biological control agents against asexual weeds arises because of their tendency towards genetic homogeneity, thus one biotype of biocontrol agent can potentially target all populations of the pest plant (Burdon and Marshall, 1981).

Plant populations which arise from small inputs, as is often the case with colonising species, almost invariably show considerably less genetic variation than those found within naturally occurring populations of the same species (Harper, 1977; Burdon and Marshall, 1981; Cronk and Fuller, 1995). For agamous and clonally reproducing plants the very limited nature of the original introduction may result in weed populations which are genetically uniform. In sexually reproducing species, although the establishment process clearly reduces the variability of the population, recombination of genes from different parents results in stands containing a range of different genotypes (Burdon and Marshall, 1981). A lack of quantitative knowledge on the population genetics of a weedy species precludes any attempt to pursue the question of its genetic diversity and thus potential susceptibility to a biocontrol agent. However, a knowledge of the breeding system of a plant species may provide some indication on its population structure. As a first approximation, it seems reasonable to assume that apomictic species will be far less variable on average than species which undergo sexual recombination every generation.

The present study

The investigation on the breeding system of a weed is not only important in terms of contributing towards an understanding of its population structure but also vital to the choice of effective biocontrol agents. As no literature has been published on the floral biology, breeding system and pollination ecology of *D. odorata* no predictions regarding the genetic structure of populations and hence the suitability of *D. odorata* to biocontrol can be made. This study attempts to address some of these aspects for *D. odorata* in southern Africa. The specific question to be addressed by this study pertains to the breeding system of *D. odorata*: Is *D. odorata* self-compatible or an obligate outbreeder? To complement the study on the breeding system, aspects of the floral biology and pollinator ecology of *D. odorata* were investigated.

Experiments were also carried out to determine whether seed set in *D. odorata* is pollen limited. In addressing the question of pollen limitation it is important to understand the breeding system of the plant. If plants are capable of producing seeds through autogamy or self-pollination, a reduction in pollen receipt may not lead to a decline in the number of seeds produced, although average seed quality may change considerably (Vaughton, 1988). Alternatively, self-incompatible species require foreign pollen for seed set.

Delairea odorata expands vegetatively as a vine through the spread of stolons and frequently sends out runners which have the potential to root at each node, each developing into a new plant. With this clonal growth habit there is the potential for stands of *D. odorata* to consist of only one or a few genotypes (Chipping, 1993). Such clonal growth is widespread among plants and clones have the potential to reach enormous sizes eg. *Eichhornia crassipes* (Martius) Solms-Laubach (Widen and Widen, 1990; Cronk and Fuller, 1995). It follows that in clonal plants as the size of the clone increases flowers will become increasingly surrounded by flowers of the same individual (Handel, 1985; Eriksson and Bremer, 1993). Observations have shown that most movements by pollinators in natural populations are between very near neighbours and therefore with increased clone size, geitonogamous pollination (pollination between different flowers of the same clone) increases (Widen and Widen, 1990). Furthermore, restricted gene flow favours the development of local structure in which neighbours on average are more similar genetically than are individuals separated by larger distances (Levin and Kerster, 1974; Arroyo, 1976; Hessing, 1988; Waser and Price, 1989). A negative correlation between fecundity per flower and plant size, attributed to insufficient out-pollination, has been reported for self-incompatible species (Carpenter, 1976; Andersson, 1988; Eriksson and Bremer, 1993). Insufficient pollination could therefore be a problem in self-incompatible species with extensive clonal growth. Furthermore, self-incompatible species are often insect pollinated and restrictions in pollen dispersal could therefore play a part in determining their fecundity, since they must outcross (Widen and Widen, 1990). This investigation aimed to determine the natural seed set of *D. odorata* in its native environment and to compare this with seed-set of hand-pollinated flowers.

It is important to note that pollen limitation of the number of seeds or fruits may occur in a number of other ways; and these must be considered in any study on pollen limitation (Goldingay and Whelan, 1990). Firstly, there may be low numbers of pollinators (Whelan and Goldingay, 1986). Secondly, there may be a suitable number of pollinators ordinarily but, due to profuse flowering at the time of the study, the pollinators become satiated (Copland and Whelan, 1989). Thirdly, pollinator visits may result in ineffective pollen transfer (Whelan and Goldingay, 1989).

3.2　Materials and methods

Study sites

To detect possible differences in the breeding system of this species, populations from three locations across KwaZulu-Natal (Harding, Dargle and Pietermaritzburg) were included in the study. Table 3.1 lists the specific location, altitude and coordinates of each study site. All three study sites fall into the mist-belt region characterised by cool, tall forests usually dominated by *Podocarpus* species (Pooley, 1993).

Table 3.1. Location and altitude of populations of *D. odorata* used in this study.

Locality	Site	Altitude (m)	Co-ordinates
Harding	Ingele Forest	1300	30°32'S, 29°43'E
Dargle	Maritzdaal	1200	29°32'S, 30°01'E
Pietermaritzburg	Ferncliffe Nature Reserve	800	29°37'S, 30°20'E

Over 70 % of the annual precipitation at the three study sites is concentrated between November and March, with a dry season from May to August. Rainfall is highest during the summer (November to February) when the average monthly temperature ranges between 19 °C and 22°C. Mean annual rainfall for all sites is above 800 mm, with a maximum of 1031 mm recorded for Ingele Forest. Dargle is the coldest location, average minimum temperatures range from 0-5 °C and frosts are frequent during June and July. Average minimum temperatures for Ingele and Ferncliffe are above 5 °C and frosts are less frequent (CCWR, 1999).

Floral biology

Observations on the development of flowers of *D. odorata* were made both in the field and in the laboratory. Flowering phenology, at the population level, was monitored at each study site. At the start of the breeding season 50 immature inflorescences were tagged and the number of capitula in each recorded. For the rest of the season, weekly records on the development of each capitulum in each synflorescence was made. For this study, four stages in the development of the capitulum were recognised: (1) young bud (all florets closed) (2) opening (some florets opening) (3) full bloom (all florets open), and (4) withering/ fruit set. Each week, for each tagged inflorescence, the number of capitula in each of these stages was recorded. Flowers damaged by herbivory or fungi were recorded in a separate category.

Experimental pollinations

All experimental pollination was done by hand in the field. Each capitulum used in experimental pollinations was protected from contamination by a fine (0.5 mm) nylon-mesh pollination bag. As the inflorescence is determinate with the central or apical capitulum maturing first, bags were frequently visited to ensure pollination occurred when the stigmas were receptive; judged by the spreading of the stigmatic lobes.

The breeding system was determined by a series of five treatments of the capitula:

1. *Control.* The capitula were tagged but otherwise unmanipulated to test for natural seed set under field conditions.

2. *Cross-pollination.* Immature capitula were bagged, as the stigmas became receptive they were pollinated with pollen from flowers collected at least 10 m away, and rebagged until fruit set. Pollen from each donor was applied by rubbing a whole capitulum of the donor plant onto the recipient capitulum, a technique similar to that used by Carr and Powell (1986) and Sobrevila (1989). As floret maturation within the capitulum is acropetal cross-pollination was carried out several times to maximise fruit set.

3. *Self-pollination.* Immature capitula were bagged, as the stigmas became receptive they were brushed with pollen from the same head and rebagged until fruit set.

4. *Apomixis.* Immature capitula were bagged, as the stigmas became receptive they were removed with a sharp razor-blade, and the flowers rebagged until fruit set (emasculation was not possible as the florets are very small).

5. *Pollinator exclusion.* The capitula were not bagged or manipulated in any manner. Plants were kept in a greenhouse out of contact with pollinators. Fruit set was determined at the end of the season.

Fruit set was determined in the laboratory with the aid of a dissecting microscope. Fertile fruits are plump and easily recognised, a pair of fine forceps was used to squeeze these achenes to confirm the presence of an embryo. Developed seeds filled the achene, while seedless achenes were characterised by the shriveled remains of the ovary.

Pollinators

Foragers on the inflorescences of *D. odorata* were netted and killed in jars charged with ethyl acetate. Fieldwork to collect pollinators was carried out at only two of the study sites, Dargle and Ferncliffe (1998 and 1999) due to logistical constraints (Harding is over 200 km away from Natal University). All pollinators were collected between 9:00 am and 4:00 pm. Following collection, the specimens were pinned, examined for the presence of pollen and kept in insect boxes for subsequent identification. To verify the presence of *D. odorata* pollen, a small piece of fuchsin jelly was rubbed over the body of each pollinator (to remove any pollen grains), placed on clean glass slide, heated and covered with a coverslip. This makes a permanent slide which can be used for qualitative and quantitative observations of pollen loads.

Pollen limitation

To test for pollen limitation of seed set in *D. odorata*, hand pollinated infructescences were compared to those which received open (natural) pollination. Zimmerman and Pyke (1988) outlined protocols for pollen limitation experiments. The tests used in this study are based on the methods they suggested but were adapted to suit the conditions of this study. Hand pollinations (HP) were performed as described in the previous section (Treatment 2: cross-pollination). Two controls were used in the investigation. On ramets with capitula which received artificial (hand) pollination (*i.e.* experimental plants) there were control capitula (EC). These were open pollinated capitula, adjacent to the hand-pollinated ones, tagged at the start of the flowering season and left unmanipulated. According to Zimmerman and Pyke (1988), if these capitula have the same seed set as those receiving an extra pollen load then seed set may be limited by resources other than pollen. However, if capitula receiving additional pollen show a higher seed number than control capitula the situation is ambiguous. Enhanced seed set in some capitula may occur at the expense of reduced seed set in other capitula on the same plant and the observed pattern may simply be the result of allocation of resources to particular flowers. It is for this reason that the second control was necessary. These were open pollinated flowers on ramets which did not have any hand pollinated flowers (i.e. control plants) (CC). If control capitula on both control and experimental plants show the same achene number then capitula receiving extra pollen have set more seeds at no expense to the other blossoms open at the same time.

Tests for pollen limitation of seed set were carried out on populations of *D. odorata*, at

Maritzdaal (Dargle) and Ferncliffe Nature Reserve, during 1999. At the end of the season fruit set for each treatment was determined by counting the number of fruits set per capitulum.

Data analysis

Kruskal Wallis ANOVA was used to test the effect of the pollination treatments on seed set. The statistical package used was Minitab© (1995).

3.3   Results

Floral Biology

Capitula of *D. odorata* are discoid, bisexual and protandrous and posses a standard number of 10 florets per capitulum. The florets are tubulate, posses a five-lobed corolla and have a uniloculate inferior ovary. The synflorescence is determinate with the central capitula maturing first as opposed to the sequence of development of florets within the capitulum where development is acropetal, the marginal florets developing first.

Floral development is very similar to its nearest ally *Senecio* L. (Lawrence, 1985) and typical of the Asteraceae. In the family the five anthers are syngenecious forming a collar around the style (Fig. 3.1). The anthers dehisce introrsely while the florets are still closed but self pollination at this stage is avoided as the receptive stigmatic surfaces are closely adpressed (Fig. 3.2). At this stage the style apex is positioned at the base of the anthers. The growing style then passes through the anther collar and brushes the pollen out of the anther cylinder by means of hairs on its outer surface (Fig. 3.3). The pollen mass extruded in this manner can then be removed by pollinators. Thus pollination can only occur after the style branches have extended beyond the collar of anthers, at which point the branches spread apart and expose the stigmatic surface (Fig. 3.4).

Flowering phenology was monitored at Ferncliffe, Dargle and Ingele (Fig. 3.5 a-c). At all three sites flowering commenced in early April (buds). This coincides with the first drop in temperature and rainfall at all sites. Peak flowering period (full bloom) occurred over the interval of late April to late May. Thereafter most of the flowers started to wither and set seed. By mid-July most of the fruits had been dispersed by wind. Approximately 18 % of flowers at each site were damaged by fungi and/or herbivory (Fig. 3.5 a-c). The breeding season thus covers approximately three months (April, May, June), through the autumn to early winter period.

Fig. 3.1. Intact anther collar, of a *D. odorata* floret, consisting of five syngenesious anthers.

Fig. 3.2. Status of the style when the *D. odorata* floret is still closed. Floret and anther collar have been prised open to show the style apex positioned at the base of the anther collar.
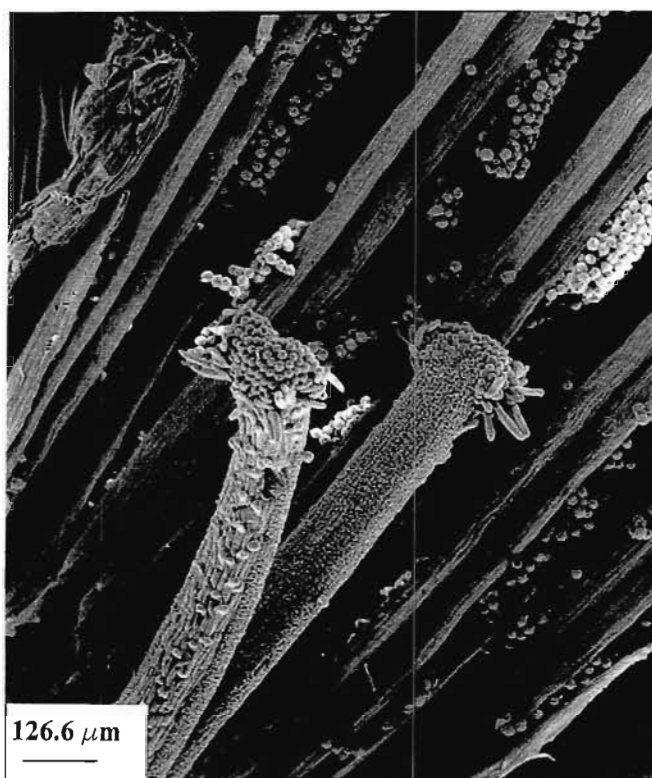
Fig. 3.3. The style pushes through the narrow anther collar of the *D. odorata* florets and removes pollen out of the cylinder by means of hairs on the outer surface.
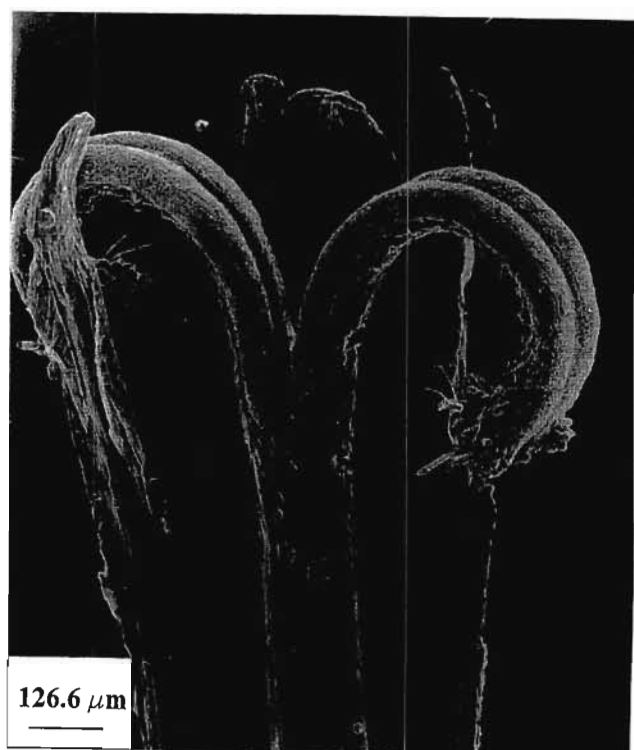
Fig. 3.4. After the style has extended beyond the anther collar, the style arms spread apart and expose the stigmatic surface.
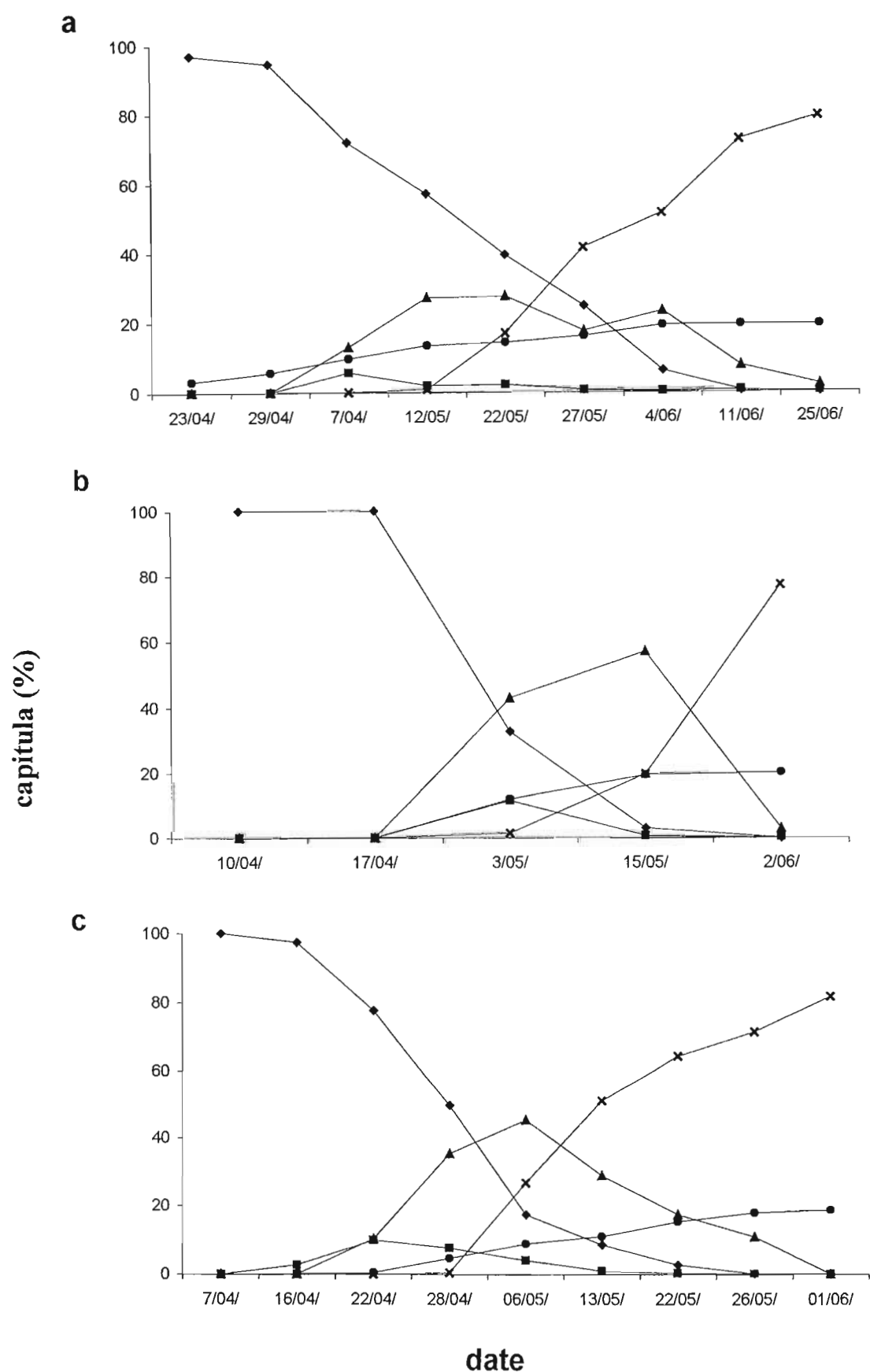
Fig. 3.5 a-c. Phenology of *D. odorata* at (a) Ferncliffe (b) Ingele and (c) Dargle

Experimental pollinations

Using individual capitula as the units of replication, fruit set of self-pollinated capitula was significantly lower than that of cross-pollinated ones at all three study sites, mean number of fruits per capitulum for the self-pollinated capitula was zero (Dargle: $p<0.05$, $H=86.41$; Ingele: $p<0.05$, $H=97.32$; Ferncliffe: $p<0.05$, $H=79.53$) (Fig. 3.6 a & b). At Dargle and Ingele only 0.5 % of the capitula in the self-pollination treatment produced one fruit while no fruit was set at Ferncliffe (Fig. 3.6 b). This contrasts strongly with the cross-pollination treatments where the percentage capitula with fruit ranged from 20-42 % at the three study sites. No fruits were produced by *D. odorata* from either the apomictic or pollinator exclusion trials.

For the cross-pollinations, the percentage capitula which set fruit and the mean number of fruits per capitulum was higher at Ferncliffe than at any other site (Fig. 3.6 a & b). Furthermore, only at Ferncliffe was the mean number of fruits per capitulum greater for cross-pollinations than for the open pollinated control flowers (natural seed set) (Fig. 3.6 a & b). Greater fruit set in the hand pollinated capitula is the expected result since these flower-heads received a greater pollen load. The population of *D. odorata* at Ingele Forest is one of the largest in Natal. High fruit set for the open pollinated (control) capitula at this site may be the product of increased pollinator activity and/or greater within patch genotypic diversity (and therefore more compatible genotypes). Low fruit set for the hand-pollinated capitula at this site could be the result of unsuccessful pollen transfers. Ingele forest is situated at Harding, over 200 km away from the University of Natal, and trips to this site could only be made every 10-14 days. It is possible that fewer visits and therefore hand pollinations at this site meant that artificial pollen transfers did not always take place when stigmas were receptive.

Pollinators

Delairea odorata is pollinated by generalist insects and the pollination system is unspecialised; the insects transfer pollen from many parts of their body as they forage throughout the inflorescences of a patch. The most frequent visitors to the inflorescences were Syrphidae (hover flies) and Calliphoridae *(Stomorhina* sp.*)* (Table 3.2). Although Apidae (bees) were less frequent visitors to the capitula than both the Syrphidae and Calliphoridae, they carried by far the most *D. odorata* pollen on their bodies (Table 3.2). A few wasps and beetles were collected foraging on the flowers, however, no pollen was collected from their bodies and they were generally

a

b
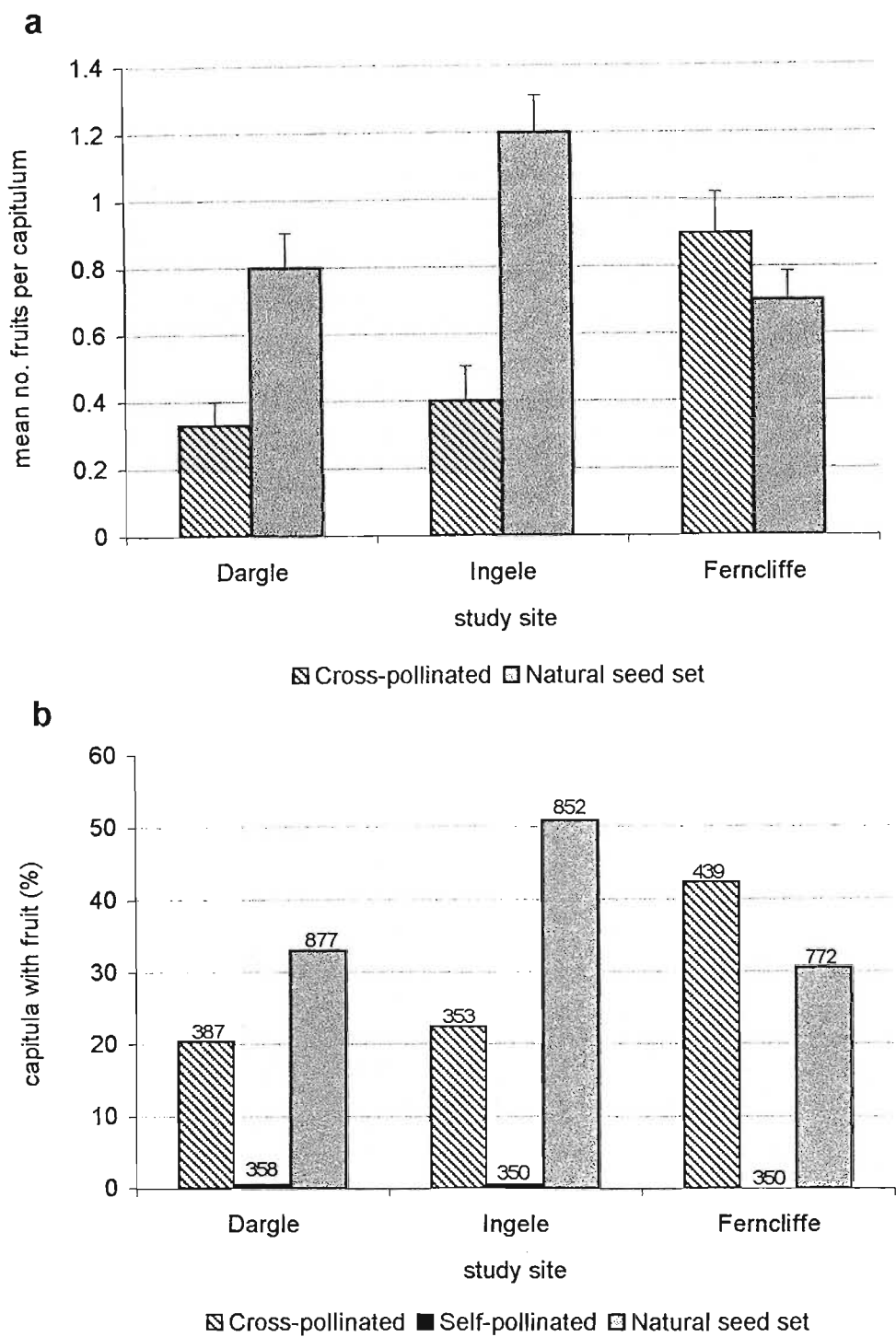
Fig. 3.6 a & b. Results of experimental pollinations on *D. odorata* capitula expressed as (a) the mean number of fruits per capitulum (error bars represent 95 % confidence intervals) and (b) the percentage capitula which set fruit (numbers refer to the total number of capitula used in the test).

Table 3.2. Details of the pollen collected from the major pollinators of *D. odorata*: the number of species of each major group (family), the number of individuals, within each family, with *D. odorata* pollen, and the average number of pollen grains per insect, is indicated (n=number of individuals collected).

Pollinator (n)	number species	*Delairea* pollen only	*Delairea* + other species	Av. number of pollen grains (±SD) per insect	Pollinator frequency
Apidae (n=12)	1	11	1	622 (250)	occasional
Syrphidae (n=33)	9	13	20	115 (100)	frequent
Muscidae (n=4)	4	1	3	20 (15)	occasional
Sarcophagidae (n=10)	1	0	1	15 (13)	occasional
Tachinidae (n=3)	3	2	1	25 (9)	rare
Calliphoridae(n=31)	1	4	27	24 (20)	frequent
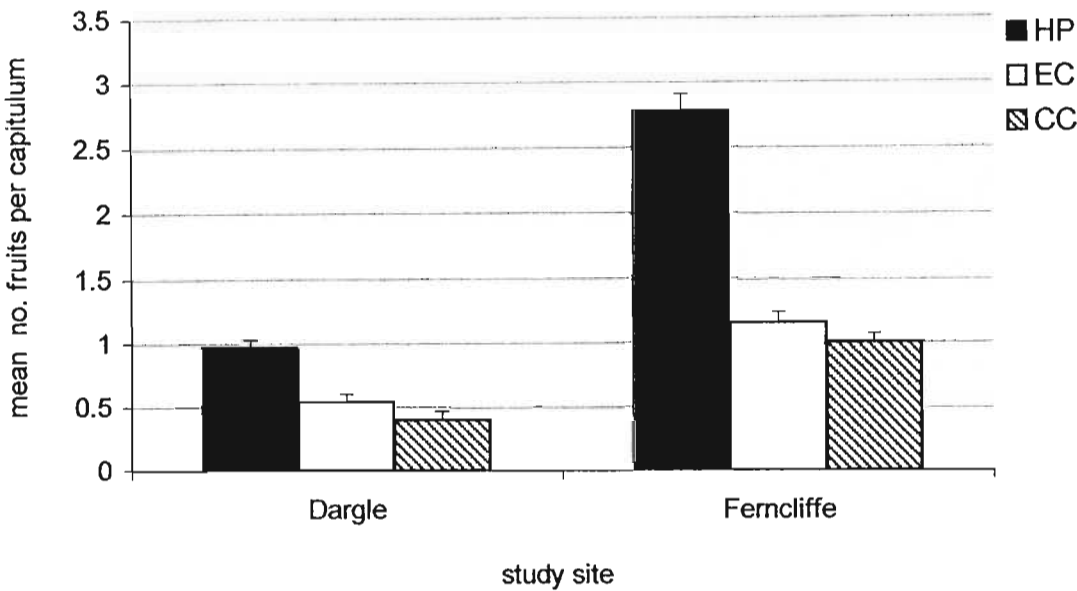
Fig. 3.7. Mean number of fruits set per capitulum for pollen limitation experiments carried out on *D. odorata* capitula. Error bar represents 95 % confidence interval. **HP**: hand-pollinated capitula; **EC**: open pollinated capitula on experimental plants; **CC**: open pollinated capitula on control plants.

infrequent visitors to *D. odorata*. Several species of butterfly (*Hyalites esebria esebria* Hewitson, *Cassionympha cassius* Godart, *Precis octavia sesamus* Cramer, *Vanessa cynthia cardui* L., *Anthene definita definita* Butler and *Belenois zochalia zochalia* Boisduval) were also observed to move from flower to flower within a patch. However, no pollen was found on their bodies and therefore their role as pollinators of *D. odorata* is questionable. The importance of bees and hoverflies in the pollination of the closely related genus *Senecio* has been reported elsewhere (Gross and Werner, 1983; Lawrence, 1985).

Pollen limitation

The results for the three pollination treatments, at each site, were significantly different (Ferncliffe: H=237.75, p<0.05; Dargle: H=63.48, p<0.05). At both study sites, the average number of fruits set per capitulum was higher for those receiving extra pollen than for control flowers on the same ramets (Fig. 3.7). The hand-pollinated flowers (HP) therefore received more pollen and also more of the plant's resources than did their respective controls. For the control flowers on experimental plants (EC) the fruit set per flower was similar to that of the control flowers on control plants (CC). The hand-pollinations therefore increased the amount of resources allocated to seeds at no expense to the other flowers . At Ferncliffe, relative to the controls, hand-pollination increased fruit set by 139 % (EC) and 173 % (CC) whilst fruit set between the two controls differed by only 14%. At Dargle fruit set for hand-pollinated flowers was 81 % greater than that of the experimental control and 145 % greater than the control flowers on control plants. The results of the open crosses can be used as measures of the maximum potential seed set of *D. odorata* at each site.

3.4   Discussion

The results of the experimental pollinations indicate that *D. odorata* is an obligate outbreeder. A self-compatibility index (SI) was calculated for each population by dividing the proportion of seeds set from self-pollinations by those in cross-pollinations. A species is self-incompatible if the index is less than 0.2 (Bawa, 1974; Zapata and Arroyo, 1978; Sobrevila, 1989). Using this measure of incompatibility, all three populations are self incompatible since their index was less than 0.2 (Dargle:0.016; Ingele:0.004; Ferncliffe:0). Furthermore, per capitulum, *D. odorata* has a high pollen ovule ratio of about 3000. High pollen ovule ratios and showy capitula are commonly regarded as indicators of outbreeding (Lawrence, 1985).

Self-incompatibility, the inability of a plant with functional gametes to produce selfed seeds, occurs widely amongst flowering plants and is known from a least 71 families. It is one of the major outbreeding mechanisms in plants, having been recorded from 250 of the c. 600 genera studied. Incompatibility results from the inhibition of pollen tube growth (Brewbaker and Majumder, 1961; Richards, 1986). This inhibition commonly occurs at one of two sites: (1) on the stigma or soon after pollen tube germination and (2) in the pistil during the first few hours of pollen tube growth. Based on the site of reaction two major types of incompatibility systems are recognised in plants (Richards, 1986): plants having the sporophytic system normally show inhibition on the stigma, while plants of the gametophytic-type have pollen tube inhibition in the style or ovary.

Members of the Asteraceae mostly have sporophytic incompatibility systems (Crowe, 1954; Brewbaker and Majumder, 1961; De Nettancourt, 1977; Lawrence, 1985; Richards, 1986; Abbot and Forbes, 1993). Several species of *Senecio* are self-incompatible (for example: *Senecio squalidus* L., *S. laurus* Urv., *S. gregorii* F. Muell and *S. odoratus* Hornem) and are likely to have a sporophytic incompatibility system (Lawrence, 1985; Abbott and Forbes, 1993). Homomorphic sporophytic systems are characterised by a single multi-allelic locus and prezygotic mechanisms (De Nettancourt, 1977). In contrast, most of the complex genetic systems such as multifactorial incompatibilities and post-zygotic mechanisms are associated with gametophytic systems (De Nettancourt, 1977). In sporophytic systems crosses between individuals will result in 0-100 % seed set, depending on whether they share the same incompatibility alleles or not. Such a contrasting pattern expected in species with sporophytic systems is more easily detected than the complex pattern expected in gametophytic systems (Sobrevila, 1989). In addition, because the expected pattern is so well defined, it might be detectable even under field conditions, where environmental factors that affect seed set cannot be easily detected (Sobrevila, 1989). For this study, it was noted that many of the capitula which were hand-pollinated set no seed at all, while for others seed set was frequently above 70 %.

Although the results of this study indicate that *D. odorata* possesses a strong sporophytic self-incompatibility system, it cannot be ruled out that under certain field conditions the effectiveness of this system will be weakened resulting in an increase in the level of self-compatibility. Breakdown of sporophytic incompatibility mechanisms can occur (Richards, 1986). Research has

shown that delayed pollination, high temperatures, high CO_2 concentrations and electrical stimuli can break incompatibility mechanisms (Richards, 1986). Roggen and van Dijk (1972) found that stigmatic abrasion with a wire brush also affected compatibility. They suggested that damage to the cuticle of the stigmatic papillae promoted penetration of selfed pollen tubes. This may account for the few seeds set in the self pollination trials of this study. Alternatively, as it is not known whether the pollination bags used completely excluded pollen, the few fruits produced by *D. odorata* from self pollination treatments may be due to inadvertent cross-pollinations or interference by small crawling insects e.g. thrips.

Delairea odorata does not conform to Baker's (1965) "ideal weed" model because it is self-incompatible. However, it is important to note that Baker (1965) based most of his analyses on agricultural weeds and while many of his "ideal weed" characters hold true for these plants they do always hold for invasive plants of natural habitats (Rejmánek, 1989; Cronk and Fuller, 1995; Mack, 1996). For example, of the environmental weeds listed by Cronk and Fuller (1995), 13% are dioecious and 11% monoecious (and the rest hermaphrodite). A similar level of dioecy is found in the native flora of New Zealand (12-13%) which is a flora considered to have a high level of dioecy (Cronk and Fuller, 1995). A rather high percentage of invasive plants thus appear to be dioecious or monoecious - two mechanisms which promote cross-pollination and therefore outbreeding. Williamson and Fitter (1996) compared attributes of the native and invasive species of the British flora to determine if any sets of characters were common to invading species. They found that most characters, particularly the "ideal weed" characters, failed to relate to invasion success.

For the pollen-limitation trials, the flowers that were given additional cross-pollen were able to produce nearly twice as many achenes per capitulum than those which were open pollinated (both EC and CC). These results indicate that seed set in *D. odorata* may be pollen limited. As previously stated, the occurrence of pollen limitation may occur in a number of ways: (1) low numbers of pollinators (2) profuse flowering so that the pollinators become satiated (3) ineffective pollen transfer, and (4) restricted gene-flow which may have severe effects on seed fitness, particularly in self-incompatible clonal species, where a single clone may occupy a large patch (Goldingay and Whelan , 1990; Widen and Widen, 1990).

Pollen limitation of seed set in natural populations has been documented in several studies (for a review see Rathcke, 1983). Widen and Widen (1990) found that distance-dependent fecundity could have severe effects on seed fitness in clonal species occurring in large patches. In the present situation pollen limitation was most likely due to low frequency of visits by pollinators and/or high density of ramets of a single clone occupying a patch. However, seed-set for the open pollinated controls, particularly at Ferncliffe, does show that patches are likely to comprise more than one genet since, despite the expected movements of pollinators between very near neighbours, some seed was set. Thus low seed set may indicate a general lack of pollinators. An isozyme analysis or genetic 'fingerprinting' would need to be carried out to confirm the genetic diversity of patches.

Chapter 4

Seed biology

4.1 Introduction

Seed biology of invasive species

Seeds are the product of sexual reproduction, and are fertilised mature ovules comprising embryos and nutritive tissue, either as endosperm or food stored in the cotyledons (Abercrombie *et al.*, 1990). Survival of most species, particularly annuals where reproduction is only by seed, depends on the production of a sufficient number of sexually produced propagules (Zimdahl, 1993). The progeny of species that reproduce from sexually produced seed may be different from their parent plants and therefore these species have the potential to extend their geographical range of distribution. Many invasive species reach reproductive maturity relatively early and produce copious amounts of seed (Zimdahl, 1993; Cronk and Fuller, 1995; Holm *et al.*, 1997). Baker (1965) associated rapid sexual maturation, high fecundity, seed vagility, unspecialised germination requirements or dormancy and seed longevity with weedy potential. Other studies have supported the "ideal weed" characters listed by Baker (1965) (Amor and Piggin, 1977; Klingman *et al.*, 1982; Stephens, 1982; Dean *et al.*, 1986; Zimdahl, 1993) and, although most of these studies were based on weeds of agricultural environments, similar reproductive traits have been found to be common to the invasive species of natural habitats. Amor and Piggin (1977) studied factors influencing the establishment and success of exotic plants in Australia and concluded that characteristics common to invasive species included rapid seed production after anthesis, high seed production under a wide range of conditions and dormancy mechanisms preventing germination under unfavourable conditions.

The Asteraceae is one of the most important weedy families worldwide (Heywood *et al.*, 1977; Heywood, 1989; Cronk and Fuller, 1995). The success of this group has been partly attributed to a high reproductive rate, the development of which is largely associated with the evolution of the capitulum (Heywood *et al.*, 1977). The aggregation of reduced flowers into a single head is sometimes associated with geitonogamy (often superimposed with agamospermy), pollinator attraction and the production of single-seeded fruits (achenes) with effective dispersal mechanisms. The one-seeded fruit often has a pappus (ring of fine hairs) or wings to aid in wind

dispersal. Hooks and spines frequently aid dispersal by animals and man (Heywood *et al.*, 1977; Heywood, 1993). Most of the weedy species of this family mature early and produce large numbers of single-seeded, dry fruits often with unspecialised germination requirements (Popay and Roberts, 1970; Lawrence, 1985; Holm *et al.*, 1997). Among the weedy members of the Asteraceae listed by Holm *et al.* (1997) many (62%) are annuals, reproducing principally by wind dispersed achenes, and some are perennials relying heavily on sexual as opposed to vegetative reproduction. For example, *Senecio vulgaris* L. (an annual) and *Chondrilla juncea* L. (a perennial), both serious economic weeds, are described as "prolific seeders" with rapid seedling establishment (Holm *et al.*, 1997). Despite a mild dormancy in a small portion of the seeds of these species, most are capable of immediate germination. Seeds of *C. juncea* and *S. vulgaris* germinate in both the light and dark and the optimum temperature for germination is 25 °C (Popay and Roberts, 1970; Holm *et al.*, 1997). Lawrence (1985) analysed characteristics of 32 *Senecio* species in Australia and found that 29 species reproduced sexually, producing over 10 000 small achenes (0.1-0.6 mg) per plant. Few species showed specific germination requirements.

General biology of weed seeds and seedlings

The germination process involves the inception of rapid metabolic activity within the seed, resulting in perceptible growth of the embryo. It is usually associated with the uptake of water and oxygen, use of stored food and normally, release of carbon dioxide (Klingman *et al.*, 1982; Zimdahl, 1993). For many investigators radicle emergence has been one of the best visible criteria identifying the initial stage of germination (King, 1966; Evetts and Burnside, 1972; Robocker, 1977; Grime *et al.*, 1981; Smreciu *et al.*, 1988). For a seed to germinate it must have an environment favourable to the germination process, factors promoting germination may include specific temperature, light, moisture and pH requirements (King, 1966; Klingman *et al.*, 1982). Generally, weeds have no special requirements for germination (Baker, 1965; Stephens, 1982; Zimdahl, 1993). However, in a few species dormancy mechanisms do occur (Zimdahl, 1993). Five environmental factors affect seed dormancy: temperature, light, moisture, oxygen and the presence of inhibitors (including allelopathic effects). Other factors directly related to the seed and its dormancy include impermeable seed/fruit coats, immature embryos and an after-ripening period (King, 1966; Grime *et al.*, 1981; Klingman *et al.*, 1982; Zimdahl, 1993; Cousens and Mortimer, 1995).

While there are great differences in the rate of seedling growth among the various weed species, the most competitive usually have rapid growth and early, extensive root development compared to the plants with which they are competing (King, 1966; Zimdahl, 1993; Cousens and Mortimer, 1995, Holm *et al.*, 1997). They grow tall quickly or gain competitive advantage by twining up larger plants. Furthermore, they are frequently tolerant of shade in that their highest carbon dioxide assimilation is not in full sunlight (Zimdahl, 1993). Certain weed seedlings have large expansive type foliar cotyledons that, through early photosynthetic function enable young seedlings to become established quickly. This is true for the Asteraceae (King, 1966). Similarly, the rate of new leaf production is important, as it affects surface area available for photosynthetic production.

The present study

In southern Africa, Hawaii and Australia *D. odorata* reproduces sexually by seed and vegetatively by stolons. However, in California, where this vine is a serious weed, no viable seed is produced and the plant reproduces only by vegetative means (Balciunas, pers. comm.). In a study conducted by the USDA Agricultural Research Service on the germination requirements of the seeds produced by *D. odorata* in California, for the entire testing procedure, only one seed germinated (USDA, 1998). The reason for the sterility of the populations in California is at present unknown (USDA, 1998). The contrasting modes of reproduction of *D. odorata* in exotic infestations raises interesting questions as to the nature of the spread of this vine. Sexual reproduction is usually an important method of propagation for weeds of the Asteraceae; they generally mature early and produce copious amounts of easily dispersed single-seeded fruits which germinate quickly or remain buried beneath the soil until germination requirements are met. Frequently, it is because of the large amount of seed produced that eradication and control of these weeds is difficult. However, if *D. odorata* is a serious invader in California despite its lack of viable seed then the importance of the seed biology of this vine in the process of invasion needs investigation.

Besides the investigation conducted by the USDA Agricultural Service (California) into seed germination, no studies have been conducted into the seed biology of the fruits of *D. odorata*. Without an understanding of the seed biology of *D. odorata* in southern Africa, Hawaii and Australia no predictions about the importance of sexual reproduction in the spread of this species

in exotic locations can be made. Furthermore, if this species does spread through sexually reproduced seed in Hawaii and Australia, knowledge of the seed biology may be important in the development and implementation of management strategies in those areas.

The present investigation was conducted to obtain a better understanding of the seed biology of *D. odorata* in southern Africa. It is a baseline study, the results of which may be used as a comparative reference for studies made on the seed biology of this species in exotic locations. A series of laboratory experiments were conducted to determine the germination characteristics of the seeds. An examination of the effect of light on seedling development was also made. Although the difficulties in relating laboratory experiments to field conditions have been pointed out (Koller and Roth, 1964; Popay and Roberts, 1970), such analyses are an essential beginning to an understanding of the behaviour of seeds/fruits in response to the complex and fluctuating conditions in the field (Popay and Roberts, 1970).

4.2   Materials and methods

All germination experiments were conducted on mature achenes of *D. odorata*. Mature infructescences, collected in June 1998 from Ferncliffe Nature Reserve, Pietermaritzburg, were brought back to the laboratory where the mature fruits were removed. The achenes were dry stored in the dark at room temperature (20 °C) for one month prior to germination trials.

Germination requirements

All germination studies were conducted in the laboratory. For all treatments, achenes were placed on moist filter paper in petri dishes. Germination was recorded every second day and was considered to have occurred once the radicle had elongated to a length of 2 mm or more. Unless otherwise stated, photoperiod was set at 16 h light/8 h dark and 10 replicates of 20 achenes each were used per treatment.

1. *Fruit weight and measurements*: As fruits are very small, average weight was determined by massing the achenes in batches of 20 (20 replicates). The average length and width of the fruits was determined using a dissecting microscope (50 achenes).

2. *Pericarp permeability*: The ability of *D. odorata* achenes to imbibe water was established by incubating the mature fruits at 25 °C on moist filter paper in petri dishes. Fruits were

blotted dry before massing at 24-hourly intervals for 12 days.

3. *Temperature requirements*: The influence of temperature upon germination rate was studied at 5 constant temperatures: 10 °C, 15 °C, 20 °C, 25 °C and 30 °C.

4. *Response to light*: Light requirements were determined by comparing germination of achenes in petri dishes wrapped in aluminum foil with those exposed to light. Achenes were germinated in the dark at 10 °C, 15 °C, 20 °C, 25 °C and 30 °C. Radicle protrusion from these fruits was examined in a growth room fitted with a green "safe light".

5. *Response to chilling*: Following one month storage in the dark at 0 °C, the effect of chilling on viability of achenes was determined by germinating achenes at 15 °C. Fruits were kept on moist filter paper in petri dishes covered with foil during chilling.

6. *Longevity*: Viability of seeds following one year dry storage, in the dark at room temperature (20 °C), was tested by germinating seeds at 10, 15 and 25 °C both in the light and dark.

7. *Depth of planting* : To establish the effect of burial depth on achene germination, achenes of *D. odorata* were placed in plastic pots and buried at depths of 0 cm, 1 cm and 5 cm below the soil surface. The pots were kept in the greenhouse maintained at 20-25 °C and the soil was kept moist. Seedling emergence was recorded after seven weeks; three replicates of 40 achenes each were used per treatment.

Seedling establishment

The effect of shading on the development of seedlings was assessed. Three light treatments, 71 %, 45 % and 17 % of PAR (photosynthetically active radiation) were employed (see Chapter 5 for full description of materials). Six replicates of 10 mature achenes were used for each treatment; achenes were placed in pots containing a 50:50 mixture of soil and composted bark and the pots were assigned to one of the three light treatments. The development of seedlings was monitored continuously; the following life history characters were recorded for each treatment:

1. days from sowing to first germination (assessed as emergence from the soil);

2. number of seeds germinating and seedling survival for the first four months following sowing and survival eight months after establishment;

3. plant height (mm) and leaf number for the first four months following sowing and eight months after germination;

4. length (mm) of the longest five leaves for each seedling eight months after germination; and

5. root, shoot and leaf dry weight (g) eight months after germination. On harvesting each seedling was separated into roots, leaves and stems and placed into paper bags. The plant components were air-dried with heater fans (± 40 °C) for approximately four days and the dry weight of roots, stems and leaves was recorded.

Data analysis

All percentage values were arcsine transformed prior to analysis (Zar, 1974). This transformation is applicable to binomial data expressed as decimal fractions or percentages, and is especially recommended when percentage values cover a wide range of values (Steel and Torrie, 1980). For all normal data one way ANOVA was used to test for significant differences between treatments. Duncan's Multiple Range Test was used to compare means. Non-normal data was analysed using the non-parametric ANOVA equivalent, the Kruskal Wallis Test (Zar, 1974). The statistical programme used was Minitab© (1995).

4.3   Results

Germination requirements

The size of the achenes of *D. odorata* was assessed by weight (mg), length (mm) and width (mm). As is the case in many members of the Asteraceae (Lawrence, 1985), the achenes of *D. odorata* are very small (Table 4.1). The water uptake study, conducted to determine the permeability of the pericarp, indicated that water was imbibed almost immediately (Fig. 4.1). Over 12 days the weight of the achenes increased from 5.1 ± 0.5 mg to 39.8 ± 13.4 mg.. All of the achenes which imbibed water germinated indicating that the fruits were non-dormant.

The germination trials conducted in the light and dark at 10, 15, 20, 25 and 30 °C indicated that germination occurred, in both the light and dark, over the temperature interval of 10-25 °C (Figs 4.2 & 4.3). No germination occurred, in the light or dark, at 30 °C (Figs 4.2 & 4.3). At the temperatures 10, 15, 20 and 25 °C there was no significant difference in final germination percentage of seeds exposed to light ($F=1.4$, $p>0.05$). In the dark, final percentage germination at 25 °C was significantly different from that at lower temperatures ($F=4.25$, $p<0.05$, Duncans Multiple Range Test). In the light, maximum germination occurred at 25 °C; an optimum temperature for the germination of many species of the Asteraceae (Lauer, 1953; Popay and Roberts, 1970; Forsyth and Brown, 1982; Holm *et al.*, 1997). Although germination was not inhibited in the dark, for these trials germination values were generally lower and the optimum

Table 4.1. Average weight and size of achenes (±s.d.) of *D. odorata*.

Measured parameter	Measurement
weight (mg)	0.3 (±0.03)
length (mm)	1.5 (±0.16)
width (mm)	0.6 (±0.09)
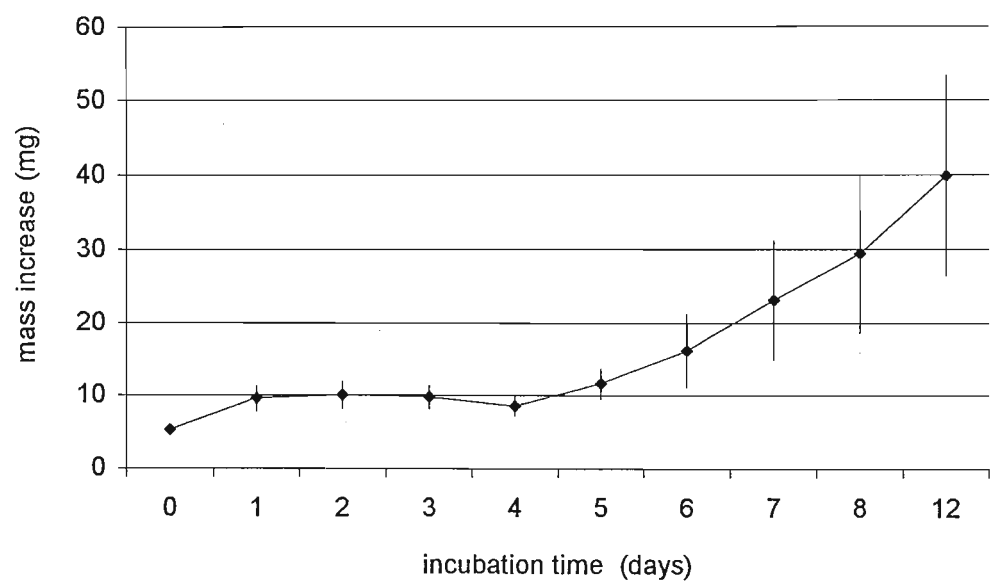
Fig. 4.1. Increase in mass (water uptake) of fruits of *D. odorata* imbibed at 25 °C for 12 days. Photoperiod was set at 16 h light/ 8 h dark. Error bars represent standard error.
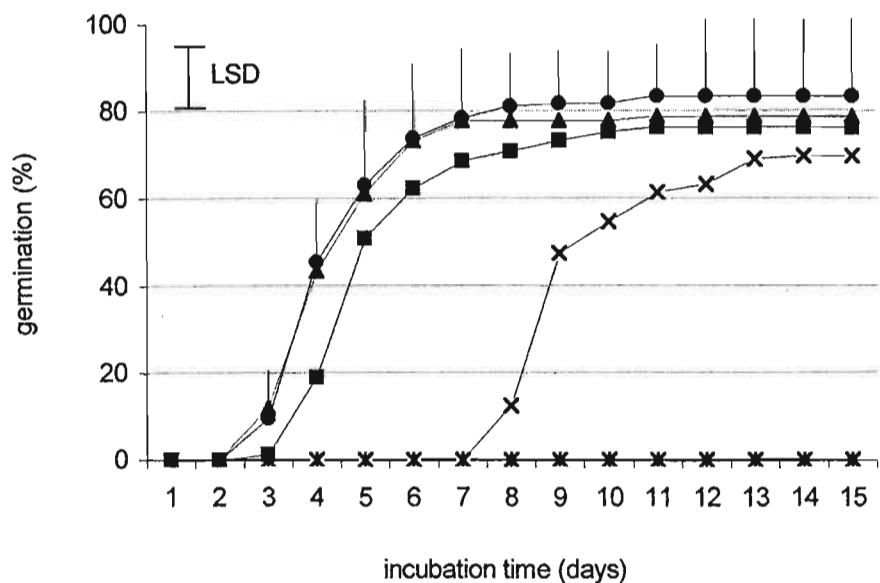
Fig. 4.2. Cumulative % germination of *D. odorata* achenes maintained for 15 days at one of five temperatures. Photoperiod was set at 16 h light/ 8 h dark. Error bars represent standard error. LSD = 12.5
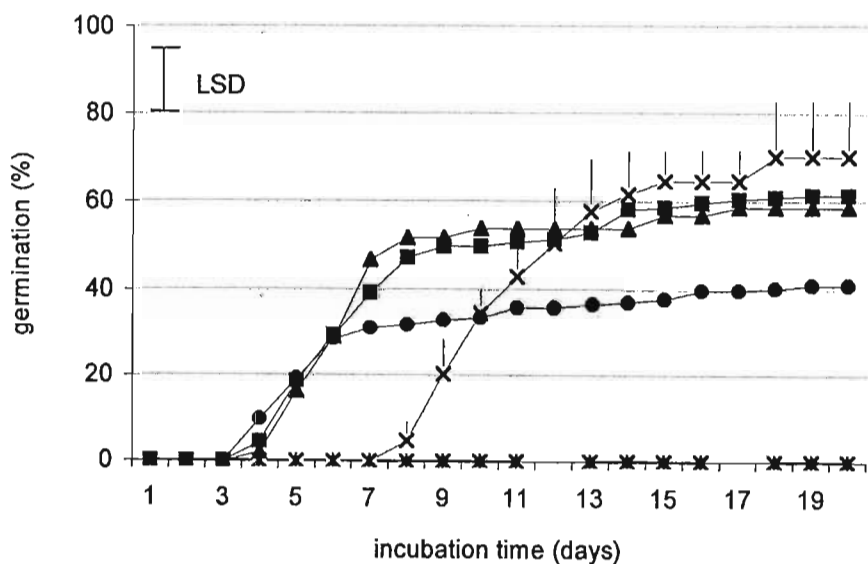
—✗— 10 °C —■— 15 °C —▲— 20 °C —●— 25 °C —✱— 30 °C

Fig. 4.3. Cumulative % germination of *D. odorata* achenes maintained for 20 days in the dark at one of five temperatures. Error bars represent standard error. LSD=12.05

—✗— 10 °C —■— 15 °C —▲— 20 °C —●— 25 °C —✱— 30 °C

temperature for germination was also decreased, maximum percentage germination was at 10 °C. Although the seeds placed at 30 °C did not germinate, when these seeds were placed at 15 °C (after 12 days exposure to 30 °C) germination occurred indicating that the seeds were still viable. Chilling for one month in the dark at 0 °C caused a slight increase in time to germination, in the light and dark, at 15 °C, but had little effect on percentage germination at this temperature (Fig. 4.4). Fruits of *D. odorata*, set in June 1998, were dry stored for one year in the dark at room temperature (20 °C) and subsequently sown, in the light and dark, at one of three temperatures - 10, 15 or 25 °C. Results show a considerable decrease in the viability of these seeds at all temperatures (Fig. 4.5). No seeds germinated at 10 °C and, for all treatments, maximum germination did not exceed 40 % (cf. Figs 4.2 & 4.3).

The results of experiments conducted to determine the effect of burial on achene germination showed that only achenes scattered on the soil surface were capable of germination (Table 4.2). This finding is in accordance with the findings of Beveridge and Wilise (1959) who recorded that larger seeds emerge more easily from greater depths in the soil than smaller seeds. With an achene mass of only 0.26 mg the result obtained for the achenes of *D. odorata* was thus expected.

Seedling establishment

In order to examine the development of *D. odorata* seedlings in response to light, trials at 71, 45 and 17 % sunlight were conducted. Barring the achenes exposed to 71 % sunlight, seedling emergence was completed within the first three weeks of planting (Fig. 4.6). Maximum percentage germination was significantly greater at 17 and 45 % sunlight than that at 71 % sunlight where only 60 % of the achenes germinated (F=5.15, $p<0.05$). For all treatments, survival decreased slightly with time from sowing. There was no significant difference in percentage survival following eight months exposure to the different light levels (F=0.17, $p>0.05$).

For the first three months, the general increase in seedling growth was arrested (Figs 4.7 & 4.8). This may be due to an experimental effect, alternatively lack of shoot development may be associated with extensive root growth, a factor frequently associated with the development of competitive weed seedlings (King, 1966; McVean, 1966; Panetta, 1977; Zimdahl, 1993; Cousens and Mortimer, 1995; Holm *et al.*, 1997). After four months growth, and until termination of the
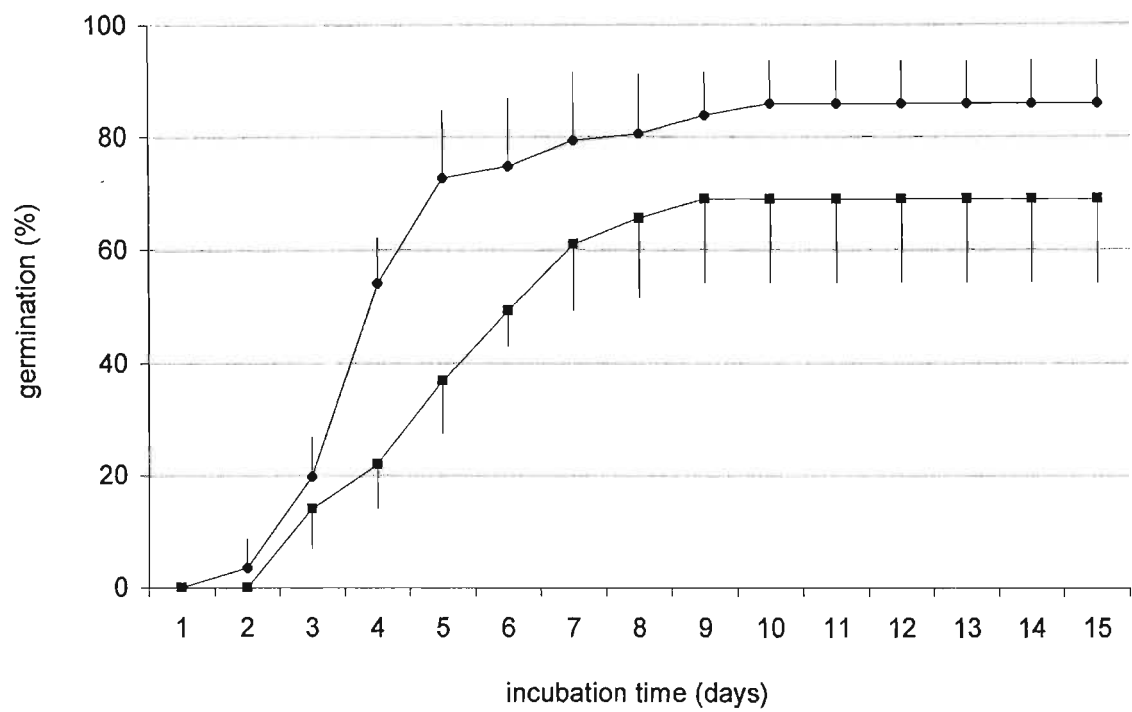
Fig. 4.4. Cumulative % germination of *D. odorata* achenes following treatment for one month in the dark at 0 °C. Achenes were germinated at 15 °C in both the light and dark. Error bars represent standard error.

—◆— light —■— dark
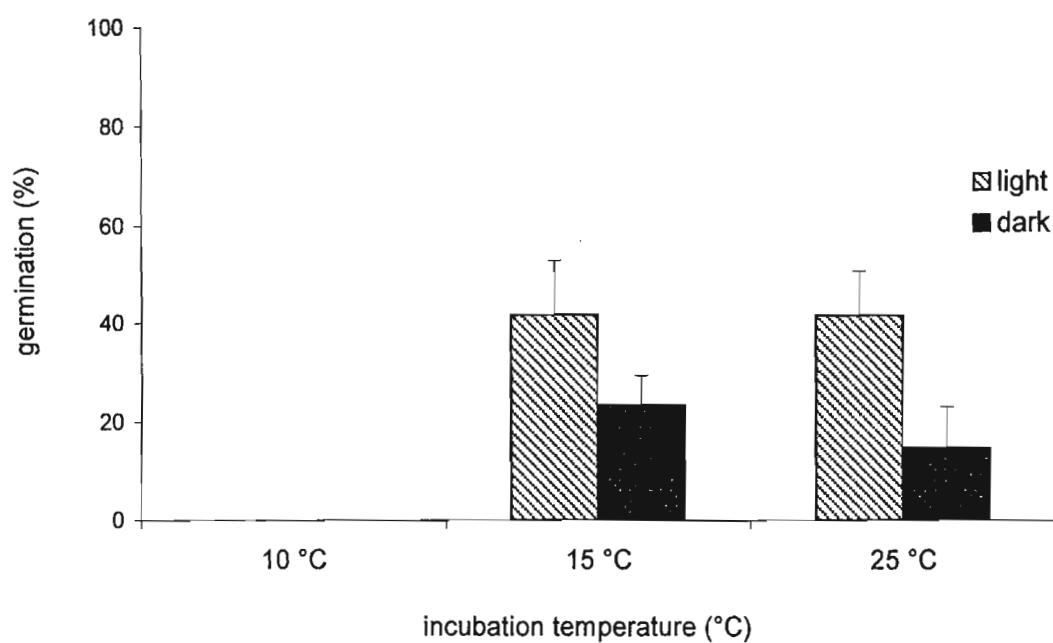
Fig. 4.5. Final % germination, in the light and dark, of dry stored (1 yr) *D. odorata* achenes, following 20 days treatment at 10, 15 and 25 °C. Error bars represent standard error.

Table 4.2. Effect of depth of planting on seedling emergence of achenes of *D. odorata*.

Planting depth (cm)	Seedling emergence (%)
0	76 (± 12)
1	0.3 (±0.5)
5	0
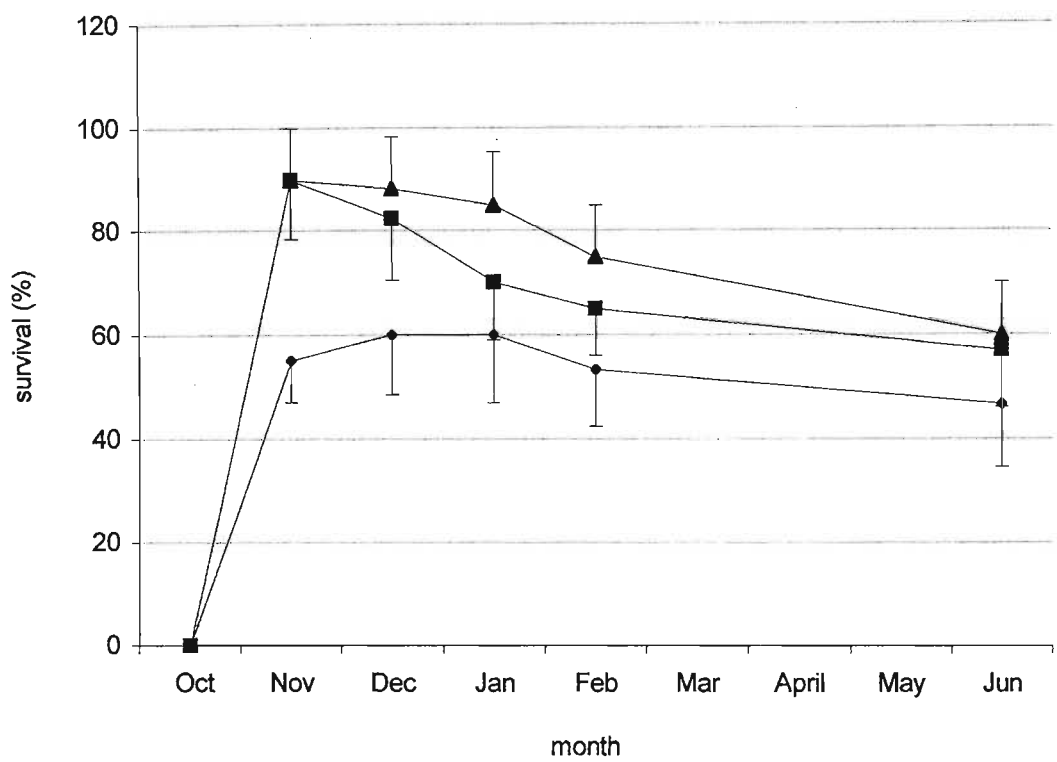
Fig. 4.6. Emergence and subsequent survival (%) of
D. odorata achenes following eight months treatment at 71, 45
and 17% sunlight. Error bars represent standard error.
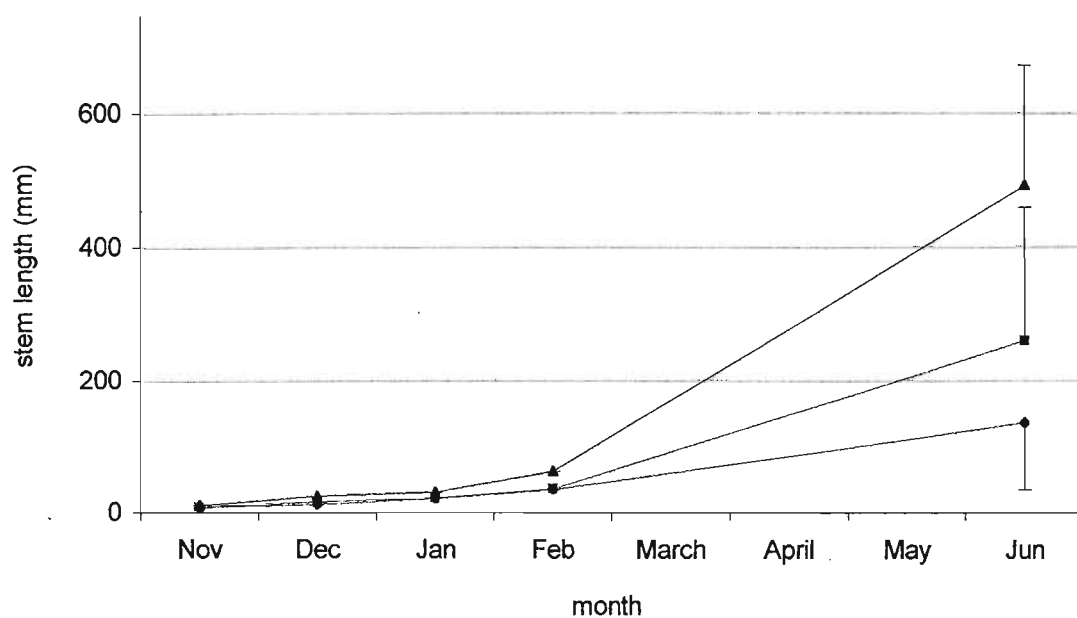
—◆— 71% —■— 45% —▲— 17%

Fig. 4.7. Monthly increase in stem length (mm) of
D. odorata seedlings exposed to 71, 45 and 17 % sunlight. Error
bars represent standard error.
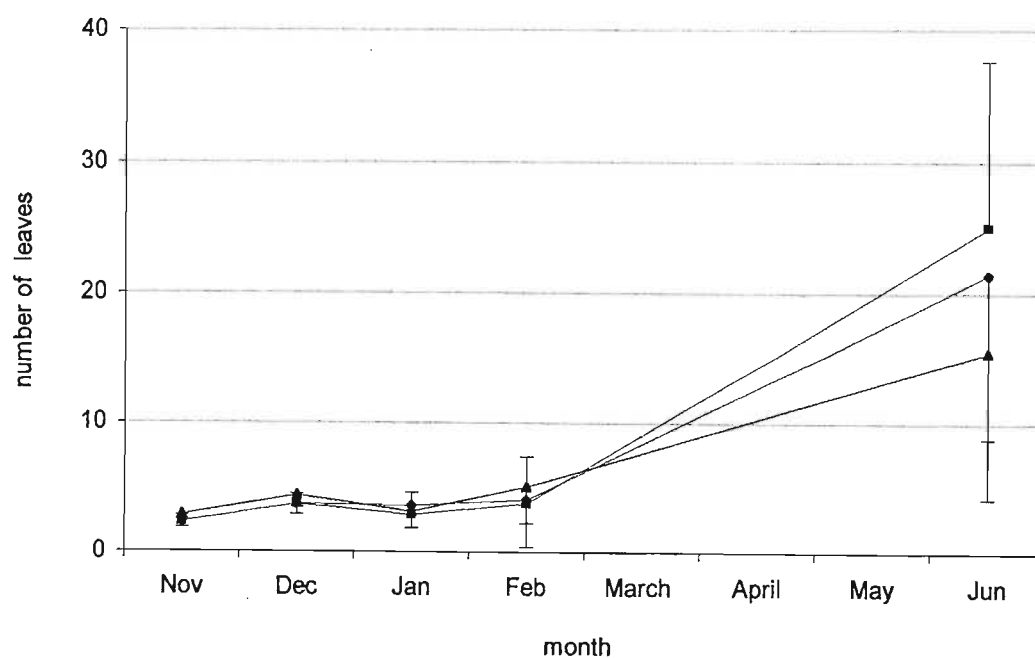
—♦—71% —■—45% —▲—17%

Fig. 4.8. Monthly increase in leaf number of *D. odorata*
seedlings exposed to 71, 45 and 17 % sunlight. Error bars
represent standard error.

—♦—71% —■—45% —▲—17%

experiment, stem length of seedlings exposed to 17 % sunlight was significantly greater than those grown at 45 and 71 % (Kruskall Wallis ANOVA, $p < 0.05$) (Fig. 4.8). Number of leaves per seedling was maintained at a relatively constant level in all treatments for the first 12-15 weeks (Fig. 4.8). However, after eight months the average number of leaves per seedling was significantly greater for those seedlings grown at 45 % sunlight ($H = 8.51$, $p < 0.05$) (Fig. 4.8). There was no significant difference in leaf size of seedlings exposed to the different light treatments, although leaves of seedlings exposed to only 17 % sunlight were generally larger than those grown at 71 % ($H = 1.29$, $p > 0.05$) (Fig.4.9).

The flowering period for *D. odorata* occurs over the months of April to June. In June 1999 the seedlings for this investigation were eight months old and they had no flowers. This is contrary to expectation as weedy *Senecio* species usually reach sexual maturity rapidly and flower within their first year of establishment (Lawrence, 1985; Abbot, 1986; Holm *et al.*, 1997). Further studies are required to confirm the result obtained for this investigation. Eight months after sowing the seedlings were harvested. Present results revealed no significant difference between treatments in the allocation of biomass to leaves, stems and roots for seedlings exposed to the different light levels (leaves; $F = 1.68$, $p > 0.05$; stems, $F = 0.5$, $p > 0.05$; roots, $F = 1.20$, $p > 0.05$) (Fig. 4.10). Maximum biomass allocation was to stems, followed by that to roots and leaves.

4.4   Discussion

Germination requirements

The results of this study corroborate those of Grime *et al.* (1981). Their analysis of the germination characteristics of several plants drawn from a local flora in northern England, found that the majority of species with small seeds germinated easily and were non-dormant. Their investigation also showed that fruits of the Asteraceae have amongst the highest capacity for immediate germination. High initial germinability of seeds of the Asteraceae was correlated with the presence of the pappus and antrorse hairs or teeth. Studies have shown that these structures are characteristic of species in which germination normally occurs in seeds lodged on the soil surface (Sheldon, 1974; Grime *et al.*, 1981). Radicle emergence and early seedling establishment of these species appears to benefit from the combined effects of seed shape and antrorse hairs or teeth, both of which tend to anchor the fruit in an upright position in loose soil bringing the radicle end into closer contact with the soil (Grime *et al.*, 1981).
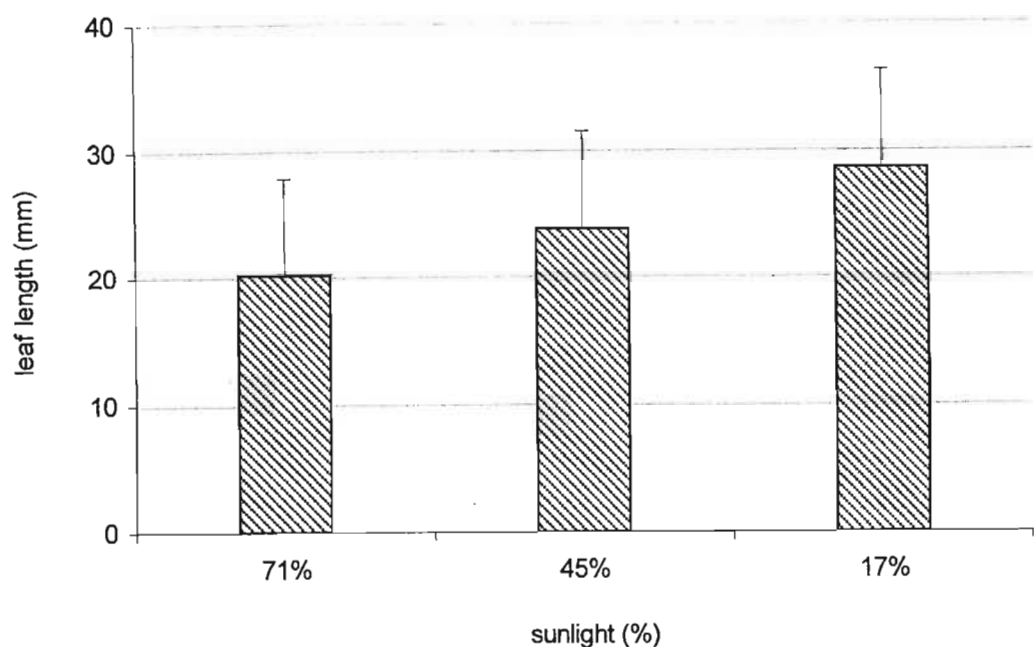
Fig. 4.9. Length of the longest leaves (mm) of *D. odorata* seedlings following eight months exposure to one of three light levels. Error bars represent standard error.
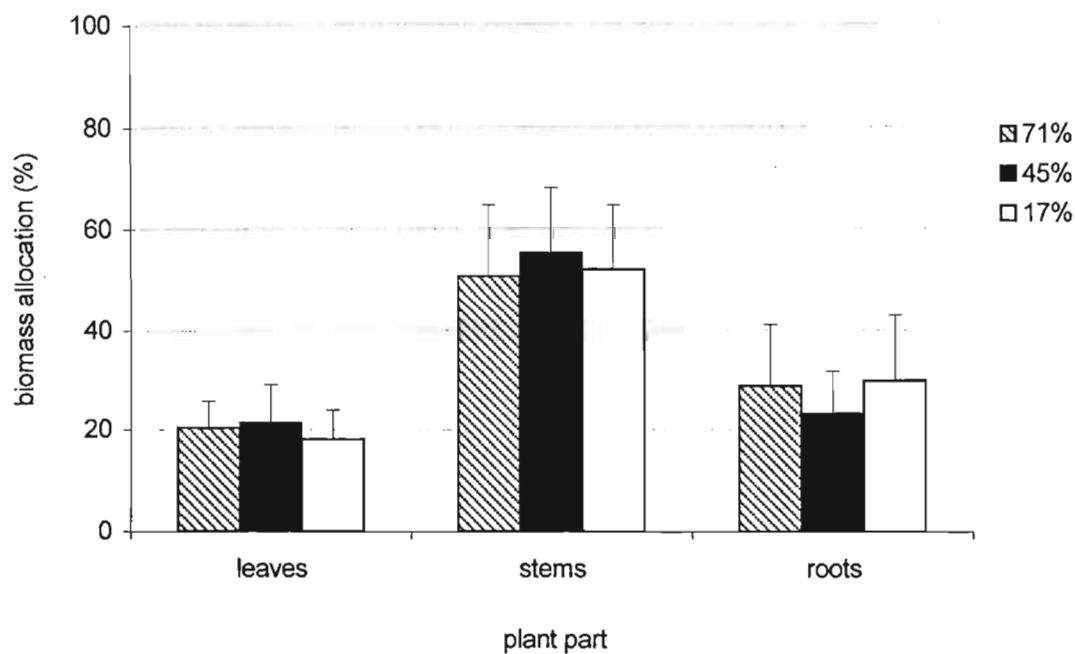
Fig. 4.10. Dry weight allocation (%) to roots, stems and leaves of *D. odorata* seedlings following eight months exposure to one of three light levels. Error bars represent standard error.

Clearly light stimulates the germination of *D. odorata* seeds. Light is one of the major factors regulating seed germination and light requirements are frequently associated with small seeds (Harper *et al.*, 1970; Mayer and Poljakoff-Mayber 1989; Bell, 1993; Plummer and Bell, 1995; Rokich and Bell, 1995). Plummer and Bell (1995) documented a light requirement for germination in several small seeded Australian Asteraceae. Several other authors have also reported a light stimulation within the Asteraceae (Popay and Roberts, 1970; Mott, 1972; Atwater, 1980; Grime *et al.*, 1981; Willis and Groves, 1991). Lack of germination of buried *D. odorata* seeds may also indicate a light requirement. Light-promoted seed germination appears to be an adaptation to enhance the chance of establishment where germination of deeply buried seeds would prove fatal. Physiologically active light flux densities rarely penetrate more than a few millimeters into the soil (Plummer and Bell, 1995). Small seeds contain limited reserves and it is advantageous for germination to occur where photosynthesis can quickly take over from stored carbohydrates (Pons, 1992; Plummer and Bell, 1995). Plummer and Bell (1995) found that Asteraceae seeds weighing less than 0.5 mg required light, whereas the response was mixed among species with heavier seed.

Low temperatures clearly have a beneficial effect on the germination of seeds of *D. odorata* when not exposed to light. Treatment with alternating temperatures or low temperature incubation has long been known to increase germination in darkness of light requiring seeds (Popay and Roberts, 1970; Bewley and Black, 1994). Grand Rapids lettuce, for example, is generally dormant in darkness above 23 °C, below this value seeds germinate without illumination (Bewley and Black, 1994). The achenes of *D. odorata* exposed to 30 °C germinated easily when moved to a lower temperature. This phenomenon is known as thermoinhibition (Horowitz and Taylorson, 1982). Thermoinhibition refers to seeds where germination fails at high temperature but proceeds upon subsequent transfer to an optimal temperature, thermoinhibition thus prevents germination when conditions are unfavourable.

It may be unwise to assume that the ability of freshly collected seed to germinate in the laboratory is a reliable indication that under field conditions germination occurs soon after fruit release. In the field, the germination of freshly dispersed seed may be prevented by limiting factors not operating in the laboratory tests (Grime *et al.*, 1981). Available literature suggests that the germination of achenes of many members of the Asteraceae coincides with the onset of wet conditions (Grime *et al.*, 1981; Holm *et al.*, 1997). In southern Africa the small wind-dispersed

fruits of *D. odorata* are set and dispersed in the dry winter months of June and July when average daily temperatures range between 5 and 20 °C (CCWR, 1999). Although the achenes of *D. odorata* have no stratification requirement, the cold dry winter conditions may prevent germination in the field. Germination of achenes may coincide with the onset of the spring rains and warmer temperatures of late September. Seedling germination, establishment and growth in the field will need to be examined to confirm these preliminary observations.

Seedling establishment

Competitive capacity of plants is governed, in part, by the efficiency with which they intercept light (Keeley and Thullen, 1978). In relation to light requirements for plant establishment and growth Grime (1966) made a distinction between shade avoiding and shade tolerant species. The observations made in this investigation indicate that the seeds of *D. odorata* germinate at both high and low light intensities, although establishment in lower light conditions (17 and 45 %) appears to be favoured. *D. odorata* is therefore likely to be a shade tolerant species. Maximum leaf production occurred at 45 % sunlight, a factor confirmed by other observations made for this project (Chapter 5). In its native environment *D. odorata* frequents forests and forest margins where variation in light intensity is typical. Furthermore, in areas where *D. odorata* creates a mat-like groundcover, extensive die back of the adult plants over winter may expose seeds to an environment of varied and changing light intensity throughout the day; an ability to tolerate a range of light intensities may thus confer a competitive advantage to the seedlings of *D. odorata*.

4.5   Concluding comments

Rapid growth through the vegetative phase to flowering, prolific seed production, unspecialised germination requirements, seed longevity and dormancy and adaptations for long and short distance dispersal have often been associated with weedy potential (Baker, 1965; Amor and Piggin, 1977; Klingman *et al.*, 1982; Stephens, 1982; Dean *et al.*, 1986; Zimdahl, 1993; Cronk and Fuller, 1995; Holm *et al.*, 1997). Furthermore, characters of late successional species (high competitive ability and shade tolerence) have been associated with species which are successful invaders of forested habitats (Cronk and Fuller, 1995). Results of this investigation show that *D. odorata* is a small seeded species with relatively unspecialised germination requirements, apparently no dormancy mechanisms and only moderate seedling growth potential (no flowers are produced during the first year of growth). However, to confirm the findings of this investigation, further studies into the seed biology of this species must be conducted. These should include an

extensive investigation into the effect of light on seed germination to determine the importance of this environmental factor in seedling establishment.

Reproduction by seed, is frequently associated with the colonisation of new habitats (Amor and Piggin, 1977). Thompson (1990) stated that for clonal dominants of tall herb vegetation, in which the main role of seed is colonisation of new habitats, small seed size and low nuclear DNA content are typical. Small mean seed mass is often associated with large seed numbers, efficient dispersal and high initial germinability (Rejmánek, 1995). Low DNA content has been positively correlated with seed size and associated with the selection for short minimum generation time (the duration of minimum period from germination until the production of the first seed) in time limited environments (Bennet, 1972; Bennet, 1987; Thompson, 1990; Rejmánek, 1996). Interestingly, among 32 species of *Senecio* analysed in Australia, three exotic invaders (*Delairea odorata* (syn. *Senecio mikanioides*), *Senecio pterophorus* L. and *Senecio vulgaris*) have significantly lower nuclear DNA content than 28 native and one non-invasive cultivated species (Lawrence, 1985).

Chapter 5

Growth and reproduction in relation to light availability

5.1 Introduction

Although weeds usually reproduce by means of seeds, they also multiply by means of vegetative or asexual methods (Leakey, 1981; Klingman *et al.*, 1982; Zimdahl, 1993). Rhizomes, stolons, tubers, roots, bulbs and bulblets are all vegetative means of reproduction. In some important perennial weeds reproduction is primarily vegetative, for example *Eichhornia crassipes* (Martius) Solms-Laubach, *Heiracium floribundum* Wimm and Grab, *Cirsium arvense* L., *Ranunculus repens* L. and *Salvinia molesta* D.S. Mitchell (Leakey, 1981; Eriksson, 1989; Zimdahl, 1993; Cousens and Mortimer, 1995; Cronk and Fuller, 1995). In an analysis of the taxonomic distribution of invasive angiosperms Daehler (1998) found rapid vegetative reproduction, especially in woody plant species, to be a good indicator of invasiveness. Vegetative structures can act as a meristem (bud) bank, akin to a seed bank, capable of producing shoots when stimulated to do so. Since a single shoot may produce many subterranean structures and each of these may produce a number of aerial shoots, an extensive clonal stand can arise from a single seed or vegetative propagule (Cousens and Mortimer, 1995). Thus, in contrast to a population of unitary plants, such as annual weeds, a clonal plant population typically consists of a limited number of genotypes, the 'genets', each represented by a number of copies, the 'ramets' (Kays and Harper, 1974; Angevine and Handel, 1986). Hypotheses on the adaptive benefits of vegetative (clonal) reproduction in plants have two general themes. One emphasises how clonal growth may allow the individual to harvest patchily distributed resources and predicts a relationship between the clonal growth form and competitive dominance (Lovett Doust 1981; De Steven, 1989). The other emphasises clonal proliferation as a means of spreading mortality risk to the individual among many vegetative propagules capable of independent survival (Cook, 1979; Cook, 1985; De Steven, 1989). Because of their greater energy reserves, shoots from vegetative organs are often better competitors than seedlings and thus allow for rapid expansion of a plant in the local environment (without the need for sexual reproduction) (Zimdahl, 1993; Cousens and Mortimer, 1995). Whereas sexual reproduction, usually associated with long distance dispersal, expands the geographic range of a species, vegetative reproduction contributes to parochial persistence of the population (Eriksson, 1992; Cousens and Mortimer, 1995).

It is common for the availability of resources, such as light and nutrients, to vary in the field and this variation can have a profound effect on both the performance of individuals and the dynamics of plant populations (Harper, 1977; Hutchings and De Kroon, 1994; Evans and Cain, 1995). Phenotypic plasticity, the capacity for differential expression of a phenotype owing to environmental influences on the genotype, has long been considered a major means of individual adaptation to environmental heterogeneity and has also been associated with invasive and weedy potential (Zimdahl, 1993; Hutchings and De Kroon, 1994; Mack, 1996; Vermeij, 1996). More recently, plasticity has also been viewed as a behavioural phenomenon allowing many vegetative (clonal) plants to arrange their ramets within the environment in a selective manner (Slade and Hutchings, 1987 a-c; Hutchings, 1988; De Kroon and Hutchings, 1995; Evans and Cain, 1995). For example, the ability of a stoloniferous clone to exploit favourable patches or avoid unfavourable patches has been described as "foraging" behaviour (Bell, 1984; Hutchings, 1988; Evans and Cain, 1995). Clonal foraging in a heterogeneous environment is controlled by plasticity in one or more of the following traits: branch production, internode distance (spacer length between ramets) and angle of rhizome growth. Morphological plasticity in these traits is hypothesised to be adaptive in environments where there is high spatial resource heterogeneity (Evans and Cain, 1995).

Many plants display extensive foraging, in terms of plasticity in growth form and deployment of biomass, in response to nutrient and light availability (Pitkella *et al.*, 1980; Slade and Hutchings, 1987 a & b; Hutchings, 1988; Sutherland and Stillman, 1988; Salonen, 1994; Hutchings and De Kroon, 1994; Evans and Cain, 1995; De Kroon and Hutchings, 1995). Light is a limiting resource in many environments and varies both spatially and temporally, affecting plant growth and reproductive output (Anderson *et al.*, 1969; Pitkella *et al.*, 1980; Slade and Hutchings 1987b; Mitchell and Woodward, 1988). The effects of light on plant morphology, physiology and resource allocation in different light environments have been extensively studied and are well understood (Bjorkman, 1980; Fitter and Hay, 1981; Mooney and Chariello, 1984). In a patchy environment, such as a forest edge or beneath the forest canopy where light is unevenly distributed, the clonal growth form is advantageous as it allows plants to place ramets selectively in bright patches.

Theoretical models predict that under low light conditions plants will show reduced branching,

longer internodes and a change in the allocation of biomass to different structural components (Slade and Hutchings, 1987b; Solangaarachchi and Harper, 1987; Sultan and Bazzaz, 1993; Salonen, 1994). The predicted adaptive response of leaves to low light is an increase in the relative biomass allocation to these organs and to produce "shade leaves", which differ in area, structure and photosynthetic capacity to "sun leaves" (Sultan and Bazazz, 1993). Light has been cited as a factor affecting allocation of biomass to sexual or vegetative reproduction with sexual reproductive effort increasing with light availability (Anderson *et al.*, 1969; Thompson and Willson, 1978; Pitkella *et al.*, 1980; Sultan and Bazzaz, 1993).

The present study

Delairea odorata expands vegetatively through the spread of stolons and frequently sends out runners in response to poor light conditions (Chipping, 1993). Along a stolon, each node has the potential to root and become a new plant, a ramet, genetically identical to the parent plant (Chipping, 1993; Archbald, 1995). In addition, thick rhizomes support regrowth when shoots are damaged or removed (Chipping, 1993; Archbald, 1995; Alvarez, 1997). Vegetative propagation of *D. odorata* is achieved by ramets which are produced at every node along a principal stolon which creeps over the surrounding vegetation or soil surface. Along the principal stolon, further stolons can develop from the axillary buds present at each node, these will grow to produce a further set of genetically identical ramets. For stoloniferous species Hutchings (1988) described the principal stolons as the primary stolons, whereas the axillary buds give rise to the secondary (and tertiary) stolons and ramets. With this kind of growth pattern the plant ultimately develops as a branched, inter-connected population of individual clones able to cover a large area in a small amount of time (Slade and Hutchings, 1987b).

Because of the usually large amount of temporal and spatial variation in light availability, plants living in forests or forest edges can be expected to respond to this environmental factor in terms of their growth and reproduction. Since *D. odorata* grows primarily in moist, shady environments along forest edges, the factors affecting its growth and reproduction are of interest, especially as very little has been published on this subject. An experiment was designed to determine the response of vegetative propagules of *D. odorata* to light intensity in terms of growth rate, morphology and resource allocation.

5.2 Materials and methods

All material used in this experiment originated from a single population of *D. odorata* located at Ferncliffe Nature Reserve, Pietermaritzburg. To eliminate the effects of genetic variation all cuttings were taken from stolons collected from a single 2 m x 2 m plot. One hundred and eighty single node vegetative cuttings (2-3 cm) were placed into plastic pots containing a 50:50 mixture of composted bark and soil and subsequently transferred to a misthouse for acclimation and establishment. After 10 days all cuttings had rooted and produced new shoots (2.5 ±0.5 cm). These established vegetative cuttings were used in the light experiment.

To determine the effect of light on growth and resource allocation patterns of *D. odorata*, vegetative cuttings, sixty per treatment, were grown at 71, 45 or 17 % available sunlight (PAR - photosynthetically active radiation). Metal frames (1.6 m x 1.3 m x 0.6 m) covered with shade cloth were placed over the pots to form shade tents which provided the required light levels. The difference in photosynthetically active radiation (PAR:μmol m^{-2}s^{-1}) incident upon transplants for each treatment was recorded with a Skye SKP 200 PAR meter. Mean midday PAR incident on transplants during the experimental period for each treatment varied between 46.9 and 189.5 μmol m^{-2}s^{-1} (Table 5.1). These light levels encompassed the range of natural light levels experienced in the field.

Table 5.1. Mean PAR (μmol m^{-2}s^{-1}) [±s.d.] incident on transplants in each treatment.

available sunlight (%)	PAR [±s.d.] (μmol m^{-2}s^{-1})
71	189 [9.1]
45	120 [5.1]
17	46 [3.7]

After 12 weeks exposure to these light regimes the following variables were recorded for each treatment: *Vegetative characters* (n = 60 for each treatment): leaf length (mm), leaf width (mm) and petiole length (mm) for 10 mature leaves per plant; number of leaves; length of primary (1°) stolon (cm), length of secondary (2°) stolon(s) (cm) and length of the first 10 sequentially produced internodes (cm). *Resource allocation analyses:* 28 transplants from each treatment were harvested, air dried for 72 hours, and weighed. The dry weights of the leaves, roots and

stolons (both 1° and 2° stolons) were determined separately for each plant. Total biomass was calculated for each plant as the summed dry weights of all plant parts. Proportional biomass components were calculated for the roots, stems and leaves. The proportion of stem biomass allocated to secondary stolon production for each transplant was also calculated.

The characters described above are viewed as growth characters indicative of plant development and fitness due to growth under different environmental conditions and have been used by other researchers (Pitkella *et al.*, 1980; Slade and Hutchings 1987b; Sultan and Bazzaz, 1993; Salonen, 1994).

To determine the effect of light on allocation of resources to sexual reproduction, plants not harvested were maintained in their respective shade tents for a further four months - until the flowering season in May-June. At peak flowering these transplants were harvested and the biomass allocated to sexual reproduction was determined.

Data analysis

For all normal data one way ANOVA was used to compare differences between treatments. Duncan's Multiple Range test was used to compare differences between means (Zar, 1974). For data which were non-normal the non-parametric (ANOVA equivalent) Kruskal Wallis test was used to compare aspects of growth under the different treatments. Data were analysed with the statistical package Minitab©.

5.3   Results

There was no significant difference in shoot length, number of leaves and leaf length of cuttings at the start of this investigation (*shoot length*: $F=1.45$, $p>0.05$; *no. leaves*: $F=0.64$, $p>0.05$; *leaf length*: $H=2.14$, $p>0.05$).

Leaves

After 12 weeks, mean leaf length and width differed significantly between plants grown at different light intensities (leaf length, $F=58.3$, $p<0.05$; leaf width, $F=45.14$, $p<0.05$). Plants grown at 45 % available sunlight produced the largest leaves, followed by plants grown under heavy shade (17 % PAR). The smallest leaves were produced by those plants exposed to 71 % available

sunlight (Fig. 5.1). The same trend was observed for petiole length (H=46.42; p<0.05) (Fig. 5.2a). Light level also had a significant effect on leaf production per plant, plants exposed to 45 % PAR had more leaves than those grown at both 71 % and 17 % available sunlight (H=31.18, p<0.05) (Fig. 5.2b).

Stolon spread

The primary stolons (1°) of *D. odorata* branch at the nodes to produce secondary (2°) stolons and in this way the plant increases occupation of the local environment. Significant differences for measured aspects of stolon growth were found between plants grown under the different light treatments (p<0.05) (Table 5.2). By the end of 12 weeks the mean length of the primary stolons for plants in all treatments had increased by at least 3000 %, some measurements indicating that several plant stolons were growing up to 3 cm per day. Many plants produced secondary stolons, so that for some individuals the summed length of 1° and 2° stolons exceeded eight metres. Both stolon length (1° and 2°) and internode length increased with decreasing light availability (Table 5.2). When the mean internode length was determined for successive nodes along the primary stolon, the full extent of the differences in internode length became apparent (Fig. 5.3). All internode lengths were longer in the more heavily shaded clones than those receiving more light. Although heavily shaded plants (17 % PAR) produced the longest 1° and 2° stolons, the proportion of plants with secondary stolons and the number of secondary stolons produced per plant in this treatment was less than for those plants receiving more light (Table 5.2). Theoretical models predict an increase in branching frequency with an increase in light availability (Slade and Hutchings, 1987b; Solangaarachchi and Harper, 1987; Sultan and Bazzaz, 1993; Salonen, 1994). Contrary to expectation, branching in *D. odorata* was greatest at 45 % PAR, thus total stolon length was greatest for these plants largely as a consequence of their profuse branching (Table 5.2).

Dry weight production

Significant differences were found between the absolute dry weights of component plant parts (roots, leaves and stems) and the proportion of biomass allocated to these parts for plants grown at different light intensities (Fig. 5.4 a-d & Fig. 5.5). The dry weight of whole plants was greatest for those plants exposed to moderate light (45 %), followed by plants exposed to 71 % available sunlight and was lowest for those plants exposed to only 17 % PAR (Fig. 5.4a). These
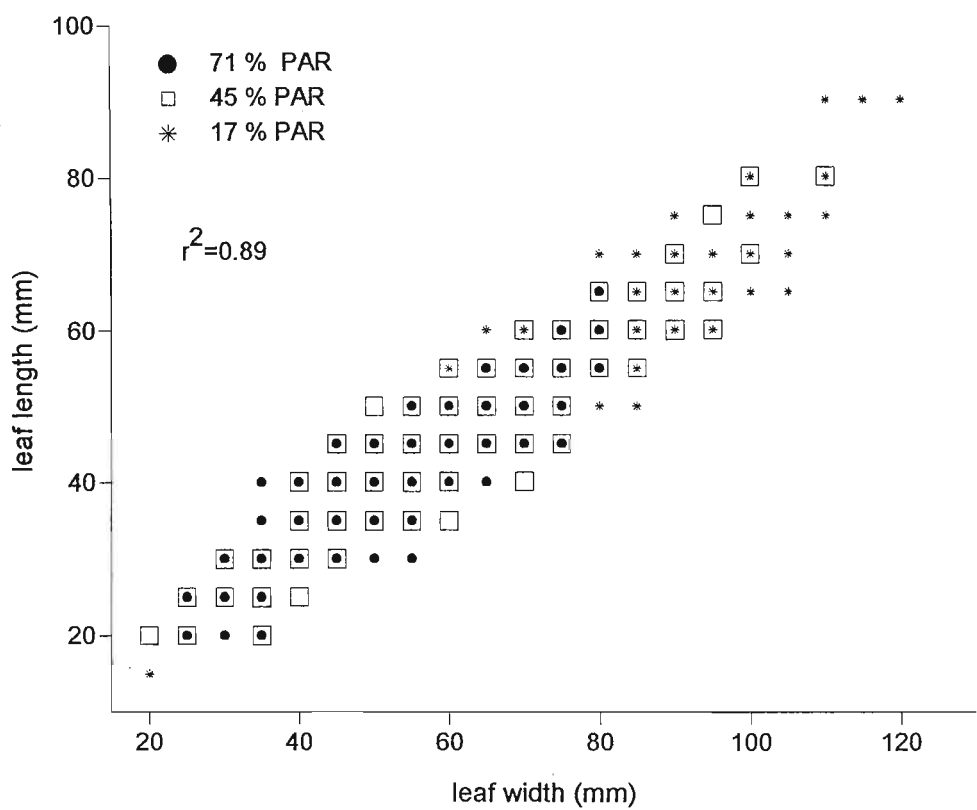
Fig. 5.1. Mean length vs. width (mm) of 10 mature leaves of *D. odorata* plants exposed to one of three light levels, 71, 45 or 17 % PAR (n=60).

a

b
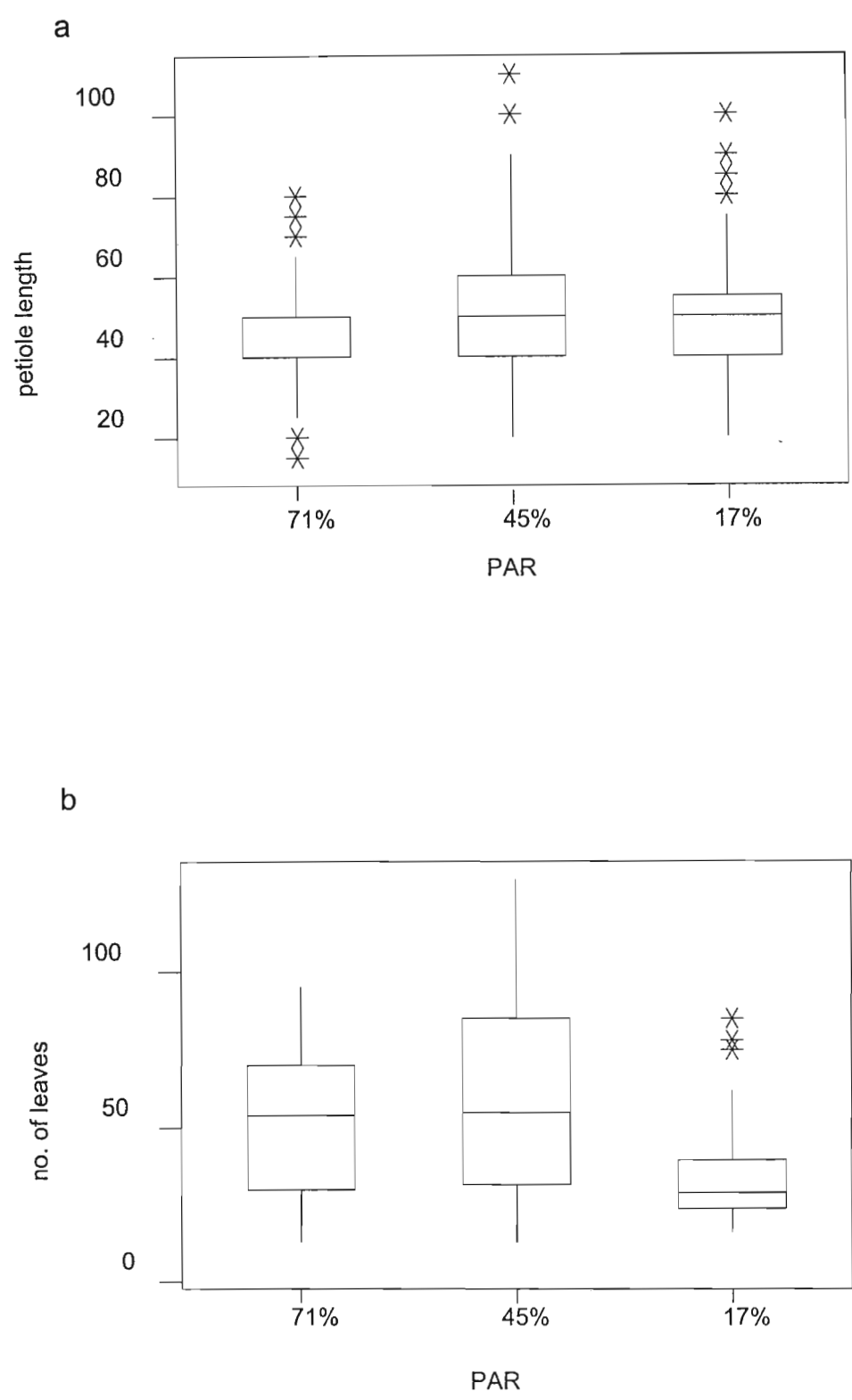
Fig. 5.2. a & b. Petiole length (a) and leaf number (b) of *D. odorata* transplants after 12 weeks exposure to one of three light levels. Horizontal bar represents the median.

Table 5.2. Summary of the results (means [±s.d.]); unless otherwise indicated) of measured characters of stolon spread for *D. odorata* plants grown at different light intensities for 12 weeks. n=60 plants measured for each character. Significantly different values are indicated with small letters (a,b,c); the difference between values with the same letter is not significant (p<0.05). CI: confidence interval.

Treatment PAR (%)	length (cm) 1° stolon	internode length (cm)	length (cm) 2° stolon	total stolon length (cm)	% plants with 2° stolon	no 2° stolon/plant [95 % CI]
17	141 [6.1]^a	5.5 [0.8]^d	80 [32]^g	173 [14.7]	36.6	0.5 [0.3-0.7]
45	122 [6.1]^b	4.4 [0.9]^e	74 [31]^g	254 [30.4]	63.3	2.1 [1.6-2.6]
71	77 [4.1]^c	3.4 [0.8]^f	26 [13]^h	108 [8.8]	61.6	1.5 [1.1-1.9]
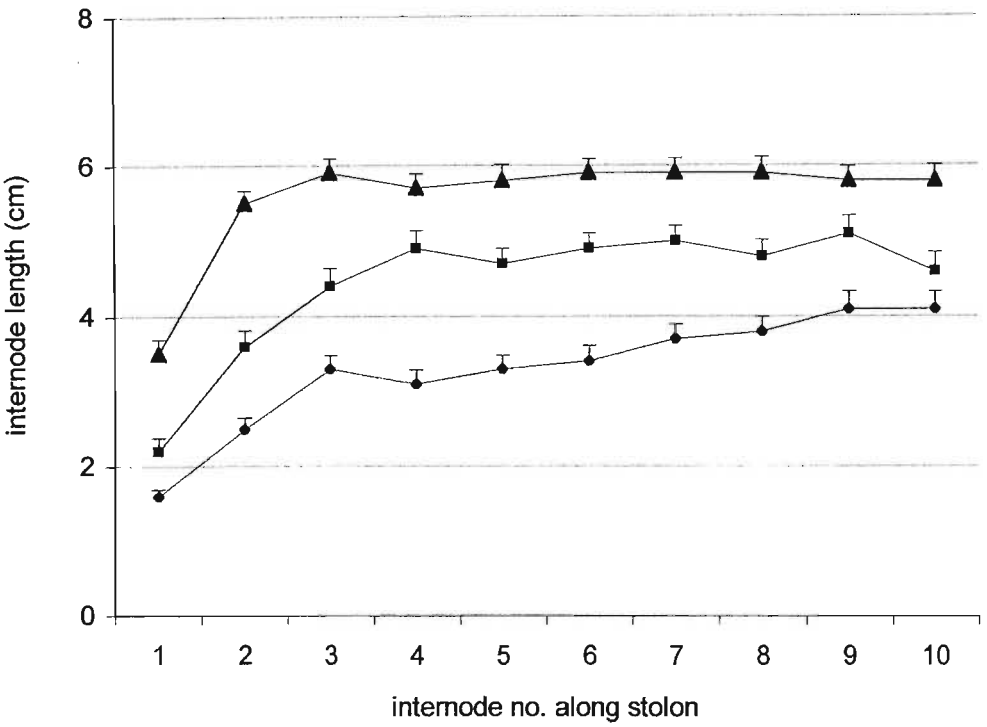
Fig. 5.3. Mean length (cm) of the first 10 sequentially produced internodes along the primary stolons of *D. odorata* cuttings, following 12 weeks exposure to one of three light levels (n=60). Error bars represent standard error.

──●── 71% ──■── 45% ──▲── 17%
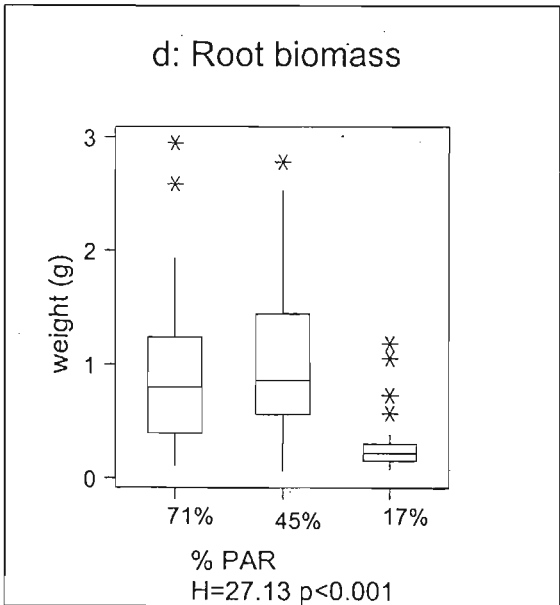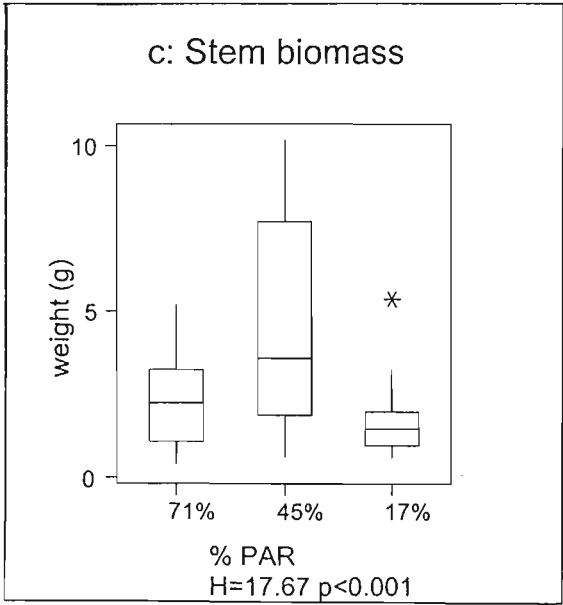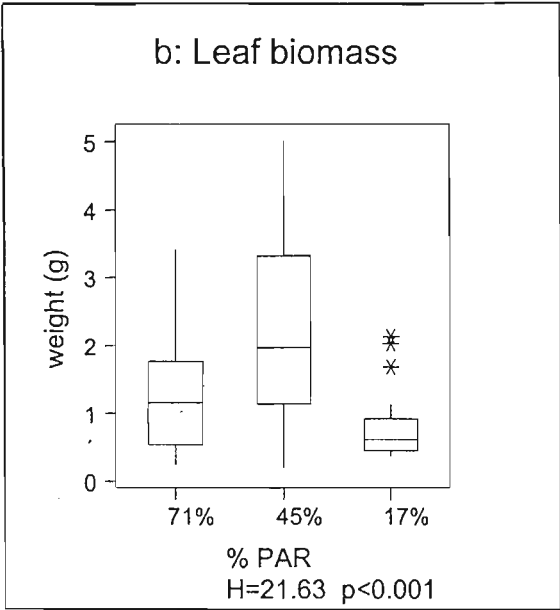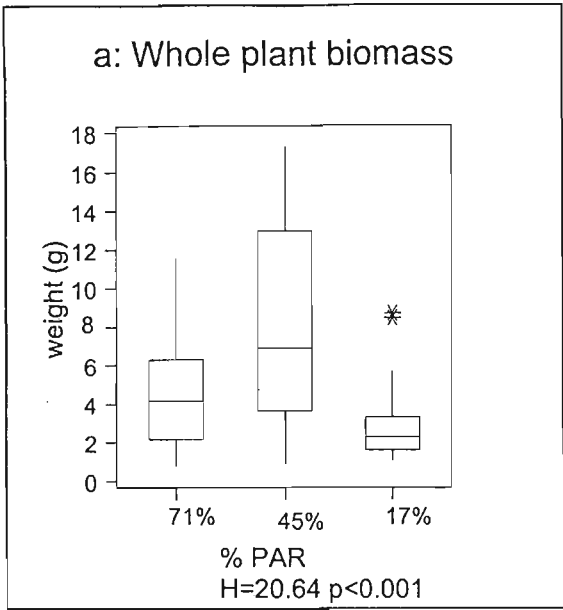
Fig. 5.4. a-d. Dry weights for whole *D. odorata* plants and component plant parts after 12 weeks exposure to different light levels (n=28). Horizontal bar represents median.
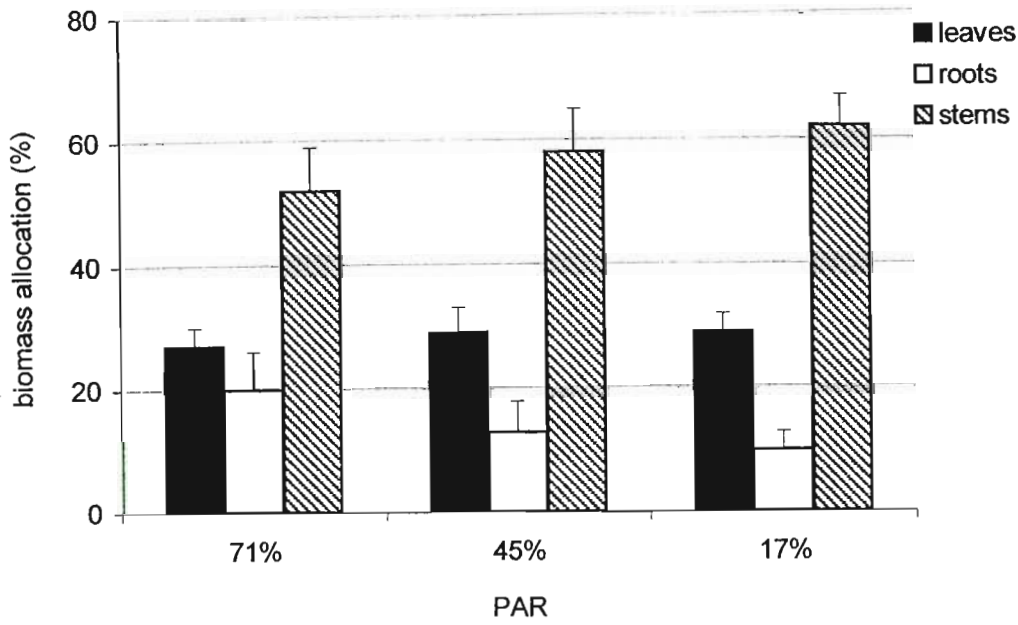
Fig. 5.5. Biomass allocation (%) to leaves, roots and stems by *D. odorata* cuttings, grown at 71, 45 or 17 % PAR (n=28). Error bars represent standard error.
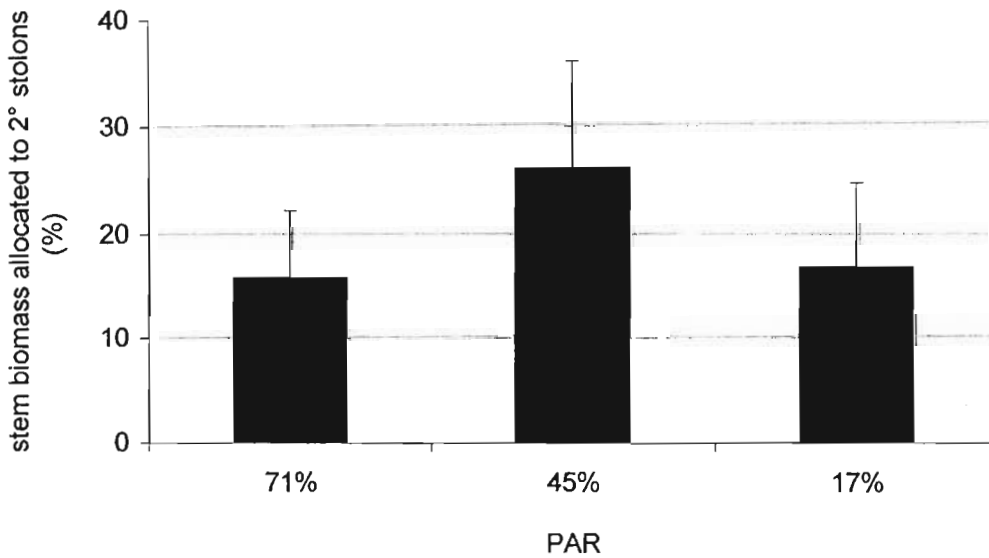
Fig. 5.6. Stem biomass (%) allocated to secondary stolons by *D. odorata* cuttings grown at 71, 45 or 17 % PAR (n=28). Error bars represent standard error.

results indicate that light intensity has an effect on average plant size. In terms of the magnitude of absolute weights, this pattern of response to available light (45 % > 71 % > 17 %) was observed for the absolute dry weights of leaves, stems and roots (Fig. 5.4. b-d).

The greatest percentage allocation of dry weight by plants in all treatments was to the stems (Fig. 5.5). Furthermore, this proportion differed significantly between treatments and increased with decreasing light levels (F=15.7; p<0.05). The proportion of biomass allocated to secondary stolon production also differed and was significantly larger for those plants maintained at 45 % PAR (F=5.95, p<0.05) (Fig. 5.6). Proportional biomass allocated to the leaves was not significantly different at the range of light intensities (F=1.26, p>0.05) despite the fact that there was a magnitude of difference in both the number and absolute dry weights of the leaves produced by the plants in each treatment (Fig. 5.2b & 5.4b). Allocation of biomass to roots for plants in all treatments was very low (<20 %) and increased with increasing light levels (Fig. 5.5).

Flowers

The flowering season for *D. odorata* is during the months of May-June. For this experiment very few individuals flowered and the experiment was terminated in late June when existing capitula were in full bloom. Although flowering was low for plants in all treatments, dry weight of flowers produced was greatest for plants grown at 45 % PAR (Fig. 5.7); 40 % (10 individuals) of the plants grown at 45 % PAR flowered while only 8 % (2 individuals) flowered at 71 % and 17% PAR. For this experiment flowering was not dependent on plant size as there was no relationship between plant size and production of flowers (Fig. 5.8).

5.4   Discussion

Delairea odorata responds strongly to a reduction in the availability of light in terms of its growth rate and resource allocation. As genetic variation was largely eliminated in our pot trial, the observed variation in plant performance can be attributed to phenotypic plasticity. After 12 weeks of growth, significant differences between plants, grown at different light levels, had developed in most measured variables. As far as absolute dry weight is concerned, the largest plants were consistently those exposed to 45 % available sunlight. Furthermore, plants grown at 45 % PAR produced the most leaves which were also larger and had longer petioles than
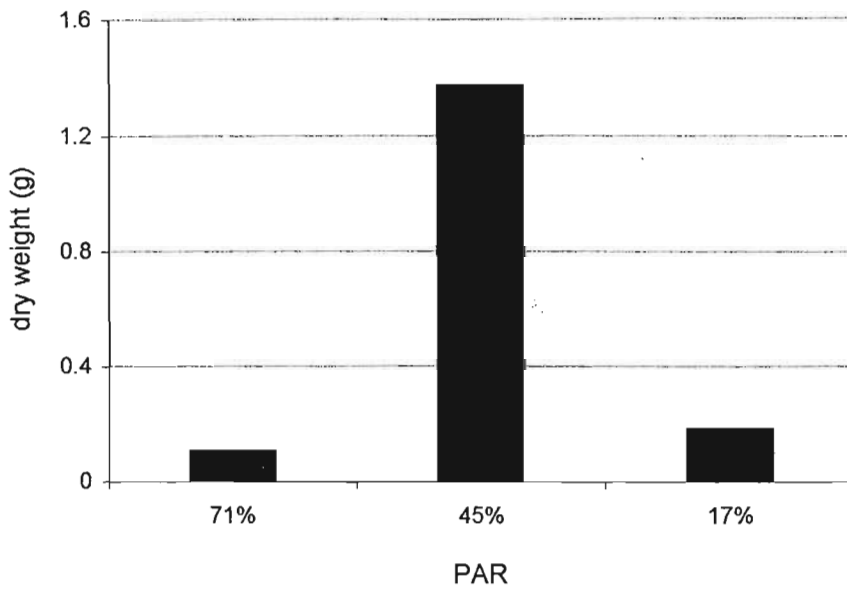
Fig. 5.7. Total dry weight (g) of inflorescences produced by *D. odorata* transplants following eight months treatment at one of three light levels.
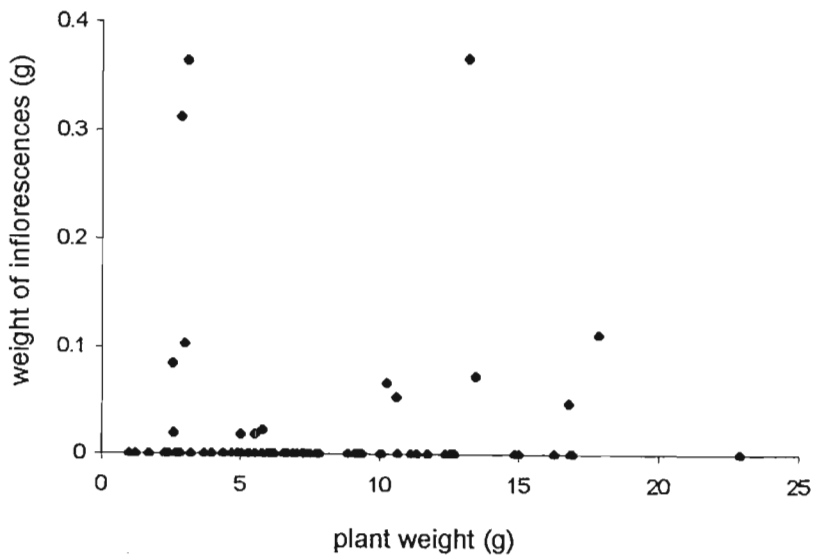
Fig. 5.8. Dry weight (g) of inflorescences produced as a function of whole plant dry weight (g). T-test showed no significant difference between weight of non-flowering plants vs. flowering plants (p>0.05).

leaves produced at 17 % and 71 % PAR. This suggests that the photosynthetic assimilation rate was highest at an intermediate light level; however, detailed physiological studies need to be conducted to confirm this observation. Zimdahl (1993) noted that weeds with great competitive ability are often shade tolerant in that their highest carbon dioxide assimilation does not occur in full sunlight. Mack (1996) suggested a viney growth habit and a low light compensation point (the point at which the amount of CO_2 given out by the respiratory process is equal to that taken up by photosynthesis) to be traits indicative of invasive potential in natural or semi-natural forests. The most consistent plastic responses to availability of light are manifested in internode elongation (etiolation) and branching (the proportion of axillary meristems which grow out) (Bazzaz and Harper, 1977; Sultan, 1987; Ellison and Niklas, 1988; Hutchings, 1988; Sutherland and Stillman, 1988; Hutchings and De Kroon, 1994; De Kroon and Hutchings, 1995). Most plants show reduced branching and internode elongation at lower flux densities (De Kroon and Hutchings, 1994), however, this is not always the case (Lovett Doust, 1987; see review by Hutchings and De Kroon, 1994). In an experiment to determine the effect of light on growth of the clonal herb *Glechoma hederacea* L., Slade and Hutchings (1987b) found that clones grown under shading had significantly longer internodes than stolons produced by unshaded clones but total stolon length of unshaded clones was over three times greater than for shaded clones, as a consequence of profuse branching. In this experiment, total stolon length, branching frequency and secondary stolon production was greatest for plants growing at an intermediate light level (45 % PAR) as opposed to those at 71 % available sunlight. Thus although *D. odorata* shows plasticity in growth response to light availability, it does not respond to changes in the light environment as predicted for plants with foraging behaviour. Instead resource acquisition appears to be consistently highest at 45 % PAR, a result consistent with observations on the success and proliferation of *D. odorata* in moist, shady environments (Fagg, 1989; Chipping, 1993; Alvarez, 1997). If internode lengths were shortest for plants exposed to 45% PAR then *D. odorata* would be foraging for intermediate light levels. However, internode length was consistently shorter at the highest light level (71 %).

The maximum weight of inflorescences was produced by plants grown at 45 % PAR. However, as only a few plants flowered the results of this experiment should be interpreted with reservation. After eight months growth, there was no relationship between plant size and sexual reproduction, a relationship which has been reported in other studies (Whigham, 1974; Werner,

1975; Pitkella *et al.*, 1980). Pitkella *et al.* (1980) found sexual reproduction in *Aster acuminatus* Michx., a forest understorey herb, to be indirectly affected by light through its effect on plant size. However, flowering is not always dependent on plant size (Salonen, 1994) and the results of this investigation indicate that flowering in *D. odorata* is independent of plant size and directly affected by light availability. Further field tests need to be conducted to confirm this observation.

Finally, following data collection, it was realized that this experiment could be improved by subjecting the stolons of *each replicate clone* to different light regimes. As stolons grow predominantly horizontally, sets of ramets along a stolon could be subjected, in experiments, to different light levels. Furthermore, to test for foraging behaviour, each set of ramets could be provided with a supply of light, nutrients and water different from that supplied to other sets of ramets. Such an experiment would test implicitly the plasticity of a clone, as all variation in morphology could be ascribed to differences in growing conditions experienced by ramets of an individual. Unfortunately there was insufficient time to conduct this experiment.

5.5 Concluding comments

The results of this investigation raise some interesting questions regarding the growth pattern of *Delairea odorata*. However, further field and laboratory studies to determine the effect of resource availability (nutrient and moisture) on clonal development need to be conducted before any conclusions regarding the growth strategy of this species can be made. Concurrent investigations into the habitat characteristics (in terms of resource availability) of areas dominated by *D. odorata* should also be made. This study shows that (1) *D. odorata* can be easily propagated from stem fragments (as short as 3 cm) (2) *D. odorata* shows plasticity in growth form and deployment of biomass in response to light intensity (3) regardless of light level, greatest allocation of biomass is to stem growth (4) growth rate is highest at intermediate light levels, and (5) sexual reproduction is affected by light availability. *Delairea odorata* proliferates most vigorously in moist shady environments (Hilliard, 1977; Fagg, 1989; Chipping, 1993; Alvarez, 1997) and seldom grows in open habitats, such as grasslands, it is therefore not surprising that growth rate is highest at intermediate light levels.

Chapter 6

The natural enemies of *Delairea odorata* and a potential biological control agent

Section 6.1.   Natural enemies

6.1.1 Introduction

Organisms which curtail plant growth or reproduction may be considered as potential biological control agents (DeBach, 1964; Harris, 1991; Shepherd, 1993). These include insects, mites, sheep, fungi, bacteria and viruses (DeBach, 1964). The search for natural enemies of a target weed should therefore encompass all organisms associated with the target plant. A high level of host-specificity is an essential attribute for any biological control agent. The first step towards biological weed control is thus the selection of an host-specific organism from a range of enemies (Harris,1991). For a classical biological programme host-specific natural enemies are collected in the country of origin of the weed (Shepherd, 1993).

Nature of the controlling action

The injury caused to a plant by an enemy may be either direct or indirect. Direct destruction pertains to natural enemies which destroy vital parts of the plant (e.g. roots, meristems and reproductive organs). An enemy may destroy a plant indirectly by facilitating infection by plant pathogens, thereby disrupting its competitive advantage (Anon, 1971; Bartlett and van den Bosch, 1971; Harris, 1973; Crawley, 1989; Harris, 1991). Since the response of a plant species to tissue damage or infection varies according to survival strategy, the crucial stage to attack with a biocontrol agent also varies (Harris, 1991). Generally, much emphasis has been placed on the action of insects which attack seeds or flowers or which bore into roots or stems (Huffaker *et al.*, 1971; McClay, 1989; Muller, 1989; Crawley, 1989; McClay and Palmer, 1995). The point of importance is that whatever the nature of the injury, a good agent is one which through direct or indirect action causes the destruction of existing host stands, thus determining the latter's abundance; reciprocally its own abundance is then adjusted to that of its host plant (Bartlett and van den Bosch, 1971; Andres *et al.*, 1976; van Driesche and Bellows, 1996).

Selection and evaluation of natural enemies

Evaluation of natural enemies should comprise the following stages (Anon, 1971):

1. Regular surveys at local populations to obtain data on the organisms attacking the weed.

2. Concentrated studies on single control agents that cover life history, distribution, importance and enemies of a potential control agent.

3. Host-specificity studies.

Surveys for potential control agents should be done over wide and ecologically diverse areas to maximise the chances of finding numerous natural enemies of broad genetic variability (Huffaker *et al.*, 1971; Andres *et al.*, 1976; Marohasy, 1989; Gillet *et al.*, 1991; McClay and Palmer, 1995). Plant species related to the weed should be included in the survey, as this provides preliminary information on the host-specificity of selected natural enemies (Muller, 1989; van Driesche and Bellows, 1996). In addition, surveys should be carried out in areas of both high and low weed density as this will enable identification of control agents with good searching ability (Bartlett and van den Bosch, 1971; Harris, 1973). The qualities of an effective agent include: (1) an ability to reduce the competitive advantage of a weed (2) good dispersal and searching capabilities (3) adaptability to varying physical conditions (4) host-specificity, and (5) ability to increase, or decrease, in number relative to the abundance of the host.

The present study

Delairea odorata infestations are presently controlled by costly mechanical and chemical means (Cudney and Hodel, 1986; Fagg, 1989; Archbald, 1995; Bossard and Benefield, 1995; Moore, 1997; Forbert, 1998). Biological control may offer a cost effective alternative to the long term management of *D. odorata* in exotic locations (Elliot, 1994; Balciunas pers. comm.). Elliot (1994) suggested the release of two insects introduced to control *Senecio jacobaeae* L. onto *D. odorata*. During 1994, experiments were conducted to test if these insects could reduce the vitality of *D. odorata* in the laboratory. Unfortunately, no follow up report on the results of these tests could be found. The alternative to using existing biocontrol agents is to search for the natural enemies of *D. odorata*. This involves the development of a classical biological control programme. Researchers from the USA are of the opinion that in southern Africa the growth of *D. odorata* is stunted, possibly due to the action of herbivores (Balciunas, pers. comm.). This has prompted surveys to determine if suitable biocontrol agents can be found in southern Africa.

To date, only one other study of the insect fauna associated with *D. odorata* has been conducted (Grobbelaar *et al.*, 1999).

The purpose of this study was to identify the natural enemies of *Delairea odorata* and to assess the potential of particular species as biocontrol agents. To this end a survey was made of the fauna associated with *D. odorata* in KwaZulu-Natal. Preliminary studies were also conducted on the life cycle and host-specificity of potential biocontrol agents (6.2 Section 2).

6.1.2 Materials and methods

From February to July and September to December (1998) surveys were made at regular intervals on populations of *D. odorata* situated at Dargle, Ingele and Ferncliffe (see Table 3.1 for location of sites). Plants at all sites were closely examined for damage by arthropods or other natural enemies. A minimum of one hour was spent collecting at each site on each occasion. The collected organisms were killed in jars charged with ethyl acetate. Later they were transferred to vials containing 70 % ethanol to preserve them for identification. Collections were biased as representatives of groups known to offer little or no prospect as biological control agents (such as crickets, grasshoppers and spiders) were not included. Any effect of these species on the rest of the insect fauna was not considered. During the flowering season, capitula were brought back to the laboratory and examined for the presence of arthropods.

In field surveys it was not possible to determine if a captured insect was actually feeding on the plant. Published faunal lists for many plants frequently do not indicate whether the species are known to feed on the plant in question or were simply collected on it. This study reports all the phytophagous species which were collected on *D. odorata*; undoubtedly the list includes some species which are generalist herbivores/casual visitors. Most specimens were submitted for identification either to specialists within the School of Botany and Zoology, University of Natal, or to taxonomists at the Transvaal Natural History Museum or the Plant Protection Research Institute (PPRI). Most specimens have been identified at least to the level of family.

6.1.3 Results and discussion

Many phytophagous species belonging to the insect orders Lepidoptera, Coleoptera, Hemiptera, and Diptera were found on *D. odorata* (Table 6.1). Most of the species are generalist

Table 6.1. Phytophagous arthropods associated with *Delairea odorata*.

Order: (Suborder)	Superfamily: Family: Subfamily	Genus & Species	No. species	Adult/ Larvae	Frequency (C/O/R)	Collection site	Feeding type
Lepidoptera Ditrysia	**Pyraloidea**: Pyralidae	*Psara vercordalis* Guenée	1	A	C	L	Ect
	Noctuoidea Noctuidae: Plusiinae	*Trichoplusia orichalcea* F.	1	A	O	S (as pupa)	Ect
	Noctuidae (larvae)	not determined	4	L	R	L	Ect
	Arctiidae: Arctiinae	*Galtara purata* Uzler	1	A	R	L	Ect
	Arctiidae (larvae)	not determined	5	L	O	L	Ect
	Geometriodea: Geometridae	not determined	1	L	C	C	Ect
	Papilionidae: Nymphalidae	not determined	1	L	R	L	Ect
Hemiptera Heteroptera	**Coreoidea**: Coreidae	not determined	2	A	O	L & S	Ect
	Pyrrhocoroidea: Pyrrhocoreidae	not determined	1	A	C	L & S	Ect
	Pentatomoidea Pentatomidae: Pentatominae	not determined	4	A	O	L & S	Ect
	Lygaeoidea: Lygaeidae	*Nysius* sp.	1	A	C	C	Ect
Homoptera	**Cercopoidea** Cercopidae: Aphrophoridae	spittle bugs	1	L	C	L & S	Ect
	Cicadelloidea: Cicadellidae	not determined	3	A	O	L	Ect

A: Adult L: larvae
Frequency : C: common   O: occasional   R: rare
Collection site: L: leaves   S: stems   C: capitula
Feeding type: Ect: ectophagous   End: endophagous

Table 6.1. continued.

Order: (Suborder)	Superfamily: Family: Subfamily	Genus & Species	No. species	Adult/ Larvae	Frequency (C/O/R)	Collection site	Feeding type
Coleoptera Polyphaga	**Chrysomeloidea** Chrysomelidae: Alticinae Chrysomelidae: Chrysomelinae Chrysomelidae: Galvercinae Chrysomelidae: Cryocerinae	*Abrarius* sp. *Ageniosa cf. badenii* Vogel *Prosmidia* sp. *Bradylema* sp.	1 1 1 1	A A & L A A	O C R R	C L L L	Ect Ect Ect Ect
	Scarabaeoidea Scarabeidae: Rutelinae (Tribe: Hopliini)	monkey beetles	2	A	O	C	Ect
	Scarabaeidae: Cetoniinae	*Leucocellis* sp.	1	A	O	C	Ect
	Cucujoidea Nitidulidae Phalacridae	not determined not determined	2 1	A A & L	C C	C C	Ect Ect
	Cleroidea Melyridae: Melyrinae	*Pagurodactylus* sp.	3	A & L	C	C	Ect
	Curculionoidea Curculionidae	not determined *Sciobius cf. bistrigicollis* Boheman *Eremnus* sp.	1 1 1	A A A	O O O	C L L	Ect Ect Ect
	Tenebrionoidea	not determined	1	A & L	C	C	Ect
Diptera Cyclorrhapha	**Tephritoidea** Tephritidae	*Parafreutreta regalis* Munro	1	A & L	O	S	End

A: Adult L: larvae
Frequency : C: common   O: occasional   R: rare
Collection site: L: leaves   S: stems   C: capitula
Feeding type: Ect: ectophagous End: endophagous

(polyphagous) herbivores not suitable as biocontrol agents. Table 6.1 indicates the frequency of collection for each specimen. Because collections were not quantitative, the assessment of whether a given species is common, occasional or rare is based on a subjective assessment of collection frequency. Species listed as common were often found at collection sites in their geographic range. Rare species were collected only on one or a few occasions, usually as single individuals. Most species are ectophagous, feeding on leaves and/or stems.

Ten species were found associated with the capitula. Most of these are small beetles belonging to the families Melyridae, Nitidulidae, Phalacridae and Scarabaeidae (Subfamily: Cetoniinae and Rutelinae), families known to possess flower feeders. The scarabaeids and the nitidulids are generally polyphagous and therefore unsuitable biocontrol agents (Scott and Way, 1990). Internal feeding on the capitula by Phalacridae, Melyrinae and Tenebrionoidea larvae was observed. One lepidopteran belonging to the family Geometridae was found associated with the capitula. The larvae of this species are bright yellow and are frequently found spinning a loose cocoon on 2-3 capitula.

Only one leaf feeder may merit further investigation - a shiny black chrysomelid, *Ageniosa cf. badenii* Vogel (Coleoptera: Chrysomelidae) (Table 6.1). Chrysomelids are generally host-specific and often completely defoliate their host. In addition, more species of Chrysomelidae have been used as weed biocontrol agents than any other family of insects (McEnvoy, 1984; James *et al.*, 1992; Julien, 1992; McEnvoy *et al.*, 1993; Syrett *et al.*, 1996). Although common, *Ageniosa cf. badenii* was only collected at Ingele Forest. The adults were frequently observed mating on *D. odorata* and larvae were found feeding heavily on the undersurface of leaves.

One endophagous feeder was found - a stem galling tephritid fly, *Parafreutreta regalis* Munro (Diptera: Tephritidae) (Table 6.1). This species was found at all study sites and was identified as the natural enemy with the greatest potential as a control agent.

Section 6.2

Studies on a potential biocontrol agent -

Parafreutreta regalis Munro (Diptera: Tephritidae)

6.2.1 Introduction

Biological control agents most frequently used against weeds of the Asteraceae belong to the family Tephritidae (see Chapter 2). Where tephritid stem-gallers have been used, outstanding control has been achieved. *Procecidochares alani* Steyskal and *Procecidochares utilis* Stone are credited with the control of *Ageratina riparia* R. King and H. Robinson and *Ageratina adenophora* (Sprengel) R. King and H. Robinson in Hawaii, both serious economic weeds (Bess and Haramoto, 1959; Dodd, 1961; Harris, 1989; Julien, 1992; Morin *et al.*, 1997). The tephritids have been employed in biocontrol programmes largely for their host-specificity (Crawley, 1989). Most of the tephritid galls are formed on plants of the family Asteraceae (Freidberg, 1984; Harris, 1989; Edwards *et al.*, 1996). Plants belonging only to seven other families (Acanthaceae, Aquifoliaceae, Goodeniaceae, Melastomataceae, Mimosaceae, Onagraceae and Verbenaceae) have been reported to bear tephritid galls and less than ten species of flies are involved (Freidberg, 1984).

Before *P. regalis* can be considered a candidate control agent it is essential that studies be conducted into its biology. This study made preliminary observations, both in the field and the laboratory, on the life cycle and host-specificity of this species.

6.2.2 Materials and methods

Field observations

In order to investigate the life cycle and host-specificity of *P. regalis*, populations, situated across KwaZulu-Natal, of *D. odorata* and its close relatives were frequently searched for mature galls. In southern Africa, closely related species to *D. odorata* include *Mikaniopsis cissampelina*, *Senecio cinarescens*, *Senecio quinquelobus* and *Senecio tamoides* (Jeffrey, 1986). These species were included in this study as a measure of the host-specificity of *P. regalis*. Stems bearing mature galls were collected and brought back to the School of Botany and Zoology, at the University of Natal, where they were potted and bagged with nylon-mesh to capture emerging insects. Data on the number of adults and pupae per gall and size of the gall at adult emergence

were recorded. Any specimens which emerged were collected for identification by Dr. R. Miller, University of Natal, Department of Entomology. Specimens are lodged at the Natal Museum.

Laboratory observations

Further observations on the life cycle of *P. regalis* were made in the laboratory in glass cages which contained, one or two, potted *D. odorata* plants. The tops of the cages were covered with 1 mm nylon-mesh to allow both adequate air-circulation into the cage and occasional mist spraying of the potted plants. As soon as the young adults emerged from mature galls they were released into the cages and observations were made on their mating and oviposition behaviour. Following this, the plants were transferred to the greenhouse and monitored continuously. The number of galls which developed per plant and their rate of development was recorded. Furthermore, the time required for larval and pupal development was noted.

Light microscopy

A light microscopy study of stem anatomy of *D. odorata* and the effects of galling thereon was carried out. This was done to provide some indication as to how *P. regalis* may be effective as a biocontrol agent. Thin transverse sections (T/S) of stems with and without galls were made for viewing with the light microscope. On galled stems sections were made of the tissue below, at and above the galls. All sections were stained with Toluidine Blue prior to viewing.

6.2.3 Results and discussion

Field observations

Stem galls were found on only two of *D. odorata*'s close relatives, *S. tamoides* and *S. quinquelobus*. The flies which emerged from these galls were *Parafreutreta felina* Munro (Diptera: Tephritidae) and *Parafreutreta conferta* Munro (Diptera: Tephritidae), respectively (Figs 6.1-6.3). Munro (1940) indicated that *P. regalis*, *P. felina* and *P. conferta* are allied in terms of their general biology and gall formation.

Figs 6.4 a-d show details of the galls collected from *D. odorata*, *S. tamoides* and *S. quinquelobus*. A total of 60 galls were collected from populations of *D. odorata*. Of these only 20 (33 %) were successfully reared to adults; loss of gall forming maggots can be attributed to immaturity at collection, failure of the stem cutting to root (and therefore death of the gall) and internal fungal infection (of the gall) (Fig. 6.5). Of the three *Parafreutreta* species collected for this
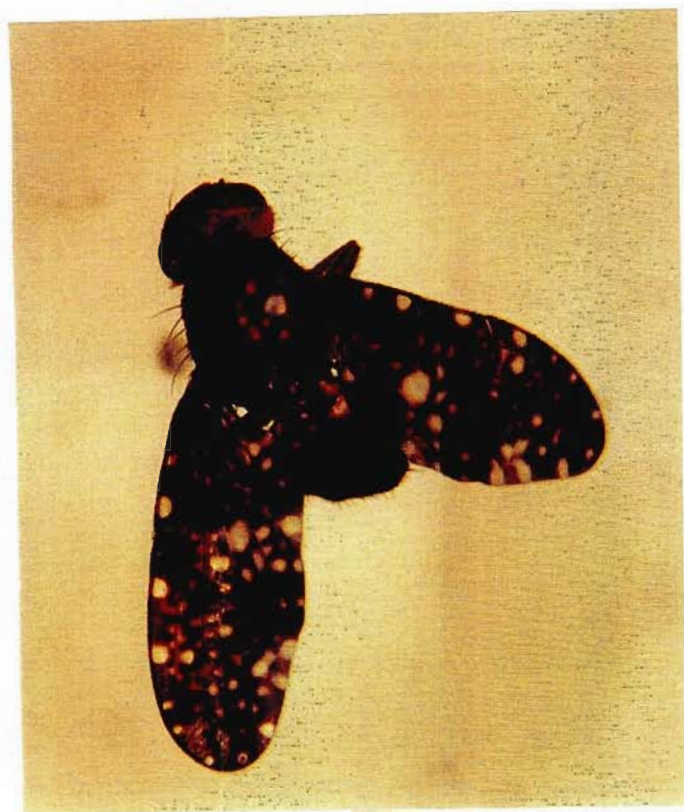
Fig. 6.1. *Parafreutreta regalis* (7.5X)

Fig. 6.2. *Parafreutreta conferta* (7X)

Fig. 6.3. *Parafreutreta felina* (6.9X)
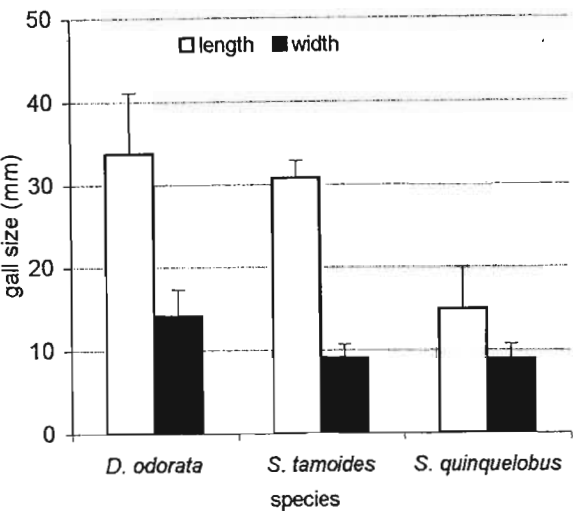
Fig. 6.4a. Average size (length and width:mm) of galls.
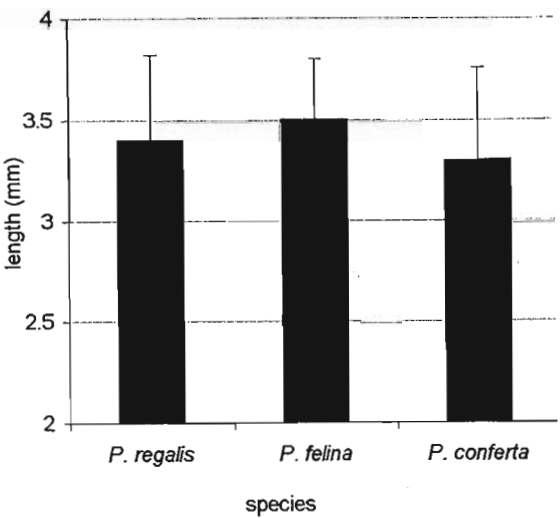
Fig. 6.4b. Average length (mm) of pupae of *P. regalis*, *P.felina* and *P. conferta*.
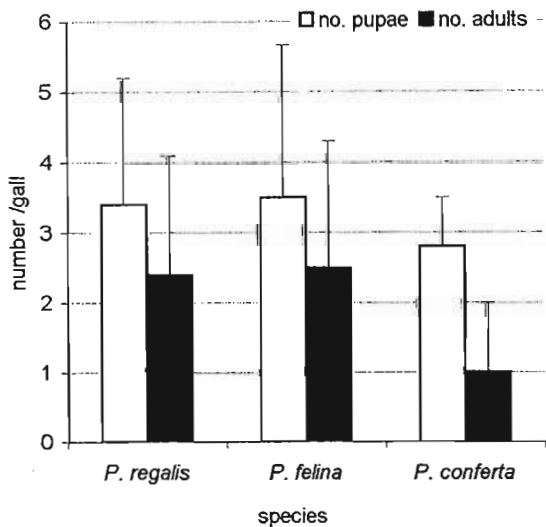
Fig. 6.4c. Average no pupae and adults per gall.
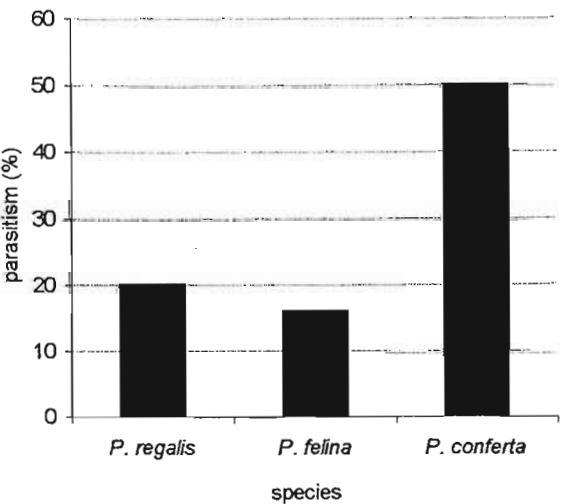
Fig. 6.4d. % parasitised galls.

Figs 6.4 a-d. Data recorded form galls collected on *D. odorata* (n=20), *S. tamoides* (n=6) and *S. quinquelobus* (n=12) for the respective *Parafreutreta* spp. All values represent the mean, error bars indicate standard error.

Fig. 6.5. Fungal infected galls (12X).

investigation, the galls formed by *P. regalis* on *D. odorata* were the largest. They are soft, irregular swellings which may occur on the stem apex (acrocecidia), at a distance from the growing tip (up to 20 cm; pleurocecidia) or on the buds in the axils of the leaves. Up to eight adults emerged from a single gall. The galls formed by *P. felina* on *S. tamoides* are more elongate and woody than those of *D. odorata* and were frequently found to contain fungal infections. The smallest galls were those formed by *P. conferta* on *S. quinquelobus*; no more than three adults emerged from a single gall. These galls were very woody and usually rounded in appearance. Generally, there seemed to be a definite tendency for larger galls to develop on younger, more succulent growth, typical of the fast growing stolons of *D. odorata*. If the plants are not growing quickly, the galls tend to be small with only one or two larvae.

Most species of *Parafreutreta* form "communal" galls where a few larvae develop together in the same cavity (Munro, 1940; Freidberg, 1984). In this study, a maximum of nine puparia were recorded from a single gall on *D. odorata*. The puparia of *P. regalis* are sub-translucent yellow and distinctly different from those of *P. felina*, which are glossy opaque black (Figs 6.6 & 6.7). The puparia of *P. conferta* are opaque yellow, the anterior end darkened, sometimes brown or almost black. According to Munro (1953) a curious aspect of the genus *Parafreutreta* is that some of the species seem to form pairs, where each of a pair of adults is very like the other but there is a marked difference in the shape and colour of their puparia; e.g. *P. regalis* and *P. conferta*.

Two species of parasitic Hymenoptera emerged from the *P. regalis* galls, both are species of Braconidae (Figs 6.8 a & b). These same species were collected from the *P. conferta* and *P. felina* galls on *S. quinquelobus* and *S. tamoides*, respectively. Up to 20 % of the *P. regalis* galls were parasitised. More galls will need to be collected to arrive at a more confident estimate of the percentage parasitism.

Laboratory investigations

In order to investigate aspects of the mating and oviposition behaviour of *P. regalis*, young adults emerging from mature galls were transferred to glass cages containing one or two potted *D. odorata* plants. Soon after this, *i.e.* within five to ten minutes, mating was initiated. Before actually mating, however, both male and female engaged in a courtship dance where the male appeared to chase the female. Throughout this dance the adults continuously flexed their wings

Fig. 6.6. Sub-translucent *P. regalis* puparia in the pith of a gall (14X).
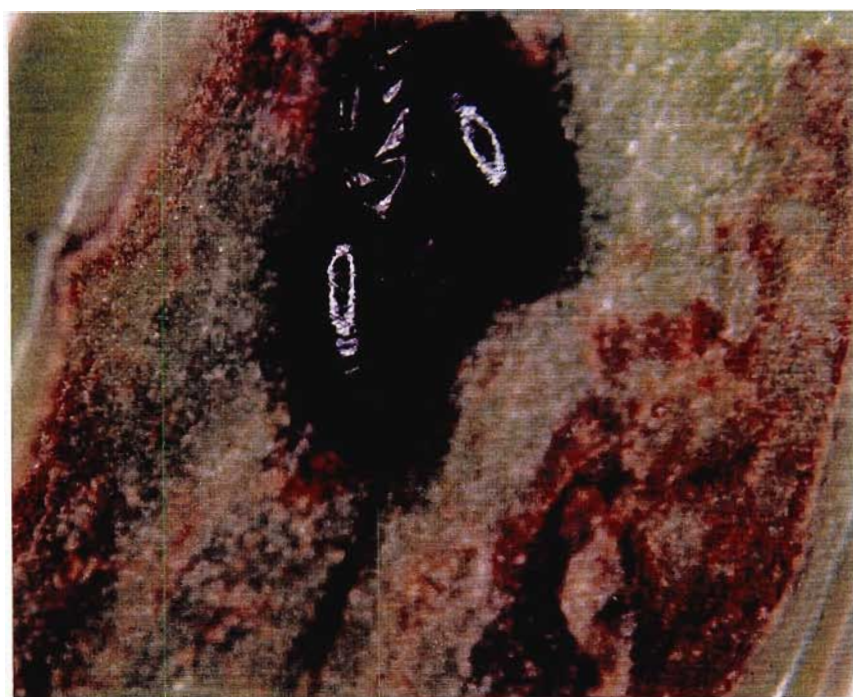
Fig. 6.7. Glossy black puparia typical of *P. felina* (12.5X).

Figs 6.8 a & b. Parasitic Hymenoptera (Braconidae) collected from *D. odorata* galls (7.5X).

and moved in semi-circular patterns. This ritualistic "rendezvous - behaviour" has been observed in other tephritid species and is associated with host plant recognition (Zwolfer, 1974; Freidberg, 1984; Harris, 1989). Meeting at the specific "rendezvous" point has been shown to be an important reproductive isolation mechanism for eight *Urophora* species (Tephritidae) that, deprived of their hosts, mate in various interspecific combinations which are rarely observed in the field (Zwolfer, 1974; Freidberg, 1984).

Following "rendezvous-behaviour" the flies mated for up to 30 minutes and within an hour the female oviposited on young, meristematic, apical shoots. The females frequently oviposited on all available growing tips, including axillary bud regions, for a period of 10-20 minutes. On a few occasions they were observed to oviposit on mature leaves and stems. However, this was usually towards the end of the breeding term and no galls from these regions were recorded. Oviposition continued for up to 10 days after emergence, but generally terminated after one week. In the laboratory breeding trials, adult longevity was approximately 14 days (Table 6.2). A similar period for adult survival has been recorded for *Procecidochares utilis* Stone, also a stem galler, belonging to the family Tephritidae (Bess and Haramoto, 1959; Dodd, 1961; Bennett, 1986).

The eggs were preferentially deposited between the youngest pair of leaves at the tip of a vegetative stem or near the axillary buds. Unfortunately, the number of eggs laid by the female on each apical tip was not recorded in this study. Early in the investigation it was hoped that it would be possible to rear enough flies to carry out more detailed life history and host-specificity studies; thus any possible source of damage to the young eggs, which may have been inflicted by counting under a light microscope, was avoided. However, due to time limits and difficulties experienced both in finding the galls in the field and in maintaining them in the greenhouse, it was not possible to continue with further investigations. Although not recorded in this study, larvae of gall forming Tephritidae usually hatch from eggs within several (3-8) days and the newly hatched larvae then enter the stem tissue (Freidberg, 1984).

Galls developed on all of the apical points of the potted *D. odorata* plants which were exposed to *P. regalis*. Only one or two galls developed on the axillary buds. Weekly measurements, made on the width and length of the developing galls, allowed data to be collected on the rate of gall development and duration of the larval and pupal life stages (Figs 6.9 a & b; Table 6.2). In

Table 6.2. Life history data collected for *P. regalis*. All measurements, except that of adult activity, based on data collected from galls initiated and raised to maturity in the laboratory (n= number of galls).

Stage in life cycle (n)	No. days/months (±s.d.)
Tot. incubation period (15)	55 (19)
Larva (10)	30.6 (3.7)
Pupa (10)	17 (3.1)
Adult longevity (10)	14 (3.2)
Adult activity (60)	September-June
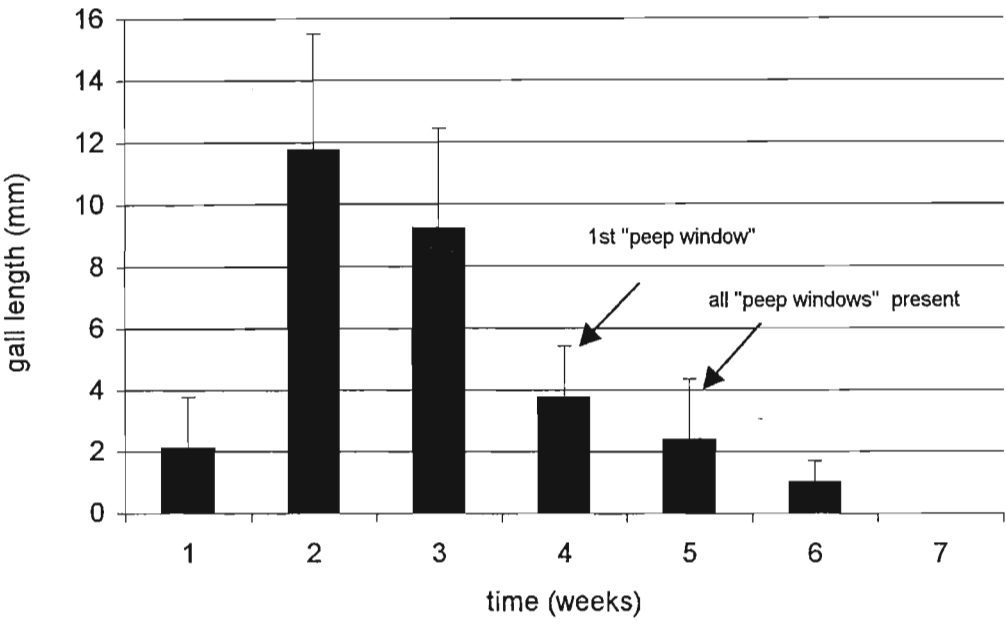
Fig. 6.9a. Weekly increase in length (mm) of developing *D. odorata* stem galls.
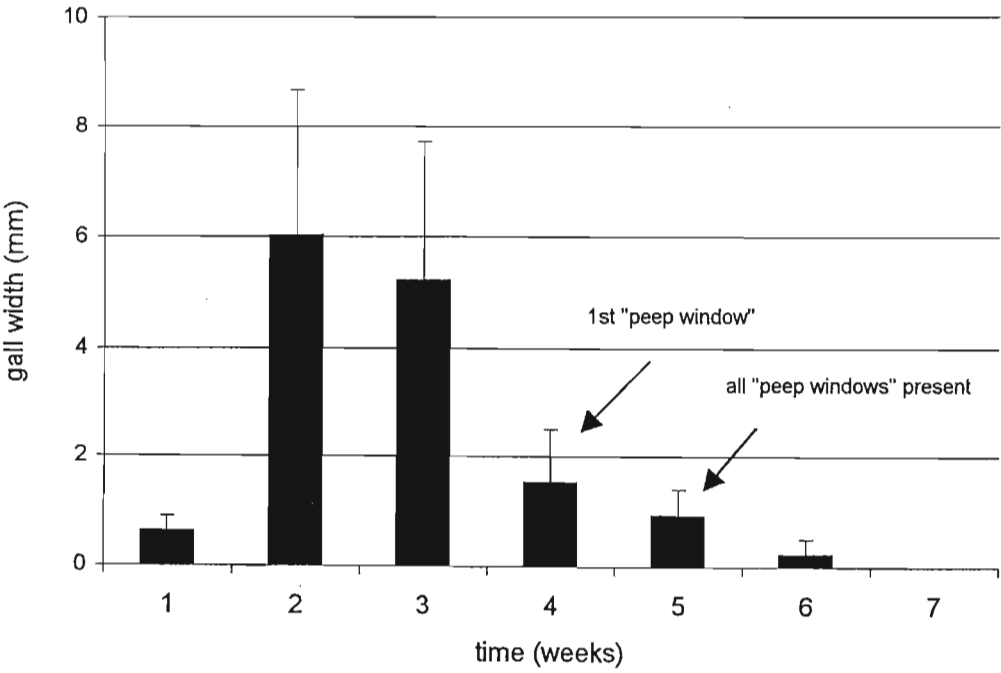
Fig. 6.9b. Weekly increase in width (mm) of developing *D. odorata* stem galls.

all cases the galls grew rapidly, some producing visible swellings within seven days of oviposition. Maximum increase in gall size occurred two to three weeks after oviposition, thereafter growth decreased rapidly terminating during the sixth week. Other studies on the life cycle of tephritids have shown that the larvae commenced feeding almost immediately after hatching and continued until they were fully grown (Dodd, 1961; Freidberg, 1984). This is corroborated in *P. regalis*. Before pupating the larva prepared an exit for the young adult, sometimes referred to as a "peep window" or an "emergence window" (Bess and Haramoto, 1959; Freidberg, 1984). The larva scraped a certain spot in the wall of the gall, leaving only a thin layer through which the young adult can escape. For *P. regalis* all "peep windows" were formed between weeks four and five, indicating that the larvae had entered into the pupal stage (Figs 6.9 a & b). Although a maximum of five "peep windows" were observed on a single gall, all adults emerged from a single window - presumably made by the first larva to pupate (Fig. 6.10). Furthermore, for all of the mature galls collected in the field (n=55) only one peep window per gall was recorded and all adults emerged from this opening. Approximately two weeks after the appearance of the "peep-windows" the adults of *P. regalis* emerged.

Parafreutreta regalis galls have been collected from *D. odorata* populations, at Dargle, Ingele and Ferncliffe, almost throughout the year, except during the very dry months of July and August when the plants undergo severe die-back. The results from this study indicated the average time to adult emergence to be 55 days. However, a minimum period of 46 days (24/10/98-9/12/98) and a maximum 101 days (22/05/98-31/08/98) were recorded. It is possible that temperature and time of year affected the duration of the life-cycle. Further data needs to be collected to confirm this observation.

Light microscopy : the anatomy of stems and the effects of galling thereon
The anatomy of young stems of *D. odorata* is typical of herbaceous dicotyledons (Fig. 6.11) (see Esau, 1960). The vascular system is eustelic. Individual vascular bundles are separated from one another but are mechanically strengthened by a band of collenchyma. There are approximately 12 vascular bundles in young stems.

Mature stems of *D. odorata* develop secondary thickening. Secondary growth results from activity of the vascular cambium and increases the amount of vascular tissues in stems. It

Fig. 6.10.   The mature larva scrapes away a spot on the wall of the gall leaving a window through which the young adult can emerge. Generally only one "peep window" is formed in the *P. regalis* galls (10X).
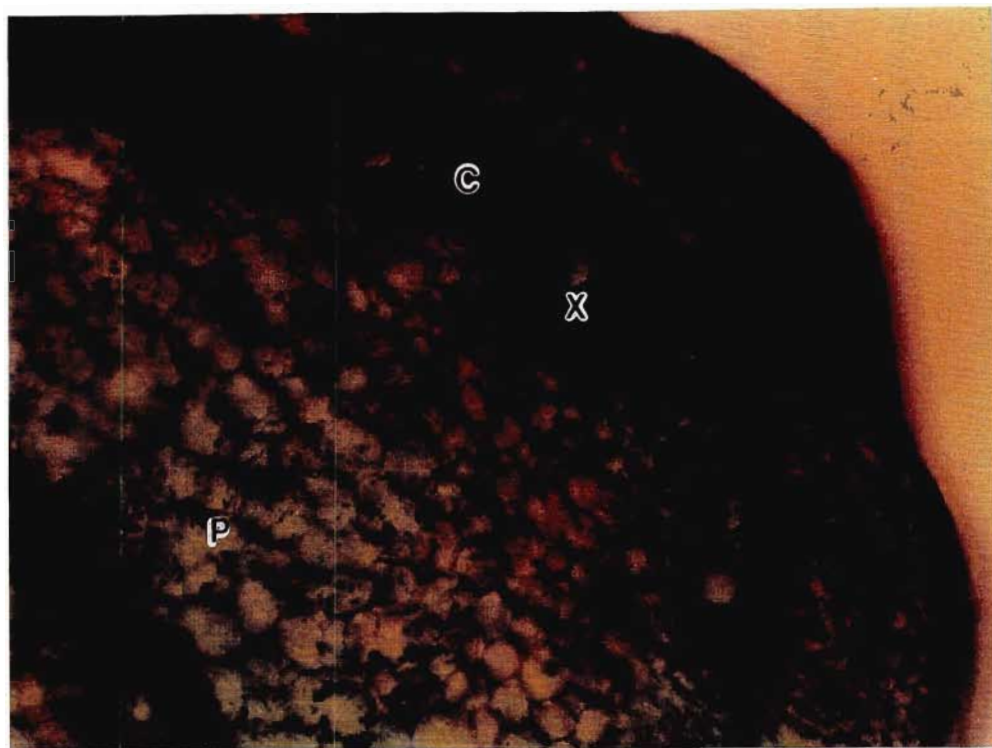
Fig. 6.11. Transverse section (T/S) through a young *D. odorata* stem (150X).

C =band of collenchyma   X= 1° xylem   P=pith
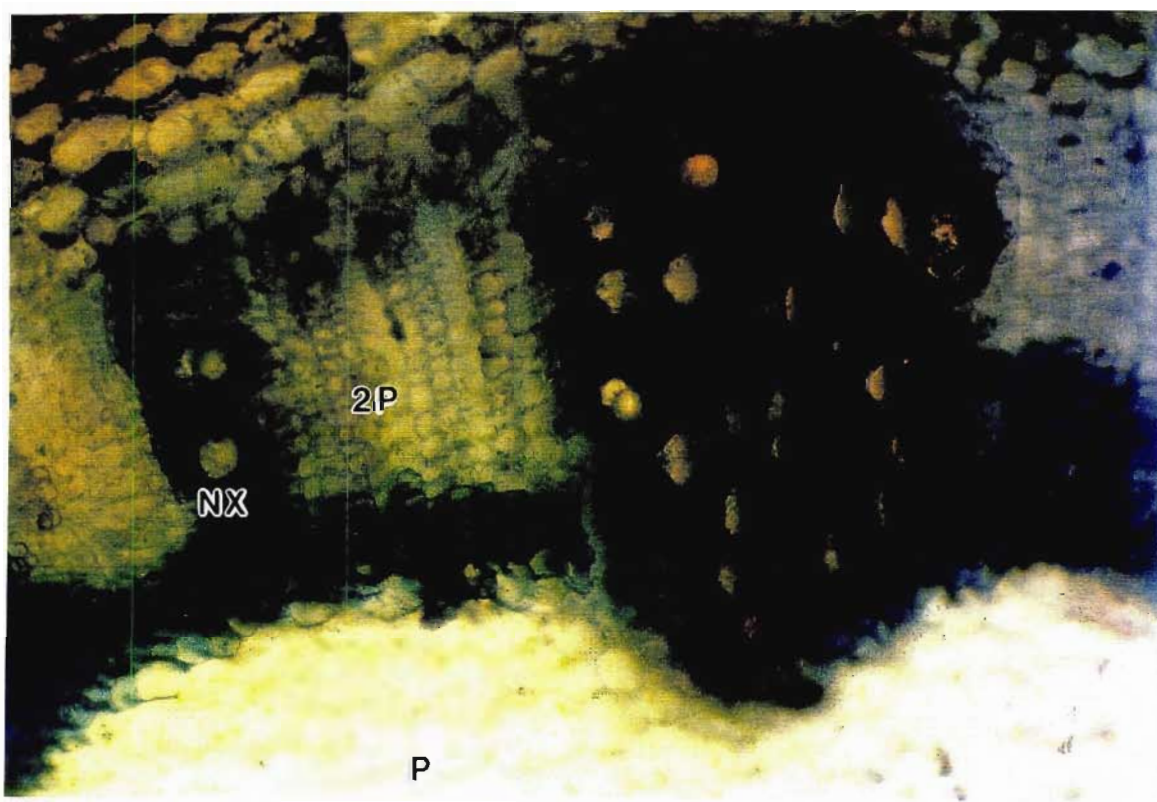
Fig. 6.12.   T/S of a mature *D. odorata* stem showing "anomalous secondary thickening" (175X).

NX=new xylem P=pith   2P=secondary parenchyma produced by the cambium

contributes to the thickness of the stem axis but not to its length (Esau, 1960). In terms of secondary growth, vines, such as *D. odorata*, differ from non-climbing plants and are characterized by the presence of "anomalous secondary thickening" (Carlquist, 1991) (Fig. 6.12). In *D. odorata*, the vascular cambium originates in the fascicular and interfascicular regions (as with normal stems) and becomes continuous. However, the interfascicular cambium produces secondary parenchyma so that wide parenchymal rays are formed in continuity with the fascicular regions and the vascular tissues are separated into wedge-like blocks with secondary parenchyma in between (Fig. 6.12). New wedges of vascular tissue may differentiate in the parenchymal rays. In *D. odorata*, young stems have approximately 12 vascular bundles while mature stems, with secondary growth, have up to 22 wedges of vascular tissue. It has been hypothesised, and proved biomechanically, that the anomalous arrangement of secondary tissues in vines greatly increases the mechanical flexibility of stems and prevents breakage when stems are twisted or coiled (Carlquist, 1991).

The larvae of *P. regalis* bore into the meristematic regions of stems and an increase in stem girth occurs, *i.e.* "gall formation", as soon as the larvae begin feeding. Tephritid larvae generally feed by slashing at tissue with their mouth-hooks and eating the cell debris (Fig. 6.13) (Christenson and Foote, 1960). Increase in the size of galled stems is the result of proliferation of the parenchymal tissue in the pith of the stem. During gall formation this tissue remains highly meristematic. Bennett (1986) noted that during the development of stem galls on *Ageratina adenophora*, formed by *Procecidochares utilis*, increase in volume of the pith region was often so great that there was lateral stretching of the cells. This was observed in the present study (Fig. 6.14). Rapid multiplication and proliferation of the cells in the pith region has been associated with high levels of cytokinins (Engelbrecht, 1971; Elzen, 1983; Lalonde and Shorthouse, 1984; Bennett; 1986). The larvae feed on the nutritive tissues of the extended pith until they pupate, at which stage cell division ceases (Bennett, 1986). Light microscopy of galled stems showed that as well as causing proliferation of pith cells, galling also causes a disruption of stem tissue below the gall (up to 3 cm) and adjacent to the gall (where galling occurs on axillary meristems) (Fig. 6.15 & Fig. 6.16). This tissue disruption may be associated with high levels of endogenous cytokinins.
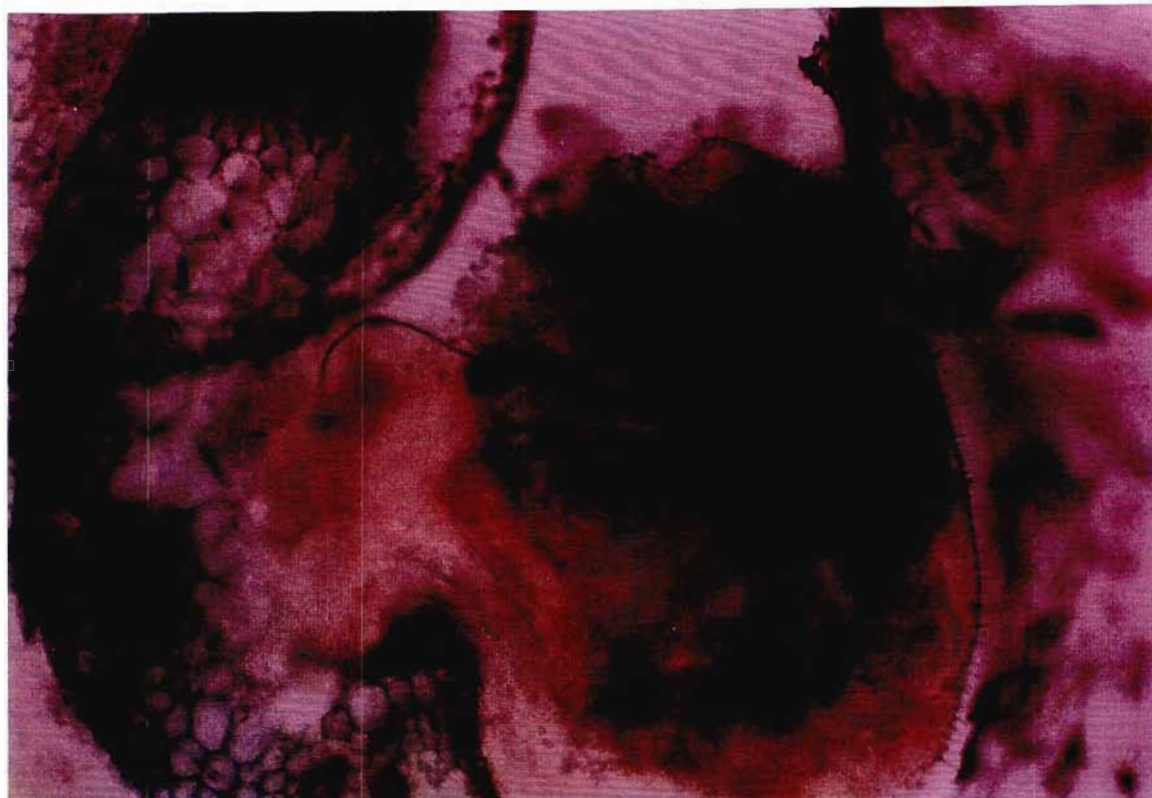
Fig. 6.13. T/S of a gall showing tephritid larva feeding on nutritive tissue. Note disruption of collenchyma tissue and vascular bundles (185X).
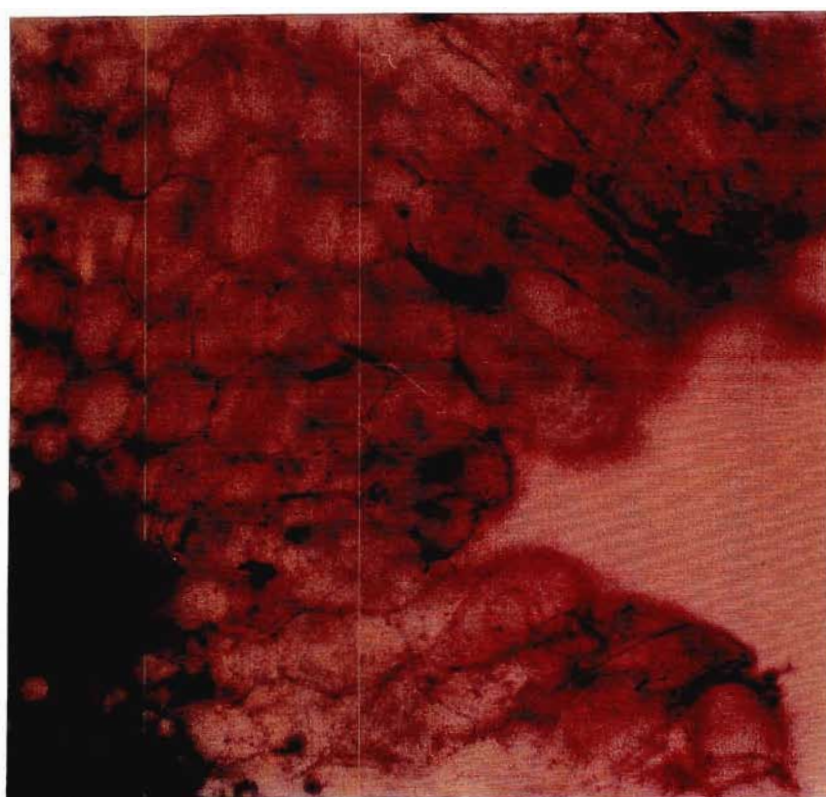
Fig. 6.14. T/S of galled stem tissue showing the highly meristematic pith region and lateral stretching of cells (167X).
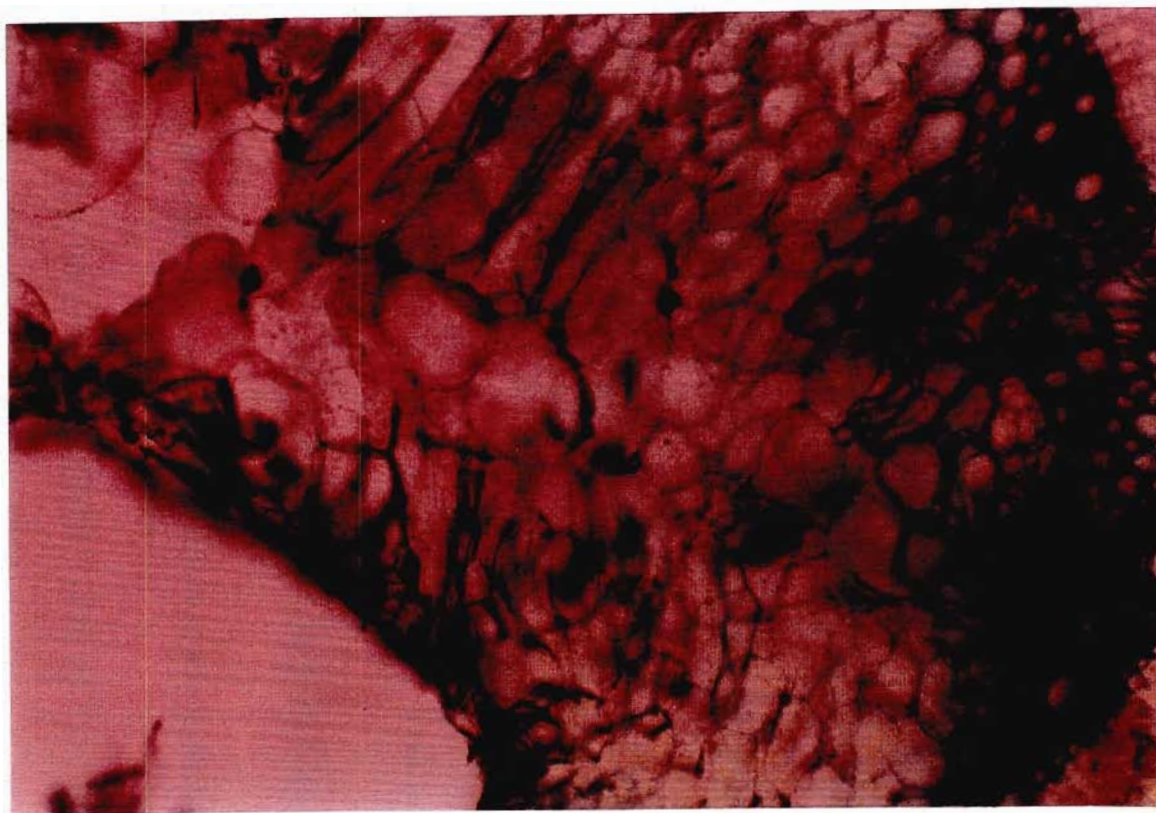
Fig. 6.15. T/S of stem tissue 3 cm below the gall (170X).
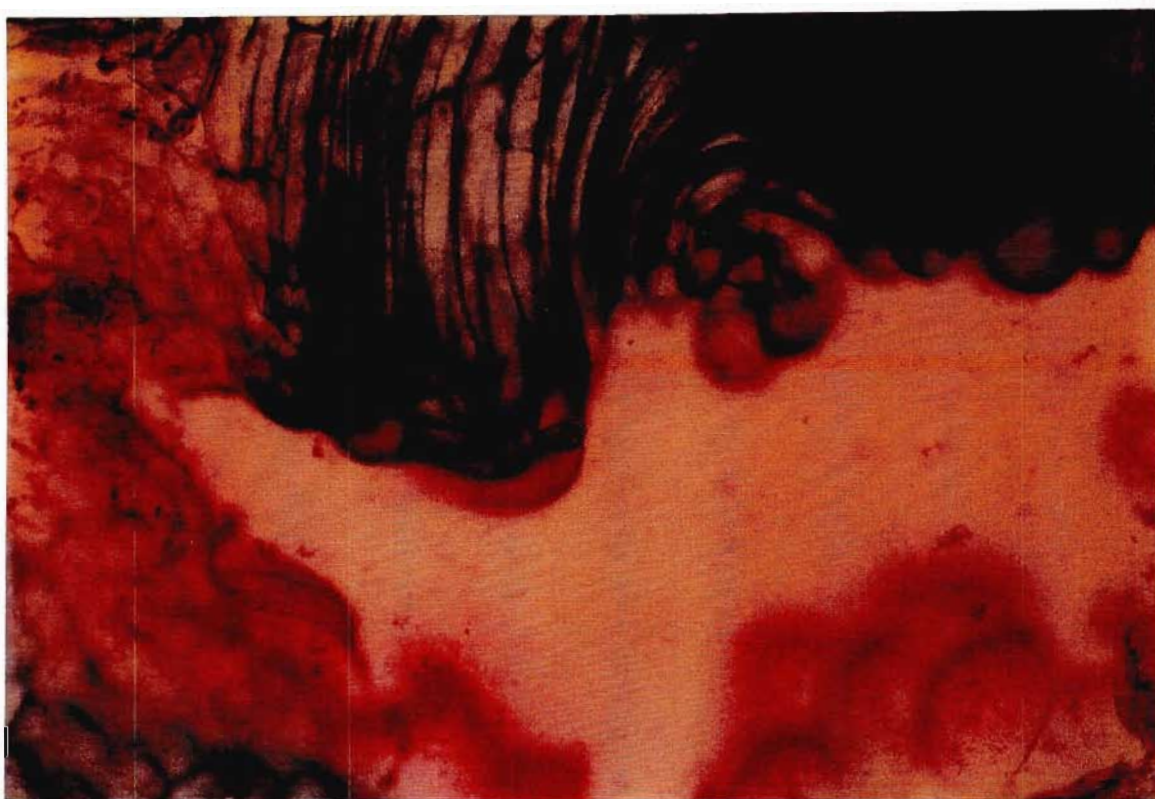
Fig. 6.16. T/S of stem adjacent to gall (when galling on axillary meristems) showing the disruptive effect of the gall (165X).

No stems with previous season's galls were ever found in the field. It is possible that these stems suffer from indirect injury, through galling, and are weakened. This could be facilitated in a number of ways. Firstly, galling causes severe disruption of the pith parenchyma and, in addition to this, once the adult flies have emerged from the gall, a large central pith cavity remains. Disruption of the pith parenchyma may have adverse side affects on the surrounding conducting tissues. If the xylem requires surrounding living tissues to act as an effective conduit then any large disruptions in the stem integrity may cause embolisms in the xylem resulting in water stress of areas distal to that point. Furthermore, during secondary thickening, when there is an increase in conducting tissues, this effect would be compounded - especially as the secondary parenchyma (produced by the interfascicular cambium) cannot replace the pith parenchyma. Secondly, emergence of the adult flies leaves a broken epidermis on the surface of the gall. This provides a site for the entry of other insect predators and fungal and bacterial pathogens which could kill the stem. Thus *P. regalis* may reduce perennation of *D. odorata* stems due to induced water stress and/or infection. Since no stems with previous season's galls were ever found in the field this seems highly probable.

6.2.4 Concluding comments

The genus *Parafreutreta* comprises 14 species all of which are cecidogenous on species of *Senecio* and their close relatives (Munro, 1952; Munro, 1953; Munro, 1957). Restriction of *Parafreutreta* to almost a single host genus, *Senecio*, as opposed to a tribe or family, indicates good specificity within this group, a valuable asset if this species is to be considered as a biocontrol agent. Furthermore, the genus *Parafreutreta* and its host plants are entirely restricted to south-central Africa (Munro, 1953; Freidberg, 1984). The lack of closely allied *Senecio* spp. in exotic locations may improve the specificity of *P. regalis* in these regions (and decrease the likely occurrence of fly predators). If the distribution of *P. regalis* in southern Africa is limited to areas where *D. odorata* occurs, then this is also a good indication of the host-specificity of this species. Presently it is not possible to map the distribution of *P. regalis* as only the type specimens (collected at Cedara, Natal) are lodged in the national collection at the Plant Protection Research Institute (Mansell, pers. comm.), and only four paratypes at the Natal Museum, Pietermaritzburg (Barraclough, pers. comm.).

Plant galls are active physiological sinks and can divert nutrients from other parts of a plant (Faucroy and Braun, 1967; Billet and Burnett, 1978; Bennett, 1986). The phenomenon of galls acting as nutrient sinks is an important component in the success achieved in programmes of biological control. If a gall can direct nutrients towards itself, or merely intercept nutrients from the translocation stream, then this could be to the detriment of the plant host. Bennett (1986) found that the galls of *Procecidochares utilis* on *Ageratina adenophora* acted as nutrient sinks, causing a significant reduction in flowering, achene production and vegetative growth. Other studies have recorded the adverse affects of galling on plant fitness (Bess and Haromoto, 1959; van Staden, Davey and Noel, 1977; Dennil, 1985). Based on the results of those studies, it can be predicted that, under heavy attack, galling of stems of *D. odorata* by *P. regalis* is likely to have a detrimental effect in the form of a reduction in sexual reproduction and vegetative growth. This prediction needs to be empirically tested.

Lastly, although tephritids have frequently been employed as biological control agents against weeds of the Asteraceae, despite one or two outstanding examples, their overall success as control agents is debatable (see Crawley, 1989; Harris; 1989; Edwards *et al.*, 1996). Poor establishment and lack of successful control by tephritids has partly been attributed to high rates of parasitisation in the new environment. It is often an expectation of biological control programmes that introduced insects will experience a lower level of parasitism than in their native environments, and thereby have a greater impact on their host plant (Edwards *et al.*, 1996). However, there remains the possibility that, in the new environment, native parasitoids will adapt to the novel host, and many examples exist to illustrate that this can occur (Cornell and Hawkins, 1993; Edwards *et al.*, 1996). Previous studies on parasitoids associated with tephritids have shown that ecological factors may be more important in determining host-parasitoid associations than phylogenetic factors, in particular the taxonomic relatedness of the tephritids (Hoffmeister, 1992). Parasitism of gall tephritids has been reported in the literature numerous times (Dodd, 1961; Freidberg, 1984) and is frequently associated with larval and pupal mortality - the most important factor regulating populations of gall tephritids (Freidberg, 1984). The following are the most important families of parasitic wasps recorded from tephritid galls: Ichneumonidae; Braconidae; Cynipidae; Eulophidae; Eupelmidae; Eurytomidae; Pteromalidae and Torymidae (Freidberg, 1984). Only two species of Braconidae were found to be associated with *P. regalis*. If there are any more natural parasitoids of this species, this will need to be determined through

extensive collection of galls of *P. regalis* from *D. odorata* across its southern African range.

If *P. regalis* is to be considered as a biocontrol agent predictions on potential parasitoids in California, Hawaii or Australia should be made. To do this a comprehensive sampling programme to survey the native parasitoids of *P. regalis* in southern Africa must be carried out to determine the species involved and their relative importance. Furthermore, a sampling programme of the parasitoids of related tephritid species occurring in the region of introduction, e.g. California, must be carried out. Based on the current status of knowledge of tephritid-parasitoid complexes, this information may give some indication as to the likelihood of parasitisation occurring in the new environment. A similar method was used by Edwards *et al.* (1996) to predict the likelihood of parasitoids attacking three *Mescolanis* species, seed head tephritids of *Chrysanthemoides monilifera* Norlindh, considered as potential biological control agents for this species in Australia.

Chapter 7

Pyrrolizidine alkaloids

7.1 Introduction

Biological activity of pyrrolizidine alkaloids

The association of plant secondary metabolism with plant-herbivore and plant-pathogen interactions is well documented (for review see Fritz and Simms, 1992; Romeo *et al.*, 1995; Hartmann, 1996; Hartmann, 1999). Secondary metabolites have been found to render plants less susceptible to herbivore attack, acting as deterrents or antifeedants (Vrieling and van Vyk, 1994). The pyrrolizidine alkaloids (PAs), a class of typical secondary compounds, are thought to serve as protective chemicals in plant-herbivore interactions (Molyneux and Ralphs, 1992; Hartmann *et al.*, 1997; Hartmann, 1999). PAs are strong feeding deterrents for most herbivores; they are hepatoxic to vertebrates (Molyneux *et al.*, 1979; Luthy *et al.*, 1981; Johnson *et al.*, 1985; Mattocks, 1986; Bicchi *et al.*, 1989; Witte *et al.*, 1993) and mutagenic to insects (Frei *et al.*, 1992).

Although plants with PAs are usually avoided by generalist herbivores, a number of insect species from diverse taxa (for example, Lepidoptera, Orthoptera, Homoptera and Coleoptera) have evolved adaptations not only to cope with these compounds but also to use them to gain protection from enemies (Mattocks, 1986; Witte *et al.*, 1993; Hartmann, 1999). These insects are usually host-specific and may use plant chemicals to locate or identify their host plants (van der Meijden, 1996). Their sexual behaviour may also be integrated in a variety of ways with host plant chemistry (for review see Landolt and Phillips, 1997). Specialist herbivores often meet, court and mate principally or exclusively on specific host plants (Landolt and Phillips, 1997). Certain insects sequester or otherwise acquire host-plant compounds and use them as sex pheromones or sex pheromone precursors. For example, certain butterflies of the subfamily Danaidinae sequester PAs from larval food plants and, as adults, use them as defense substances or pheromones (Rothschild, 1973; Hartmann and Zimmer, 1986; Mattocks, 1986). Host plant chemistry thus plays an important role in the feeding and sexual communication of a number of phytophagous insects. Since PAs are frequently implicated in such interactions the PA profile of many plants is an important consideration in the determination of feeding patterns and host ranges of natural enemies, particularly that of specialist herbivores.

Chemistry of the pyrrolizidine alkaloids

The PAs encompass a diverse group of about 360 structures with a restricted occurrence in certain higher-plant taxa. They were first isolated from the genus *Senecio* (Compositae) but they have also been found in the Santalaceae, Boraginaceae, Rhizophoraceae, Poaceae, Orchidaceae and Fabaceae (Robins, 1982; Hartmann, 1996; Hartmann *et al.*, 1997). The PAs are ester alkaloids consisting of a necine base (amino alcohol moiety), which is esterified to a necic acid (acid moiety) and are frequently accompanied in the plant by a variable proportion of their N-oxides. They may occur as monoesters, open chain diesters or macrocyclic diesters (Mattocks, 1986; Witte *et al.*, 1993). Esters of unsaturated necine bases (having a 1,2 double bond, e.g. retronecine) are referred to as "unsaturated PAs", whereas PAs with a saturated necine moeity (e.g. platynecine) are called "saturated PAs", even if there is unsaturation in the acid moiety (Mattocks, 1986) (see Base A & B). A plant does not usually contain a single PA, more often several alkaloids are present and these may vary considerably within a plant species with stage of growth, season and provenance (Borstel *et al.*, 1988; Witte *et al.*, 1993; van Dam and Frieling, 1994). Molyneux and Ralphs (1992) suggested the biosynthesis of a number of alkaloids to be a response to different insect enemies.

The present study

One of the most important considerations in biological control is the host-specificity of the control agent as it is important to demonstrate that on introduction to a new area it will not damage plants of economic or ecological importance (Huffaker, 1964; Zwolfer and Harris, 1971; Wapshere, 1974; van Driesche and Bellows, 1996). Since host-specificity in insects is largely dependent on host-plant morphology (e.g. presence of spines or burrs) and/or chemistry (e.g. alkaloids or essential oils), prior to any biological control programme it is equally important to investigate the degree to which the host plant is characterised by the presence of ecotypes or biotypes. Extensive intraspecific variation in the target weed can make biological control difficult as the ecotypes may differ in their susceptibility to the biocontrol agent (Andres *et al.*, 1976; McClay, 1989; Shepherd, 1993). Furthermore, it may be difficult to identify effective biocontrol agents for a particular ecotype, and if several forms are present, several biotypes of the control agent may have to be introduced (McClay, 1989; Dennil and Hokkanen, 1990; van Dreische and Bellows, 1996).

One of the aims of this study was to assess the suitability of *D. odorata* to biocontrol. Since several studies have already been conducted on the PAs of this species (Adams and Gianturco,1956; Culvenor and Geissman, 1961; Stelljes and Seiber, 1990; Stelljes *et al.*, 1991) a study of the profile of these compounds for *D. odorata* across its native range will enable a comparison of alkaloids to be made. If *D. odorata* is characterised by a high degree of variation in alkaloid profile this may affect the complement of host-specific enemies associated with different populations. Furthermore, perhaps the breeding behaviour in exotic locations favours the formation of genetically isolated populations which may preserve specific or unique chemical characters. If so, this could have important implications for a biological control programme.

The most extensive investigation into the alkaloids of *D. odorata* was made by Stelljes *et al.* (1991) on plants collected in California. Using GC-MS these authors detected the presence of 12 PAs in *D. odorata* (Table 7.1).

7.2   Materials and methods

7.2.1 Development of an analytical technique

Before an analysis of the PAs of *D. odorata* could be undertaken, literature and laboratory investigations had to be carried out to determine a suitable extraction and isolation method for these compounds.

Extraction and isolation

The PAs may be extracted either with methanol or dilute acid. Methanolic Soxhlet extraction is commonly used (Mattocks, 1967; Molyneux *et al.*, 1979; Luthy *et al.*, 1981; Johnson *et al.*, 1985; Hartmann and Zimmer, 1986; Witte *et al.*, 1993; Krebs *et al.*, 1996) and has been recommended as the first general procedure for complete extraction of a PA source of unknown composition. Typically, methanolic extraction is used for the extraction of alkaloids from large samples (200 g-1 kg) (Koekemoer and Warren, 1951; Hartmann and Zimmer, 1986; Krebs *et al.*, 1996). For smaller samples (<10 g) and routine preparation of many samples, aqueous acidic extraction is recommended (Witte *et al.*, 1993). As PAs occur both as their water soluble N-oxides and as tertiary bases, an excess of zinc dust must be added to the acidic extract to convert all the N-oxides into their GC-volatile tertiary bases. Following extraction, an Extrulet® column or a solvent partitioning method is used to isolate the alkaloids from the crude extract (Hartmann and Zimmer, 1986; Hartmann and Toppel, 1987; Stelljes and Seiber, 1991; Biller *et al.*, 1994;

Table 7.1. Pyrrolizidine alkaloid (PA) profile for *Delairea odorata* as determined by GC-MS (Stelljes *et al.*, 1991). Type refers to the alkaloid properties (type of necine base). R: retronecine P: platynecine M: monoester D: diester.

Alkaloid	Type	Percent of Total PA
anhydroplatynecine	-	6
7-tiglylretronecine	RM	trace amount
9-tiglylretronecine	RM	22
7-angelylplatynecine	PM	4
9-angelylplatynecine	PM	39
7-tiglylplatynecine	PM	4
9-tiglylplatynecine	PM	8
sarracine	PD	5
sarranicine	PD	7
triangularicine	RD	1
neosarracine	PD	2
neosarranicine	PD	4

Hartmann *et al.*, 1997). The Extrulet® method is suitable for extraction and quantification of PAs from small amounts of plant material. However, it may not be quantitative if very polar PAs (free necine bases and esters with more than 2-3 free hydroxyl groups) are involved (Witte *et al.*, 1993). Partitioning against an organic solvent has been used by a number of investigators to isolate alkaloids (Hartmann and Zimmer, 1986; Stelljes and Seiber, 1990; Stelljes *et al.*, 1991; Witte *et al.*, 1993). Following zinc reduction, the chlorophyll and waxes are removed by extraction of the aqueous acidic extract with petroleum benzene and/or diethyl ether. After basification of the acidic extract, the alkaloids are extracted into dichloromethane. The solvent is then removed and the residue resuspended in methanol.

Preliminary studies indicated acidic extraction combined with organic solvent partitioning to be the most reliable and practical methods with the highest yield of alkaloids.

Separation and identification of alkaloids

Techniques for separation and qualitative and quantitative analysis of the PAs include: paper, gas and thin layer chromatography, high performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS), spectrophotometry and carbon-13 NMR (nuclear magnetic resonance) spectroscopy (Chalmers *et al.*, 1965; Mattocks, 1967; Molyneux *et al.*, 1979; Luthy *et al.*, 1981; Mattocks, 1986; Roeder, 1990; Krebs *et al.*, 1996). Because of the variation in structural properties of alkaloids, some techniques are better applied to some groups of PA's than others. Spectrophotometric determination of alkaloids is a sensitive and reliable method but it is only effective for the identification and quantification of PAs with an unsaturated necine moiety. Capillary gas chromatography combined with mass spectrometry (GC-MS) is the most widely used technique for the analysis of PA mixtures (Hartmann and Toppel, 1987; Biller *et al.*, 1994; Witte *et al.*, 1993). When used in combination with the selective and sensitive nitrogen detector (NPD - Nitrogen Phosphorus specific Detector) GC-MS is a powerful high resolution technique (Hartmann and Zimmer, 1986). The retention indices (RI) in combination with $[M]^+$ and type specific MS fragmentation patterns can be used for identification of most PAs, including geometrical isomers.

Both NPD-detection and GC-MS were used for the identification of alkaloids. Furthermore, since Stelljes *et al.* (1991) had detected the presence of 12 alkaloids in *D. odorata* (collected in California) with GC-MS, it was assumed the present study would provide comparable data.

7.2.2. Methods used for the extraction, isolation and identification of alkaloids

Sample collection and preparation

To detect any differences in the alkaloid profile of local populations, plants from three locations across Natal (Harding, Dargle and Pietermaritzburg) were included in this study (plants were collected from two sites at each locality) (Table 3.1 lists the altitude and coordinates of each location). All plant material used in this analysis was collected in late May (1999) when *D. odorata* was in full bloom. To account for any diurnal variation in alkaloid accumulation plants were harvested between noon and 1:00 pm. The plant material, collected from each site, was separated into leaves, stems and flowers, air dried and then frozen (-18 °C) until analyses started.

Alkaloid extraction and isolation

The plant material (10 g dried leaves/stems/flowers) was ground in a Wareing Blender for 3-5 minutes in 100 ml 0.5 N HCl, and extracted overnight with occasional stirring. After filtering the extract through glass wool, an excess of zinc dust was added to reduce all N-oxides to their corresponding tertiary bases. To allow for reduction, the mixture was stirred for 8-10 hours at room temperature. After re-filtering through glass wool, to remove zinc dust, 20 ml of the aqueous acidic phase was extracted five times with 60 ml diethyl ether and subsequently alkalinized with 5 ml saturated aqueous $NaHCO_3$. This basic aqueous phase was extracted four times with 60 ml dichloromethane . The solvent was evaporated under vacuum at 40 °C and the residue redissolved in 3 ml methanol.

Gas-Chromatography (GC)

GC analysis was carried out using a Varian 3300 instrument fitted with a Nitrogen-Phosphorus specific Detector (NPD) and a 25 m, BP-5 bonded column (SGE, Australia) of 0.53 mm internal diameter and 1μm film thickness. The injector temperature was set at 240 °C and the detector at 300 °C. The attenuation of the detector was set at 4×10^{-12} mV/V. Integration of the detector was by a Hewlett Packard 3395 integrator. The temperature programme for the analysis of samples was: 100 °C-170 °C at 15 °C/min, 170 °C-250 °C at 8 °C/min followed by an 8 minute hold at 250 °C.

Standard curve

The chromatograms were evaluated quantitatively using an external standard. Since pure samples of the alkaloids known to occur in *D. odorata* could not be obtained (limited funds) hyoscyamine

($C_{17}H_{23}NO_3$) was used to construct a standard curve. Hyoscyamine contains a single nitrogen in the molecule as do the PAs, and the detector response is therefore comparable.

A standard curve of detector response vs concentration was prepared for a hyoscyamine dilution series. Six successive replicates of each concentration of the dilution series were analysed by injection of 1μl onto the GC. A straight line plot (regression analysis) was used to determine the relationship of peak area to concentration (Appendix A. Fig. 1).

Gas Chromatography/Mass spectrometry (GC-MS)

GC-MS identification of the alkaloids was carried out with an HP 5988A instrument fitted with a 15 m, HP-1 column of 0.25 mm internal diameter and 1μm film thickness. The injector temperature was set at 220 °C. The temperature programme for the analysis was : 40 °C-270 °C at 10 °C/min.

7.3   Results and Discussion

The alkaloids of Delairea odorata

Gas chromatography of the purified alkaloid extracts of *D. odorata* revealed the presence of at least nine possible nitrogenous compounds (Fig. 7.1). GC-MS indicated the presence of nine pyrrolizidine alkaloids (Fig. 7.2, Table 7.2). However, as both the structure and polarity of the column on the GC (BP-5) were different from that on the GC-MS (HP-1) and instrument sensitivity differed, most of the pyrrolizidine alkaloid peaks identified by GC-MS could not be correlated with the nine peaks obtained on the GC with the NPD-detector. Only the most prominent GC-peaks, GC peak 2 (10.883 min) and GC peak 3 (11.106 min) (Fig. 7.1), could be tentatively identified as GC-MS peak 4 (9.56 min) and GC-MS peak 5 (9.61 min) (Fig. 7.2, Table 7.2; for review on problems experienced with peak resolution when using different column types see Witte *et al.* (1993)).

When analysing mass spectral data the fragmentation pattern unequivocally helps to distinguish between esters of unsaturated necine bases (e.g. retronecine) and esters of saturated necine bases (e.g. platynecine) as well as O^7- and O^9- monoesters (e.g. 7-angelylplatynecine and 9-angelyl-platynecine) (Mattocks, 1986; Witte *et al.*, 1993). Monoesters of saturated necines, such as platynecine, typically have an $[M]^+$ 239 and give fragments in the ranges m/z 95-97, 122-123 and 138-140; there is a characteristic base peak at m/z 82 or m/z 95 for the O^7 (e.g. 7-tiglyl-
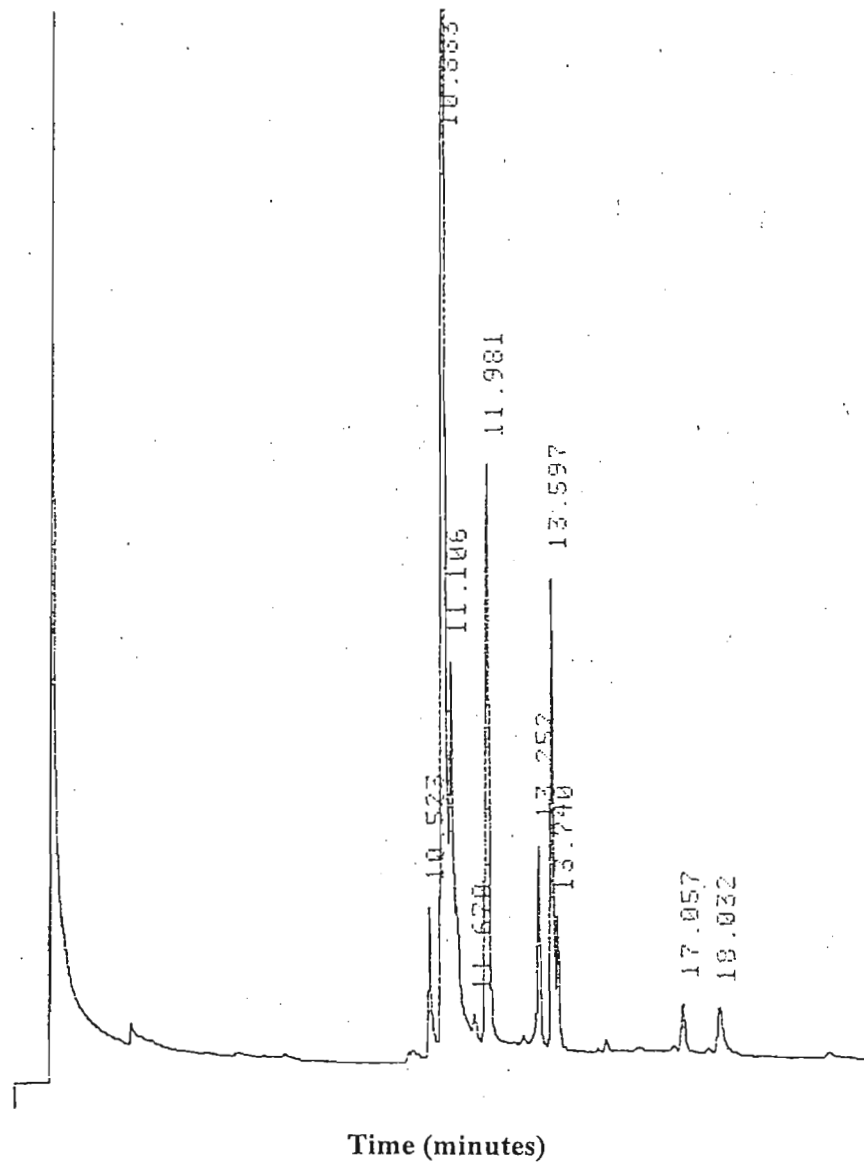
Fig. 7.1. Separation of an alkaloid extract from *Delairea odorata* by GC.
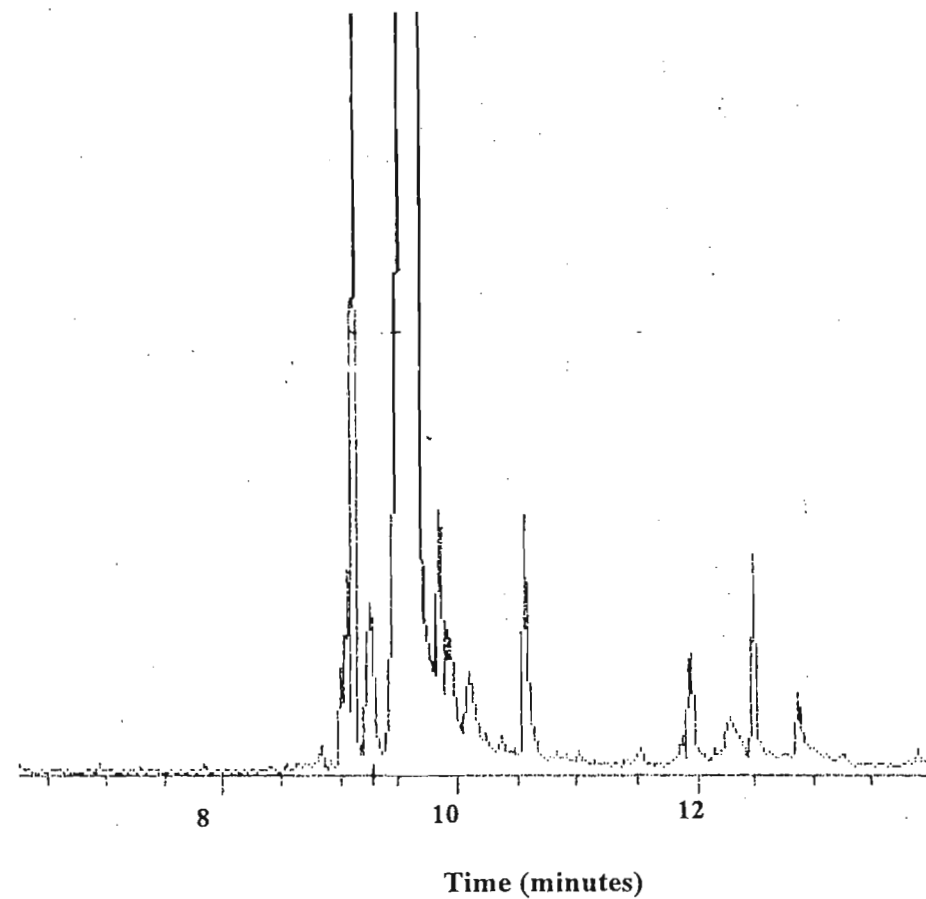
Fig. 7.2. GC-MS of alkaloid extract.

Table 7.2. Mass spectra of pyrrolizidine alkaloids extracted from *Delairea odorata* collected in Natal.
([ ] indicate relative abundance of ions)

Peak	Rt	[M]+	Other characteristic base ions														
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	9.01	237	80 [100]	111 [49]	93 [44]	106 [40]	94 [34]	137 [29]	136 [18]	138 [15]	81 [14]	124 [14]	120 [11]	108 [10]	71 [10]	83 [9]	237 [5]
2	9.07	237	93 [100]	138 [53]	94 [38]	80 [32]	137 [24]	136 [14]	111 [12]	106 [8]	155 [5]	193 [3]	124 [4]	108 [4]	237 [3]	154 [2]	
3	9.25	237	80 [100]	106 [43]	111 [36]	137 [29]	94 [27]	124 [24]	136 [18]	81 [13]	83 [12]	237 [3]	154 [2]				
4	9.56	239	80 [100]	93 [78]	137 [66]	106 [59]	94 [49]	111 [47]	83 [37]	124 [35]	136 [35]	138 [22]	154 [18]	155 [12]	193 [3]		
5	9.61	239	93 [100]	137 [33]	94 [25]	138 [23]	83 [19]	136 [18]	155 [17]	154 [17]	80 [17]	108 [4]	106 [3]	124 [2]	193 [4]		
6	9.85	237	93 [100]	149 [90]	109 [84]	81 [68]	127 [60]	139 [56]	94 [39]	71 [42]	80 [46]	69 [39]	95 [37]	83 [34]	136 [31]	106 [20]	111 [19]
7	9.87	237	93 [100]	136 [68]	83 [52]	94 [51]	80 [35]	119 [26]	137 [21]	120 [16]	106 [14]	81 [10]	111 [8]	118 [8]	154 [8]	117 [8]	
8	10.5	285	93 [100]	193 [63]	83 [16]	94 [14]	80 [9]	194 [8]	67 [6]	92 [4]	285 [4]	106 [3]	82 [3]				
9	11.9	251	220 [100]	136 [58]	93 [41]	83 [37]	120 [32]	94 [29]	119 [20]	221 [17]	80 [15]	251 [10]	121 [9]	137 [9]	118 [9]	106 [8]	

platynecine) or O^9 (e.g. 9-tiglylplatynecine) esters respectively (Mattocks, 1986; Witte *et al.*, 1993). Esters of unsaturated necines have an $[M]^+$ 237, the O^7-esters (e.g. 7-angelylretronecine) show key fragments at m/z 80, 106 and 111 and the respective O^9-esters (e.g. 9-angelylretronecine) fragment at m/z 93, 136,137 and 138 (Witte *et al.*, 1993).

Using plants collected in California, Stelljes *et al.* (1991) found 12 pyrrolizidine alkaloids in *D. odorata* (Table 7.1). Table 7.3 shows the mass spectral data of all the alkaloids isolated by these authors. The major alkaloid was 9-angelylplatynecine which comprised 39 % of the total alkaloid content (Table 7.1). 9-Tiglylretronecine was also present in large amounts but was mixed with lesser amounts of 7-tiglylretronecine (Table 7.1). Thus both saturated (platynecine based) and unsaturated (retronecine based) PAs were present in the material analysed by Stelljes *et al.* (1991), although the saturated PAs were by far the most abundant.

The fragmentation patterns of the GC-MS peaks of material analysed in this study did not correlate with that of Stelljes *et al.* (1991) (compare Tables 7.2 & 7.3). Most of the alkaloids identified by those authors were platynecine monoesters or diesters with a characteristic base peak at m/z 82/95 (Table 7.3). With the exception of the alkaloid identified at peak 9, all of the alkaloids identified in this study have a base ion of m/z 80/93 (Table 7.2). This indicates that most of the alkaloids present in material analysed for this study are unsaturated retronecine (O^7 and O^9) monoesters. Key fragments at 220, 93-95, 119-121 and 136 are all characteristic of retronecine diesters (Toppel *et al.*, 1987), fragments obtained for GC-MS peak 9 correspond to this. No platynecine based (saturated) alkaloids were present in *D. odorata* collected in Natal.

GC-MS peaks 4 and 5 have a $[M]^+$ 239, and a base peak at m/z 80 and 93 respectively (Table 7.2). This is unusual as the base peak fragments (and others) are key fragments of retronecine monoesters yet characteristically retronecine esters have an $[M]^+$ 237. This indicates that for peaks 4 and 5 there has either been incomplete separation and two compounds are eluting as a single peak, or that unknown alkaloids are present in the plant material. Only one pair of unsaturated monoesters was found by Stelljes *et al.* (1991), 7 and 9-tiglylretronecine (Table 7.1 & Table 7.3), the angelate esters of retronecine were not detected in their extract. Interestingly, the fragmentation pattern for GC-MS peak 2 corresponds exactly to that of 9-angelylretronecine published by the same authors for *Senecio hydrophilus* Nutt (Table 7.4). This may indicate the presence of these isomers in locally distributed plants.

Table 7.3. Mass spectra of pyrrolizidine alkaloids extracted from *Delairea odorata* by Stelljes *et al.* (1991). ([ ] indicates relative abundance of ions)

Alkaloid	Rt	[M]+	Other characteristic base ions										
			1	2	3	4	5	6	7	6	8	9	10
anhydroplatynecine	8.79	139	82 [100]	139 [29]	138 [34]	96 [15]	110 [9]	95 [9]	120 [5]	122 [4]			
7-tiglylretronecine	23.24	237	80 [100]	93 [61]	94 [44]	106 [34]	137 [32]	136 [23]	111 [23]	138 [20]	124 [17]	154 [9]	108 [7]
9-tiglylretronecine	23.30	237	93 [100]	80 [49]	94 [43]	138 [37]	137 [36]	136 [20]	106 [15]	154 [17]	124 [10]	111 [9]	108 [9]
7-angelylplatynecine	24.37	239	82 [100]	139 [59]	83 [48]	156 [38]	140 [37]	138 [20]	114 [12]	122 [8]	95 [7]	239 [7]	96 [6]
9-angelylplatynecine	25.71	239	82 [100]	95 [99]	96 [46]	122 [14]	83 [13]	221 [11]	140 [9]	239 [6]	138 [5]	139 [4]	156 [2]
7-tiglylplatynecine	26.16	239	82 [100]	139 [53]	156 [27]	138 [22]	83 [20]	95 [12]	114 [11]	96 [8]	140 [6]	122 [5]	239 [1]
9-tiglylplatynecine	27.68	239	95 [100]	82 [74]	221 [15]	96 [14]	83 [11]	122 [6]	140 [5]	138 [4]	139 [3]	156 [1]	
sarracine	48.59	337	138 [100]	82 [65]	122 [54]	83 [38]	96 [37]	139 [35]	123 [29]	237 [20]	222 [17]	121 [15]	97 [9]
sarranicine	49.25	337	138 [100]	82 [58]	95 [43]	122 [41]	139 [30]	96 [29]	123 [27]	83 [26]	121 [16]	237 [16]	222 [12]
triangularicine	49.68	335	83 [100]	93 [68]	136 [66]	94 [64]	120 [46]	119 [37]	80 [35]	121 [33]	81 [23]	220 [21]	137 [16]
neosarracine	50.05	337	138 [100]	82 [78]	122 [62]	95 [45]	96 [42]	139 [38]	83 [37]	123 [34]	222 [18]	237 [18]	121 [17]
neosarranicine	50.68	337	138 [100]	82 [76]	95 [56]	122 [53]	139 [38]	123 [36]	96 [36]	83 [34]	121 [21]	237 [19]	222 [17]

Table 7.4. Mass spectral data for 9-angelylretronecine (Stelljes *et al.*, 1991).

Alkaloid	[M]+	1	2	3	4	5	6	7	8	9	10	11
9-angelylretronecine	237	93	138	94	80	137	136	111	106	124	108	154
relative abundance		[100]	[49]	[46]	[44]	[36]	[15]	[10]	[10]	[9]	[6]	[3]

It was expected that similar compounds to that isolated by Stelljes *et al.* (1991) would be present in plants collected in Natal and that GC-MS fragmentation patterns would enable identification of alkaloids. However, obviously different fragmentation patterns were obtained. Based on the fragmentation patterns alone none of the alkaloids obtained for this study can be positively identified. To do this, more analytical data needs to be collected - this includes isolation of individual alkaloids and an HPLC analysis. Nuclear magnetic resonance (NMR) can be used to identify unsaturated PAs and when used in combination with GC-MS is an effective technique to elucidate structural isomers (Hartmann and Zimmer, 1986). Many of the alkaloids in *D. odorata* (in Natal) are retronecine esters, a NMR analysis may help to identify the individual alkaloids. Since it was anticipated that known alkaloids would be found an exhaustive analysis to identify compounds falls out of the scope of this study. All purified alkaloid samples have therefore been pooled and this sample will be used to positively identify alkaloids at a later stage.

As well as providing information on the types of alkaloids in *D. odorata* collected from Natal, this study also examined the alkaloid profiles of locally distributed plants. To this end plants were collected from two sites at each of three locations across Natal. The distribution of alkaloids within the plant was also investigated. Prior to analysis plants were separated into flowers, leaves and stems.

Distribution of alkaloids within plants and between populations
The same alkaloids were extracted from plants collected at Ingele, Ferncliffe and Dargle (Fig. 7.3 a-f). For all plants, and their component parts, the most abundant alkaloid was that occurring at GC-peak 2 (10.883 min, Fig. 7.1) (a retronecine) (Fig. 7.3 a-f). This alkaloid comprised at least 70 % of the total alkaloid content of all samples. At all sites, the flower heads contained the highest concentration of alkaloids and more than 80 % of the total alkaloids of plants in full bloom were present in the capitula (Fig. 7.3 a-f & Fig. 7.4). Much lower concentrations of alkaloids were present in both the stems and leaves (Figs 7.3 a-f & Fig. 7.4). Hartmann and Zimmer (1986) and Borstel *et al.* (1988) found PAs in *Senecio vulgaris* L. and *S. vernalis* L. to accumulate mainly in the inflorescences. Hartmann and Zimmer (1986) found concentrations of

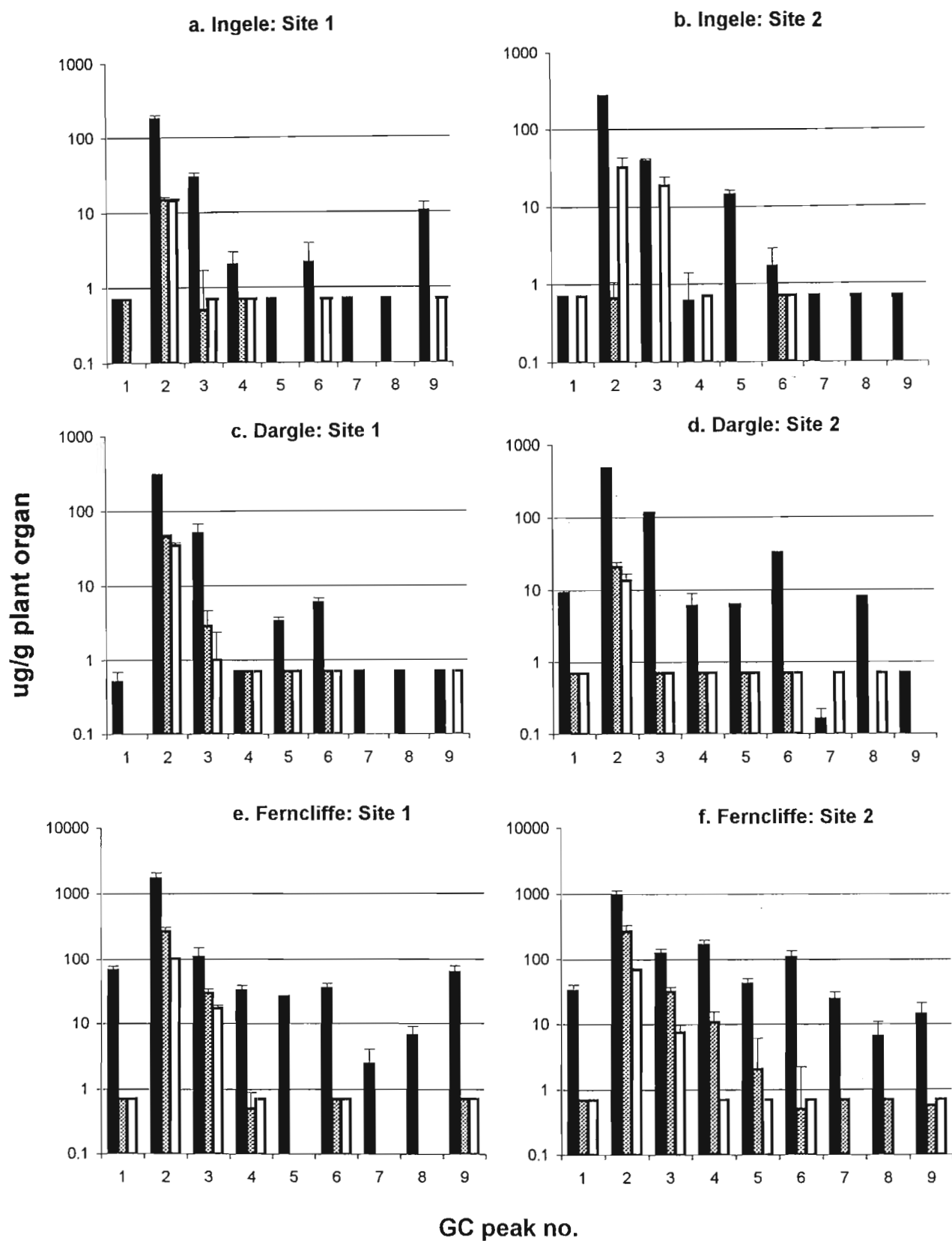**Fig. 7.3 a-f.** Pyrrolizidine alkaloids (ug/g) found in flowers, leaves and stems of *D. odorata* plants collected at two sampling sites at 3 localities; Ingele (a&b), Dargle (c&d) and Ferncliffe (e&f).
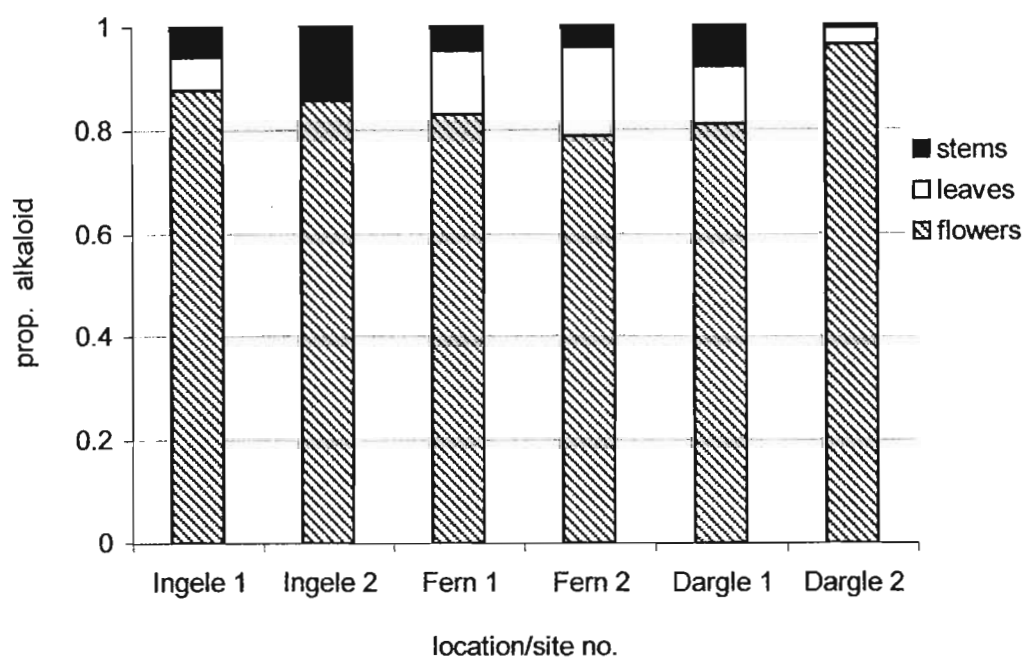
flowers ■ leaves ▨ stems ☐

Fig. 7.4. Relative distribution of pyrrolizidine alkaloids within different *D. odorata* plant parts. Plants collected from two sampling sites at three localities: Ingele, Ferncliffe and Dargle.

alkaloids in mature flower heads of *S. vernalis* to exceed that of vegetative organs 5-10 fold and to account for up to 80 % of total plant alkaloids.

Plants collected from Ferncliffe had by far the highest concentration of alkaloids (Fig. 7.5 a-d). Therefore, although alkaloid profiles of plants collected from the three populations were similar, in terms of concentration of alkaloid, plants collected at Ferncliffe had more alkaloid than those at Dargle and Ingele. Variation in alkaloid concentration between populations, and even individual plants, is not uncommon (Johnson *et al.*, 1985; van Dam and Vrieling, 1994).

7.4   Concluding comments

Two basic types of alkaloids can be distinguished from the plant material collected in KwaZulu/Natal - retronecine monoesters and one diester. The results differ markedly with previous studies which have found principally saturated alkaloids, either sarracine (platynecine diester) (Culvenor and Geissman, 1961; Stelljes and Seiber, 1990) or 9-angelylplatynecine (monoester), to be the major alkaloids. It is interesting to note that a repeated study by the same authors (Stelljes and Seiber, 1990; Stelljies *et al.*, 1991) yielded a different major alkaloid. Stelljes *et al.* (1991) suggest isomerisation of sarracine to neosarracine, sarranicine and neosarranicine during sample storage to account for some of the variation observed in sarracine content from 1990 to 1991. Sarracine is an apparently rare alkaloid reported only in *D. odorata* and *Senecio atratus* Greene (Stelljes and Seiber, 1990), the absence of this alkaloid from the material analysed in this study is therefore noteworthy.

The results of this study suggest that the alkaloid profile of locally distributed plants differs from those occurring in California and possibly even Australia (Culvenor and Geissman, 1961; Stelljes and Seiber, 1990; Stelljes *et al.*, 1991). The fact that the same alkaloids, with similar profiles, were extracted from plants distributed across KwaZulu-Natal serves to reinforce the apparent difference in alkaloid profile between local plants and those in California. These results indicate that chemotypes of *D. odorata* may be present. However, a more exhaustive comparative analysis including seasonal material collected from throughout South Africa and from Australia and California needs to be conducted to confirm these findings. Furthermore, this data should be collected prior to any biological control initiatives.

Pyrrolizidine alkaloids are present in all parts of *D. odorata,* however, there is a conspicuous accumulation of alkaloids in the flower heads. As already noted, the pyrrolizidine alkaloids play
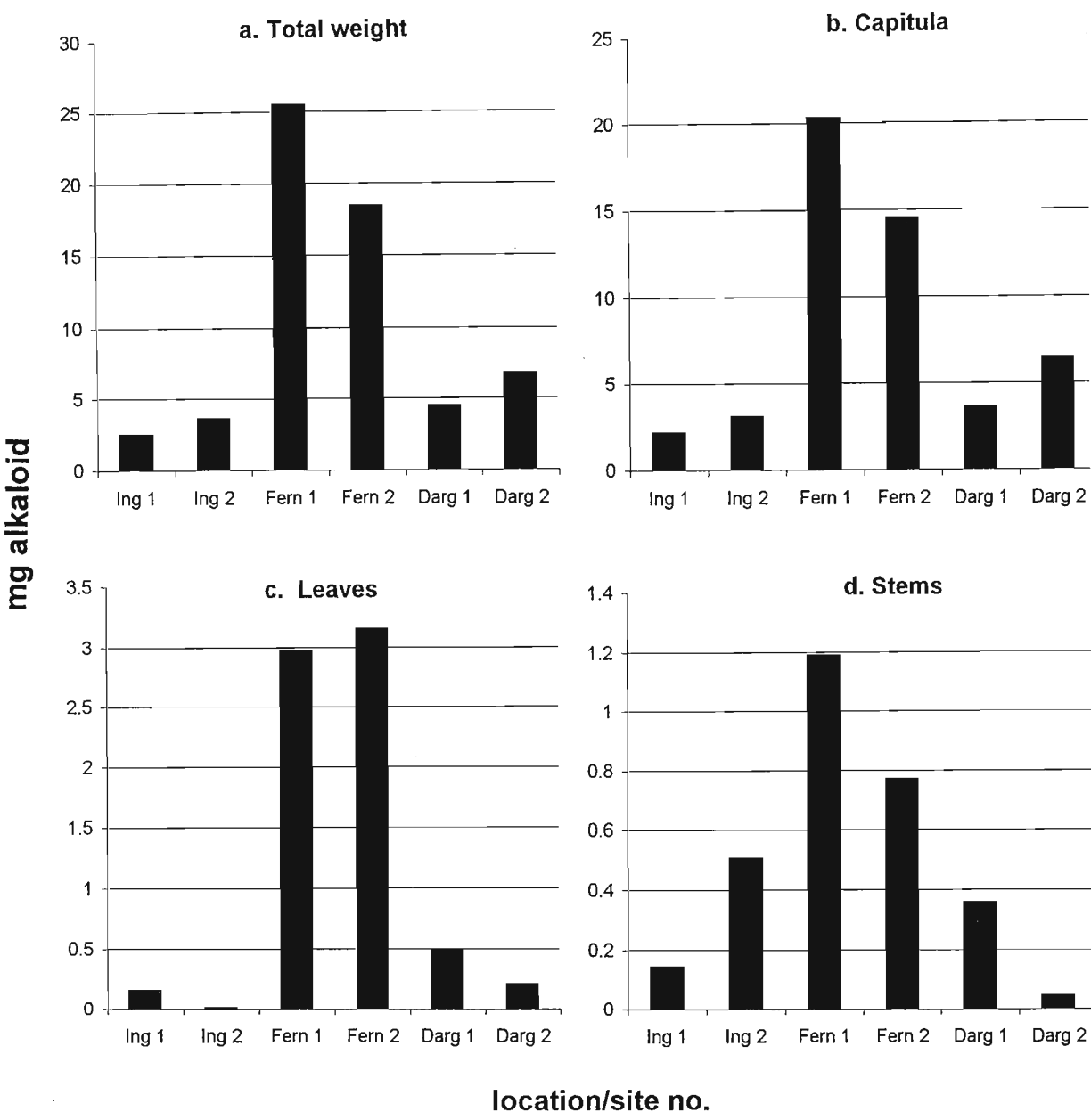
Fig. 7.5 a-d. Weight (mg/10 g dry plant material) of pyrrolizidine alkaloid extracted from (a) whole plant (leaves, flowers and stems) (b) capitula (mg/10 g) (c) leaves (mg/10 g), and (d) stems (mg/10 g) of *D. odorata*, collected at two sites for three geographical localities: Ingele, Ferncliffe and Dargle.

an important part in plant-insect interactions. The predominance of alkaloid accumulation in flowers may simply be interpreted as effective protection of a very valuable plant organ against herbivory.

Chapter 8
Summary and conclusions

Applicability of biological control to any weed problem depends on the nature of the weed, its taxonomic position, the degree of control required, the availability of suitable enemies and the stability and complexity of the plant community which is invaded (Andres *et al.*, 1976; van Driesche and Bellows, 1996). Generally, successful biological control has occurred mainly in families containing few plants of economic or ecological importance (Andres *et al.*, 1976; Shepherd, 1993; van Driesche and Bellows, 1996). Mode of reproduction is also an important determinant of the success or failure of a biological control programme. Burdon and Marshall (1981) noted the greater success of biological control programmes against asexual weeds.

Delairea odorata is an important weed of many nature conservation areas (Fagg, 1989; Scott and Delfosse, 1992; Alvarez and Cushman, 1997) and biological control is a potential method for the management of this species in exotic locations. As *Delairea* is a monotypic genus, no closely related crop or ecologically important species, which may be targeted by introduced enemies, have yet been identified. This makes biological control of this species a viable option. For this investigation emphasis was placed on the reproductive biology, the natural enemies and the chemical ecology of *D. odorata*. All of these attributes were recognised as key aspects in determining the suitability of *D. odorata* to biological control.

The surveys for natural enemies of *D. odorata*, in KwaZulu-Natal, indicate that this species has a wide range of enemies, although many appear to be generalist herbivores. One potential control agent was identified, a stem galling tephritid fly, *P. regalis*. The preliminary observations made in this study indicate this species to be host-specific, a valuable asset if it is to be used in a biocontrol programme. Furthermore, as *D. odorata* proliferates extensively by means of stem regeneration and elongation, galling of these growing points by *P. regalis* may limit stolon spread in exotic locations. Further studies to determine the potential of *P. regalis* as a biocontrol agent must, however, be carried out. These should include controlled greenhouse and field tests designed to evaluate (1) fly population growth (2) the effect of galling on stem regeneration, and (3) effect of galling on sexual reproductive effort. Conclusions regarding the limiting effect of galling on plant population spread and dispersal may then be drawn and the suitability of *P. regalis* as a biocontrol agent determined.

The pyrrolizidine alkaloid analysis shows that the same alkaloids are present in populations of *D. odorata* scattered across KwaZulu-Natal. However, these alkaloids appear to be different from those occurring in plants found in California and possibly even Australia (Culvenor and Geissman, 1961; Stelljes and Seiber, 1990; Stelljes *et al.*, 1991). This is an interesting and important result which indicates that chemotypes of *D. odorata* may be present, a factor which may affect the likelihood of natural control agents establishing on exotic plant populations. Further studies to confirm these preliminary findings are therefore essential. For veritable interspecific populational comparisons to be made, follow up investigations should include an analysis of the pyrrolizidine alkaloids of plants collected from throughout South Africa, Hawaii, California and South Australia. These results will indicate, conclusively, whether chemotypes of *D. odorata* are present or not. An examination of the seasonal and ontogenetic change in alkaloid profiles should also be made. A record of seasonal change (if any) in alkaloid profiles will indicate at what time of year the plants are most susceptible to enemies as this may be important regarding the timing of release of specific control agents in exotic locations. Furthermore, juvenile plants may be found to contain high proportions of alkaloids, as compared to adults, protecting them from enemies. This information will be useful to any control initiative.

This investigation has shown that *D. odorata* is a shade tolerant, self-incompatible species which reproduces sexually by seeds and asexually by stolons. Typically, the most suitable breeding system for colonising new areas is "self-compatible" (Baker, 1965). Selfing provides obvious benefits to a colonist allowing it to reproduce when mates are absent or at low density, as is often the case during the initial stages of colonisation. An association in plants between colonising success and uniparental reproduction has frequently been reported (Barret and Richardson, 1986; Abbot and Forbes, 1993). However, as is the case with *D. odorata*, not all successful colonisers are capable of uniparental reproduction and this raises interesting questions as to how they overcome the constraints imposed by the requirements for mates during the initial stages of colonisation. Baker (1974) suggested that for perennial weeds, where self-compatibility is less certain to be found, prolific vegetative reproduction is often present and this achieves the same end, *i.e.* the rapid multiplication of individuals with appropriate genotypes. This observation was supported by Vogt Anderson (1995) who found vegetative reproduction to be more important than sexual reproduction for invaders of natural habitats. *D. odorata* reproduces extensively by stolons which have the potential to produce a new plant at each node. In addition, thick rhizomes

support regrowth of aerial shoots when they are damaged or removed. Vegetative reproduction thus plays an important role in the local spread of this species and has facilitated invasion in exotic locations. One other possible answer to the question of overcoming mate requirements during the initial stages of colonisation was suggested by Abbot and Forbes (for perennial species; 1993). Following arrival at a new site perennials will survive to flower for more than one year. This population of colonising individuals could expand in numbers and become established via seed immigration over two years or more before engaging in sexual reproduction. However, this theory requires colonisation by more than one individual.

In California, no viable seed has yet been collected from *D. odorata* (Balciunas, pers comm.; Alvarez, 1997). This may indicate that most of the plants in California spread vegetatively by stolons and that the lack of seed set is due to clonal populations. Pollination experiments to determine whether geographically separated Californian populations have the potential to reproduce by seed are presently being carried out (Robison, 1999). If, as present information suggests, the Californian populations are largely clonal, this has a number of important implications with regard to the biological control of *D. odorata* in this region. Asexual weeds with genetic homogeneity may potentially be controlled by a single agent; a valuable asset if biological control is to be attempted. However, it is important to remember that interpopulation variation in a plant species may arise due reproductive isolation - be that geographic, seasonal or temporal (Housard and Escarre, 1995). Due to reproductive isolation exotic populations of *D. odorata* may be distinct ecotypes of their native counterparts. Although further testing is required, results of the pyrrolizidine alkaloid analysis indicate that chemotypes may be present. This could present problems in the development of a control programme.

In Australia and Hawaii, *D. odorata* apparently spreads both vegetatively and by wind blown diaspores (Lawrence, 1985; Jacobi and Warshauer, 1992; Cox, 1998). As previously mentioned, the nature of the contrasting modes of reproduction of *D. odorata* in exotic locations raises some interesting questions as to the genetic attributes and the nature of the spread of populations in each exotic location. This will influence the measures taken for its control in each of these regions. Natural enemy surveys carried out for this study revealed several species associated with the capitula of *D. odorata*. Although most appear to be generalist herbivores further, and more detailed, studies on some of these enemies may yield useful control agents. A good control agent

is one which targets the life strategy of the host plant. Furthermore, good control of weeds of the Asteraceae has been achieved when multiple enemy introductions have been made (e.g. control programme against *Senecio jacobaeae*). Introducing both seed and shoot enemies may limit the spread of *D. odorata* in these regions.

Although numerous studies have been conducted to characterise attributes common to invasive species (Baker, 1974; Lawrence, 1980; Rejmánek, 1989; Rejmánek *et al.*, 1991; Perrins *et al.*, 1992; Vogt Anderson, 1995; Pysek, *et al.*, 1995; Rejmánek, 1995; Mack, 1996; Williamson and Fitter, 1996; Vermeij, 1996) as yet no list of defining characters has been published. Amongst a suite of other characters, climate (homoclines), habitat requirements and absence of natural enemies have been cited as important factors regulating the spread of plant populations in new environments. South Africa is overall an extremely dry country with sporadic moist patches. Here, *D. odorata* has a limited distribution and occurs only in scarp forests 800 m above sea level, which are dominated by cool climate with high annual rainfall (the mist-belt region). In southern Africa the spread of *D. odorata* may be limited to scattered wet areas, hence its patchy distribution. In South Australia, California and Chile, *D. odorata* occurs along the coastal belt. Interestingly, all of these areas have high annual rainfall, precipitation being predominant in the winter months (Mediterranean climates). In Hawaii, *D. odorata* occurs between 500 m and 2500 m above sea level in areas with 1250 mm to 2500 mm average annual rainfall. Invasion by *D. odorata* thus appears to have occurred only in locations with a high annual rainfall (and consequent moist conditions). Ideal growing conditions coupled with a lack of natural enemies may account for the invasive nature of this species in exotic regions.

Chapter 9

References

Abbot, R. J. (1986). Life history variation, polymorphism and outcrossing in *Senecio vulgaris* L. *Heredity* **6**, 381-391.

Abbot, R. J. & Forbes, D. G. (1993). Outcrossing rate and self-incompatibility in the colonizing species *Senecio squalidus*. *Heredity* **71**, 155-159.

Abercrombie, M., Hickman, M., Johnson, M. L. & Thain, M. (1990). *The New Penguin Dictionary of Biology*. Penguin Books Ltd., London.

Adair, R. J. & Scott, J. K. (1991). Distribution, life history, host specificity and suitability of an undescribed *Chrysolina* species (Coleoptera) for biological control of *Chrysanthemoides monilifera* (Compositae). *Bulletin of Entomological Research* **81**, 235-242.

Adams, R. & Gianturco, M. (1956). *Senecio* alkaloids, the alkaloid from *Senecio mikanioides*. *Journal of the American Chemical Society* **79**, 166-169.

Altmann, J. & Campbell, C. L. (1977). Effects of herbicides on plant disease. *Annual Review of Phytopathology* **15**, 361-385.

Alvarez, M. E. (1997). Management of Cape Ivy (*Delairea odorata*) in the Golden Gate National Recreation Area. In *Proceedings of the Exotic Pest Plant Council Symposium*, vol. 3 (ed. M. Kelly, E. Wagner and P. Warner), pp. 91-95, California.

Alvarez, M. E. & Cushman, J. H. (1997). Influence of an invasive plant on species richness and composition of three coastal plant communities. *Supplement to the Bulletin of the Ecological Society of America* **78**, 46-52.

Amor, R. L. & Piggin, C. M. (1977). Factors influencing the establishment and success of exotic plants in Australia. In *Proceedings of the Ecological Society of Australia*, vol. 10 (ed. D. Anderson), pp. 10-23. The Ecological Society of Australia, Melbourne.

Anderson, R. C., Loucks, O. L. & Swain, M. (1969). Herbaceous response to canopy cover, light intensity and through fall precipitation in conifer forests. *Ecology* **50**, 256-263.

Andersson, S. (1988). Size dependent pollination efficiency in *Anchusa officinalis* (Boraginaceae): causes and consequences. *Oecologia* **76**, 125-130.

Andres, L. A., Davies, L. J., Harris, P. & Wapshere, A. J. (1976). Biological control of weeds. In *Theory and practice of biological control* (ed. C. B. Huffaker and P. S. Messenger), pp. 481-500. Academic Press, New York.

Angevine, M. W. & Handel, S. N. (1986). Invasion of forest floor space, clonal architecture, and population growth in the perennial herb *Clintonia borealis*. *Journal of Ecology* **74**, 547-560.

Anon. (1971). *Principles of plant and animal pest control*, vol 2. Subcommittee on weeds, Committee on Plant and Animal Pests, Agricultural Board, National Research Council. National Academy of Sciences.

Anon. (1996). Exotic pest plants of greatest ecological concern in California as of August 1996. In *CalEPPC News*. California Exotic Pest Plant Council.

Archbald, G. (1995). Biology and control of German Ivy. California Department of Fish and Game Pesticide Seminar.

Archbald, G. (1997). Letter to David Chipping summarizing Cape Ivy fish toxicity experiments by Dr. Carla Bossard at St. Marys College. Conservation Projects, Golden Gate National Parks Association, San Francisco, California 94123.

Arnold, T. H. & de Wet, B. C. (1993). *Plants of southern Africa*. National Botanical Institute, South Africa.

Arroyo, M. T. K. (1976). Geitonogamy in animal pollinated tropical angiosperms. A stimulus for the evolution of self-incompatibility. *Taxon* **25**, 543-548.

Atwater, B. R. (1980). Germination, dormancy and morphology of the seeds of herbaceous ornamental plants. *Seed Science and Technology* **8**, 523-573.

Baker, H. G. (1955). Self-compatibility and establishment after "long distance" dispersal. *Evolution* **9**, 347-348.

Baker, H. G. (1965). Characteristics and modes of origin of weeds. In *The genetics of colonising species* (ed. H. G. Baker and G. L. Stebbins), pp. 588. Academic Press, New York.

Baker, H. G. (1974). The evolution of weeds. *Annual Review of Ecology and Systematics* **5**, 1-24.

Balciunas, J. (pers. comm.). Exotic and Invasive Weed Management Research Unit, USDA-ARS Western Regional Research Centre, California.

Barraclough, D. (pers. comm.). Natal Museum, 237 Loop Street, Pietermaritzburg, Natal.

Barret, P. & Richardson, M. (1986). Genetic attributes of invading species. In *Ecology of biological invasions* (ed. R. H. Groves and J. J. Burdon), pp 10-22. Cambridge University Press, London.

Bartlett, B. R. & van den Bosch, R. (1971). Foreign exploration for beneficial organisms. In *Biological control of insect pests and weeds* (ed. C. B. Huffaker), pp. 283-345. Plenum Press, New York.

Bawa, K. S. (1974). Breeding system of tree species of a lowland tropical community. *New Zealand Journal of Botany* **28**, 85-92.

Bazzaz, F. A. & Harper, J. L. (1977). Demographic analysis of growth of *Linum usitatissimum*. *New Phytologist* **78,** 193-208.

Bell, A. D. (1984). Dynamic morphology: a contribution to plant population ecology. In *Perspectives on plant population ecology* (ed. R. Dirzo and J. Sarukhan), pp. 48-65. Sinauer Associates, Massachusetts.

Bell, D. T. (1993). The effect of light quality on the germination of eight species from sandy habitats in Western Australia. *Australian Journal of Botany* **41,** 321-326.

Bennet, M. B. (1987). Variation in genomic form in plants and its ecological implications. *New Phytologist* **106,** 177-200.

Bennet, M. D. (1972). Nuclear DNA content and minimum germination time. *Proceedings of the Royal Society of London* **181,** 109-135.

Bennett, P. H. (1986). An investigation into the biological control of crofton weed, *Ageratina adenophora* (K.& R.) (syn. *Eupatorium adenophorum* Spreng.) by the gall fly *Procecidochares utilis* Stone. PhD thesis, University of Natal.

Bess, H. A. & Haramoto, F. H. (1959). Biological control of Pamakani, *Eupatorium adenophorum*, in Hawaii by a tephritid gall fly, *Procecidochares utilis*. 2. Population studies of the weed, the fly, and the parasites of the fly. *Ecology* **40,** 244-249.

Beveridge, J. L. & Wilise, C. P. (1959). Influence of depth of planting, seed size and variety on emergence and seedling vigour in alfalfa. *Agronomy Journal* **51,** 731-734.

Bewley, J. D. & Black, M. (1994). *Seeds: Physiology of development and germination*, 2nd edition. Plenum Press, New York.

Bicchi, C., Caniato, R., Tabacchi, R. & Tsoupras, G. (1989). Capillary gas chromatography/ positive negative ion chemical ionisation mass spectrometry on pyrrolizidine alkaloids of *Senecio inaequidens* using ammonia and hydroxyl ions as the reagent species. *Journal of Natural Products* **52**, 32-41.

Billet, E. E. and Burnett, J. H. (1978). The host parasite physiology of the maize smut fungus *Ustilago maydis*. II. Translocation of ^{14}C-labelled assimilates in smutted maize plants. *Physiological Plant Pathology* **12**, 103-112.

Biller, A., Boppre, M., Witte, L. & Hartmann, T. (1994). Pyrrolizidine alkaloids in *Chromalaena odorata*: chemical and chemoecological aspects. *Phytochemistry* **35**, 615-619.

Bjorkman, O. (1980). Responses to different flux densities. In *Encyclopedia of plant physiology*, vol. 12A (ed. A. Pirson and M. H. Zimmerman), pp. 57-107. Springer, Berlin.

Blossey, B., Schroeder, D., Hight, S. D. & Maleck, R. A. (1994). Host-specificity and environmental impact of the weevil *Hylobius transversovittatus*, a biological control agent of purple loosestrife (*Lythrum salicaria*). *Weed Science* **42**, 128-133.

Borstel, K., Witte, L. & Hartmann, T. (1988). Pyrrolizidine alkaloid patterns in populations of *Senecio vulgaris, S. vernalis* and their hybrids. *Phytochemistry* **28**, 1639-1643.

Bossard, C. & Benefield, C. (1995). War on German Ivy: Good news from the front. In *Proceedings of the California Exotic Pest Plant Council, Symposium* (ed. J. E. Lovich, J. Randall and M. D. Kelly), pp. 63-64, California.

Brewbaker, J. L. & Majumder, S. K. (1961). Incompatibility and the pollen grain. *Recent Advances in Botany* **2**, 1503-1508.

Brown, A. H. D. & Marshall, D. R. (1980). The evolutionary genetics of colonising plants. In *Proceedings of the Second International Congress of Systematics and Evolutionary Biology* (ed. G. E. C. Scudder and J. L. Reveal), pp. 351-363. Hunt Institute for Botanical Documentation, Pittsburgh.

Burdon, J. J. & Marshall, D. R. (1981). Biological control and the reproductive mode of weeds. *Journal of Applied Ecology* **18,** 649-658.

Carlquist, S. (1991). Anatomy of vine and liana stems: a review and synthesis. In *The biology of vines* (ed. F. E. Putz and H.A. Mooney), pp 53-73. Cambridge University Press, New York.

Carpenter, F. L. (1976). Plant-pollinator interactions in Hawaii: pollination energetics in *Metrosideros collina* (Myrtaceae). *Ecology* **57,** 1125-1144.

Carr, G.D. & Powell, E. A. (1986). Self-incompatibility in the Hawaiian Madiinae (Compositae): an exception to Baker's rule. *Evolution* **40,** 430-434.

Catalano, S., Luschi, S., Flamini, G., Ciono, P. L., Neri, E. M. & Morelli, I. (1996). A xanthone from *Senecio mikanioides* leaves. *Phytochemistry* **42,** 1605-1607.

CCWR (1999). Computing Centre for Water Research. University of Natal, Private Bag X01, Scottsville, Pietermaritzburg.

Chalmers, A. H., Culvenor, C. C. J. & Smith, L. W. (1965). Characteristics of pyrrolizidine alkaloids by gas, thin-layer and paper chromatography. *Journal of Chromatography* **20,** 270-277.

Chipping, D. (1993). German Ivy infestation in San Luis Obispo. In *CalEPPC News*, vol. 1, pp. 15-16.

Christenson, L. D. & Foote, R. H. (1960). The biology of fruit flies. *Annual Review of Entomology* **5**, 171-192.

Clement, S. L. & Cristofaro, M. (1995). Open-field tests in host-specificity determination of insects for biological control of weeds. *Biocontrol Science and Technology* **5,** 395-406.

Cook, R. E. (1979). Patterns of juvenile mortality and recruitment in plants. In *Topics in plant population biology* (ed. O. T. Jain, G. B. Johnson and P. H. Raven), pp. 207-231. Macmillan Press, London.

Cook, R.E. (1985). Growth and development in clonal plant populations. In *Population biology and evolution of clonal organisms* (ed. J. B. C. Jackson, L. W. Buss and R.E. Cook), pp. 59-78. Yale University Press, New Haven.

Copland, B. J. & Whelan, R. J. (1989). Seasonal variation in flowering intensity and pollination limitation of fruit-set in four co-occurring *Banksia* species. *Journal of Ecology* 77, 509-523.

Cornell, H. V. & Hawkins, B. A. (1993). Accumulation of native parasitoid species on introduced herbivores: a comparison as hosts as natives and host as invaders. *American Naturalist* 141, 847-865.

Cousens, R. & Mortimer, M. (1995). *Dynamics of weed populations*. Cambridge University Press, Cambridge.

Cox, D. (1998). Letter to Ramona Robison regarding *Delairea* in Australia. Department of Natural Resources and Environment. Victoria, Australia.

Crawley, M. J. (1989). The successes and failures of weed biocontrol using insects. *Biocontrol News and Information* 10, 213-223.

Cronk, Q. C. B. & Fuller, J. L. (1995). *Plant invaders: the threat to natural ecosystems*. Chapman and Hall, London.

Crowe, L. (1954). Incompatibility in *Cosmos bipinnatus*. *Heredity* 8, 1-11.

Cudney, D. W. & Hodel, D. (1986). German Ivy control in coastal sage scrub regions of southern California. Report, Western Society of Weed Science Research, San Diego.

Culvenor, C. C. J. & Geissman, T. A. (1961). The alkaloids of *Senecio mikanioides* Otto. Sarracine and Sarracine –oxide. *Journal of Organic Chemistry* 26, 3045-3050.

De Bach, P. (1964). *Biological control of insect pests and weeds*. Chapman and Hall, London.

Daehler, C. C. (1998). The taxonomic distribution of invasive angiosperm plants: ecological insights and comparison to agricultural weeds. *Biological Conservation* **84**, 167-180.

De Kroon, H. & Hutchings, M. J. (1995). Morphological plasticity in clonal plants: the foraging concept reconsidered. *Journal of Ecology* **83**, 143-152.

De Nettancourt, D. (1977). *Incompatibility in Angiosperms*. Springer-Verlag, Berlin.

De Steven, D. (1989). Genet and ramet demography of *Oenocarpus mapora* a clonal palm of a Panamanian tropical moist forest. *Journal of Ecology* **77**, 579-596.

Dean, S. J., Holmes, P. M. & Weiss, P. W. (1986). Seed biology of invasive alien plants in South Africa and Namibia. In *Proceedings of the National Symposium of the Ecology of Biological Invasions* (ed. L. A. McDonald, F. J. Kruger and A. A. Ferrar), pp 157-178. Oxford University Press, Cape Town.

Dempster, J. P. (1971). The population ecology of the cinnabar moth, *Tyria jacobaeae* L. (Lepidoptera: Arctiidae). *Oecologia* **7**, 26-67.

Dennil, G.B. (1985). The effect of the gall wasp *Trichilogaster acacialongifoliae* (Hymenoptera: Pteromalidae) on reproductive potential and vegetative growth of the weed *Acacia longifolia*. *Agriculture, Ecosystems and Environment* **14**, 53-61.

Dennil, G. B. & Moran, V. C. (1989). On insect-plant associations in agriculture and the selection of agents for weed biocontrol. *Annals of Applied Biology* **114**, 157-166.

Dennil, G. B. & Hokkanen, H. M. T. (1990). Homeostasis and success in biological control of weeds - a question of balance. *Agriculture, Ecosystems and Environment* **33**, 1-10.

Dodd, A. P. (1961). Biological control of *Eupatorium adenophorum* in Queensland. *Australian Journal of Science* **23**, 356-365.

Dunn, P. H. & Campobasso, G. (1993). Field test of the weevil *Hadroplonthus trimaculatus* and the fleabeetle *Psylliodes chalcomera* against musk thistle (*Carduus nutans*). *Weed Science* **41,** 656-663.

Edwards, P. B., Adair, R. J. & Holtkamp, R. H. (1996). Seedhead tephritids and their parasitoids-can predictions be made about likely parasitism of tephritids introduced into Australia for biological control of Asteraceae. In *Proceedings of the IX International Symposium on Biological Control of Weeds* (ed. V. C. Moran and J. H. Hoffmann), pp. 153-164. University of Cape Town, Stellenbosch, South Africa.

Elliot, W. (1994). German Ivy engulfing riparian forests and heading for the uplands. In *CalEPPC News*, vol. 2, pp. 9.

Ellison, A. M. & Niklas, K. J. (1988). Branching patterns of *Salicornia europaea* (Chenopodiaceae) at different successional stages: a comparison of theoretical and real plants. *American Journal of Botany* **75,** 501-512.

Elzen, G. W. (1983). Cytokinins and insect galls. *Comparative Biochemistry and Physiology* **76,** 17-19.

Engelbrecht, L. (1971). Cytokinin activity in larval infested leaves. *Biochemie und Physiologie der Plansen* **162,** 9-27.

Entwistle, P. F. (1983). Control of insects by virus diseases. *Biocontrol News and Information* **4,** 203-220.

Eriksson, O. (1989). Seedling dynamics and life histories in clonal plants. *Oikos* **55,** 231-238.

Eriksson, O. (1992). Evolution of seed dispersal and recruitment in clonal plants. *Oikos* **63,** 439-448.

Eriksson, O. & Bremer, B. (1993). Genet dynamics of the clonal plant *Rubus saxatilis*. *Journal of Ecology* **81,** 533-542.

Esau, K. (1960). *Anatomy of seed plants*. John Wiley and Sons, New York.

Evans, J. P. & Cain, M. L. (1995). A spatially explicit test of foraging behaviour in clonal plants. *Ecology* **76**, 1147-1155.

Evetts, L. L. & Burnside, G. C. (1972). Germination and seedling development of common milkweed and other species. *Weed Science* **20**, 371-374.

Fagg, P. C. (1989). Control of *Delairea odorata* (Cape Ivy) in native forest with the herbicide clopyralid. *Plant Protection Quarterly* **4**, 107-110.

FAO (1997). Code of conduct for the import and release of exotic biological control agents. *Biocontrol News and Information* **18**, 119-124.

Faucroy, M. and Braun, C. (1967). Observations sur la galle de l' *Aulax glenchamae* L. sur *Glenchoma hederacea* L. - seconde partie-histologie et role physiologique de la coque sclrifie. *Marcellia* **34**, 3-30.

Fisher, A. (1997). The effects of *Senecio mikanioides* on riparian invertebrate populations in Redwood Creek and Elk Creek in the Golden Gate National Recreation Area. *Unpublished paper*.

Fitter, A. H. & Hay, R. K. M. (1981). *Environmental physiology of plants*. Academic Press, London.

Forbert, M. (1998). Some tips on herbicide treatment of Cape Ivy (*Delairea odorata*). In *CalEPPC News*, vol. 6, pp. 4.

Forsyth, C. & Brown, N. A. C. (1982). Germination of *Bidens pilosa* L. *The New Phytologist* **90**, 150-164

Freeman, T. E. & Charudattan, R. (1984). Conflicts in the use of plant pathogens as biocontrol agents for weeds. In *Proceedings of the VI International Symposium on the Biological Control of Weeds* (ed. E. S. Delfosse), pp. 351-357. Agriculture Canada, Vancouver.

Frei, H., Luthy, J., Brauchli, J., Zweifel, U., Wurgler, F. E. & Schlatter, C. (1992). Structure/activity relationships of the genotoxic potencies of sixteen pyrrolizidine alkaloids assayed for induction of somatic mutation and recombination in wing cells of *Drosophila melanogaster*. *Chemical and Biological Interactions* **83**, 1-22.

Freidberg, A. (1984). Gall Tephritidae (Diptera). In *The biology of gall insects* (ed. T. N. Ananthakrishnan), pp. 129-167. Edward Arnold, New Delhi.

Fritz, R. S. & Simms, E. L. (1992). *Plant resistance to herbivores and pathogens - ecology, evolution and genetics*. University of Chicago Press, Chicago.

Gillet, J. D., Harley, K. L. S., Kassulke, R. C. & Miranda, H. J. (1991). Natural enemies of *Sida acuta* and *S. rhombifolia* (Malvaceae) in Mexico and their potential for biological control of these weeds in Australia. *Environmental Entomology* **20**, 882-888.

Goeden, R. D. (1988). A capsule history of biological control of weeds. *Biocontrol News and Information* **9**, 55-61.

Goldingay, R. L. & Whelan, R. J. (1990). Breeding system and tests for pollen-limitation in two species of *Banksia*. *Australian Journal of Botany* **38**, 63-71.

Grime, J. P. (1966). Shade avoidance and shade tolerance in flowering plants. *British Ecological Society Symposium* **6**, 187-207.

Grime, J. P., Mason, G., Curtis, A. V., Rodman, J., Band, S. R., Mowforth, M. A. G., Neal, A. M. & Shaw, S. (1981). A comparative study of germination characteristics in a local flora. *Journal of Ecology* **69**, 1017-1059.

Grobbelaar, E., Neser, S. & Neser, O. C. (1999). Biological control of *Delairea odorata* in California: a survey for its natural enemies in South Africa. In *Proceedings of the Twelfth Entomological Congress* (ed. J. B. J. van Rensburg and J. van den Berg), pp. 52. Entomological Society of Southern Africa, Potchefstroom.

Gross, R. S. & Werner, P. A. (1983). Relationships among flowering phenology, insect visitors, and seed set of individuals: experimental studies on four co-occurring species of goldenrod (*Solidago*: Compositae). *Ecological Monographs* **53**, 95-117.

Groves, R. H. (1986). Plant invasions of Australia - an overview. In *Ecology of biological invasions* (ed. R. H. Groves and J. J. Byrdon), pp. 155-163. Cambridge University Press, New York.

Handel, S. N. (1985). The intrusion of clonal growth patterns on plant breeding systems. *American Naturalist* **125**, 367-384.

Harper, J. L. (1977). *Population biology of plants*. Academic Press, London.

Harper, J. L., Lovell, P. H. & Moore, K. G. (1970). The shapes and sizes of seeds. *Annual Review of Ecology and Systematics* **1**, 327-356.

Harris, P. (1973). The selection of effective agents for the biological control of weeds. *Canadian Entomologist* **105**, 1495-1503.

Harris, P. (1989). The use of Tephritidae for the biological control of weeds. *Biocontrol News and Information* **10**, 7-16.

Harris, P. (1991). Classical biocontrol of weeds: its definition, selection of effective agents, and administrative-political problems. *Canadian Entomologist* **123**, 827-849.

Harris, P. & Zwolfer, H. (1968). Screening of phytophagous insects for biological control of weeds. *Canadian Entomologist* **100**, 295-303.

Hartmann, T. (1996). Diversity and variability of plant secondary metabolism: a mechanistic view. *Entomologia Experimentalis et Applicata* **80**, 177-188.

Hartmann, T. (1999). Chemical ecology of pyrrolizidine alkaloids. *Planta* **207**, 483-495.

Hartmann, T. & Toppel, G. (1987). Senecionine –oxide the primary product of pyrrolizidine alkaloid biosynthesis in root cultures of *Senecio vulgaris*. *Phytochemistry* **26,** 1639-1643.

Hartmann, T. & Zimmer, M. (1986). Organ-specific distribution and accumulation of pyrrolizidine alkaloids during the life history of two annual *Senecio* species. *Journal of Plant Physiology* **122,** 67-80.

Hartmann, T., Witte, L., Ehmke, A., Theuring, C., Rowell-Rahier, M. & Pasteels, J. M. (1997). Selective sequestration and metabolism of plant derived pyrrolizidine alkaloids by chrysomelid leaf beetles. *Phytochemistry* **45,** 498-497.

HEAR (Hawaiian Ecosystems at Risk Project©) (1986). Alien species map index. University of Hawaii, Botany Department, Hawaii.

Hendricks, J. D., Sinnhuber, R. O., Henderson, M. C. & Buhler, D. R. (1981). Liver and kidney pathology in rainbow trout (*Salmo gairdneri*) exposed to dietary pyrrolizidine (*Senecio*) alkaloids. *Experimental and Molecular Pathology* **35,** 170-183.

Hessing, M. B. (1988). Geitonogamous pollination and its consequences in *Geranium caespitosum*. *American Journal of Botany* **75,** 1324-1333.

Heywood, V. H. (1989). Patterns, extents and modes of invasions by terrestrial plants. In *Biological invasions: a global perspective* (ed. J. A. Drake, H. A. Mooney, F. Di Castri, R. H. Groves, F. J. Kruger, M. Rejmának and M. Williamson), pp. 31-43. John Wiley, Chichester.

Heywood, V. H. (1993). *Flowering plants of the world*. Oxford University Press, New York.

Heywood, V. H., Harbourne, J. B. & Turner, B. L. (1977). *The biology and chemistry of the Compositae*, vol. 1. Academic Press, New York.

Hilliard, O. M. (1977). *Compositae of Natal*. University of Natal Press, Pietermaritzburg.

Hoffmeister, T. (1992). Factors determining the structure and diversity of parasitoid complexes in tephritid fruit flies. *Oecologia* **89,** 288-297.

Holm, L. G., Doll, J., Holm, E., Pancho, J. & Herberger, J. (1997). *World weeds: natural history and distribution.* John Wiley and Sons Inc., London.

Holm, L. G., Pancho, J. V., Herberger, J. P. & Plucknett, D. L. (1979). *A geographical atlas of world weeds.* Wiley Interscience, New York.

Horowitz, M. & Taylorson, R. B. (1982). Effect of high temperatures on imbibition, germination, and thermal death of velvetleaf (*Abutilon theiphrasti*). *Canadian Journal of Botany* **61,** 2269-2276.

Houssard, G. & Escarre, J. (1995). Variation and covariation among life history traits of *Rumex acetosella* from a successional old-field gradient. *Oecologia* **102,** 70-80.

Huffaker, C. B. (1964). Fundamentals of biological weed control. In *Biological control of insects pests and weeds* (ed. P. DeBach), pp. 631-663. Chapman and Hall, London.

Huffaker, C. B., Messenger, P. S. & DeBach, P. (1971). The natural enemy component. In *Biological Control* (ed. C. B. Huffaker), pp. 16-68. Plenum Press, New York.

Hutchings, M. J. (1988). Differential foraging for resources and structural plasticity in plants. *Trends in Ecology and Evolution* **3,** 200-204.

Hutchings, M. J. & De Kroon, H. (1994). Foraging in plants: the role of morphological plasticity in resource acquisition. *Advances in Ecological Research* **25,** 159-238.

Jacobi, J. D. & Warshauer, F. R. (1992). Distribution of six alien plant species in upland habits on the island of Hawaii. In *Alien plant invasions in native ecosystems of Hawaii: Management and research* (ed. C. P. Stone, C. W. Smith and J. T. Tunison), pp. 155-180. University of Hawaii Press, Honolulu.

James, R. R., McEvoy, P. B. & Cox, C. S. (1992). Combining the cinnabar moth (*Tyria jacobaeae*) and the ragwort fleabeetle (*Longitarsus jacobaeae*) for control of ragwort (*Senecio jacobaeae*): an experimental analysis. *Journal of Applied Ecology* **29**, 589-596.

Jeffrey, C. (1986). The Senecioneae in east tropical Africa. *Kew Bulletin* **41**, 873-937.

Jeffrey, C. & Chen, Y. L. (1984). Taxonomic studies in the tribe Senecioneae (Compositae) of Eastern Asia. *Kew Bulletin* **39**, 264-446.

Johnson, A. E., Molyneux, R. J. & Merrill, G. B. (1985). Chemistry of toxic range plants. Variation in pyrrolizidine alkaloid content of *Senecio*, *Amsinckia*, and *Crotolaria* species. *Journal of Agriculture and Food Chemistry* **33**, 50-55.

Julien, M. H. (1989). Biological control of weeds worldwide: trends, rates of success and the future. *Biocontrol News and Information* **10**, 299-306.

Julien, M. H. (1992). *Biological control of weeds: a world catalogue of agents and their target weeds*, 3rd edition. CAB International, Wallingford.

Kays, S. & Harper, J. L. (1974). The regulation of plant and tiller density. *Journal of Ecology* **62**, 97-105.

Keeley, P. E. & Thullen, R. J. (1978). Light requirement of yellow nutsedge (*Cyperus esculentus*) and light interception by crops. *Weed Science* **26**, 10-16.

King, L. J. (1966). *Weeds of the world: biology and control*. Interscience Publishers Inc., New York.

Klingman, K. C., Ashton, F. M. & Noordhoff, L. J. (1982). *Weed science: principles and practices*, pp. 450. John Wiley and Sons, New York.

Koekemoer, M. J. & Warren, F. L. (1951). The *Senecio* alkaloids. Part VIII. The occurrence and preparation of the N-oxides. An improved method of extraction of the *Senecio* alkaloids. *Journal of the Chemical Society* **5,** 66-68.

Koller, D. & Roth, N. (1964). Studies on the ecological and physiological significance of amphicarpy in *Gymnarrhena micrantha* (Compositae). *American Journal of Botany* **51,** 26-35.

Krebs, H. C., Carl, T. & Habermehl, G. (1996). Pyrrolizidine alkaloid composition in six Brazilian *Senecio* species. *Phytochemistry* **43,** 1227-1229.

Kruger, F. J. (1977). Invasive woody plants in the Cape fynbos with special reference to the biology and control of *Pinus pinaster*. In *Proceedings 2nd National Weed Conference of South Africa* (ed. A. A. Balkema), pp. 57-74. Cape Town, South Africa.

Lalonde, R. G. and Shorthouse, J. D. (1984). Developmental morphology of the gall of *Urophora cardui* (Diptera, Tephritidae) in the stems of Canada thistle (*Cirsium arvense*). *Canadian Journal of Botany* **62,** 1372-1384.

Landolt, P. J. & Phillips, T. W. (1997). Host plant influences on sex pheromone behaviour of phytophagous insects. *Annual Review of Entomology* **42,** 371-391.

Lauer, E. (1953). Uber die Keimtemperatuur van Ackerunk-rautern und derern einfluss auf die Eusammetisetzung van Unkrautgesellschaften. Flora oder Algemeine. *Botanische Zeitung* **140,** 551-595.

Lawrence, M. E. (1980). *Senecio* L. (Asteraceae) in Australia: chromosome numbers and the occurrence of polyploidy. *Australian Journal of Botany* **28,** 151-165.

Lawrence, M. E. (1985). *Senecio* L. (Asteraceae) in Australia: reproductive biology of a genus found primarily in unstable environments. *Australian Journal of Botany* **33,** 197-208.

Lawton, J. H. (1984). Ecological theory and choice of biological control agents. In *Proceedings of the VI International Symposium on the Biological Control of Weeds* (ed. E. S. Delfosse), pp. 13-26. Agriculture Canada, Vancouver.

Leakey, R. R. B. (1981). Adaptive biology of vegetatively regenerating weeds. *Advances in Applied Biology* **6,** 57-90.

Levin, D. A. & Kerster, H. W. (1974). Gene flow in seed plants. *Evolutionary Biology* **7,** 139-220.

Lovett Doust, L. (1981). Intraclonal variation and competition in *Ranunculus repens*. *New Phytologist* **89,** 495-502.

Lovett Doust, L. (1987). Population dynamics and local specialisation in a clonal perennial (*Ranunculus repens*). III. Responses to light and nutrient supply. *Journal of Ecology* **75,** 555-568.

Luthy, J., Zweifel, U., Karlhuber, B. & Schlatter, C. (1981). Pyrrolizidine alkaloids of *Senecio alpinus* L. and their detection in feedstuffs. *Journal of Agriculture and Food Chemistry* **29,** 302-305.

Mack, R. N. (1996). Predicting the identity and fate of plant invaders: emergent and emerging approaches. *Biological Conservation* **78,** 107-121.

Maddox, D. M. & Sobhain, R. (1987). Field experiment to determine host-specificity and oviposition behaviour of *Bangasternus orientalis* and *Bangasternus fausti* (Coleoptera: Curculionidae), biological control candidates for yellow starthistle and diffuse knapweed. *Environmental Entomology* **16,** 645-648.

Mansell, M. (pers. comm.). Plant Protection Research Institute, Private Bag X134, Pretoria 0001, South Africa.

Marohasy, J. J. (1989). A survey of fireweed (*Senecio madagascariensis* Poir) and its natural enemies in Madagascar with a view to biological control in Australia. *Plant Protection Quarterly* **4**, 139-140.

Mattocks, A. R. (1967). Spectrophotometric determination of unsaturated pyrrolizidine alkaloids. *Analytical Chemistry* **39**, 443-447.

Mattocks, A. R. (1986). *Chemistry and toxicology of pyrrolizidine alkaloids*. Academic Press Inc., London.

Mayer, A. M. & Poljakoff-Mayber, A. (1989). *The germination of seeds*, 4th edition. Pergamon Press, New York.

McClay, A. S. (1989). Selection of suitable target weeds for classical biocontrol in Alberta. PhD thesis, Alberta Environmental Centre.

McClay, A. S. & Palmer, W. A. (1995). Phytophagous arthropods associated with *Parthenium hystophorus* (Asteraceae) in North America. *Environmental Entomology* **24**, 796-810.

McEnvoy, P. B. (1984). Depression in ragwort (*Senecio jacobaeae*) abundance following introduction of *Tyria jacobaea* and *Longitarsus jacobaeae* on the central coast of Oregon. In *Proceedings of the VI International Symposium on the Biological Control of Weeds* (ed. E. S. Delfosse), pp. 57-64. Agriculture Canada, Vancouver.

McEnvoy, P. B., Rudd, N. T., Cox, C. S. & Huso, M. (1993). Disturbance, competition, and herbivory effects on ragwort *Senecio jacobaeae* populations. *Ecological Monographs* **63**, 55-75.

McVean, D. (1966). Ecology of *Chondrilla juncea* L. in South Eastern Australia. *Journal of Ecology* **54**, 345-365.

Minitab© (1995). Minitab for Windows. Minitab Inc., 3081 Enterprise Drive, State College, PA 16801-3008.

Mitchell, P. L. & Woodward, F. L. (1988). Responses of three woodland herbs to reduced photosynthetically active radiation and low red to far-red ratio in shade. *Journal of Ecology* **76**, 807-825.

Molyneux, R. J., Johnson, A. E., Roitman, J. N. & Benson, M. E. (1979). Chemistry of toxic range plants. Determination of pyrrolizidine alkaloid content and composition in *Senecio* species by nuclear magnetic resonance spectroscopy. *Journal of Agriculture and Food Chemistry* **27**, 494-499.

Molyneux, R. J. & Ralphs, M. H. (1992). Plant toxins and palatability to herbivores. *Journal of Range Management* **45**, 13-18.

Montenego, G., Teillier, S., Arce, P. & Poblete, V. (1991). Introduction of plants into Chile. In *Biogeography of mediterranean invasions* (ed. R. H. Groves and F. D. Di Castri), pp. 115-131. Cambridge University Press, New York.

Mooney, H. A. & Chiariello, N. (1984). The study of plant function: the plant as a balanced system. In *Perspectives on plant population ecology* (ed. R. Dirzo and J. Sarukhan), pp. 305-323. Sinauer, Massaschussets.

Moore, K. (1997). Battling the Kudzu of the west: controlling Cape Ivy (formerly German Ivy) by hand removal. In *CalEPPC News*, vol. 5, pp. 4.

Morin, L., Hill, R. L. & Matayoshi, S. (1997). Hawaii's successful biological control strategy for mist flower (*Ageratina riparia*) - can it be transferred to New Zealand? *Biocontrol News and Information* **18**, 77-87.

Mortimer, A. M. (1990). The biology of weeds. In *Weed control handbook: principles* (ed. R. J. Hance and K. Holly), pp. 1-42. Blackwell Scientific Publications, Oxford.

Mott, J. J. (1972). Germination studies on some annual species from an arid region of Western Australia. *Journal of Ecology* **60**, 293-304.

Muller, H. (1989). Structural analysis of phytophagous insect guilds associated with the roots of *Centaurea maculosa* Lam., *C. diffusa* Lam and *C. vallesiaca* Jordon in Europe. *Oecologia* **78**, 41-52.

Muller-Scharer, H., Lewinsohn, T. M. & Lawton, J. H. (1991). Searching for weed biocontrol agents - when to move on? *Biocontrol Science and Technology* **1**, 271-280.

Munro, H. K. (1940). Further South African gall forming Trypetidae (Diptera) with description of new species. *Journal of the Entomological Society of South Africa* **3**, 76-87.

Munro, H. K. (1952). A remarkable new gall forming trypetid (Diptera) from southern Africa and its allies. *South African Department of Agriculture Entomological Memoirs* **2**, 329-341.

Munro, H. K. (1953). Records of some Trypetidae (Diptera) collected on the Bernard Carp Expedition to Barotseland, 1952, with a new species from Kenya. *Journal of the Entomological Society of South Africa* **16**, 217-226.

Munro, H. K. (1957). Trypetidae. *Ruwenzori Expedition* **9**, 853-1054.

Noble, I. R. (1989). Attributes of invaders and the invading process. In *Biological Invasions: a global perspective* (ed. J. A. Drake, H. A. Mooney, F. Di Castri, R. H. Groves, F. J. Kruger, M. Rejmánek and M. Williamson), pp 301-313. John Wiley, Chichester.

O'Brien, C. W. (1995). Curculionidae as premier biocontrol agents (Coleoptera: Curculionidae). *Memoirs of the Entomological Society of Washington* **14**, 119-128.

Panetta, F. D. (1977). The effects of shade on seedling growth in the groundsel bush (*Baccharis halimifolia* L.). *Australian Journal of Agricultural Research* **28**, 681-690.

Panetta, F. D. & Mitchell, N. D. (1991). Homocline analysis and the prediction of weediness. *Weed Research* **31**, 273-284.

Perrins, J., Williamson, M. & Fitter, A. (1992). A survey of differing views of weed classification: implications for regulation of introduction. *Biological Conservation* **60,** 47-56.

Pitkella, L. F., Stanton, D. S. & Peckenham, M. O. (1980). Effects of light and density on resource allocation in a forest herb, *Aster acuminatus* (Compositae). *American Journal of Botany* **67,** 942-948.

Plummer, J. A. & Bell, D. T. (1995). The effect of temperature, light and gibberellic acid (GA_3) on the germination of Australian everlasting daises (Asteraceae, tribe Inuleae). *Australian Journal of Botany* **43,** 93-102.

Pons, T. L. (1992). Seed responses to light. In *The ecology of regeneration in plant communities* (ed. M. Fenner), pp. 259-284. CAB International, Wallingford.

Pooley, E. (1993). *The complete field guide to trees of Natal,* 1st edition. Natal Flora Publication Trust, Durban.

Popay, A. I. & Roberts, E. H. (1970). Factors involved in the dormancy and germination of *Capsella bursa-pastoris* (L.) Medik. and *Senecio vulgaris* L. *Journal of Ecology* **58,** 103-122.

Prach, K. & Wade, P. M. (1992). Population characteristics of expansive perennial herbs. *Preslia* **64,** 45-51.

Price, S. C. & Jain, S. K. (1981). Are inbreeders better colonisers? *Oecologia* **49,** 283-286.

Pysek, P. (1995). On the terminology used in plant invasion studies. In *Plant invasions-general aspects and special problems* (ed. P. Pysek, K. Prach, M. Rejmánek and M. Wade), pp. 71-81. SPB Academic Publishing, Amsterdam.

Pysek, P., Prach, K. & Smilauer, P. (1995). Relating invasion success to plant traits: an analysis of the Czech alien flora. In *Plant invasions - general aspects and special problems* (ed. P. Pysek, K. Prach, M. Rejmánek and M. Wade), pp. 30-43. SPB Academic Publishing, Amsterdam.

Rathcke, B. (1983). Competition and facilitation among plants for pollination. In *Pollination Biology* (ed. L. Reel), pp. 305-329. Academic Press, New York.

Rejmánek, M. (1989). Invasibility of plant communities. In *Biological invasions. A global perspective* (ed. J. A. Drake, H. A. Mooney, F. Di Castri, F. Groves, R. H. Kruger, M. Rejmánek and M. Williamson), pp. 369-388. John Wiley, Chichester.

Rejmánek, M. (1995). What makes a species invasive? In *Plant invasions-general aspects and special problems* (ed. P. Pysek, K. Prach, M. Rejmánek and M. Wade), pp. 3-13. SPB Academic Publishing, Amsterdam.

Rejmánek, M. (1996). A theory of seed plant invasiveness: the first sketch. *Biological Conservation* **78,** 171-181.

Rejmánek, M. & Richardson, D. M. (1996). What attributes make some plant species more invasive ? *Ecology* **77,** 1655-1659.

Rejmánek, M., Thomsen, C. D. & Peters, I. D. (1991). Invasive vascular plants of California. In *Biogeography of mediterranean invasions* (ed. R. H. Groves and F. Di Castri), pp. 81-101. Cambridge University Press, New York.

Richards, A. J. (1986). *Plant breeding systems.* Allen & Unwin, London.

Robins, D. J. (1982). The pyrrolizidine alkaloids. *Natural Product Reports* **41,** 115-203.

Robison, M. (1999). Proposal for the control of *Delairea odorata* (Cape Ivy) in California. PhD thesis proposal, University of California, Davis.

Robison, M. (pers. comm.). University of California, Davis, CA 95616, California, USA.

Robocker, W. C. (1977). Germination of seeds of common yarrow (*Achillea millefolium*) and its herbicidal control. *Journal of Range Management* **25,** 456-458.

Roeder, E. (1990). Carbon-13 NMR spectroscopy of pyrrolizidine alkaloids. *Phytochemistry* **29,** 11-29.

Roggen, H. P. & van Dijk, A. J. (1972). Breaking incompatibility in *Brassica oleracea* L. by steel brush pollination. *Euphytica* **21,** 48-51.

Rokich, D. P. & Bell, D. T. (1995). Light quality intensity effects on the germination of species from the Jarrah (*Eucalyptus marginata*) forest of Western Australia. *Australian Journal of Botany* **43,** 169-179.

Romeo, J. T., Saunders, J. A. & Barbosa, P. (1995). Phytochemical diversity and redundancy in ecological interactions. *Recent Advances in Phytochemistry* **30,** 1-319.

Rothschild, M. (1973). Secondary plant substances and warning colouration in insects. In *Insect/plant relationships* (ed. H. F. van Emden), pp. 59-83. Blackwell, Oxford.

Salonen, V. (1994). Growth and reproduction of *Rubus saxatilis* L. in relation to availability of light. *Acta Ecologica* **15,** 484-493.

Schroeder, D. (1985). The search for effective biological control agents in Europe. 1. Diffuse and spotted knapweed. In *Proceedings of the 6th International Symposium on the Biological Control of Weeds* (ed. E. S. Delfosse), pp. 103-119. Agriculture Canada, Vancouver.

Schroeder, D. & Goeden, R. D. (1986). The search for arthropod natural enemies of introduced weeds for biological control - in theory and practice. *Biocontrol News and Information* **7,** 147-155.

Scott, J. K. & Way, M. K. (1990). A survey in South Africa for potential biological control agents against capeweed, *Arctotheca calendula* (L.) Levyns (Asteraceae). *Plant Protection Quarterly* **5,** 31-33.

Scott, J. K. & Delfosse, E. S. (1992). Southern African plants naturalised in Australia: a review of weed status and biological control potential. *Plant Protection Quarterly* **7,** 70-80.

Sheldon, J. C. (1974). The behaviour of seeds in soil. III. The influence of seed morphology and the behaviour of seedlings on the establishment of plants from surface lying seeds. *Journal of Ecology* **62,** 47-66.

Shepherd, R. C. H. (1993). The role of biological control in weed management. *Plant Protection Quarterly* **8,** 144-146.

Slade, A. J. & Hutchings, M. J. (1987a). The effects of nutrient availability on foraging in the clonal herb *Glechoma hederacea. Journal of Ecology* **75,** 95-112.

Slade, A. J. & Hutchings, M. J. (1987b). The effects of light intensity on foraging in the clonal herb *Glechoma hederacea. Journal of Ecology* **75,** 639-650.

Slade, A. J. & Hutchings, M. J. (1987c). Clonal growth and plasticity in foraging behaviour in *Glechoma hederacea. Journal of Ecology* **75,** 1023-1036.

Smreciu, E. A., Currah, R. S. & Toop, E. (1988). Viability and germination of herbaceous perennial species native to the southern Alberta grasslands. *Canadian Field Naturalist* **102,** 31-38.

Sobrevila, C. (1989). Effects of pollen donors on seed formation in *Espletia schultzii* (Compositae) populations at different altitudes. *Plant Systematics and Evolution* **166,** 45-67.

Solangaarachchi, S. M. & Harper, J. L. (1987). The effect of canopy filtered light on the growth of the white clover *Trifolium repens. Oecologia (Berl.)* **72,** 372-376.

Stelljes, M. E. & Seiber, J. N. (1990). Pyrrolizidine alkaloids in an overwintering population of monarch butterflies (*Danaus plexippus*) in California. *Journal of Chemical Ecology* **16,** 1459-1470.

Steel, R.G.D. & Torrie, J.H. (1980). *Principles and procedures of statistics: a biometrical approach.* McGraw-Hill International Editions, Singapore.

Stelljes, M. E., Kelley, R. B., Molyneux, R. J. & Seiber, J. N. (1991). GC-MS determination of pyrrolizidine alkaloids in four *Senecio* species. *Journal of Natural Products* **54,** 759-773.

Stephens, R. J. (1982). *Theory and practice of weed control.* The Macmillan Press Ltd, London.

Sultan, S. E. (1987). Evolutionary implications of phenotypic plasticity in plants. *Evolutionary Biology* **21,** 127-176.

Sultan, S. E. & Bazzaz, F. A. (1993). Phenotypic plasticity in *Polygonum persicaria.* 1. Diversity and uniformity in genotypic norms of reaction to light. *Evolution* **47,** 1009-1031.

Surles, W. & Kok, T. T. (1978). *Carduus* thistle seed destruction by *Rhinocyllus conicus. Weed Science* **26,** 264-269.

Sutherland, W. J. & Stillman, R. A. (1988). The foraging tactics of plants. *Oikos* **52,** 239-244.

Syrett, P. (1985). Host-specificity of the ragwort flea beetle *Longitarsus jacobaeae* (Waterhouse) (Coleoptera : Chrysomelidae). *New Zealand Journal of Zoology* **12,** 335-340.

Syrett, P., Fowler, S. V. & Emberson, R. M. (1996). Are chrysomelid beetles effective agents for biological control of weeds? In *Proceedings of the IX international symposium on biological control of weeds* (ed. V. C. Moran and J. H. Hoffmann), pp. 399-407. University of Cape Town, Stellenbosch, South Africa.

Takahashi, N. (1979). A concept of dormancy, germination and longevity in cereal seeds. *Science Reports Research Institutes Tohoku University* **30,** 21-27.

Thompson, J. N. & Willson, M. F. (1978). Disturbance and the dispersal of fleshy fruits. *Science* **200,** 1161-1163.

Thompson, K. (1990). Genome size, seed size and germination temperature in herbaceous angiosperms. *Evolutionary Trends in Plants* **4,** 113-116.

Timbilla, J. A. & Braimah, H. (1996). The menace of *Chromolaena odorata*: a case study in the Brong Ahafo region of Ghana. In *Proceedings of the Third International Workshop on the Biological Control and Management of* Chromolaena odorata (ed. U. K. Prasad, P. Muniappan, P. Ferrar and J. P. Aeschlimann), pp. 67-75. Orstam, Abijan, Cóte d'Ivoire.

Toppel, G., Witte, L., Riebesehl, B., Borstel, K. & Hartmann, T. (1987). Alkaloid patterns and biosynthetic capacity of root cultures from some pyrrolizidine alkaloid producing *Senecio* species. *Plant Cell Reports* **6,** 466-469.

Trujilo, E. E. (1984). Biological control of hamakua pamakani with *Cercosporella* sp. in Hawaii. In *Proceedings of the VI International Symposium on the Biological Control of Weeds* (ed. E. S. Delfosse), pp. 661-671. Agriculture Canada, Vancouver.

USDA (United States Department Of Agriculture) (1998). Germination enhancement trials with Cape Ivy seeds. Agricultural Research Service Invasive Weed Seed Laboratory, Reno.

van Dam, N. M. & Vrieling, K. (1994). Genetic variation in constitutive and inducible pyrrolizidine alkaloid levels in *Cynoglossum officinale* L. *Oecologia* **99,** 374-378.

van der Meijden, E. (1996). Plant defence, an evolutionary dilemma: contrasting effects of (specialist and generalist) herbivores and natural enemies. *Entomologia Experimentalis et Applicata* **80,** 307-310.

van Driesche, R. G. & Bellows, T. S. (1996). *Biological control*. Chapman and Hall, New York.

van Staden, J., Davey, J. E. and Noel, A.R A. (1977). Gall formation in *Erythrina latissima*. *Zeitschrift fur Planzenphysiologie* **84,** 283-294.

Vaughton, G. (1988). Pollination and seed set of *Banksia spinulosa*: evidence for autogamy. *Australian Journal of Botany* **36,** 633-642.

Vermeij, G. (1996). An agenda for invasion biology. *Biological Conservation* **78,** 3-9.

Vogt Anderson, U. (1995). Comparison of dispersal strategies of alien and native species in the Danish flora. In *Plant Invasions - general aspects and special problems* (ed. P. Pysek, K. Prach, M. Rejmánek and W. Wade), pp. 45-57. SPB Academic Publishing, Amsterdam.

Vrieling, K. & van Vyk, A. M. (1994). Estimating costs and benefits of the pyrrolizidine alkaloids of *Senecio jacobaeae* under natural conditions. *Oikos* **70**, 449-454.

Wan, F., Harris, P., Cai, L. & Zhang, M. (1996). Host-specificity of *Altica carduorum* Guer. (Chrysomelidae: Coleoptera), a defoliator of *Cirsium arvense* (L.) Scop. (Asteraceae) from north-western China. *Biocontrol Science and Technology* **6**, 521-530.

Wapshere, A. J. (1974). A strategy for evaluating the safety of organisms for biological weed control. *Annals of Applied Biology* **77**, 201-211.

Wapshere, A. J. (1989). A testing sequence for reducing rejection of potential biological control agents for weeds. *Annals of Applied Biology* **114**, 515-526.

Waser, N. M. & Price, M. V. (1989). Optimal outcrossing in *Ipomopsis aggregata*: seed set and offspring fitness. *Evolution* **43**, 1097-1109.

Watson, A. K. (1984). Host-specificity of plant pathogens in biological weed control. In *Proceedings of the VI International Symposium on the Biological Control of Weeds* (ed. E. S. Delfosse), pp. 577-586. Agriculture Canada, Vancouver.

Wells, M. J. & Stirton, C. J. (1982). South Africa. In *Biology and ecology of weeds* (ed. W. Holzner and M. Numata), pp. 339-345. Junk, The Hague.

Werner, P. A. (1975). Predictions of fate from rosette size in teasel (*Dipsacus fullonum*). *Oecologia* **20**, 197-201.

Whelan, R. J. & Goldingay, R. L. (1989). Factors affecting fruit-set in *Telepea speciosissima* (Proteaceae): the importance of pollen-limitation. *Journal of Ecology* **77**, 1123-1134.

Whelan, R. J. & Goldingay, R. L. (1986). Do pollinators influence seed-set in *Banksia paludosa* Sm. and *Banksia spinulosa* R. Br.? *Australian Journal of Ecology* **77**, 1123-1134.

Whigham, D. (1974). An ecological life history study of *Uvularia perfoliata* L. *American Midland Naturalist* **91**, 343-359.

Widen, B. & Widen, M. (1990). Pollen limitation and distance-dependent fecundity in females in the gynodioecious herb *Glechoma hederacea* (Lamiaceae). *Oecologia* **83**, 191-196.

Williamson, M. H. & Fitter, A. (1996). The characters of successful invaders. *Biological Conservation* **78**, 163-170.

Willis, A. J. & Groves, R. H. (1991). Temperature and light effects on the germination of seven native forbes. *Australian Journal of Botany* **39**, 219-228.

Witte, L., Rubiolo, P., Bicchi, C. & Hartmann, T. (1993). Comparative analysis of pyrrolizidine alkaloids from natural sources by gas chromatography-mass spectrometry. *Phytochemistry* **32**, 187-196.

Woodburn, T. L. (1993). Host-specificity testing, release and establishment of *Urophora solstitialis* L. (Diptera: Tephritidae), a potential biological control agent for *Carduus nutans* L., in Australia. *Biocontrol Science and Technology* **3**, 419-426.

Yano, K. & Heppner, J. (1983). Description of hamakua pamakani plume moth from Hawaii (Lepidoptera: Pterophoridae). *Proceedings of the Hawaiian Entomological Society* **24**, 335-339.

Zapata, T. R. & Arroyo, M. T. K. (1978). Plant reproductive ecology of secondary deciduous tropical forests in Venezuela. *Biotropica* **10**, 221-230.

Zar, J.H. (1974). *Biostatistical analysis*. Prentice Hall Inc., London.

Zimdahl, R. L. (1993). *Fundamentals of weed science*. Academic Press Inc., New York.

Zimmerman, M. & Pyke, G. H. (1988). Reproduction in *Polemonium*: assessing the factors limiting seed set. *American Naturalist* **131,** 723-738.

Zwolfer, H. (1974). Das Treffpunkt-Prinzip als Kommunikationsstrategie and Isolations mechanismus bei Bohrfliegen (Diptera: Trypeidae). *Entomologica Germanica* **1,** 11-20.

Zwolfer, H. & Harris, P. (1971). Host-specificity determination of insects for biological control of weeds. *Annual Review of Entomology* **16,** 159-178.

APPENDIX A

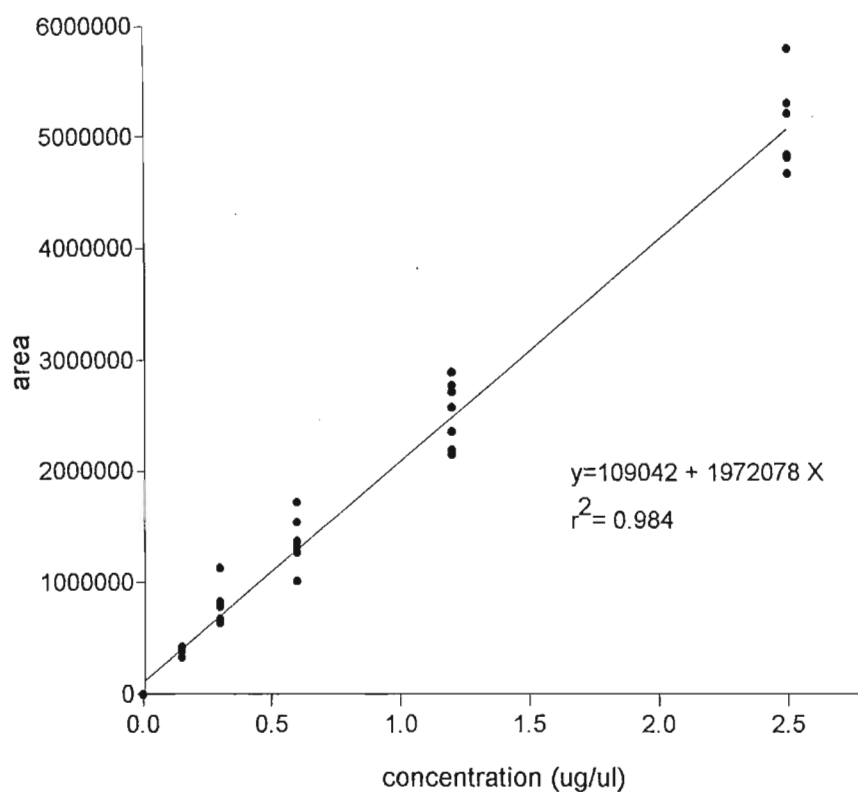**Fig. 1**. Standard curve prepared from a hyoscyamine dilution series used to estimate the PA concentrations in aerial plant parts.