THE ECOLOGY OF *HYPERICUM GRAMINEUM* WITH REFERENCE TO BIOLOGICAL CONTROL OF *H. PERFORATUM* BY THE MITE, *ACULUS HYPERICI*

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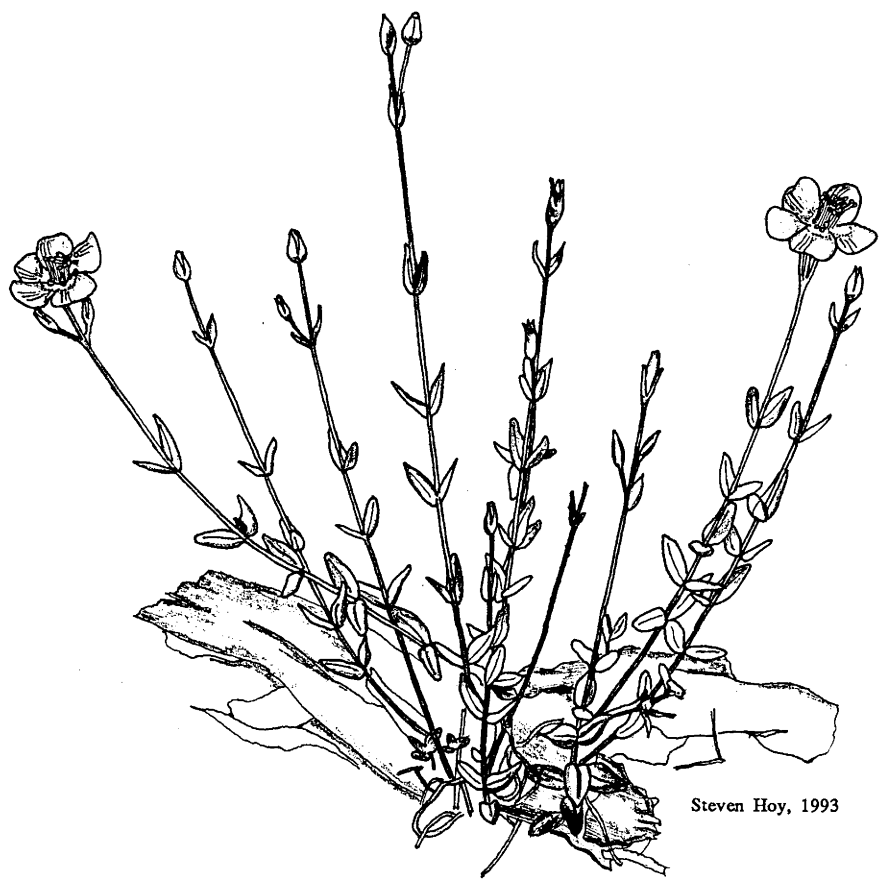
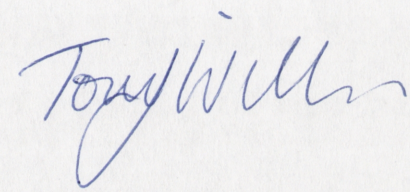


Hypericum gramineum Forst.

DECLARATION

The research presented in this thesis is my original and independent work. Specific contributions and assistance by others are referred to in the text and acknowledgments.



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ABSTRACT

A major concern with biological control is that deliberately introduced organisms may have damaging effects on non-target species. Introductions of some animal species to control invertebrates have proved devastating to native ecosystems, but similar problems have not arisen in biological weed control. Experience has shown that utilisation of a host in laboratories does not necessarily translate into severe impacts in the field. Biological weed control agents have been observed on non-target species in the field. Utilisation of such non-target species does not appear to have led to reductions in populations of the novel host. In no case, however, has a non-target indigenous species been monitored to determine the impact of control agents on plant growth and population dynamics, nor have the impacts in glasshouses and the field been compared. Critical questions are why utilisation does not translate into population reductions, and whether the lack of reported impacts may simply reflect the absence of precise monitoring. These issues are addressed in this thesis, which investigates the impact of a biological control herbivore, *Aculus hyperici* Liro, on both the target weed, *Hypericum perforatum* L., and a non-target species, *H. gramineum* Forster. In so doing, a comparative approach is adopted, whereby the effect of *A. hyperici* on the non-target species is compared with its effect on the target weed in both glasshouse and field studies, both experimentally and by monitoring populations. Limited study of other *Hypericum* species was also undertaken. The plant-herbivore system is examined in the context of current theories of herbivore-plant interactions.

Hypericum perforatum L. is a European perennial herb which became naturalised in Australia. The species spread rapidly throughout south-eastern Australia, reducing the capacity of grasslands to provide grazing for livestock and lowering the floristic diversity of infested areas. Despite several attempts to biologically control *H. perforatum*, it remains weedy in much of south-eastern Australia. Concern about the spread of *H. perforatum* prompted further research into biological control of this species. In May 1991, *A. hyperici* was released into populations of *H. perforatum*, despite evidence that it is able to feed and reproduce on the indigenous *H. gramineum* under laboratory conditions.

There are no published accounts of the ecology of *H. gramineum*. One aim of this thesis, therefore, has been to provide basic ecological information about the native study species. To judge the impact of mites on populations of the non-target native, demographic aspects of the species were investigated. Experiments on *H.*

gramineum's reproductive ecology indicated that the species is not apomictic and that more seeds are produced following cross-pollination than selfing. Seeds require an after-ripening period of about 6 months. Germination, which is strongly temperature and light-dependent, increased when parental plants were infested by mites. Recruitment from seeds occurs in autumn or spring, providing that innate seed dormancy has been overcome and that light, soil temperature and soil moisture are not limiting. Seeds are incorporated into a persistent seed bank, since few such diaspores (< 20%) are removed by predators. A model of decay of seeds in the seed bank suggests that about 63% survive each year.

A field survey of *H. gramineum* demonstrated that populations consist of seedlings, juveniles, vegetatively produced ramets, single-stemmed adults and multi-stemmed adults. Annual adult mortality approximated 12%, but increased to 65% during periods a drought. A stage-structured transition matrix simulation suggests that populations are relatively insensitive to reductions in growth but sensitive to changes in mortality and seed production.

Investigation of *A. hyperici* dispersal indicated that the probability of mites establishing on the non-target is significantly less than on the target species. This may reflect a preference for the target weed, but is likely to be strongly affected by the greater 'apparency' of *H. perforatum*. Mite establishment declines inversely with distance, which suggests that sparse populations are likely to be infested less rapidly. Density-dependence is thus likely to reflect the square-root of host density.

Host-choice tests across a range of species raise methodological issues. Recently, it has been argued that in comparative studies, statistical assumptions of independence are violated by ignoring the phylogenetic relationship of taxa. Re-analyses of CSIRO data using a nested ANOVA to reflect *Hypericum*'s infra-generic phylogeny did not reveal clear phylogenetic differences in the suitability of *Hypericum* species as *A. hyperici* hosts. Analyses concurred, however, with CSIRO predictions that mite growth and reproduction is higher on *H. perforatum* than on other species. It appears that host suitability reflects traits which vary independently of phylogeny.

Having determined that mites infest populations of *H. gramineum*, experiments were conducted to quantify the impact of mites on plant growth and populations. The effects of nutrient limitation, plant competition, water limitation and aphid herbivory were combined with mite herbivory. Mites reduced most indices of absolute growth, usually by about 20 - 40%, though many of these decreases were not statistically significant. This lack of significance may reflect variability in the mite infestations.

Consistently, roots were more adversely affected than shoots, thereby altering the ratio of root:shoot biomass. Mites had similar, though proportionally greater effects on *H. perforatum*. Differences between growth of *H. perforatum* and *H. gramineum* were usually insignificant. There was no indication that plant tolerance of herbivory decreased under stress. Rather, plant growth under combinations of such stresses was roughly the product of growth under each stress alone. This conclusion lends little support to current theories of herbivore plant interaction, which variously hypothesise that either stressed plants or vigorously growing plants are better sources of nutrition for herbivores, leading to greater impact.

Reductions in growth of *H. gramineum* attributable to *A. hyperici* were modelled in a projection matrix, simulating density-dependent plant infestation. At typical densities of 3 - 5 plants m^{-2}, the modelled population declined at about 6% per annum, the impact declining as densities fell. Drought caused a faster proportional decline in populations.

The thesis concludes by indicating that *A. hyperici* is likely to infest populations of *H. gramineum*, though the non-target species will probably support smaller mite populations than *H. perforatum*. In combination with the greater suitability of *H. perforatum* as a host, this conclusion is consistent with Feeney's (1976) hypothesis of plant 'apparency'. At typical plant densities, mites are likely to have negative effects on populations of the indigenous species. Under conditions of sustained herbivory over several years, however, the rates of population decline are likely to be slow. The target weed species, *H. perforatum* is likely to suffer mite impacts at similar, relatively low-levels.

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SECTION A:

GENERAL INTRODUCTION

This section of the thesis comprises an introductory chapter, providing an outline of the methods and current concerns of biological weed control and background information on the plants and mite being studied. The chapter concludes with a summary of the broad aims of the thesis and an outline of its structure.

CHAPTER 1

GENERAL INTRODUCTION

1.1. Weeds

Weeds are plants '...not valued for use or beauty, growing wild and rank, and regarded as cumbering the ground or hindering the growth of superior vegetation' (Humphries *et al.* 1991). Batra (1982) indicates that weeds are potentially the most damaging agricultural pests and may pose a greater threat to crops than insect pests. Annual expenditure on chemical weed control exceeds that of insect pest control. In 1992, for example, Australian farmers spent about $460 million on herbicides and about $130 million on insecticides (Australian Bureau of Agricultural and Resource Economics 1993). Clearly, weeds are of great economic importance. The environmental effects of weeds are more difficult to cost, but invasive plants can have significant impacts on floristic diversity and community ecology (Vitousek 1990; Humphries *et al.* 1991).

In Australia, naturalised foreign plants, accidentally or deliberately introduced for a variety of aesthetic, horticultural, agricultural and ecological reasons, now comprise up to 15% of the continent's flora and locally may dominate the vegetation (Humphries *et al.* 1991). In excess of 1000 species are naturalised in temperate regions. While many do not present major economic problems, they are potentially aggressive components of the flora, posing serious environmental threats. Of the 825 naturalised species in Victoria, 412 are weeds of native vegetation and of these, almost 23% are considered a serious threat to communities of native plants (Humphries *et al.* 1991). Long-term control of these weeds is a major aim of conservation in Australia. To achieve specificity in controlling weeds and minimise the impact of control measures on the native flora, the use of biological control seems most promising.

1.2. Herbivory and the control of weeds

Herbivores, including taxa as diverse as mammals, molluscs, insects and mites, can have dramatic impacts on the biology of their host plants. By grazing, sucking,

mining, boring and generally deforming their host tissues, herbivores may destroy or impair the functions of organs and remove photosynthate, nutrients, water and other plant resources (Crawley 1983; New 1988). Herbivores may also have indirect effects on plant fitness and survival by transmitting pathogens and facilitating infection (Kennedy *et al.* 1962; Burdon 1987).

In reducing plant growth, reproduction and host fitness, herbivory has the potential to alter plant population dynamics and ultimately plant communities (Crawley 1989; Huntly 1991). Perhaps the clearest evidence of this aspect of herbivory comes from the literature on the biological control of exotic weeds. Herbivores introduced from the host plant's native range for control of aggressive exotic weeds such as *Lantana camara* (Verbenaceae) in Hawaii, the aquatic fern, *Salvinia molesta* (Salviniaceae) in Papua New Guinea and prickly pear, *Opuntia* spp. (Cactaceae), in Australia, have caused large scale reductions in the severity of infestations and the rate of population expansion of these species (Crawley 1989, and references therein).

Successful biological control is not limited to any particular taxon of herbivore or mode of feeding, though photosynthetic and reproductive structures are generally targeted. In summarising some of the most successful biological weed control projects up to 1980, Crawley (1989) notes, for example, that chrysomelid and curculionid beetles, noctuid and pyralid moths, a tephritid fly, a cecidomyiid midge, wasps, hemipterans and a range of other herbivorous taxa have been used. More recently, phytophagous mites have also been employed (Gerson 1990). Illustrating the diversity of feeding modes, these herbivores variously graze leaves, bore and mine stems and roots, consume seeds and fruit, and cause galling of their hosts' shoots.

Many cases of attempted biological weed control have been less successful, for a variety of reasons, including that the introduced herbivores are less fit, have less impact or disperse poorly in the new environment, or that control may require the combined effects of several herbivores and/or environmental stress. These 'failures' cannot, however, be considered as evidence for the unimportance of herbivory. Rather, there is a complex interplay between host, herbivores and the environment.

Interactions between the environment and the physiological condition of plants have been the focus of many hypotheses concerning the nature of herbivore-plant interactions. Such hypotheses have generally fallen into two groups: those emphasising the importance of phago-stimulatory compounds, and those

highlighting the role of plant defensive chemistry. Theories from the former group are largely centred on observations that outbreaks of many insect herbivores are correlated with relatively higher concentrations of utilisable plant nitrogen, which ordinarily limits insect growth (Mattson 1980; Mattson and Haack 1987a,b). Moreover, such increases in plant nitrogen concentration have been associated with moderate levels of plant stress, and in particular, water limitation (Mattson 1980; White 1984; Mattson and Haack 1987a,b; Larsson 1989). The 'plant stress hypothesis' thus links increased herbivory to plant stress.

In contrast to the 'plant stress' hypothesis, Price's (1991) 'plant vigour' hypothesis argues that herbivores benefit from vigorously growing plants. The hypothesis is based on observations that herbivores achieve most rapid growth and population expansion on rapidly growing plants and plant organs that are young and fast growing (Waring and Price 1990; Price 1991). By comparison, hypotheses surrounding the importance of phago-inhibitory compounds have centred on the ability of plant secondary metabolites to cause toxic effects and/or in combination with complex organic compounds, to deter insect herbivores by reducing the digestibility of plant tissue (Ehrlich and Raven 1964; Feeney 1976; Rhoades and Cates 1976; Rhoades 1983; Gershenzon 1984; Coley *et al.* 1985; Zangerl and Berenbaum 1993). Coley *et al.* (1985) speculate that in resource-rich environments, where growth is rapid, plant organs are expendable and plants are likely to rely on metabolically cheap toxic defences which protect against key herbivores. In resource-poor environments, where growth is slower, they argue that plants are likely to rely more heavily on large quantities of digestibility-reducing compounds which, though metabolically expensive, remain with the plant organ for its duration and give better protection against a wider range of phytophagous arthropods. The hypothesis suggests that plant species may show a 'trade-off point' where, for example, rapidly growing seedlings defended by simple, toxic deterrent compounds, give way to tissues/organs characterised by slower growth and defended by larger quantities of digestibility-reducing compounds.

These theories of herbivore-plant interaction are not mutually exclusive (Price 1991). It is likely that there is a continuum of herbivore responses to plants associated with interactions between environmental stress, plant health and chemistry, and the mode of herbivore feeding (Larsson 1989). From a plant's perspective, feeding herbivores may induce stress by removing plant resources. Therefore, it may be useful to treat herbivory as a form of stress rather than focussing too much on the diversity of feeding behaviour.

Practitioners of biological weed control have emphasised the importance of plant stress in the interaction between herbivore and host. In releasing several herbivores into populations of weedy species, the different agents are generally targeted at distinct plant tissues and often in different seasons. In so doing, it is argued that the stresses imposed by herbivory are sustained through different seasons and limit the ability of the weed to regrow when one of the herbivores is less active, or the season promotes vigorous plant growth. Harris (1980) advocates releasing biological control agents into weed populations physically stressed by, for example, water, or nutrient limitation. He suggests that such stressed plants are less tolerant of herbivores and, therefore, more likely to succumb to their debilitating effects. Biological weed control presents opportunities to investigate the nature of some of the interactions outlined above.

1.3. Principles and methods of biological weed control

Biological control of weeds involves attempts to reduce plant populations to non-noxious levels of abundance by introducing or promoting plant-attacking organisms (Wapshere 1982). The density-dependent relationships between herbivore and plant generally mean that dense plant populations are severely affected, while at low density some plants escape herbivory. Biological control thus limits, rather than eliminates the target.

Recently, reviewing the advantages and disadvantages of biological weed control, Wapshere *et al.* (1989) recognised four approaches: (1) 'classical' or inoculative biological control, (2) inundative/augmentative biological control, (3) broad-spectrum control and (4) conservative biological control.

1.3.1. Classical biological control

Classical biological weed control was the first technique employed and has been responsible for the successful management of several species including *Opuntia* spp. and one form of skeleton weed, *Chondrilla juncea* (Asteraceae) in Australia, *Hypericum perforatum* (Hypericaceae) in California and *Lantana camara* in Hawaii (see Wapshere *et al.* 1989). The process involves importation of natural enemies of a weed from its native range to the country of introduction. After demonstrating host-specificity of the agents, they are released into field populations of the weed, whereupon they self-perpetuate and disseminate.

1.3.2. Inundative biological control

In the approach of inundative biological control, large numbers of a control agent are reared and released into populations of the weed at artificially high levels. One advantage of this technique is that native organisms occurring on the weed in the area of infestation can be used, even when at more natural densities they have only marginal impacts on weed growth (Wapshere *et al.* 1989). This control method has been relatively successful in managing infestations of the water hyacinth, *Eichhornia crassipes* (Pontederiaceae), in the south-eastern USA; two applications of the native fungal pathogen, *Cercospora rodamnii*, in combination with freezing temperatures killed many plants (see Wapshere *et al.* 1989).

1.3.3. Conservative control

To date, conservative biological control has not been thoroughly investigated and has not been used in any weed biological control program. Proponents of the technique argue that by reducing the number of parasites, predators and diseases that attack naturally-occurring phytophagous arthropods, the ability of the latter to reduce weed growth is enhanced. The necessary procedures are, however, unclear.

1.3.4. Broad spectrum control

In broad-spectrum control, land managers manipulate the numbers of generalist herbivores within a defined area, thereby reducing the degree of weed infestation to more acceptable levels. A clear example is in the use of livestock such as sheep and goats to limit weed abundance in pastures. In a similar way, phytophagous fish such as *Tilapia* spp. have also been used against aquatic weeds (Wapshere *et al.* 1989). One problem with this method of control is that non-target species are also likely to be utilised and potentially damaged by the generalist agents. Attempts are made to limit such undesirable side effects by restricting the herbivores to defined areas. This is achieved more easily with large mammalian grazing agents than, for example, fish.

1.3.5. Conflicts of interest in the biological control of weeds

Regardless of the form of biological control implemented to manage weeds, there is increasing awareness that biological control may generate conflicts of interest between community groups (Andres 1980; Andres 1981; Howarth 1983; Pimentel *et al.* 1984; Pemberton 1985; Turner 1985; Cullen 1989). Plants viewed as weeds

by some may be valued by others for, among other reasons, their aesthetic appeal, their horticultural or medicinal potential, their use as fodder or as a source of nectar for honey-bees (Andres 1981; Syrett *et al.* 1985; Turner 1985). Increasingly, questions regarding the impact of biological control agents on non-target species are also being asked (Andres 1981; Turner 1985). A perennial concern with classical biological control is that an introduced agent may significantly affect the growth and population structure of non-target species, perhaps by expanding its host range after field-release. It is this concern which lead to the current project.

1.3.5.1. Testing of biological control agents on non-target species

The early protocol for classical biological control of weeds was that release of agents followed demonstration of their potency on target taxa, and demonstration that economically important non-target taxa, such as fruit or cereal species, were unable to support agent-feeding or oviposition (Huffaker 1957; Huffaker 1959; Wilson 1964; Zwölfer and Harris 1971). Recognising the short-comings of this procedure in identifying potentially susceptible species, the selection of plants for host-specificity screening was broadened to include plants closely related to the target species (but not necessarily of any economic value), plant species attacked by taxonomically similar agents, and taxa on which the agents had been previously recorded (Wapshere 1974). The process was formalised into a 'centrifugal phylogenetic testing method' (Wapshere 1974), in which phylogenetically close relatives of the target weed are tested first and progressively, plants of greater taxonomic diversity or greater cladistic/phylogenetic distance are screened. Most host-specificity testing has followed this strategy in recent years. In Australia, a strong emphasis on testing closely related indigenous species has also developed recently, especially for composite weeds (R. Groves, pers. comm.).

Despite all testing precautions, Zwölfer and Harris (1971) noted that there is no fully reliable test for predicting the host-specificity or host-range of biological control agents. There are many reasons for this associated with complex interactions between the physiologies, ecologies and behaviour of the biological control agents and their hosts. In addition, biological control herbivores are likely to interact with predators, parasites and pathogens, naturally occurring in the new range of the agents. All such interactions may be affected by the environment of quarantine laboratories or glasshouses, in which most host-specificity screening occurs. Thus, most testing is either upon detached plant organs or upon plants grown under relatively unstressed conditions.

1.3.5.2. Host-range expansion in herbivores

Glasshouse trials may reveal the physiological host range of a biological control agent, i.e., the plants on which growth and completion of an agents life cycle is/is not physiologically possible (Cullen 1989) and eliminates many potential non-target species. However, expansion of host-ranges remains possible. Incorporation of novel hosts into the diet of herbivores and variation in the preference-ranking of host plants has been documented on many occasions in agricultural and natural systems (Rausher 1983; Rausher 1984; Thomas *et al.* 1987; Courtney and Forsberg 1988; Fry 1989; Karowe 1990; Berenbaum and Zangerl 1991; Bowers *et al.* 1992). The lace bug *Teleonemia scrupulosa* (Hemiptera: Tingidae), for example, was introduced into southern Africa to control *Lantana camara* (Verbenaceae) and was soon found infesting nearby sesame (*Sesamum indicum*: Pedaliaceae) crops (see Turner 1985): a shift in host family. Native non-target species have also been included in the diet of biological control herbivores, but the significance of such findings is more controversial and more difficult to quantify than similar events on cultivated plants (Turner 1985). For example, the leaf beetle, *Lema cyanella*, introduced into Canada to control the thistle, *Cirsium arvense*, has been found on populations of native *Cirsium* species (Peschken 1984). Similarly, Rees (1977) reported that the Canadian native, *Cirsium undulatum*, was attacked by *Rhinocyllus conicus* (Coleoptera: Curculionidae), introduced to assist in controlling thistles in the *Carduus nutans* complex: a shift in genus. Andres (1985) located populations of the North American native, *Hypericum concinnum*, infested by *Chrysolina quadrigemina* (Coleoptera: Chrysomelidae), originally introduced to control *H. perforatum*. This beetle has also been observed on the Australian native, *H. gramineum* (pers. obs.). Evidently, the expansion of host ranges both within a genus and more broadly may occur. Attempts to control weeds biologically therefore provides unique opportunities to investigate the evolution of herbivory, host-range expansion, and the dynamics of herbivore-plant interactions.

1.3.5.3. Utilisation versus impact

A distinction has been drawn between control agents utilising non-target species, and having a significant impact on the growth and population dynamics of those species (Andres 1981; Turner 1985). In each of the above cases, for example, the biological agents detected on non-target natives are considered to have had minimal impact on the abundance of their novel hosts. Moreover, Groves (1989) found no documented cases in which a natural enemy, deliberately introduced to control a weed, affected reductions in the population of a native plant species. In no case,

however, has a native non-target species been monitored or studied in relation to the agent. Minor, and perhaps major impacts, could have been overlooked.

With growing community awareness of the importance of biological communities, research into biological control must demonstrate the safety, not only of economically important species, but increasingly, the safety of native floras. Howarth (1983) expresses concern over indiscriminate biological introductions being a form of 'biological pollution'. He observes, for instance, that the extinction of an endemic Hawaiian snail was apparently caused by introducing a predator intended to control another snail species. In comparison with introductions for invertebrate biological control, however, biological weed control has a relatively 'clean slate'. In future, assessment of the success or failure of weed biological control programs must include the impact of agents on non-target species, and not merely whether the target pest was controlled adequately (Howarth 1983).

Frequently, screening trials in glasshouses indicate that a potential biological control agent will feed and even reproduce on non-target native species. None the less, it is considered that the impact of the agent on natural populations of these indigenous taxa will be slight or undetectable. Past experience, albeit anecdotal, confirms this view, but little effort has been made to verify these claims, investigate the magnitude of the impacts, or indeed, explain the lack of effects.

This project was undertaken to investigate more fully the impact of a control agent on both a target and non-target species. The plant-herbivore system examined comprises the target weed, *Hypericum perforatum* L., the biological control mite, *Aculus hyperici* Liro, and the non-target indigenous Australian species, *Hypericum gramineum* Forster. Glasshouse trials revealed both *Hypericum* species to be a potential food source for the mite (CSIRO 1991), but past experience suggested that only the target weed would suffer damaging levels of herbivory. Emphasis has been placed on investigating the impact of *A. hyperici* on the growth and population dynamics of the native Australian non-target species. In so doing, a comparative approach has been adopted whereby the effect of *A. hyperici* on *H. gramineum* is contrasted with its impact on *H. perforatum*. The project commenced before release of the mite to allow monitoring of its impact. The study was, therefore, given a broad focus, since it was not clear where effects might be most severe.

In the remainder of this chapter the biology of *H. gramineum* and *H. perforatum* will be summarised, followed by a brief discussion of attempts to control this weed in Australia. Finally, the aims of the thesis are detailed more precisely.

1.4. Biology of *H. gramineum*

Hypericum is a fairly diverse genus, ranging from trees to annual herbs. Robson (1977) recognises about 380 species within the genus, distributed world-wide, with a probable centre of origin in Africa. He classifies the Australian native perennial herb, *H. gramineum* Forster along with the second indigenous Australian species, *H. japonicum* Thunb., also a perennial herb, in section *Trigynobrathys* (Robson 1990). This generic section, comprising about 52 species, has extant representatives in South America and Australasia.

Hypericum gramineum is native to New Zealand, parts of Papua New Guinea and south-east Asia as well as Australia. Within Australia, *H. gramineum* is distributed from Tasmania to northern Western Australia. The species is most prolific in the south-east of the continent where typical populations occur at densities of about 10^4 plants ha^{-1}. *Hypericum gramineum* flowers from spring (November) till late summer (February), generally dying back to one or two short, over-wintering vegetative shoots in autumn (March - May). The 5 - 15 mm diameter golden flowers (Plate 1) are borne at the apices of dichasial inflorescences which, by the end of summer, are typically 25 - 30 cm in height and bear 10 - 20 fruit (capsules; about 3 x 8 mm) which each contain about 200 small (0.5 x 0.25 x 0.25 mm) seeds. The species also produces subterranean rhizomes facilitating vegetative reproduction. In south-eastern Australia, *H. gramineum* is a common component of native grasslands and woodlands, but rarely achieves a cover of more than 1 - 2%. Other than in taxonomic works, there are no published accounts of the ecology of this species.

1.5. Biology of *H. perforatum*

1.5.1. Taxonomy and distribution of H. perforatum

Robson (1977) places *H. perforatum* in the generic section *Hypericum*, which achieves its greatest diversity in Eurasia. This section is relatively distantly related to section *Trigynobrathys* (N. Robson, pers. comm.), in which the Australian *Hypericum* species are classified. *Hypericum perforatum*, the type specimen for the section, is native to western Asia, Europe and northern Africa, though it has become a naturalised invasive weed in South Africa, North America, Hawaii, parts of South America, New Zealand and Australia (Clausen 1978).

Plate 1 (a) and (b) Examples of *Hypericum gramineum* growing in woodland, and (c) grassland environments; (d) detail of an *H. gramineum* flower. Scale bar represents 2 cm.

Commonly known as 'St. John's Wort', *H. perforatum* is a multi-stemmed perennial herb which grows to about 60 cm in height (Plate 2a). Upright flowering shoots elongate from the over-wintering rosette of relatively short, procumbent, leafy shoots in early spring. The numerous bright yellow flowers, 15 - 25 mm in diameter (Plate 2b), are clustered in terminal cymes borne at the extremities of the upright summer growth. These upright, relatively woody stems gradually die over autumn and winter, when the re-shooting vegetative rosette again becomes the dominant form.

1.5.2. Ecology of H. perforatum

Hypericum perforatum was deliberately introduced, as a horticultural plant, into the Bright region of north-eastern Victoria, Australia, in the 1800s, though it was also cultivated in Melbourne and Adelaide (Groves 1989). It quickly became a naturalised invasive plant, spreading throughout Victoria, New South Wales and South Australia. In south-eastern Australia, populations of *H. perforatum* often achieve densities of at least 10^5 plants ha^{-1} (pers. obs.).

As an aggressive species generally avoided by livestock, *H. perforatum* quickly spreads in pastures (Clausen 1978), reducing the capacity of grasslands to provide grazing for livestock and altering the floristic composition of infested areas (Plate 2c; Groves 1989). In common with many congeners, *H. perforatum* contains a photo-sensitive pigment, hypericin, which, if ingested in sufficient quantity, causes un-pigmented skin to become hyper-sensitive to light (Clausen 1978) and may detrimentally affect the central nervous system of livestock (Dodd 1920). When exposed to light, un-pigmented skin becomes irritated, blistered and may be rubbed raw. *Hypericum* poisoning of sheep, cattle, horses and goats has been reported from North and South America, Europe, Africa and Australia (see Clausen 1978, and references therein), though Tisdale *et al.* (1959) note that *H. perforatum* is primarily of concern as an aggressive weed, rather than a toxic species.

Tisdale *et al.* (1959) indicate that *H. perforatum* produces about 30 000 small (1 x 0.5 x 0.5 mm) seeds per plant per year. Sticky at capsule dehiscence, these seeds appear well-adapted to dispersal by passing animals (and traffic), which facilitate dispersal of the seeds over wide distances. With time or leaching, both of which lead to removal of a chemical germination inhibitor in the seed coat, about 70% of seeds are germinable in light at 15°C (Campbell 1985). Without removing the chemical inhibitor, and in the absence of appropriate temperature and lighting

Plate 2 (a) Example of a flowering individual of *Hypericum perforatum* and (b) detail of *H. perforatum* flowers. Scale bar represents 2.5 cm. (c) An infestation of *H. perforatum* near Kambah Pool, on the banks of the Murrumbidgee River, ACT, in November 1992.

regimes, germination of *H. perforatum* is limited (Campbell 1985). Clark (1953) suggests that the initial establishment and rapid spread of *H. perforatum* in Australia was possible because of its high seed productivity and success as a pioneer species on disturbed sites, such as gold dredgings. The extensive root system of this species is capable of vegetative reproduction via adventitious lateral roots, and the resultant ramets are quickly detached from the parent plant (Tisdale *et al.* 1959). Procumbent rosette shoots may also take root, thereby providing a second means of vegetative spread.

The combination of the high seed productivity and dispersability of *H. perforatum*, its efficient vegetative reproduction, its invasiveness into the native flora and its potential toxicity to grazing livestock render St. John's Wort a serious weed of economic and ecological importance. Campbell (1985) estimates that *H. perforatum* infests 500 000 ha of south-eastern Australia. Shepherd (1983) indicates that the species is now mainly a weed of bushland, open sclerophyll forests and plantations, rather than a weed of agricultural areas, though poorly managed pasture may also become heavily infested (Shepherd 1983; Campbell 1985).

1.5.3. Management of H. perforatum

Two stages of the life cycle of *H. perforatum* must be targeted to achieve successful control. In the short term, plant growth must be limited, thereby reducing replenishment of root reserves, from which *H. perforatum* is able to reproduce vegetatively. Long term control of St. John's Wort requires seed production to be significantly reduced (Groves 1989). Successful control of *H. perforatum* can be achieved in pasture with appropriate management techniques, including establishment of competitive pasture species such as clover, *Trifolium subterraneum*, and perennial grasses (Davey 1919; Moore and Cashmore 1942; Moore *et al.* 1989) and/or in combination with applications of appropriate herbicides (Campbell and Delfosse 1984). Nevertheless, such methods are labour-intensive, expensive and may not be practical in many of the wooded areas in which *H. perforatum* is weedy. Moreover, the process can have severe adverse effects on native flora.

1.5.3.1. Biological control of *H. perforatum*

Previous attempts at controlling *H. perforatum* biologically in Australia are well documented and have met with mixed success (Huffaker 1967; Harris *et al.* 1969; Groves 1989). Delfosse and Cullen (1981) indicated that eight out of twelve herbivorous insects imported into Australia from Britain and Europe were liberated into populations of *H. perforatum*. Of these, those that established and spread from their points of release most successfully were the beetle, *Chrysolina quadrigemina* Suffrain, the aphid, *Aphis chloris* Koch (Homoptera: Aphididae), and the gall midge, *Zeuxidiplosis giardi* Kieff. (Diptera: Cecidomyiidae). Despite successful establishment and dissemination, populations of the latter two species never increased to damaging levels (Delfosse and Cullen 1981). The chrysomelid beetle, by comparison, has been more successful.

1.5.3.2. Biological control with *Chrysolina quadrigemina*

Chrysolina quadrigemina, the first biological control herbivore to be released for *H. perforatum*, was initially responsible for controlling large infestations of the weed and continues to exert control in parts of southern and western Australia. In south-eastern Australia, however, its success was, and continues to be limited for several reasons: Firstly, the slow rate of increase in *C. quadrigemina* populations allows recovery of previously heavily-attacked stands of *H. perforatum* (Huffaker 1967). This appears to be because the beetles lose synchrony with the climate and seasons in south-eastern Australia leading to premature emergence from aestivation and ultimately death of adults (Huffaker 1967; Delfosse and Cullen 1981). In California, successful control of *H. perforatum*, locally termed 'Klamath weed', was achieved because the northern Californian climate was more like the European 'Mediterranean' type, and did not enforce asynchrony of beetle life-cycles with the environment (Huffaker 1967). Moreover, the absence of summer rains in California kills plants previously defoliated by *C. quadrigemina*, whereas in many Australian infestations of the weed, summer rainfall promotes regrowth and enables them to survive (Groves 1989). In combination, these factors have limited the efficiency of *C. quadrigemina* as a biological control agent in eastern Australia. Because of this, *H. perforatum* continues to infest and spread into many areas of Victoria and New South Wales, typically those of higher altitude and rainfall, dominated by *Eucalyptus* woodlands and forests (Delfosse and Cullen 1981; Wapshere 1984).

1.5.3.3. Biological control with *Aculus hyperici*

Ongoing concern about the spread of *H. perforatum* prompted continued research into potential biological control agents for this weed. The latest such agent, the mite, *Aculus hyperici* (Acari: Eriophyidae), was released into field infestations of *H. perforatum* in Victoria, New South Wales (NSW) and the Australian Capital Territory (ACT) in May 1991. This occurred despite the potential for *A. hyperici* to feed and reproduce, albeit at significantly lower rates, on several other species of *Hypericum*, including the Australian native species *H. japonicum*, and particularly *H. gramineum*.

Permission to release *A. hyperici* was granted by the Australian Plant Quarantine and Inspection Service, as it was believed that the advantages of controlling *H. perforatum* outweigh the risk that non-target species may be affected, and because the mite offers the best chance of controlling St. John's Wort in eastern Australia (Wapshere 1984). Sustained biological control of *H. perforatum* might, thereby, be possible (CSIRO 1991). That *A. hyperici* may have negative impacts on non-target species remains, however, of some concern. In particular, the potential impact of mites on *H. gramineum* warrants careful examination given that this indigenous congener of *H. perforatum* is sympatric with the weed in much of its range. Clearly, this increases the chance of *A. hyperici* encountering, utilising and possibly affecting individuals and populations of *H. gramineum*.

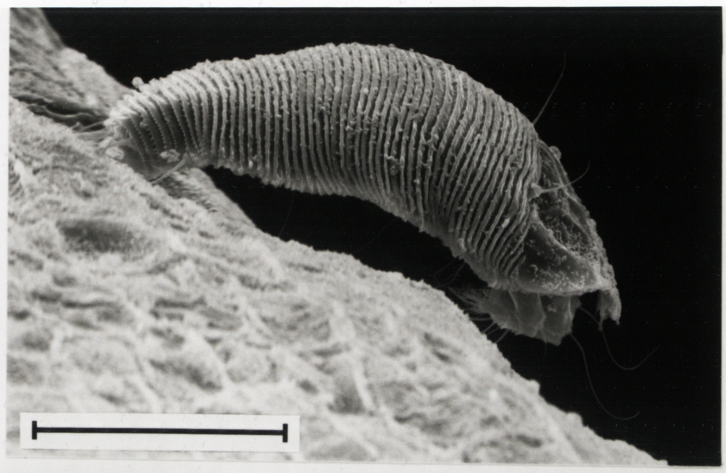
1.6. Biology of *A. hyperici*

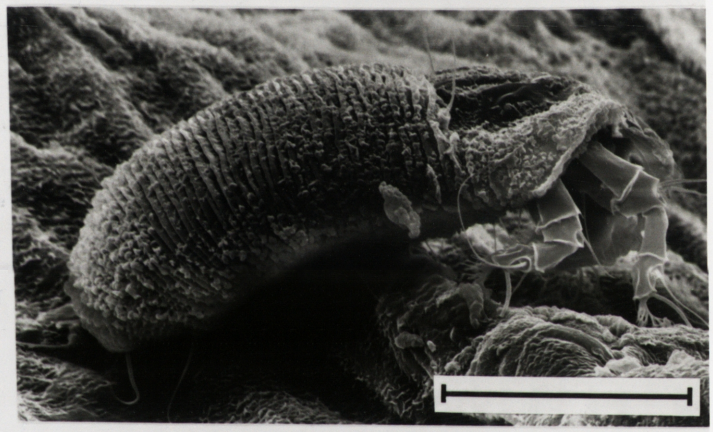
Aculus hyperici (Plate 3) is not an agricultural pest. Except for various taxonomic descriptions (see CSIRO 1991), it has received little attention from biologists. The only detailed investigations of its biology appear to be those conducted during host-specificity screening of the mite (CSIRO 1991), prior to its field release. The following summarises this work, drawing also from observations made during this study.

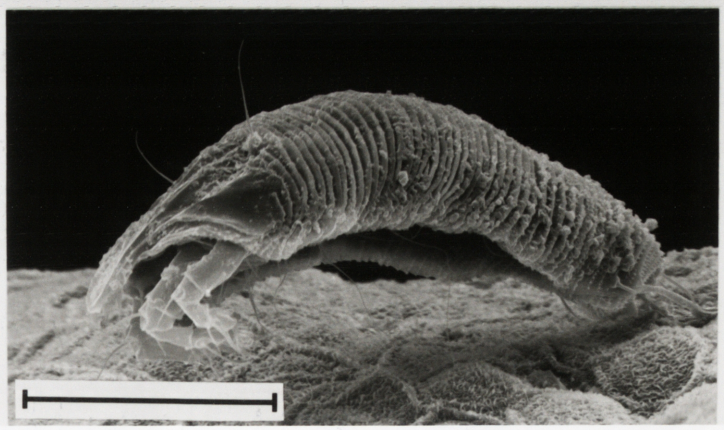
1.6.1. Life cycle

Adult females of *A. hyperici* lay eggs singly on the surface of plants, mainly between leaves around the apical meristems, though in dense infestations, oviposition may also occur on stems and petioles. After hatching, the life cycle is characterised by two larval instar phases, each separated by a short inactive phase,

Plate 3. Examples of several *Aculus hyperici* adults. Scale bar represents 25 μm.







before maturation to adulthood. Males and females are common, but when females are unable to locate spermatophores or mature males, only males are produced. Such arrhenotoky, the production of males from unfertilised eggs, appears common in other plant-inhabiting mites (Jeppson *et al.* 1975).

In the laboratory at 20°C, the minimum generation time of *A. hyperici* (egg to first oviposition) is about 13 days. Adult longevity is approximately 14 days, in which time, about 25 larvae may be produced per adult female. Although there does not appear to be a resting stage, populations seem to peak in spring and autumn (CSIRO 1991). All growth stages can be found throughout the year.

1.6.2. Feeding and symptoms of infestation

Feeding by *A. hyperici* is achieved by piercing the host's epidermal cells with cheliceral stylets (Jeppson *et al.* 1975; Krantz 1978). There appears to be relatively little mechanical damage associated with such feeding in eriophyids, since the stylets remain in position, at the point of penetration, as long as the mite feeds. This contrasts with the feeding mode of other acarine taxa such as spider mites (Tetranychidae), which thrust and retract their stylets to maintain lubrication with salivary fluid (Jeppson *et al.* 1975).

Some eriophyids possess growth regulators which, when injected into host cells at the time of feeding, induce localised galling or production of papillae (erinea) within and among which, the mites live (Jeppson *et al.* 1975). *Aculus hyperici* produces no such symptoms of infestation, though occasionally apical leaves may become contorted and somewhat blistered. More typically, however, heavy infestations of *A. hyperici* result in dwarfing of the shoots by shortening the internodes, and localised chlorosis of tissues. The generic host-specificity of *A. hyperici*, in combination with its ability to reduce growth of *H. perforatum*, suggest that it is potentially a useful biological control herbivore for this weed. To determine the effectiveness of the mite in reducing populations of *H. perforatum*, growth and population spread in Australia requires glasshouse and field experimentation and monitoring.

1.6.3. Mites as biological control agents for weeds

Because phytophagous mites are highly host-specific and are capable of reducing plant growth and reproduction, they are potentially excellent agents for biological control of weeds. Jeppson (1975) indicates that the Eriophyidae, in particular, are

highly host-specific, often limited to a single species or genus. Possibly because relatively little is known about the ecology and taxonomy of these arthropods, they have not been utilised more widely in weed biological control. This lack of knowledge may stem, in part, from the small size of many eriophyids, which makes them relatively difficult to study. *Aculus hyperici*, for example, measures 70 - 100 x 20 - 50 µm. Further, plant-feeding mites, especially the eriophyids, are vectors of some plant viruses, including wheat-streak mosaic virus (transmitted by *Eriophyes tulipae*), currant reversion virus (transmitted by *Cecidophyopsis ribis*) and latent plum virus (transmitted by *Aculus fockeui*) (Krantz 1978). Despite host-specificity, the potential exists for mites to transmit viruses to new species.

1.6.3.1. Examples of mites used in biological weed control

Following host-specificity testing and demonstration that *Aceria chondrillae* (Eriophyidae) was not a vector of any known viruses, this gall mite was among the first deliberate mite introductions for biological control of a weed. Released in Australia and parts of the United States to augment other agents in the biological control of *Chondrilla juncea* (Cullen *et al.* 1982; Andres 1983; Gerson 1990), mite feeding induced gall formation, stunted shoot growth, reduced root growth and decreased fruit production (Cullen *et al.* 1982). Rosenthal (1983) documents consideration of *Aceria convolvuli* (Eriophyidae) for biological control of field bindweed, *Convolvulus arvensis*, but field-release of this species has not yet occurred. Several other eriophyids have also been used, or considered for biological control of weeds including knapweed, *Centaurea repens*, and ragweed, *Ambrosia* spp., with variable success (see Gerson 1990).

Interestingly, Dodd (1929, 1936, 1940, in Hill and Stone 1985) observed that during one of the most dramatic examples of biological weed control, that of prickly pear, *Opuntia* spp., in Queensland, a mite, *Tetranychus desertorum* (Tetranychidae) was released inadvertently. Probably introduced into Australia with shipments of insects for the control of *Opuntia* spp., *T. desertorum* thinned about 360 ha of land dominated by *O. inermis*. This mite caused 75% reduction in the abundance of cacti in two years until it was out-competed by more efficient insect introductions.

The host-specificity of phytophagous mites combined with their ability to reduce plant growth and ready dispersability are characteristics that promise further use of these arthropods in biological weed control.

1.7. Aims and structure of the thesis

1.7.1. Aims of the project

As noted, Howarth (1983) recently asserted that the success of weed biological control programs must consider the impact of agents on non-target species as well as their effect on the target weed. A central issue is, why should a non-target species which is utilised under laboratory conditions not suffer a population decline under field conditions? This is a critical question, not just for biological control but relevant to assessment of the impact of biologically altered or engineered organisms in an ecosystem. Answers to such questions will indicate whether current practises are adequate for assessing the impact of novel biological agents in ecosystems.

The broad aim of this thesis is to examine the impact of the mite, *Aculus hyperici*, introduced to biologically control the weed *Hypericum perforatum*, on the non-target native species, *H. gramineum*. This is treated both as a study in its own right as well as an example of a more general problem. To address this aim, a series of experiments and field surveys have been employed to assess the impact of *A. hyperici* on growth of *H. gramineum*, and to examine the effects of introducing this mite into Australia for field populations of the non-target species. Some of the specific questions the thesis attempts to answer can be summarised as follows:

- Is *A. hyperici* likely to disperse to and infest field populations of *H.* gramineum?

- If *A. hyperici* infests populations of *H. gramineum*, is growth of individual plants affected, and in what ways?

- Do the effects of mite-herbivory of *H. gramineum* affect the population dynamics of the non-target native species?

- How does herbivory by *A. hyperici* interact with other environmental plant stresses, and do such stress combinations render *H. gramineum* more sensitive to mite-infestations compared with unstressed individuals?

To answer these questions, a comparative approach has been adopted, whereby the effect of mites on *H. gramineum* are contrasted with those on the target species, *H. perforatum*. In doing so, the thesis examines theories of plant-herbivore

interactions through several glasshouse experiments aimed at investigating combinations of herbivory and various physical and biological stresses. The results of these investigations are then contrasted with those from a series of field experiments in order to better understand the nature of the *H. perforatum-A. hyperici-H. gramineum* plant-herbivore system. Field work (experiments and surveys) was conducted among populations of *H. gramineum* near Beechworth in north-eastern Victoria and among populations of *H. gramineum* distributed in the Canberra region of the Australian Capital Territory and south-eastern New South Wales. Selection of these disjunct populations enabled the ecology of the non-target native species to be assessed in much of its eastern range and facilitated comparisons between populations occurring in a wide diversity of habitats. A conceptual model of factors that are potentially important in the *Hypericum-Aculus* system and summarising the areas of research addressed in this thesis is provided in figure 1.1.

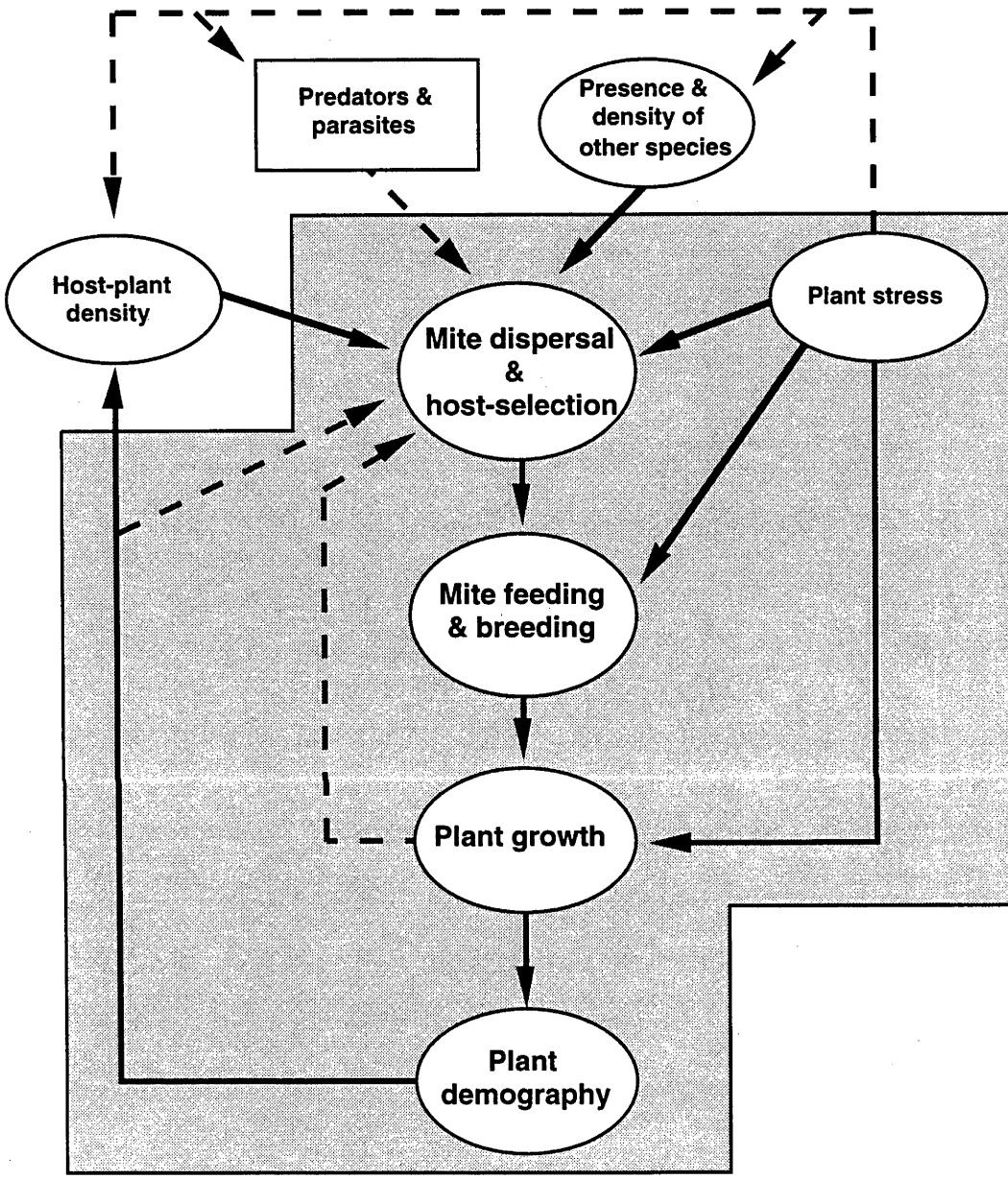


Fig. 1.1 Conceptual model of proposed interactions between *A. hyperici* and populations of *Hypericum* spp. in Australia. Processes emphasised in this thesis are enclosed by a shaded box. Those that have been directly examined are encircled by ovals and linked by solid lines. Potentially important interactions that have not been examined are indicated by dashed lines.

1.7.2. Structure of the thesis

This thesis has been divided into five sections (A - E), each comprising one or more chapters. In sections B - D each chapter has an introduction to the theoretical issues investigated and their practical implications for the *A. hyperici-Hypericum* system. This is followed by the specific research aims, materials and methods, results and a discussion of the findings. In the absence of any accounts of the ecology of *H. gramineum*, Section B: 'Plant Demography' explores the reproductive ecology (chapter 2) and population dynamics (chapter 3) of *H. gramineum*, providing a framework, within which, the potential impact of *A. hyperici* on the non-target native species can be assessed. Section C: 'Dispersal and Host-Preference of *Aculus hyperici*' explores ecological aspects of the mite associated with its dispersal to target and non-target species (chapter 4) and its host preferences (chapter 5). In Section D: 'Herbivory of *Hypericum* species by *A. hyperici*', the impact of the mite on growth of *H. gramineum* is compared with its effect on growth of the target, *H. perforatum* and several other non-target species of *Hypericum* (chapter 6). The possibility that in combination, plant stress and herbivory by *A. hyperici* might affect growth of *H. gramineum*, where singly, their effects are minor, is explored in a series of glasshouse-based experiments (chapter 7). The impact of *A. hyperici* on field-grown *H. gramineum* is assessed in chapter 8. In the final section, Section E: 'General Discussion' (chapter 9), results presented in the preceding chapters are integrated into a more general discussion of the likely impact of *A. hyperici* on *H. gramineum*, in comparison with its effect on *H. perforatum*.

SECTION B:

PLANT DEMOGRAPHY

This section of the thesis explores the demography of *H. gramineum*, in the absence of *A. hyperici*. Chapter 2 focuses on the reproductive ecology of the species by examining the pollination biology and aspects of its seed germination biology through a series of glasshouse and field experiments. Chapter 3 describes the population dynamics and mortality of adult plants located in three populations of *H. gramineum*, based on a three-year survey in which tagged plants were regularly monitored. This section concludes with a Leslie projection matrix, based on the results of chapters 2 and 3, simulating changes in *H. gramineum* populations under varying levels of herbivory by *A. hyperici*.

2.2. Materials and Methods

2.2.1. Pollination experiments

2.2.1.1. Cultivation of plant material

Hypericum gramineum seeds were collected from field populations in the Canberra region of south-eastern Australia (35°S, 149°E) during November 1990, sown on potting mixture and germinated under a misting system in a glasshouse with a temperature range from 15° to 30°C. Once germinated, seedlings were transplanted to pots and grown in a shade house. When the first floral buds appeared, after about 6 weeks, 30 plants were transferred to the glasshouse for pollination experiments. In September 1990, individuals of *H. perforatum* were transplanted from field populations to pots and grown in a shade house till floral buds appeared, at which point, they were also moved into the glasshouse for experimentation.

2.2.1.2. Hand-pollination experiments

Five pollination treatments were performed on *H. gramineum* and *H. perforatum*: an untreated control (C), self-pollination (flowers pollinated with pollen from the same flower, S), cross-pollination (flowers pollinated with foreign pollen, from flowers of a separate plant, X), emasculation (E) and emasculation followed by cross-pollinating the same flower (EX). Self-pollinations were carried out by removing all stamens from a flower with forceps and touching dehiscing anthers onto the stigmatic surface of the same flower. Cross-pollinations were performed in the same way, but using the androecium from the flower of different plants. In emasculated treatments, the undehisced stamens were removed with forceps and discarded. Emasculated and crossed flowers (EX) were similarly treated, but cross-pollinated with foreign pollen. A pilot experiment indicated that 'bagging' flowers to exclude pollinators was not necessary, since unbagged flowers set no seed and the glasshouse was free of pollinating insects. Bagged controls were, therefore, not included in the experimental design.

Treatments were randomly applied within plants to flowers occupying different positions along the axes of inflorescences. All inflorescences received two

replicates (treated flowers) of each pollination treatment. In total, each treatment was replicated 15 - 20 times and was performed on between 7 and 10 individuals. After 4 weeks, the mature, undehisced fruit capsules were harvested and the number of seeds resulting from each treatment tallied.

Numbers of seed produced by naturally pollinated flowers on field-grown individuals (F) collected in the local Canberra region were also scored, by randomly selecting four fruit from each of five infructescences.

2.2.2. *Seed germination experiments*

Seeds used in the germination and predation experiments were collected in the Canberra region of south-eastern Australia during December and January of 1990/91 and 1991/92. They were stored in paper bags at room temperature (approximately 22°C) until used, no later that 10 months after the collection date. Germination experiments were carried out in growth cabinets, a glasshouse and at various field sites (Table 2.1).

2.2.2.1. Growth cabinet experiments

Dormancy and the effect of temperature on germination of *H. gramineum* seeds were assessed in the following tests. These trials were supplemented by examining the germinability of seeds from *H. gramineum* infested with *A. hyperici.*

Unless stated otherwise, all petri dishes (replicates) contained 0.005 g of seed (300.5 seeds ± 4.8 s.e., n = 15) scattered over a Watmans No. 1 filter paper, which had been moistened previously with 6 mL of distilled water (or gibberellic acid - see below). Dishes were then sealed and incubated in an artificially lit growth cabinet with a 30°C day (8 hours, with a photon flux density of 70 - 110 $\mu mol\ m^{-2}\ s^{-1}$) and a 20°C night (16 hours) until completion of the experiment, 20 days later.

Table 2.1 Location of study sites used in various experiments investigating aspects of the seed ecology of *H. gramineum*.

Experiment	Habitat	Site	Site latitude & longitude
Field germination	Grassland	Border East, ACT	35° 10' S, 149° 09' E
		Border West, ACT	35° 10' S, 149° 09' E
		Horse park, ACT	35° 10' S, 149° 08' E
		Radio CY, ACT	35° 13' S, 149° 02' E
		Smith's Paddock G1, ACT	35° 17' S, 149° 05' E
		Smith's Paddock G2, ACT	35° 17' S, 149° 05' E
	Woodland	Border North, ACT	35° 10' S, 149° 09' E
		Border South, ACT	35° 10' S, 149° 09' E
		Honeysuckle Creek, ACT	35° 35' S, 149° 00' E
		Kowen Forest, NSW	35° 16' S, 149° 15' E
		Smith's Paddock W1, ACT	35° 17' S, 149° 05' E
		Smith's Plantation, ACT	35° 17' S, 149° 05' E
Viability after field-exposure	Grassland	Beechworth G1, Victoria	36° 23' S, 146° 44' E
		Beechworth G2, Victoria	36° 23' S, 146° 44' E
		Mt. Ainslie G1, ACT	35° 17' S, 149° 10' E
		Mt. Ainslie G2, ACT	35° 17' S, 149° 10' E
		Smith's Paddock G1, ACT	As above
		Smith's Paddock G2, ACT	As above
	Woodland	Beechworth W1, Victoria	36° 23' S, 146° 43' E
		Beechworth W2, Victoria	36° 23' S, 146° 43' E
		Mt. Ainslie W1, ACT	35° 17' S, 149° 10' E
		Mt. Ainslie W2, ACT	35° 17' S, 149° 10' E
		Smith's Paddock W1, ACT	As above
		Smith's Paddock W2, ACT	35° 17' S, 149° 05' E
Seed bank	N/A	Beechworth 1, Victoria	36° 23' S, 146° 44' E
		Beechworth 2, Victoria	36° 23' S, 146° 43' E
		Farrer Ridge 1, ACT	35° 23' S, 149° 05' E
		Farrer Ridge 2, ACT	35° 23' S, 149° 05' E
		Mt. Taylor, ACT	35° 23' S, 149° 04' E
		Smith's Paddock, ACT	As above
Seed predation	Grassland	Smith's Paddock G1	As above
		Mt. Taylor	As above
		Farrer Ridge 1	As above
		Farrer Ridge 2	As above
	Woodland	Smith's Paddock W1	As above
		Smith's Plantation	As above
		Farrer Ridge W	35° 23' S, 149° 05' E
		Mt. Ainslie W1	As above

(a) Dormancy

The existence of dormancy in *H. gramineum* seed, and the means by which any such dormancy could be broken, was tested by subjecting seeds to five treatments during, and/or prior to germination:

(1) After-ripening - seeds were germinated at 4-weekly intervals, from the date of collection, for 24 weeks. Subsequently, germinations were conducted after 40, 55 and 77 weeks.

(2) Gibberellic acid - seeds were germinated on filter paper moistened with a 500 ppm solution of gibberellic acid (GA_3).

(3) Cold stratification - seeds were placed on moistened filter paper and stored at 4°C in the dark for 3 weeks.

(4) High temperature - seeds were stored between 40° and 50°C in the dark for 3 weeks.

(5) Washing - seeds were washed for 24 hours in running tap water.

(b) Temperature and darkness

Germination trials were conducted using 6-month old seeds at alternating day/night temperature regimes of 15/5, 20/10, 25/15, 30/20 and 35/25°C. Petri dishes used in the dark treatments were randomly interspersed amongst light treatments of a given temperature regime. They therefore received the same temperature alternations but were wrapped in aluminium foil for the duration of the experiment.

(c) Herbivory

Seeds from plants subjected to herbivory by *A. hyperici* in a glasshouse were compared with seeds from plants simultaneously propagated under identical conditions, but kept free of infestation by *A. hyperici*. Seeds were randomly selected from those produced by five mite-infested plants (+mites) and two mite-

free plants (-mites). Each of 15 petri dishes for each seed source contained 20 seeds and were treated with a 500 ppm solution of gibberellic acid, as above.

2.2.2.2. Field experiments

(a) Field germination experiment

Seeds of *H. gramineum* were sown into rings of PVC piping, 12 cm diameter x 3 cm high and pushed into the soil of field sites leaving a 1 cm high rim protruding above the soil surface, thereby shielding the seeds from wind and rainwash. The experiment had a factorial design with six treatments (2 sowing times x 2 habitats x 2 micro-environments) applied to two species, as follows:

(1) Sowing season - two seasons: seeds sown in autumn (late May), or in spring (early October) 1992. Seeds for the spring germinations were sown adjacent to those sown in autumn.

(2) Habitat - two habitats: native grassland (dominated by *Themeda triandra*) or woodland (dominated by various combinations of *Eucalyptus blakelyi*, *E. macrorhyncha*, *E. melliodora*, *E. nortonii* and *E. polyanthemos*, with a grass understorey comprising *T. triandra*, *Poa sieberiana*, *Danthonia* spp. and native forbs).

(3) Micro-environment - seeds were sown onto undisturbed, usually bare soil in two micro-environments: either sheltered on the southern (shady) side of a large grass tussock, or in an inter-tussock gap of 15 - 30 cm diameter.

(4) Seeds - seed batches of two types were sown: (i) 0.005 g of pure *H. gramineum* seeds, and (ii) 0.005 g of *H. gramineum* seeds mixed with 25 *H. perforatum* seeds. Seed-free controls (for chance germinations within plots) confirmed that negligible germinations occurred unless seeds were sown into the plots.

Six grassland and six woodland field sites were used in the experiment, each separated from one another by at least 500 m. All but one site were within the Australian Capital Territory (Table 2.1). The total number of germinations, including those of seedlings that subsequently died, was scored every four - six

weeks for the nine months of the experiment, commencing two weeks after the 'autumn sowing'.

(b) Measurement of light and soil temperature regimes

Summer surface soil temperature (°C) and light intensity (photosynthetically active radiation, μmol $m^{-2}s^{-1}$) were measured adjacent to grass tussocks and in inter-tussock gaps, in woodland (*Eucalyptus* spp.) and grassland (*Themeda triandra*) habitats representative of field sites used in the field germination experiment. Measured on a sunny day in mid-summer (December) 1993, these measurements quantified the likely temperature and light regimes experienced by *H. gramineum* seeds in the two environments. Twenty-six measurements were taken in each: thirteen adjacent to grass tussocks and thirteen in inter-tussock gaps. The data were compared by 2-way analysis of variance using Statview-4 (Abacus Concepts, Statview 1992), after logarithmically transforming the data. Un-transformed arithmetic means are presented in the results.

(c) Seed viability after field-exposure

H. gramineum seeds (0.005 g) were enclosed in fine mesh (< 0.25 x 0.25 mm holes) nylon bags, randomly assigned to a treatment within a factorial design and pegged to the soil surface, though many became buried by litter with time. The experiment comprised 11 treatments (3 field sites x 4 time periods x 2 habitats x 2 micro-environments), as follows:

(1) Field region - three regions: Beechworth (Victoria), Mt. Ainslie and Black Mountain (ACT; see Table 2.1 for locations).

(2) Time - seed bags were retrieved from the field after 3, 6, 12 and 18 months.

(3) Habitat - two habitats (grassland and woodland), each twice replicated at each site.

(4) Micro-environment - two micro-environments: either sheltered on the southern side of a large grass tussock, or in an inter-tussock gap.

After collecting seed-bags from the field, their contents were germinated *in vitro*, using the same techniques employed in the growth cabinet experiments. Comparisons of the number of germinations were made between treatments. For each time period, the mean number of germinations was also compared with laboratory-stored seed of the same age, though this comparison was not included in the statistical analyses.

2.2.2.3. Glasshouse experiments

(a) Seed bank survey

(1) Survey Design: This factorial design survey comprised two soil depths, at locations beneath or distant to a plant, replicated four times at each of six field sites (see Table 2.1). The samples were taken using a 125 mm x 150 mm quadrat. One location was sampled immediately beneath a reproductively mature *H. gramineum* individual and the other, one metre away from the same and surrounding conspecifics, in an arbitrarily selected direction. Two soil depths, 0 - 1 cm and 1 - 3 cm, at each location within the site were sampled. Paired locations were separated from other replicates by 6 - 10 m.

(2) Procedure: Samples were oven-dried for 5 days at 40° - 45°C before recording the mass of each sample. They were then evenly spread within 13 x 7 x 4.5 cm trays and arranged in a fully randomised experimental design within a glasshouse, and watered daily with tap water. After 30 days, the number of *H. gramineum* seedlings germinating from each sample was recorded. All seedlings were then removed and the soil turned and watered for a further 25 days to check for further germinations. The experiment was terminated after this second period, since few germinations occurred. The total number of germinations from both periods in each soil sample was combined and used in analyses.

(b) Effect of seed burial on germination

Germination of *H. gramineum* seeds sown at four soil depths (3, 1, 0.5 and 0 cm) was compared. Seeds (0.005 g) were evenly spread onto potting soil within 9 cm x 9 cm square pots of 8.5 cm depth. In all but the surface treatments, in which seeds were sown onto the soil surface, seeds were then covered with soil to the

appropriate depth, ensuring that the soil surface was consistently 1.5 cm below the rim of the pots. Pots were placed under a misting spray within a glasshouse at 20 - 30°C, having randomly assigned one pot from each soil depth to each of 12 blocks within a randomised block design. After 40 days, the number of germinations in each pot was scored.

2.2.3. Seed predation (removal) experiment

2.2.3.1. Experimental design

Predation (removal) of *H. gramineum* and *H. perforatum* seeds by terrestrial arthropods (e.g. ants) was studied during the summer of 1992 (January 13 - 21) in the Australian Capital Territory.

A nested factorial design was employed to compare removal of *Hypericum* seeds from grassland and woodland habitats over time. Experimental treatments (2 habitats x 3 time periods x 2 seed-source distances) were applied to seed caches comprising *H. gramineum* or *H. perforatum* seeds, as follows:

(1) Habitat - seeds placed into two habitats: either grassland or woodland

(2) Time - seeds placed into the field, then retrieved after 1, 3 and 7 days.

(3) Distance - Seeds placed at two distances from a fruiting *H. gramineum* individual: either immediately beneath an individual, or about 1 m from the same plant.

Each treatment combination was replicated in four grassland and four woodland habitats, providing a total of 96 seed caches. As a control for wind dispersal, all treatment combinations were also applied to caches of sand grains of similar size to *H. gramineum* seeds, yielding a further 48 replicates. In total, 144 sand/seed baits were placed into the field.

2.2.3.2. Location of study sites

Woodland and grassland field sites used in the study were located in various nature reserves within Canberra (Table 2.1). Study sites were located at least 500 m apart, though were often separated by 5 - 10 km. *Eucalyptus* spp. dominated the woodland sites, usually with an understorey of *T. triandra*. All grassland sites were dominated by *T. triandra*, interspersed with other native and introduced grass and forb species.

2.2.3.3. Preparation of seeds and study sites

Seed mass rather than number, was used in the experiment since *H. gramineum* seeds are small (approximately 0.5 x 0.25 x 0.25 mm). Large numbers of seeds (> 300) were used in each replicate. Although *H. perforatum* seeds are larger (approximately 1 x 0.5 x 0.5 mm), seed mass was also used in this species. Previously, it had been determined that 0.005 g of the *H. gramineum* seed yielded 327.7 ± 7.9 s.e. seeds (n = 15), while 0.005 g of the *H. perforatum* seeds yielded 37.8 ± 0.4 s.e. seeds (n = 12).

Seeds were placed into weighed glass vials (5 cm deep x 1.8 cm opening - to enable easy entry and exit of arthropods) and desiccated over silica gel crystals for one week at room temperature (22°C). Washed sand of grain-size about that of *H. gramineum* seed was treated similarly.

Following desiccation, the filled vials were re-weighed. For *H. gramineum*, the mean seed mass per vial was 0.0051 g ± 0.0002 s.e. The mean seed mass for *H. perforatum* was 0.0084 g ± 0.0002 s.e., while mean sand mass for the controls was 0.0127 g ± 0.0003 s.e.. In all cases, n = 48.

To prevent seeds from being blown away, they were left within the vials when placed at the study sites. Vials were laid on their sides, the necks flush with the soil surface, and the seed massed at the vial's closed end. Wooden spatulas formed a bridge from the seed mass to the external soil, in case potential seed predators were unable to traverse the clean glass surface.

All vials were shielded with an inverted plastic petri-dish such that any rain drops would splash and flow away from the open vial mouths. In fact, no rain was

recorded at the experimental sites during the experiment. The mean maximum and minimum temperature during the study was 28.9°C and 13.1°C, respectively: a temperature range suitable for foraging by most local arthropods.

Vials were collected from the study sites after 1, 3, and 7 days, their exteriors cleaned with warm water and detergent, and returned to the desiccation jar for another week. All vials were then re-weighed and the difference between the weight of vials containing seeds at the start of the experiment, and their weights at the end of the experiment was determined.

2.2.3.4. Collection and trapping of local ant species

To assess the most common ants present at each study site, two pitfall traps were randomly positioned at least 10 m from each other. The 10 cm deep traps, made from disposable plastic cups with 8 cm diameter mouths flush with the soil surface, were filled with soapy water. After 7 days, the contents were collected and stored in 100% ethanol before identification.

2.2.4. *Analyses of data*

All statistical analyses were performed using Genstat 5 algorithms (Lane *et al.* 1987; Digby *et al.* 1989). Unless indicated otherwise, data were logarithmically transformed to satisfy assumptions of the statistical models. Untransformed, arithmetic means are presented for all experiments.

2.2.4.1. Pollination experiments

The number of seeds produced following each pollination treatment were compared by one-way analysis of variance (ANOVA).

2.2.4.2. Seed germination experiments

The number of seeds germinating were analysed by ANOVA. Square-root transformations were necessary for the temperature/light trial (growth cabinet experiments) and the seed bank trial (glasshouse experiments). Data-transformations were not necessary for the other experiments. In the seed bank

trial, dried soil mass was included as a covariate. Since most of the field trials yielded zero germinations, binary data (zero germinations or ≥ 1) were analysed using logistic regression procedures with logit link and binomial errors, fitting the terms habitat, micro-environment, treatment, season and their interactions.

When presented, percentages, are estimated as the mean number of seeds germinating per 300, multiplied by 100. In such cases, standard errors refer to the standard error of the arithmetic mean.

2.2.4.3. Seed predation experiment

Sand was included in all analyses of main experimental factors and factor interactions, as a treatment within the main effect of species. In subsequent references to the seed predation experiment, the main factor 'species' includes sand, unless stated otherwise.

Final seed and sand mass (after field exposure) was compared using ANOVA and fitting initial mass (prior to field exposure) as a covariate. The data were analysed as survival, rather than removal, because, in the latter format, assumptions of the statistical model were violated and could not be overcome by data-transformation. To emphasise predation, however, results are presented as the percentage of the original mass removed.

2.3. Results of pollination experiments

Glasshouse pollination treatments significantly affected the number of seeds set in both *H. gramineum* and *H. perforatum*. In both species, more seeds matured following natural field-pollinations (treatment F), than in any of the hand-pollination treatments (Fig. 2.1). In *H. perforatum*, the difference between the field seed set (F) and all other treatments was highly significant ($P < 0.001$). In *H. gramineum*, field seed set was significantly ($P < 0.001$) greater than all treatments except cross-pollinations (X), which yielded marginally fewer seeds.

Untreated *H. gramineum* and *H. perforatum* controls (C) both produced few seeds, indicating that mechanical selfing does not normally occur and that seeds resulting from pollination treatments were generally attributable to the manipulations, and not errors such as accidental insect- or wind-pollination. That

the emasculated flowers (E) set significantly fewer seeds than other treatments underlines the latter assertion and suggests that apomixis is unlikely, though pseudogamy is not excluded. Results from the emasculation treatments should be

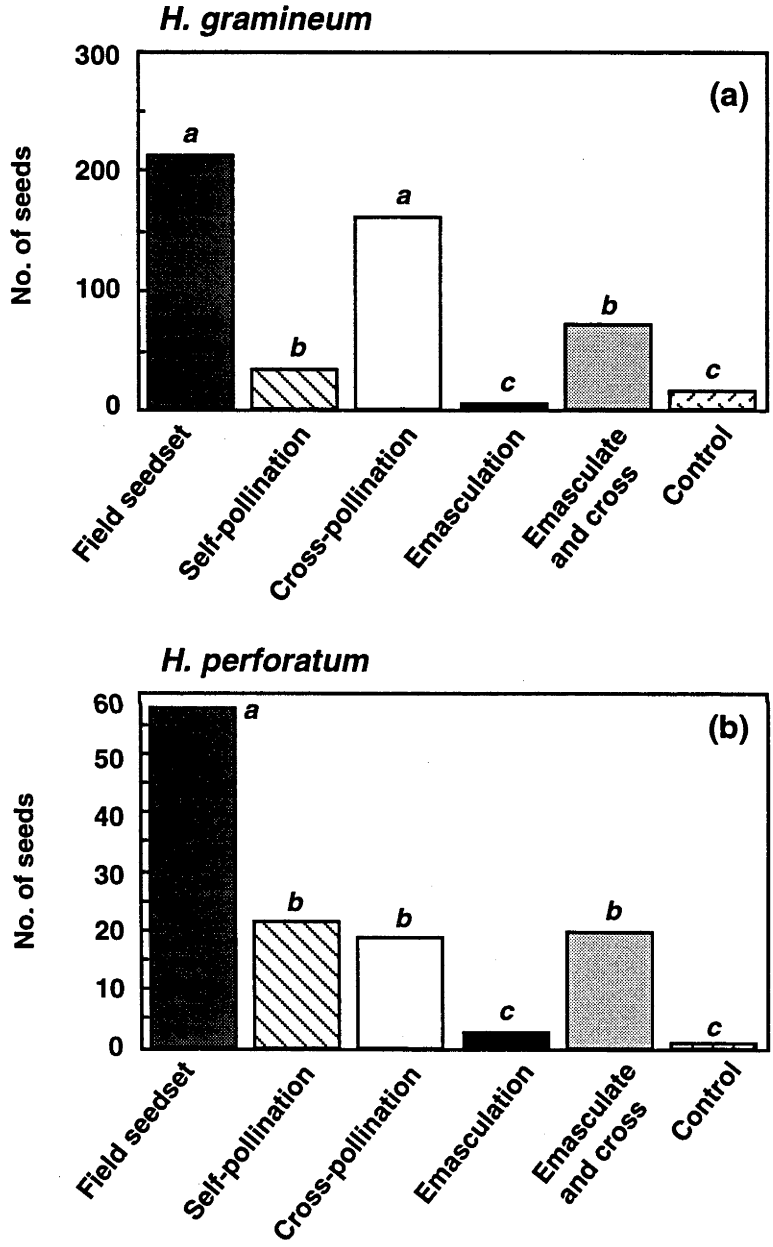


Fig. 2.1 Mean number of seeds produced per flower by *H. gramineum* (a) and *H. perforatum* (b) after various pollination treatments. Within each species, columns with different letters differ significantly ($P < 0.05$). The overall F-tests were highly significant ($P < 0.001$).

treated with some caution as the process of emasculation appears to have affected seed maturation: In both species, seed production was significantly lower in emasculated crossed flowers (EX) than in unemasculated crosses (X), although the result may reflect some additional selfing in the latter treatment.

Self-pollinations (S) in *H. gramineum* produced fewer seeds than simple cross-pollinations (X) and the emasculated crosses (EX), but only the former differed significantly. Although self-pollination produced slightly more seeds than did cross-pollination in *H. perforatum*, the difference was non-significant ($P > 0.05$). In *H. perforatum* also, control flowers set few seeds suggesting that, although a few 'accidental' self-pollinations may produce seed, mechanical self-pollination is rare. Combined with low seed production in emasculated replicates (E), these data imply that few seeds result from apomixis in *H. perforatum*.

2.4. Results of germination experiments

2.4.1. Growth cabinet experiments

After-ripening is evident in *H. gramineum* seeds: germinability increased with time, from 32% three days after harvest, to 92% after 16 weeks. Germinations then slowly declined, though the difference in germinations after 55 and 77 weeks (49%, and 52% respectively) was not significant ($P > 0.05$, Fig. 2.2). Variance was very high in the initial sampling periods, but decreased markedly with time, infringing the homogeneity of variance assumption of ANOVA. Thus, although the significant tests of the ANOVA may be biased, the trends are clear.

Differences between the number of germinations following the dormancy-breaking treatments compared with the untreated control further suggest that *H. gramineum* seeds are innately dormant, requiring after-ripening immediately following dispersal from fruit. Separately, either an application of gibberellic acid or washing the seeds in running water overcame this requirement and significantly ($P < 0.001$) increased seed germinability (Fig. 2.3). Heating the seeds to 40° - 50°C prior to imbibing water also increased germinability significantly ($P < 0.05$). Cold-stratification of the seeds had no significant effect.

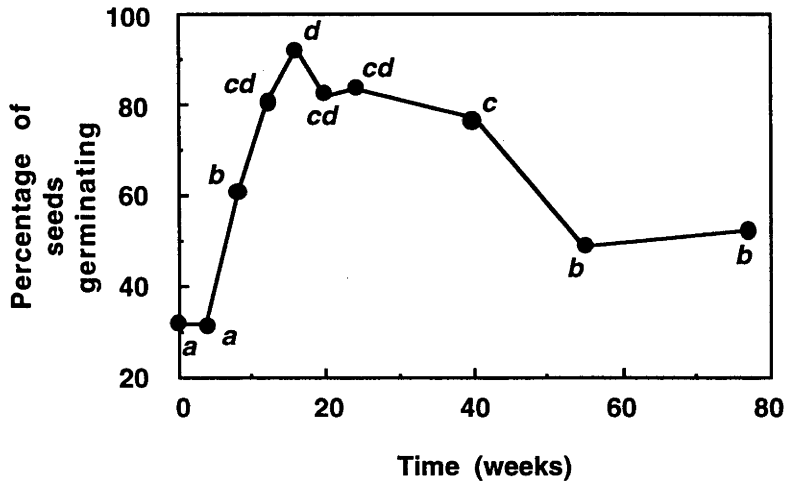


Fig. 2.2 After-ripening in *H. gramineum* seeds, reflected as an increase in the percentage of germinating seeds with time. ANOVA suggests that germination levels vary significantly ($P < 0.05$) between time periods marked with different letters, and that the overall F-test was highly ($P < 0.001$) significant. However, homogeneity of variance cannot be assumed between the early and late samples.

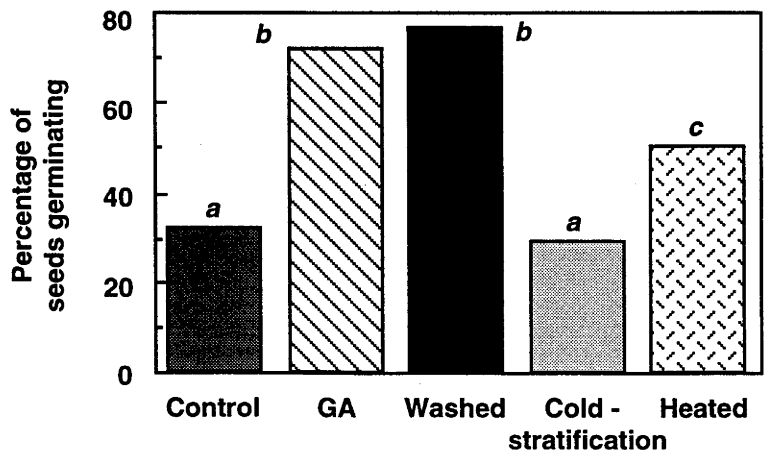


Fig. 2.3 Percentage of *H. gramineum* seeds germinating after various dormancy-breaking treatments; GA = gibberellic acid; columns with different letters differ significantly ($P < 0.05$).

Two-way analysis of variance indicates that temperature, light and the interaction between these factors have significant ($P < 0.001$) effects on the number of germinating *H. gramineum* seeds. Germination was greatest at the higher temperature regimes, and although the 35/25°C regime had slightly more germinations, the difference was not significantly greater than at 30/20°C (Fig. 2.4). Darkness strongly inhibited germination at all temperatures. A significant ($P < 0.001$) interaction was caused by the higher number of germinations in the dark at 35/25°, compared with other temperatures (Fig. 2.4). This relatively high temperature, however, did not overcome the strong light-requirement of seeds. Indeed, at 35/25° in the dark, significantly fewer germinations occurred than at 15/5° in light.

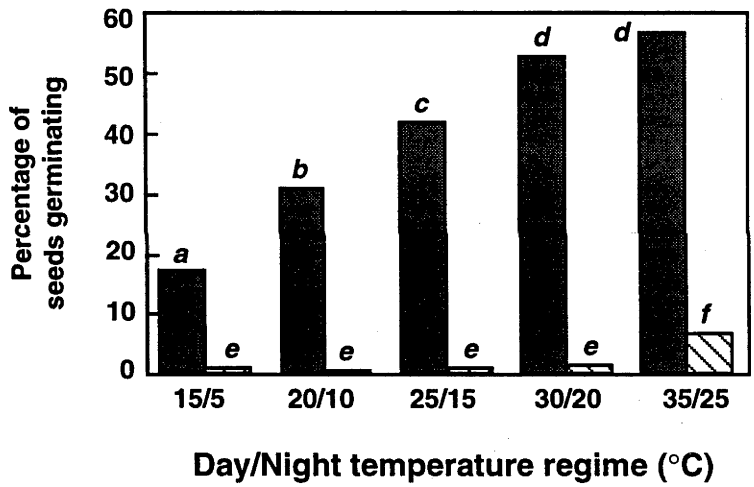


Fig. 2.4 Germination of *H. gramineum* seeds under various temperature (°C) and light regimes. Shading indicates germination in the light, while hatching indicates germination in the dark. Columns with different letters differ significantly ($P < 0.05$). The overall F-test was highly significant ($P < 0.001$).

Herbivory of *H. gramineum* shoots by *A. hyperici* appeared to increase germinability in seeds produced by infested plants. Analysis of variance indicated that the difference was significant ($F_{1, 28} = 8.573$; $P = 0.007$): Of seeds from parental plants free of infestation, 50% germinated, compared with 68% from mite-infested parents (Fig. 2.5). Since '+mite' and '-mite' seeds used in the trial

derived from only five and two plants respectively, such analysis of variance over-estimates the actual degrees of freedom, because parentage of seeds within treatments was unknown. It was, therefore, not possible to accurately determine the appropriate degrees of freedom for the analysis. An attempt to minimise this discrepancy in the degrees of freedom was achieved by randomly allocating three '+mite' petri dishes to each of five hypothetical parents, seven '-mite' dishes to another hypothetical parent and the remaining '-mite' dishes to a seventh parent plant. These data were re-analysed by ANOVA, partitioning 1 degree of freedom to mite-infestation treatments, leaving five residual degrees of freedom in the 'plant stratum' of the analysis. With 23 degrees of freedom allocated to the plant x dish stratum, the re-assigned degrees of freedom totalled 29. Partitioning the degrees of freedom and re-analysing the data in this way still suggests that seeds from mite infested plants germinate more readily, although the difference between mite-infested and mite-free treatments is less significant (P = 0.029).

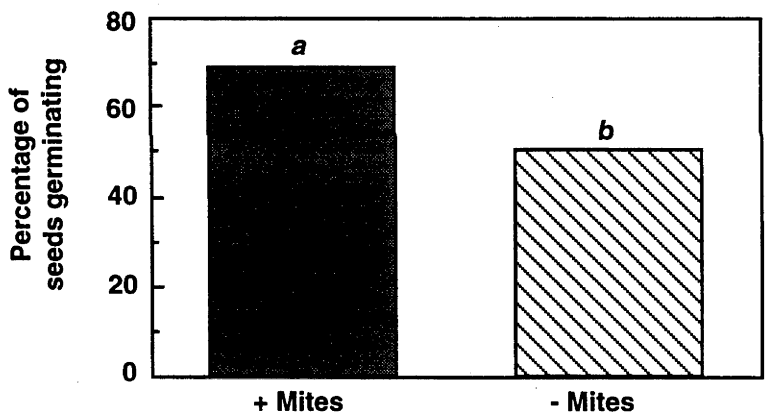


Fig. 2.5 Germination of *H. gramineum* seeds produced by parents either infested with mites (*A. hyperici*), or free of infestation. The treatments differ significantly (P = 0.007 - 0.029).

2.4.1.1. Field experiments

Very few germinations (1.2 ± 0.24 s.e., n = 138; about 0.4%) occurred in any of the field germination treatments (Fig. 2.6a). When germinations were successful, many fewer (about 1%) occurred than in the growth cabinet trials. Logistic

regression indicates that the probability of *H. gramineum* germinating is significantly (P = 0.01) affected by habitat and seed-type: The probability of ≥ 1

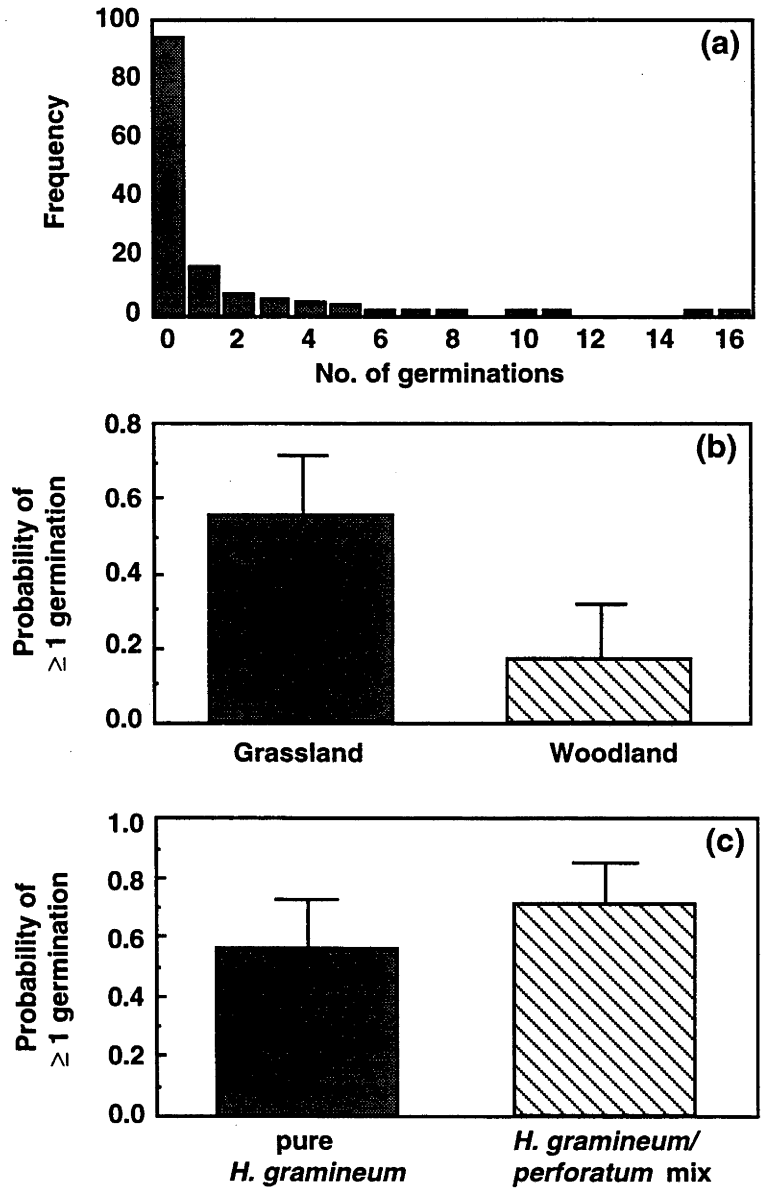


Fig 2.6 Frequency with which ≥1 *H. gramineum* germination occurred in all trials of the field germination experiment (a), and the probability of ≥ 1 *H. gramineum* seed germinating in grassland and woodland plots, each containing ca. 300 seeds (b) and in pure and mixed combinations of *H. gramineum* seeds (c). Error bars indicate 95% confidence intervals.

seed germinating in a grassland site was greater (55%) than germinating in a woodland (17%, Fig. 2.6b). Analysis confirmed that significantly ($P \leq 0.01$) fewer germinations occurred in the control plots, suggesting that the successful germinations resulted from the sown seeds. While more germinations of *H. gramineum* occurred from seed mixtures of the native forb and *H. perforatum*, the difference was not significant ($P > 0.05$, Fig. 2.6c). Sowing seeds on either the shady, southern side of grass tussocks, or in inter-tussock gaps did not significantly ($P > 0.05$) affect the probability of germinating. The probability of germinating in spring (35%) was slightly higher than the probability of germinating in autumn (30%) though the difference was not significant.

In vitro germination of *H. gramineum* seeds retrieved from the field after varying periods of exposure confirms that seed germinability is strongly ($P < 0.001$) affected by time (Fig. 2.7a). Germinability of field-exposed seeds was consistently much lower than in seeds of the same age stored *in vitro* at room temperature (Fig. 2.7a). In both the field-exposed seeds and those stored *in vitro*, germinations increased to a peak after 6 months, then decreased to a relatively constant level after 12 and 18 months. Across all time periods, region and time provided the only significant ($P = 0.030$) factor interaction (Fig. 2.7b). This complex interaction was largely due to the high germinability of seeds from Mt. Ainslie after 3 months, and the relatively low germinability of seeds from the same region after 18 months. Environmental factors in the experiment (region, habitat and micro-environment) had no significant ($P > 0.05$) effect.

2.4.1.1.1. Measurement of light and soil temperature

There were highly significant ($P < 0.001$) differences between habitats (woodland and grassland) and between micro-environments (tussocks and gaps) for both light (photon flux density) and soil temperature (°C; Table 2.2). Overall, the surface soil temperature and the photosynthetically active radiation (PAR) were higher in grassland than woodland, and higher in gaps than adjacent to tussocks. Much higher readings of both light and temperature in the gaps of grasslands resulted in significant ($P < 0.001$) interactions between habitat and micro-environment for each of measured parameter.

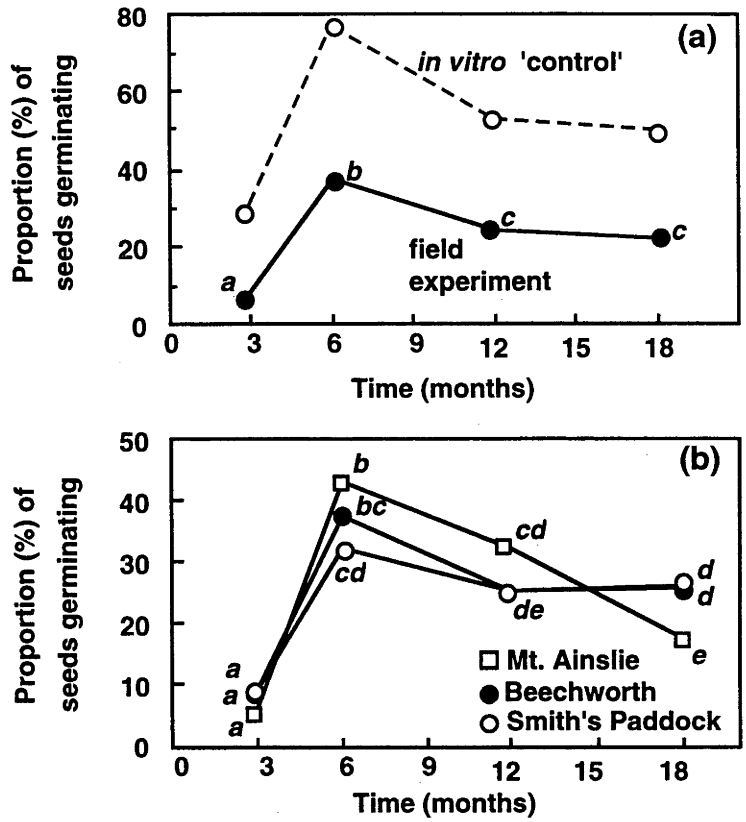


Fig. 2.7 Germinability of (a) field-exposed and laboratory-stored seeds over time and the significant interaction between region and time (b). Within each figure, different letters indicate that germination levels differ significantly ($P < 0.05$), although the F-tests for the effect of time (fig. a) and the region x time interaction (fig. b) are more significant ($P < 0.001$ and $P = 0.03$, respectively).

Table 2.2 Summer photosynthetically active radiation (PAR [light], μmol $m^{-2}s^{-1}$) and surface soil temperature (°C) at midday in various combinations of habitat and micro-environment, with standard errors of the arithmetic mean indicated. In a 2-way ANOVA, there were significant differences between grassland and woodland habitats ($P < 0.001$), between gap and tussock micro-environments ($P < 0.001$) and in the habitat x micro-environment interaction ($P < 0.001$). In all measures, n = 13.

Measure	Grassland		Woodland	
	Gap	Tussock	Gap	Tussock
Light	2109 ± 11	104 ± 12	219 ± 25	42 ± 4
Temperature	51.3 ± 0.8	23.5 ± 0.6	22.1 ± 0.6	18.5 ± 0.4

2.4.1.2. Glasshouse experiments

(a) Seed bank

Soil mass as a covariate did not significantly ($P \geq 0.122$) affect the ANOVA of germinations in the seed bank experiment. The number of germinations were, however, affected ($P = 0.007$) by sampling distance from *H. gramineum*. Averaged across all plots at all sites, more germinations occurred from soil adjacent to plants (53.8 germinations ± 11.3 s.e.) than from soil 1 m from the same plants (25.1 ± 4.5 s.e., Fig. 2.8a). Overall, soil depth was not significant ($P = 0.381$). A significant ($P = 0.009$) site x distance interaction is attributable to the much higher number of germinations that occurred in soil sampled adjacent to *H. gramineum* individuals at Mt. Taylor and Smith's Paddock (Fig. 2.8b). The 3-way interaction ($P = 0.008$) between site x distance x soil-depth appears to result from a higher number of germinations at Mt. Taylor and Smith's Paddock, compared with the other sites. At these sites, significantly more germinations occurred from the top 1 cm of the soil than from soil 1 - 3 cm deep. Such differences were insignificant ($P > 0.005$) at other sites.

(b) Seed burial

Differences between the number of germinations from seeds sown onto the soil surface, compared with seeds sown at varying depths beneath the surface in the seed burial experiment were obvious (Fig. 2.9), and did not require statistical analysis: very few of the buried seeds germinated (0.03% - 0.19%) compared with about 60% in the surface-sown seeds.

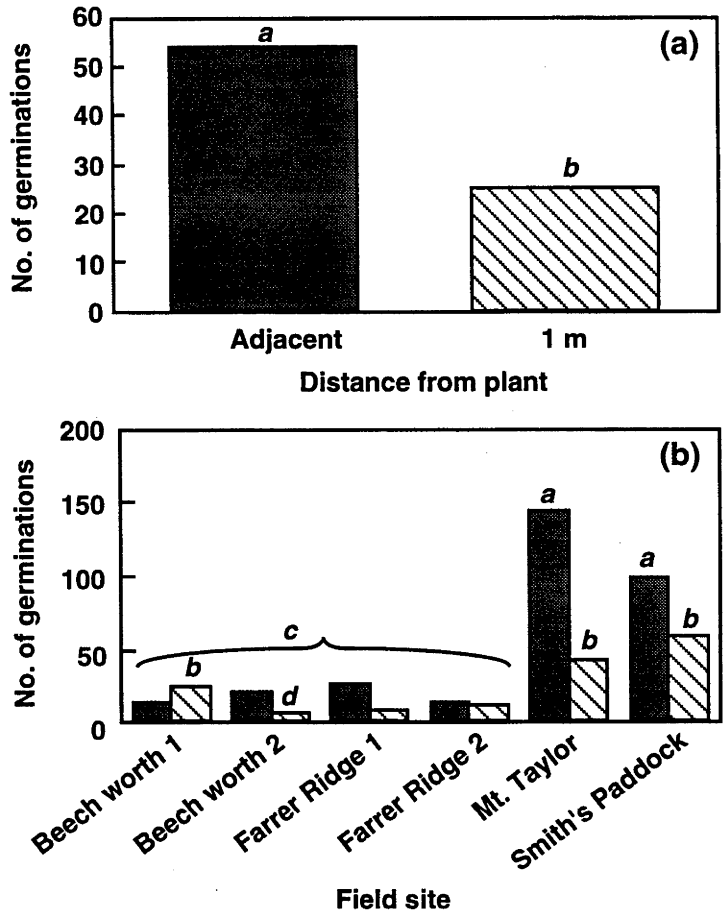


Fig. 2.8 Germination of *H. gramineum* seeds from soil sampled adjacent to *H. gramineum* individuals, and 1 m from individuals, averaged across all plots at all sites (a) and illustrating the complex interaction (P = 0.008) between site and sampling distance (b). Columns with different letters differ significantly. Shading indicates samples adjacent to an *H. gramineum* individual, while hatching indicates samples 1 m from an individual.

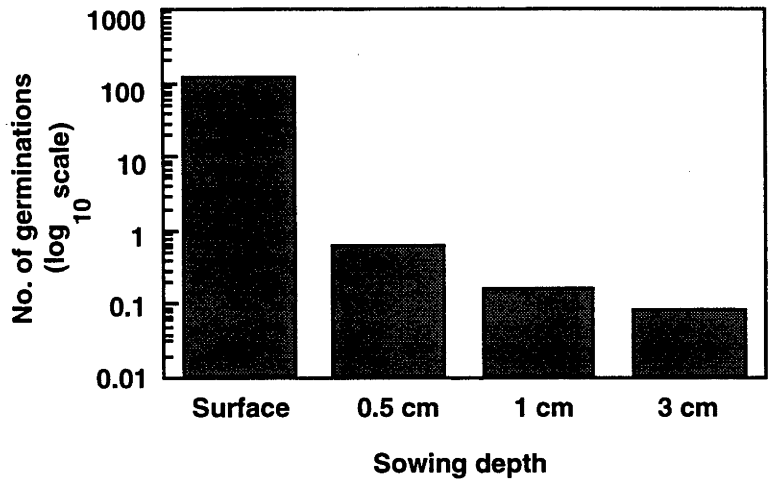


Fig. 2.9 Germination of *H. gramineum* after sowing seeds at various soil depths.

2.5. Results of seed predation experiment

2.5.1. Seed predation

Initial mass of the three seed lots (*H. gramineum*, *H. perforatum* and sand) differed significantly ($P < 0.001$) at the start of the experiment. Initial mass was also a significant covariate ($P \leq 0.021$) when comparing main effects within the analysis.

Removal of seeds and sand was highly variable in all treatments. In some replicates all of the seeds were removed, while in others, none was harvested. This high variability may explain why only two of the main factors in the experimental design, habitat and species (comprising seeds of both *Hypericum* species and sand), were significant ($P = 0.031$ and < 0.001, respectively): Higher proportions of initial seed and sand mass were removed from woodland (20%) than from grassland (7%) and proportionally more seed was removed than sand (16%, 22% and 3%, of *H. gramineum*, *H. perforatum* and sand, respectively, Fig. 2.10a and b). While time was not significant ($P = 0.782$), there was a clear trend towards increased seed removal with duration of field exposure, though most removal was within the first day (Fig. 2.10c). Distance from a fruiting plant was

also non-significant (P = 0.116), although seed lots located 1 m from an *H. gramineum* individual tended to suffer higher predation (Fig. 2.10d). There were no significant (P ≥ 0.073) factor interactions in the analysis.

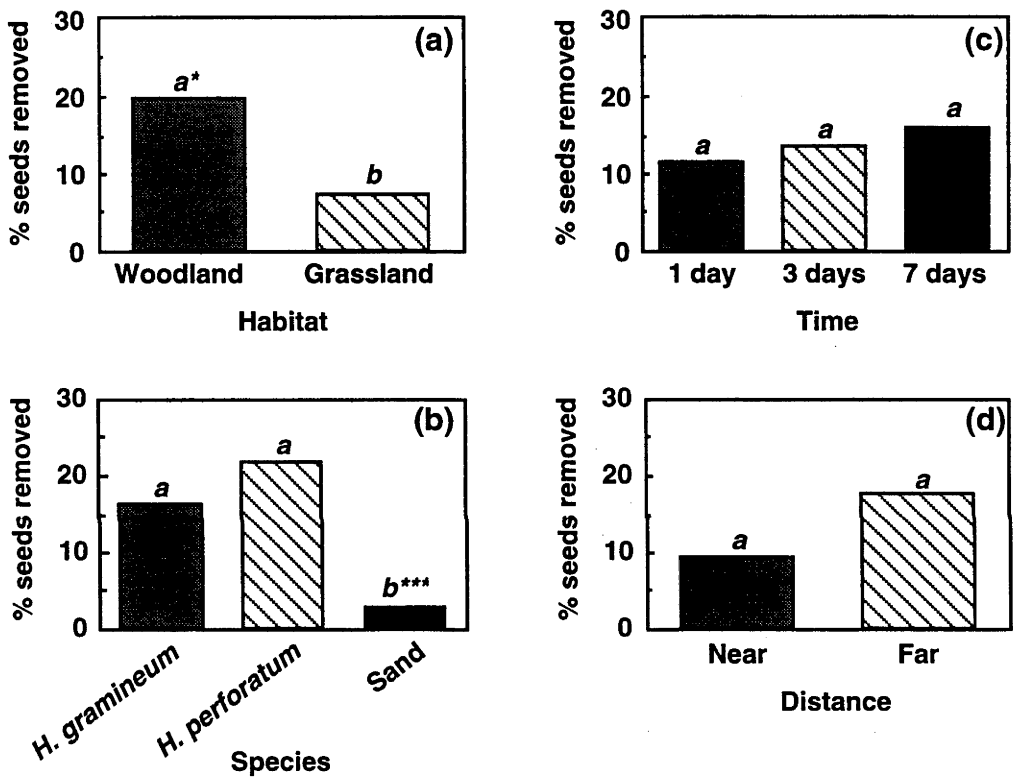


Fig. 2.10 Histograms of the proportion of *H. gramineum* seeds removed by ants, as affected by the main factors of habitat (a), species (including sand, b), time (c) and distance from a fruiting individual (d), and analysed by ANOVA. Within histograms of main factors, columns with different lettering differ significantly (P < 0.05*, P < 0.001***).

2.5.1.1. Identification of ants

Many Australian ant species cannot yet be named at the species level. Nomenclature of ants in the present study therefore follows that of Andersen (1988, 1991).

In terms of species diversity, pitfall trapping of ants yielded generally consistent results between study sites, though no attempt was made to quantify the observations. Species of *Iridomyrmex*, *Camponotus*, and *Rhytidoponera*, were common to all grassland and woodland sites, while taxa, such as *Pheidole* were common to most (Table 2.3). Ants in these genera have previously been reported as seed harvesters (Andersen 1989; Hughes and Westoby 1992a,b) and on several occasions in the present study, species of *Iridomyrmex* and *Camponotus* were observed exiting vials carrying seeds.

2.6. Discussion

2.6.1. Pollination biology of *H. gramineum*

Hypericum gramineum is evidently self-compatible, as it is able to produce seeds in the absence of pollen from other conspecifics. Seedset following self-pollination is, however, only 30 - 50% as successful as that resulting from cross-pollination (treatment X) and emasculation and crossing (treatment EX). The reduction in seedset following self-pollination suggests that post-zygotic abortion of self-fertilised ovules may occur in *H. gramineum*, although to verify such a process, the extent of stigmatic and stylar incompatibility, if any, and the success of self-pollen tubes in entering self-ovules requires investigation. Such research was beyond the scope of this thesis. Several Australian taxa are known to possess such post-zygotic abortion to minimise the number of seeds produced by self-fertilisation (Willis and Ash 1990, and references therein).

Table 2.3 Ant species trapped in grassland and woodland field sites during the seed predation experiment. Nomenclature follows that of Andersen (1991).

Site	Habitat	Species
Farrer Ridge	Grassland	*Camponotus 'consobrinus'* (Erichson)
		Iridomyrmex 'bicknelli' Emery
		Iridomyrmex 'foetans' Clark
		Iridomyrmex 'gracilis' (Lowne)
		Pheidole sp.
		Rhytidoponera 'metallica' (F. Smith)
Mt. Taylor	Grassland	*Camponotus 'consobrinus'*
		Iridomyrmex 'bicknelli'
		Iridomyrmex 'gracilis'
		Pheidole sp.
		Rhytidoponera 'metallica'
Smith's Paddock	Grassland	*Camponotus 'consobrinus'*
		Camponotus 'intrepidus' (Kirby)
		Iridomyrmex 'gracilis'
		Iridomyrmex 'purpureus'
		Myrmecia pulchra Clark
		Rhytidoponera 'metallica'
Farrer Ridge	Woodland	*Camponotus 'intrepidus'*
		Iridomyrmex 'bicknelli'
		Iridomyrmex 'foetans'
		Melophorus sp.
		Notoncus 'ectatomoides' (Forel)
		Rhytidoponera 'metallica'
Mt. Ainslie	Woodland	*Camponotus 'consobrinus'*
		Camponotus 'nigroaeneus' (F. Smith)
		Iridomyrmex 'purpureus'
		Pheidole sp.
		Plagiolepis sp.
Smith's Paddock	Woodland	*Camponotus 'consobrinus'*
		Camponotus 'intrepidus'
		Crematogaster sp.
		Iridomyrmex 'gracilis'
		Iridomyrmex 'purpureus'
		Notoncus 'ectatomoides'
		Rhytidoponera 'metallica'

Although apparently self-compatible, unbagged control treatments (C) set few seeds, implying that mechanical selfing in *H. gramineum* is rare. This implies that at most, a few seeds were produced by apomixis and probably these resulted from accidental crossing or contamination. With the exception of pseudogamy, apomicts generally set all seed when bagged.

It is noteworthy that despite attempts to maximise seed set in all hand-pollination treatments by covering the stigmatic surface in pollen, natural field pollination yielded significantly more seeds. This may be because pollen in the hand-pollination treatments was inviable, perhaps because of high humidity, in the glasshouse. The difference between field and glasshouse results might also be because in the field, *H. gramineum* flowers are open for several hours and may attract numerous pollinators, thereby enabling repeated pollination and increasing the potential for seed production. In the glasshouse, they were only hand-pollinated once.

Any damage that *Aculus hyperici* inflicts on *H. gramineum* depends on the mite successfully encountering the native non-target species. This is considered in detail in chapters 6 - 8, but it is of relevance to the current study that pollinators might act as dispersal agents for *A. hyperici* and facilitate rapid transfer between *H. perforatum* and *H. gramineum* plants, whose floral phenologies overlap. Attempts were made to capture pollinators from mite-infested populations of *H. perforatum* to determine if they were inadvertent mite vectors, but proved unsuccessful. However, previously introduced *H. perforatum* biological control herbivores including the beetle, *Chrysolina quadrigemina* and the aphid, *Aphis chloris*, were captured and found to be carrying *A. hyperici*. To date, *A. chloris* has not been observed on field populations of *H. gramineum* (Briese 1989), but *C. quadrigemina* was observed on *H. gramineum* during this study. Clearly, such arthropods are capable of transferring *A. hyperici* between target and non-target species, highlighting a further important, but unexplored aspect of pollination biology as it applies to weed biological control.

2.6.2. Pollination biology of *H. perforatum*

In contrast to *H. gramineum*, it appears that *H. perforatum* seeds may be produced by self-pollination in similar quantities to cross-pollination. However, low seed set in unpollinated controls and emasculations suggests that relatively

few seeds, if any, were produced by this means. As with *H. gramineum*, more seeds were produced in naturally pollinated flowers, possibly implying a reliance on insect pollinators. Robson (1968) reports that in Europe, 97% of *H. perforatum* seeds are produced by apomixis, although no details of the experimental methods or type of apomixis are provided. It is, therefore, possible that Australian populations of *H. perforatum* differ from those in Europe in producing a comparatively high proportion of seeds by sexual means, including self-pollination. This contradicts the generalisation that invasive weeds are often apomictic lineages, whereas the source populations are often more sexual (W. D. Hamilton, pers. comm.).

Handel (1983) observes that at low plant densities, self-incompatible species should have a decline in seed set, since frequency of pollinator visits declines with inter-floral distances (Harper 1977). Such trends, however, may be complicated by seed predation, which is also likely to be density-dependent. In *Cassia biflora*, for example, seed set was highest in dense populations and pollinator activity was positively correlated with the high density. Infestation of seed pods by seed predators, however, was also higher (Silander 1978).

Locally, field populations of *H. perforatum* in Australia are often much denser (e.g. 10^5 shoots ha^{-1}) than populations of *H. gramineum* which comprise relatively sparsely distributed individuals (e.g. 10^4 shoots ha^{-1}; see chapter 1). This is partly due to the rapid vegetative spread of the former, which can produce vegetative 'daughters' from rhizomes and the over-wintering shoot growth. Thus, the populations are strongly clonal and most neighbouring flowers will be of the same genotype. If *A. hyperici* is able to reduce population densities of *H. perforatum*, it may affect the probability of the plants selfing. Nevertheless, it does not appear that the pollination ecology of *H. perforatum* would be severely affected, since the high self-compatibility of *H. perforatum* would ensure continued sexual success. It seems, therefore, that the pollination biology of *H. perforatum* will neither enhance nor limit the success of *A. hyperici* as a biological control agent, as the species is largely self-compatible. Assuming adequate pollinator visitation, seeds may be produced by self-pollination. Populations of *H. gramineum*, by comparison, may be more adversely affected since their greater reliance on cross-pollination might decrease the seed productivity of individuals, if population density is reduced by A. *hyperici*. Clearly, this has implications for both the genetic diversity and abundance of the native and could result in a decline in population viability. In the past, such indirect impacts of biological control agents on non-target species, and indeed,

target weeds, have not been considered, probably because the importance of pollination biology to biological weed control has been neglected. Careful field-testing of such hypotheses are needed, but were not possible in this study.

2.6.3. Germination of *H. gramineum* seeds

Following wind/gravity-dispersal of seeds in late spring and summer, a majority of *H. gramineum* seeds are innately dormant. If this dormancy is overcome by, for example, after-ripening, some may germinate. If not, seeds that are not removed by seed-harvesting ants can be incorporated into a seed bank, from which they may germinate at a later date if various physiological requirements are met. Such requirements are affected by the seed's broader physical and biological environment.

In vitro, innate dormancy of *H. gramineum* seeds can be broken with gibberellic acid, by washing the seeds in running water, or storing the seeds at high temperature. This suggests that in *H. gramineum*, seed dormancy results from chemical inhibition, which, in the field, may be ameliorated with time, or by physical factors such as rain water diluting, or high temperatures denaturing the inhibitor. Both leaching and comparable high temperatures may be encountered in the field, the latter in open bare soil (Table 2.2). Seeds of several species re-enter dormancy if, after their first season in the field, they fail to germinate, and may subsequently ripen again the following year (Baskin and Baskin 1989; Baskin *et al.* 1992; Baskin *et al.* 1993). Following an initial increase, then decline in the *in vitro* germinations, after 18 months, there was no indication of a similar cyclical increase in germinability of *H. gramineum* diaspores.

Hormonal influences on germination have been reported in many studies (see, for example, Hagon 1976; Groves *et al.* 1982; Campbell 1985; Baskin and Baskin 1989; McIntyre 1990; Willis and Groves 1991; Baskin *et al.* 1992; Lewandowska and Szczotka 1992; Ni and Bradford 1993). Campbell (1985) and Clark (1953) observed low germinability of freshly harvested *H. perforatum* seeds and suggested that a seed capsule exudate inhibits germination. Campbell (1985) speculates that the exudate may contain abscisic acid, a known hormonal inhibitor, though this was not tested. As in the present study, he demonstrated that with time or irrigation, the inhibition may be alleviated.

Germination was strongly affected by light and burial. Even covering seeds with 0.5 cm of soil reduced germination rates to < 1%. Most *in vitro* germinations of *H. gramineum* occurred at 35/25°C, while germination was least successful at the coldest temperature regime (15/5°C). It appears, therefore, that when water is not limiting, germination of *H. gramineum* increases with increasing temperature, although above a temperature regime of about 30/20°C, the number of germinations may begin to plateau. These responses seem to favour germination of *H. gramineum* seeds in unshaded bare soil. Several other Australian native forbs have similar light and temperature requirements for germination (Mott 1972; Hitchmough *et al.* 1989; McIntyre 1990; Willis and Groves 1991; Bell *et al.* 1993).

In the field germination experiment, the season in which *H. gramineum* seeds were sown did not significantly affect the number of germinations, despite clear temperature requirements for their germination *in vitro*. This result probably occurred for two reasons: Firstly, the optimum temperature for germination of *H. gramineum* implies that given sufficient moisture, most field germinations should occur in January, the hottest month (mean maximum air temperature of 27.7°C in the ACT; Bureau of Meteorology and Department of Arts 1991), but in most years soil moisture may then be limiting. Secondly, the germinability of both laboratory-stored and field-exposed seeds peaks about 4 months after dispersal, so most germinations by the current seed cohort are not expected until autumn (March - May). Thus, it is possible that the lack of a strong seasonal effect on field germinations results from cool autumn temperatures (the average autumn air temperature ranges from 3.1 to 24.4°C; Bureau of Meteorology 1991) limiting germination, while in the summer, field-germination may not reach its *in vitro* potential because seeds are dormant. If seeds are no longer dormant, possibly because of dispersal the previous year and subsequent after-ripening, or because high soil temperatures overcame the dormancy, few germinations are likely because water is limited. Overall, the after-ripening and temperature requirements for germination of *H. gramineum* seeds suggest that in the field, germinations are likely to occur in autumn following rains, or in spring (September - November) as soil temperatures begin to warm, but before the soil is completely dried by hot summer conditions. At lower altitudes than Canberra, germination may be more continuous through autumn to spring. This mixture of controls may account for the lack of observed seasonality.

In the field germination trials, the probability of at least one germination was greater in grassland than woodland. This is probably because grassland sites used

in the study were low-lying and poorly drained relative to the woodland sites. Moister soil in the grasslands during the trial may, therefore, have met the germination requirements of *H. gramineum* more successfully than soil in the woodlands. In addition, grasslands provide hotter surface soil and higher levels of photosynthetically active radiation than woodlands; as noted, germination of *H. gramineum* is increased at higher temperatures and is strongly light-dependent.

Because germination of *H. gramineum* seeds is strongly influenced by temperature and light, the chance of germinating adjacent to shady grass tussocks was expected to differ from that of germinating in inter-tussock gaps. This expectation stemmed from observations that seeds sown adjacent to tussocks received significantly lower levels of light and were placed onto cooler soils than those sown in gaps. Despite these expectations, differences between the tussock and gap micro-environment did not explain a significant amount of the experimental variance. This may be because the biotic 'safety' (*sensu* Grubb 1977) of the micro-environments was more influential to seed survival and germination than the physical safety of the sites. Nevertheless, others have noted the importance of a seed's physical micro-environment for successful germination and establishment (Harper *et al.* 1965; Harper 1977; Silvertown 1982; Sindel *et al.* 1993).

Germination in the field was much lower (0.4 - 5.3%) than the potential indicated in the growth cabinet trials (92% after 4 months in the after-ripening trial). Possibly, this is because soil moisture was limiting and restricted the number of germinations, or because the seed population was depleted. Some seeds may also have been blown or washed away after sowing. Alternatively, fungal pathogens may have limited the viability of dispersed seeds, they may have become covered in litter or soil and entered the seed bank, or they may have germinated between census periods but failed to establish and, therefore, not been scored.

Herbivory of parent plants during seed production apparently increased germinability of mature *H. gramineum* diaspores. Following fungal infection by *Cercospora heliotropii-bocconii* Sred., germinability of *Heliotropium europaeum* L. (Boraginaceae) seeds also increases (A. Sheppard, pers. comm.). Crawley (1983, 1989) noted several studies demonstrating that arthropod herbivory may lead to the production of smaller seeds, although germinability of the seeds was not tested. That mite herbivory increased germinability of seeds in this study was surprising, as others have found that shoot herbivory leads to re-direction of

resources from reproduction to compensatory plant growth (Crawley 1983, 1989, and references therein).

It is possible that the size and mass of *H. gramineum* seeds was decreased by *A. hyperici* herbivory, as in the above studies. Because the germination trials were conducted with replicates of seed mass rather than seed numbers, greater numbers of smaller, lighter seeds might have constituted replicates of +mite treatments, while the -mite treatments may have comprised fewer, heavier seeds. Differences in the number of germinations resulting from the +mite and -mite treatments may, therefore, be spurious, reflecting differences in the number of seeds tested, rather than a positive effect of *A. hyperici* on the germinability of diaspores. Clearly, the observation of increased germinability following herbivory requires further investigation.

2.6.4. Predation of *H. gramineum* seeds

In addition to the possible limitations to field germination outlined above, seed predators may also have restricted germination in the field by removing dispersed diaspores before they were able to germinate. Seed predation by arthropods is a common occurrence in many ecosystems (see, for example, Hughes and Westoby 1992a,b; Reader 1993). In much of Australia, ants are the predominant seed predators of small-seeded species (Ashton 1979; Andersen and Ashton 1985; Wellington and Noble 1985; Andersen 1987; Andersen 1988; Andersen 1989). This predominance of ants contrasts studies in North America, Israel and the rainforests of northern Queensland, where rodents and/or birds were the predominant seed predators (Abramsky 1983; Morton 1985; Osunkoya 1992). Since many of the ants trapped during the survey are known seed harvesters (Andersen 1989; Andersen 1991; Hughes and Westoby 1992a,b), it seems reasonable to assume that they represented at least part of the seed removing fauna. In addition, species of *Iridomyrmex* and *Camponotus*, common to all sites and widespread throughout south-eastern Australia were observed selectively removing whole seeds and leaving chaff, confirming that at least some of the trapped ants were active seed harvesters. It is recognised, however, that pitfall trapping indicates, but does not precisely measure the abundance or activity of seed harvesting arthropods

Removal of seeds can affect seedling recruitment and plant community structure. Seed removal may affect the recruitment of *H. gramineum* and *H. perforatum* as

there was an obvious, though marginal, increase in the proportion of seeds removed over time. Reader (1993) observed that in Canadian pastures, recruitment of *H. perforatum* was determined, in part, by the activity of seed predators. The predators were not identified, although they were probably small arthropods such as ants, as they were successfully excluded by plastic tubes around the field plots.

In south-eastern Australia, higher proportions of *Hypericum* seeds are removed in woodland, than grassland, paralleling Andersen and Ashton's (1985) observation that more seed-removal occurs in woodland than heathland. This may be because in woodlands, the grass and forb understorey is less dense than in grassland or heathland, facilitating easier location and access to seeds.

In the majority of replicates, small amounts of sand were removed, perhaps indicating that the sand and seeds were blown, or in some other way, accidentally displaced. Careful positioning of vials with their necks flush to the soil surface minimised the possibility that the baits were accidentally disturbed in this way. Moreover, if this had occurred, the chaff remnants, mentioned above, would also have been dislodged, and there would have been no clear sign of predator-selectivity. This suggests that ants were removing sand as well as the seeds, but in much smaller amounts. Most ant species are known to move sand grains and even small stones. These are then employed in nest construction (Dean and Yeaton 1993).

Seed 'baiting' experiments, in which seeds are artificially placed into the field and their removal monitored, as in the above study on *H. gramineum* should be interpreted with care. Andersen and Ashton (1985) found that in the short term (3 days) the size of seed clumps in such experiments may influence the rate of seed removal. This is because social foragers, which recruit workers to assist in seed-harvesting after locating a seed-source (e.g. *Pheidole* spp.), are more likely and better able to harvest clumps of seed, than they are to remove similar numbers of dispersed individual seeds, which need to be located individually. Conversely, if solitary foragers such as *Rhytidoponera* spp. are involved, seed-removal requires independent location of baits. On a weekly basis, results from the present study suggest that about 90% of seeds survive in grassland and about 80% in woodland. If these rates continued, then after 16 weeks, the survival would be only 0.9^{16} - 0.8^{16}. It seems from the data, however, that these rates may not be sustained and long-term removal rates may be much less, perhaps 5 - 10% per week. This suggests that 0.95^{16} and 0.9^{16} seed survive in the grassland and woodland

respectively. Since ant activity is highly seasonal, removal rates may be even lower.

2.6.5. The *H. gramineum* seed bank

With the aid of rainwash, litterfall and soil organisms, dispersed *H. gramineum* seeds may be incorporated into a soil seed bank. Once incorporated, their strong light requirement prevents germination, unless the soil is disturbed. This expectation is supported by results from the experiment investigating the effect of seed burial on germination (section 2.4.3.), in which buried seeds were generally unable to germinate. Campbell (1985) noted similar inhibition of germination in buried *H. perforatum* seeds.

Thompson and Grime (1979) classify seed banks into two broad groups: transient and persistent. In the former, all viable seeds in the soil germinate or die within a year. In the latter, some viable seeds survive in the soil for at least one year before germinating. The seed bank experiment conducted in this chapter suggests that *H. gramineum* forms a persistent seed bank, since viable seeds were detected in soil sampled from *H. gramineum* populations. These seeds must have entered the bank at least one year prior to sampling, because at the time the soil samples were removed, no plants in the population had set fruit for that season. Many small seeded perennial herbs establish persistent seed banks (Thompson and Grime 1979; Silvertown 1982; Baker 1989; Reisman-Berman *et al.* 1991; Cavers *et al.* 1992; Forcella *et al.* 1992; Callihan *et al.* 1993; Davis *et al.* 1993; Thompson *et al.* 1993).

Germinability of field-exposed, but bagged, *H. gramineum* seeds is consistently 30 - 50% less than in laboratory-stored seeds of the same age. In the field, few (< 1%) seeds germinated during this experiment. As all were protected from seed-harvesting predators by their enclosing nylon bag, differences in the number of germinations are likely to result from pathogenic decay of seeds and/or accelerated age-related mortality. Between the 6-month peak in germinability and the final census at 18 months, the decline in viability caused by decay or mortality of *H. gramineum* diaspores in the field (as indicated by the proportion of bagged, but field-exposed seeds germinating in the laboratory) can be modelled by a curve of form:

$$y = e^{(4.37 - 0.43 \ln t)}, r^2 = 1.0,$$

where y = the proportion of viable seeds remaining at time t (months; Fig. 2.11, Model A). The rate of decline in their viability is approximately 37% per year, although this slows with time. Unless the seed bank is disturbed, which could lead to germination or predation of seeds, about 24% of diaspores are likely to remain viable within the seed bank, after 18 months.

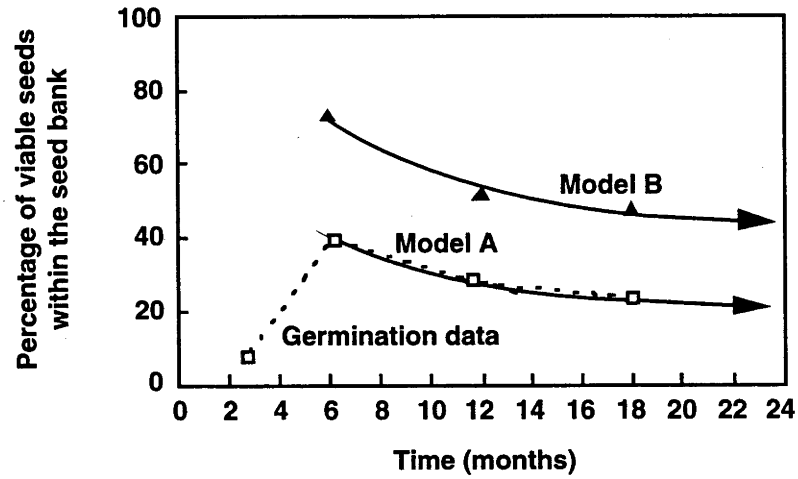


Fig. 2.11 Models showing exponential decline in the viability of *H. gramineum* seeds exceeding 6 months of age. Model A indicates the field-exposed seeds, while Model B indicates the laboratory-stored seeds. The rate of decline of both is approximately 37% year^{-1}. Squares and triangles indicate the observed values on which Models A and B, respectively, were based. The observed germination of bagged, but field-exposed seeds is depicted by the dashed line.

2.6.6. Reproductive ecology of *H. gramineum*

The above experiments permit parameters to be estimated for a model of the reproductive ecology of *H. gramineum*, as summarised below (Fig. 2.12). In brief, a large majority of seeds are formed sexually, usually by cross-pollination, although some diaspores may also be produced by self-pollination (Fig. 2.12, Box 1). Seed production by apomixis appears unlikely (≤ 3%), but requires further investigation. Following maturation and dispersal of the diaspores during late spring and summer, a majority germinate the following autumn or spring,

after overcoming innate dormancy (Fig. 2.12, Box 2). Few seeds, probably < 20% are harvested by predators. Seeds that are not killed by pathogens enter a persistent seed bank, ready to germinate when, and if released from both enforced and innate dormancy (*sensu* Harper 1977). A proportion of such seeds may become inviable because of age-related mortality. Detailed studies of the seed and germination ecology of other species in the Australian forb flora are generally lacking, but may reveal patterns similar to those detected in *H. gramineum*.

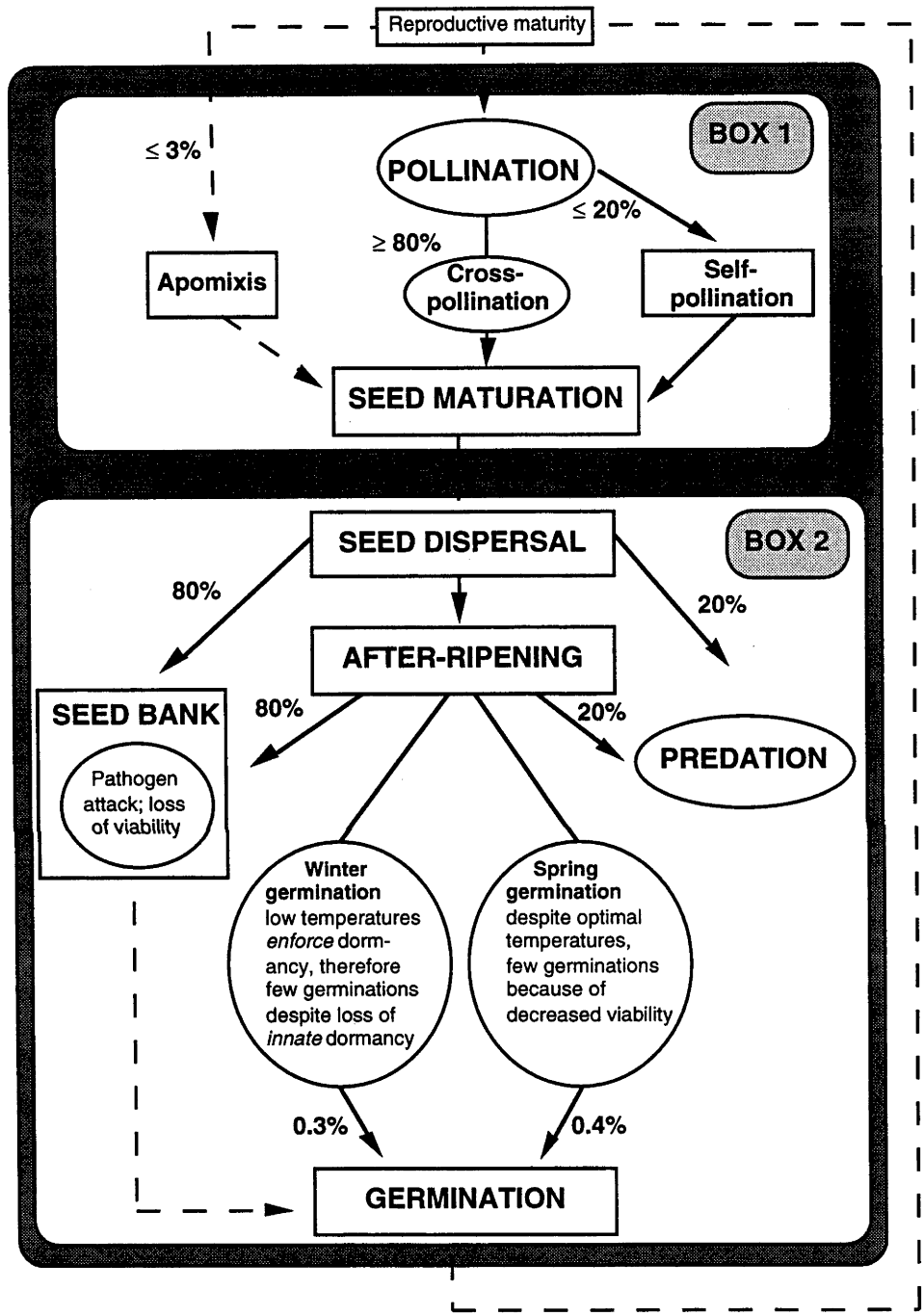


Fig. 2.12 Proposed model of the reproductive ecology of *H. gramineum*. Topics examined in this chapter occur within the large dark-shaded box. Percentages indicate the probability of that process. Aspects of the pollination biology of *H. gramineum* are delineated in Box 1, while Box 2 delineates the major features of the germination of *H. gramineum* seeds. Of the processes investigated, those that are primarily plant-mediated are placed within rectangles. Those that are mediated by other biotic/abiotic factors occur within ovals. Processes that have not been quantified in this chapter are indicated by dashed lines. Mortality of plants and seeds is possible at all stages.

2.7. Summary

Several aspects of the reproductive ecology of the native perennial forb, *H. gramineum*, were examined in a series of pollination, germination and seed predation experiments. While self-pollination may result in some mature seeds, more are produced by cross-pollination. Seeds require after-ripening and light for germination. The optimal temperature regime for germination was about 35/25°C, and is possibly higher. Fresh seeds were innately dormant. These two results suggest that in the field, *H. gramineum* is more likely to germinate in spring or autumn, although field experiments did not indicate a strongly seasonal effect. Seeds produced by individuals infested with the mite, *A. hyperici*, germinated more readily than seeds from mite-free plants, although this result requires further investigation, as the experiment had few replications. *H. gramineum* seeds that failed to germinate after dispersal may be incorporated into a persistent seed bank (roughly 80%), or removed by seed predators (roughly 20%). A model summarising factors influencing the seed ecology of *H. gramineum* is provided. In the following chapter, results of the present studies will be included in a transition matrix simulating a population of *H. gramineum*.

CHAPTER 3

THE POPULATION DYNAMICS OF ESTABLISHED *HYPERICUM GRAMINEUM*

3.1. Introduction

Knowledge of life history processes such as germination, flowering and fruiting facilitate more detailed analysis of plant population demography and are necessary to understand population regulation and viability (Eriksson 1988; Boeken 1989). Many investigations of plant demography, have involved annual or biennial species because single seedling cohorts, typical of such taxa, enable precise descriptions of plant age and growth stage (Werner 1975; Werner and Caswell 1977; Fone 1989). Other authors describe the population biology of clonal plants, which pose unique questions associated with recruitment and population stability (Eriksson 1988; Navas and Garnier 1990). In general, of all plant life forms, perennial herbs have been relatively well studied (see for example, Hutchings 1987a,b; Matlack 1987; Solbrig *et al.* 1988; Boeken 1989; Fone 1989; Watkinson 1990; Hegazy 1992; Windig 1993), though few investigations document the population biology of native Australian forbs.

Classical biological control is ultimately concerned with altering weed population dynamics and may also affect the stability of populations of indigenous taxa. The latter undesirable out-come is difficult to assess without prior knowledge of the population ecology of native species that are considered at potential risk. This chapter aims to investigate and describe the demography and life history of established adult plants in natural populations of *Hypericum gramineum*. A transition matrix model is used synthesise the findings of chapters 2 and 3 to make predictions about the population dynamics of this forb. In so doing, it is hoped to provide the basis for an assessment of the impact, if any, that *Aculus hyperici* has on *H. gramineum*.

3.2. Study sites

Three study sites were chosen representing a range of typical *H. gramineum* habitats, from open woodland to grassland. At the time of selection (October, 1990), populations of *H. gramineum* at all sites appeared vigorous and healthy.

3.2.1. Site 1: Beechworth

This site was located about 5 km from Beechworth, NE Victoria (36° 23'S, 146° 43'E), in the Mt. Pilot Nature Reserve. The site comprised an open *Eucalyptus polyanthemos* and *E. macrorhyncha* woodland dominated by an understorey of *Hypericum perforatum*, *Brachyloma daphnoides* and *Hibbertia riparia* growing on well drained, coarse granitic soil. Of fifty 1 m x 1 m quadrats randomly placed within a 30 x 20 m area, 62% contained at least one *H. gramineum* adult. Where present, the mean above-ground density of established *H. gramineum* was 7.8 m^{-2}. Above-ground herbaceous biomass of all species, estimated in November 1991 (by harvesting, oven-drying at 60°C for 5 days and weighing samples from 30 randomly placed 12 x 10 cm quadrats), was about 35 g m^{-2} (0.4 g 120 cm^{-2} ± 0.1 s.e.).

3.2.2. Site 2: Mt. Ainslie

This site was located at the foot of Mt. Ainslie, near the Canberra Shooters Club, in urban Canberra (35° 17'S, 149° 10'E). The vegetation comprised a *E. mannifera* and *E. melliodora* woodland with a predominantly grass understorey including *Themeda triandra* and *Poa sieberiana*, but including forbs, mainly *Pomax umbellata*. The site was poorly drained, straddling a water course of winter runoff from the Mt. Ainslie summit. It became permanently waterlogged during winter, but dry over summer. On average, there were 5.5 established *H. gramineum* adults m^{-2} in the 44% of sampled quadrats (n = 50) in which it occurred. The above-ground herbaceous biomass in November 1991 was 465 g m^2 (5.6 g 120 cm^{-2} ± 0.6 s.e., n = 30).

3.2.3. Site 3: Smith's Paddock

This grassland site was located adjacent to Rani Drive at the foot of Black Mountain, ACT (35° 17'S, 149° 05'E). The site was dominated by *T. triandra*, *P.*

sieberiana and *Carex appressa*. Like Mt. Ainslie (site 2), it was poorly drained and waterlogged in winter, but the silty soil dried out over spring and summer. The above-ground herbaceous biomass in November 1991 was 670 g m^{-2} (8.1 g 120 cm^{-2} ± 1.1 s.e., n = 30). Where present, the mean density of *H. gramineum* adults was 9.3 m^{-2}. Established plants occurred in 74% of 1 m^2 quadrats (n = 50).

3.3. Materials and Methods

3.3.1. Sampling procedure

At the beginning of October 1990, a 30 m x 20 m grid was laid down at each site. Twenty individuals of *H. gramineum* with the longest shoot 20 to 90 mm long (small plants), 15 with the longest shoot 100 to 140 mm long (medium plants) and 15 with shoot length > 150 mm (large plants) were randomly selected from within the grid, and their location mapped. Seedlings less than 20 mm in height were excluded from the survey because of difficulties in correctly distinguishing such plants from co-occurring forbs of similar habitat, size and shoot architecture. All selected plants were tagged with plastic labels fixed to the ground with a tent peg. The base of each shoot system was marked with a plastic pin, enabling relocation of the plants the following season, after dying back over winter. Plants were censused approximately every 4 weeks during the ensuing spring and summer of 1990/91 (season 1), by estimating the mean shoot length, counting the number of shoots, scoring whether the plants had flowered, counting the number of mature fruits, determining the number of leaves (or leaf scars, where leaves had senesced or been grazed) per shoot, scoring plants for damage caused by grazing herbivores (1 = no damage to 4 = severe damage, 50 - 70% of total leaf area removed) and at the final census for the season, scoring for the presence of over-wintering vegetative 'rosettes', usually comprising 2 - 3 short (about 15 mm) shoots originating from the parental shoot base. Finally, the general vigour of plants was ranked from 1 (healthy) to 4 (no plant or remains visible; it was assumed all such plants had died, as they failed to re-appear). Four-weekly censuses were also conducted from October 1991- April 1992 (season 2), and from October 1992- April 1993 (season 3).

During seasons 2 and 3, the number of rhizomatously produced vegetative daughter plants was also estimated: At the end of season 2, the connective rhizomes between adults and putative daughters were excavated to 2 cm depth to confirm the origin of 'daughters'. In some cases the rhizomes appeared to have rotted and it was not

possible to determine whether daughters were true ramets, or distinct genets. In such cases, plants were considered vegetative progeny if they occurred within a 20 cm radius of the 'parent'.

3.3.2. Analysis of data

3.3.2.1. Plant growth

During the 1990/91 season (season 1), sites 1 (Beechworth) and 3 (Smith's Paddock) were vandalised with the labels from up to 40% of plants being removed. The loss of these data early in the survey coupled with natural plant mortality rendered inter-site comparisons difficult because of the low sample number at Smith's Paddock. Plant growth analyses are therefore based on data pooled across all sites. Growth, measured as shoot height, shoot number, and the number of fruit and leaves produced, was compared between size classes and season by restricted maximum likelihood estimation (REML, Genstat 5 algorithms: Lane *et al.* 1987; Digby *et al.* 1989), after logarithmic transformation of the data to satisfy assumptions of the statistical model. Untransformed arithmetic means are, however, presented to summarise the results.

3.3.2.2. Adult demography

Yearly growth of *H. gramineum* individuals was divided into three stages: (1) flowering, (2) fruiting and, towards the end of the season, (3) production of a vegetative rosette. Pooling plant size and data from the two Canberra sites, the populations were compared with the Beechworth data by examining the proportions of plants progressing from one growth stage to another. Plants lost due to vandalism were removed from all comparisons, including those of season 1.

3.4. Results

3.4.1. Plant growth

Measures of shoot growth generally varied significantly ($P \leq 0.016$) between seasons and between plants of different sizes (Fig. 3.1). For most growth indices, small plants were significantly ($P \leq 0.03$) less productive than large plants. While a trend towards intermediate productivity in medium-sized plants is apparent, differences between small and medium, and between medium and large plants are not significant. Fruit production was similar ($P = 0.085$) in all size classes (Fig. 3.1f). Production of vegetative daughter plants was slightly higher in small plants, and least in large plants, although differences were not significant (Fig. 3.1g, $P = 0.271$). Herbivory seemed slightly more severe on medium plants, which averaged a ranking of 2.54, than on small (1.89) or large plants (2.26).

Season had a major effect on average shoot length, measured at both the start of the growing period in October (initial shoot length, Fig. 3.1a) and at its conclusion in early April the following year (final shoot length, Fig. 3.1b, $P < 0.001$). In season 2, shoots were significantly shorter at the end of the season than at the end of seasons 1 or 3, probably because of a drought early in the season and associated decreases in soil moisture levels (see below). A dry 1990/91 season may also account for the low numbers of shoots that year relative to seasons 2 and 3 ($P = 0.016$, Fig. 3.1c). The number of leaves per shoot was similar ($P > 0.05$) in all three seasons (Fig. 3.1d), although the total number of leaves per plant, estimated as the number of leaves per shoot x the number of shoots, was significantly ($P = 0.007$) lower in season 1, possibly because of a drought, than in subsequent years (Fig. 3.1e). The production of fruit increased in the last two years of the survey, relative to season 1 ($P < 0.001$, Fig. 3.1f). In comparison, the number of daughter plants was similar ($P > 0.05$) in seasons 2 and 3 (Fig. 3.1g). Grazing damage was similar (2.20, 2.21 and 2.30 in seasons 1, 2 and 3, respectively; $P = 0.769$) in all three years.

Season and plant size interacted significantly ($P \leq 0.035$) for initial and final shoot length, and the number of leaves per shoot, and fruit per plant. In all cases, the trend was similar to that for initial and final shoot length (Fig. 3.2a and b) in which, by season 2 or 3, small plants did not differ ($P > 0.05$) from large plants.

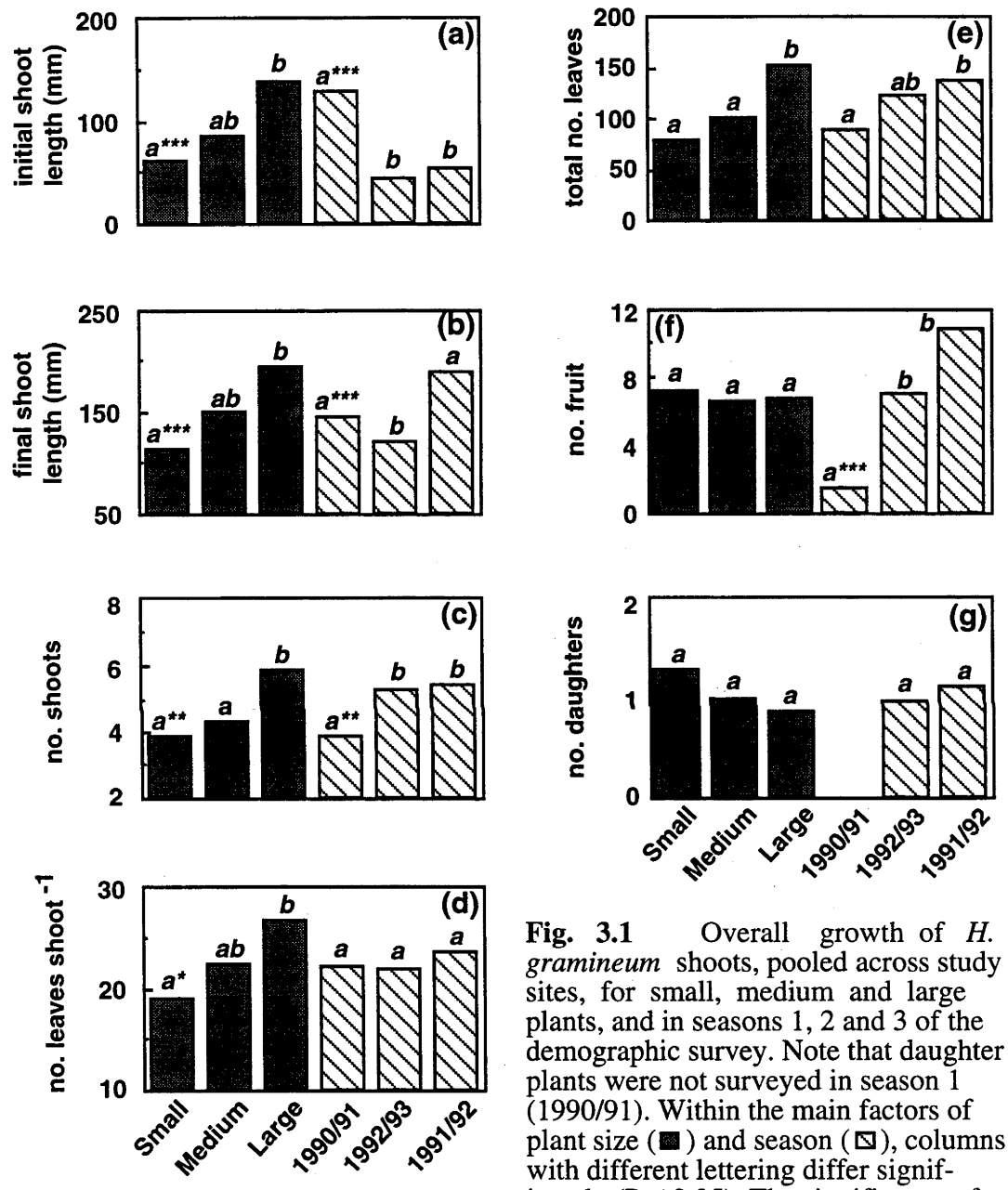


Fig. 3.1 Overall growth of *H. gramineum* shoots, pooled across study sites, for small, medium and large plants, and in seasons 1, 2 and 3 of the demographic survey. Note that daughter plants were not surveyed in season 1 (1990/91). Within the main factors of plant size (■) and season (▨), columns with different lettering differ significantly ($P \leq 0.05$). The significance of F-tests between treatments of the main factors is indicated ($P \leq 0.05$*, $P \leq 0.01$**, or $P \leq 0.001$***).

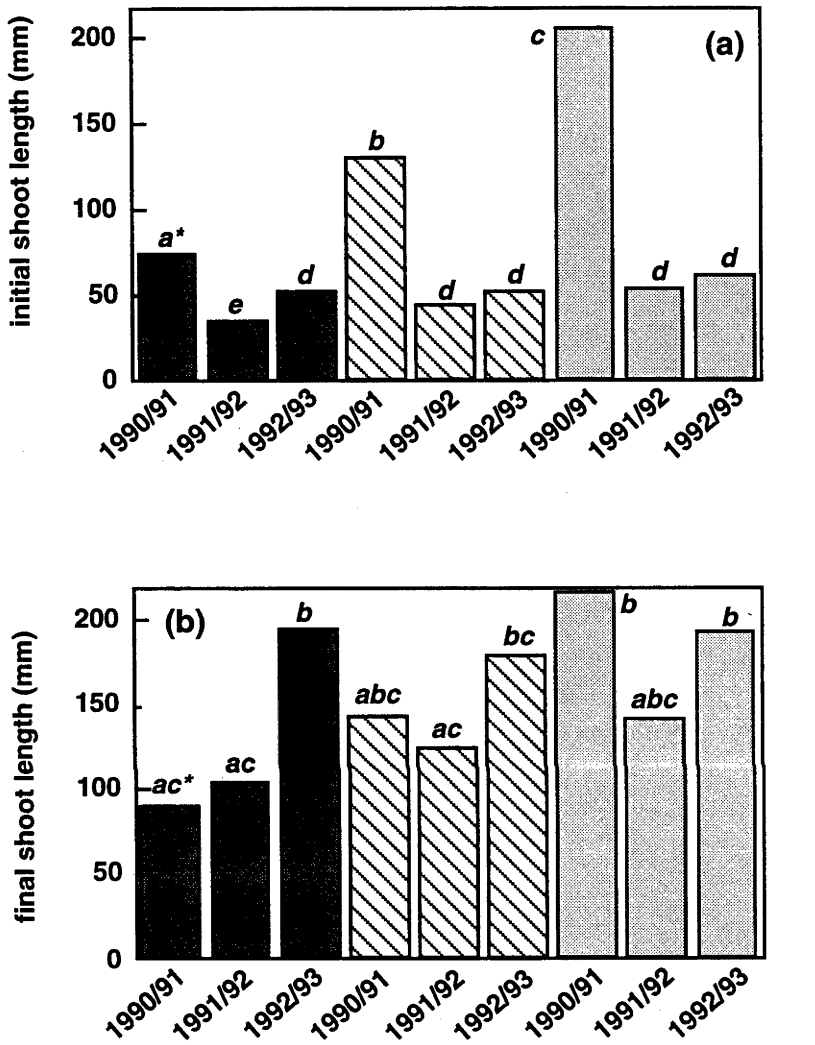


Fig. 3.2. Mean initial shoot length of *H. gramineum* (length at the season's start; a) and final length (at the season's conclusion; b) illustrating the plant size x season interaction. Columns with different lettering differ significantly ($P \leq 0.05$). The overall F-test for both initial and final shoot lengths was highly significant ($P < 0.001$). ■ = small plants, ▨ = medium plants and ▩ = large plants.

3.4.2. Demography of established plants

Canberra populations of *H. gramineum* performed similarly to the Beechworth population, as illustrated in figure 3.3, although there were some notable differences, particularly in the proportion of mortalities in each of the regions.

3.4.2.1. Site 1 - Beechworth

(a) Seasonal demography

In the first season, a majority (66%) of plants at Beechworth produced flowers, most (78%) of which continued to set at least one fruit (Fig. 3.3a). Although a small proportion (4%) of these then died, about half of the fruiting plants produced a rosette by the end of the season. Regardless of whether fruiting plants produced a rosette, about 87% survived the winter of 1991 till the spring of season 2. A few flowering plants (18%) failed to mature any fruit, but subsequently grew as well as the fruiting plants, a majority (82%) surviving the winter, at least till spring of season 2.

Thirty-four per cent of plants set no flowers in season 1. Most of these (88%) did not produce a rosette, but almost all successfully over-wintered. Twelve per cent of the non-flowering plants produced a rosette and all of these survived the winter.

As summarised in figure 3.3a, similar patterns were observed in seasons 2 and 3, although the proportion of flowering, and subsequently, fruiting plants, increased. By the end of seasons 2 and 3, fewer plants appeared to form an over-wintering rosette, although this did not seem to increase mortality.

(b) Mortality

Mortality at Beechworth was fairly constant (about 12% $year^{-1}$) over the three years (Fig. 3.4). Seventeen per cent of all deaths occurred during season 1, and 15% during season 2. At termination of the survey in late March 1993, no further deaths had occurred. Plant mortality was highest in winter (72% of all deaths).

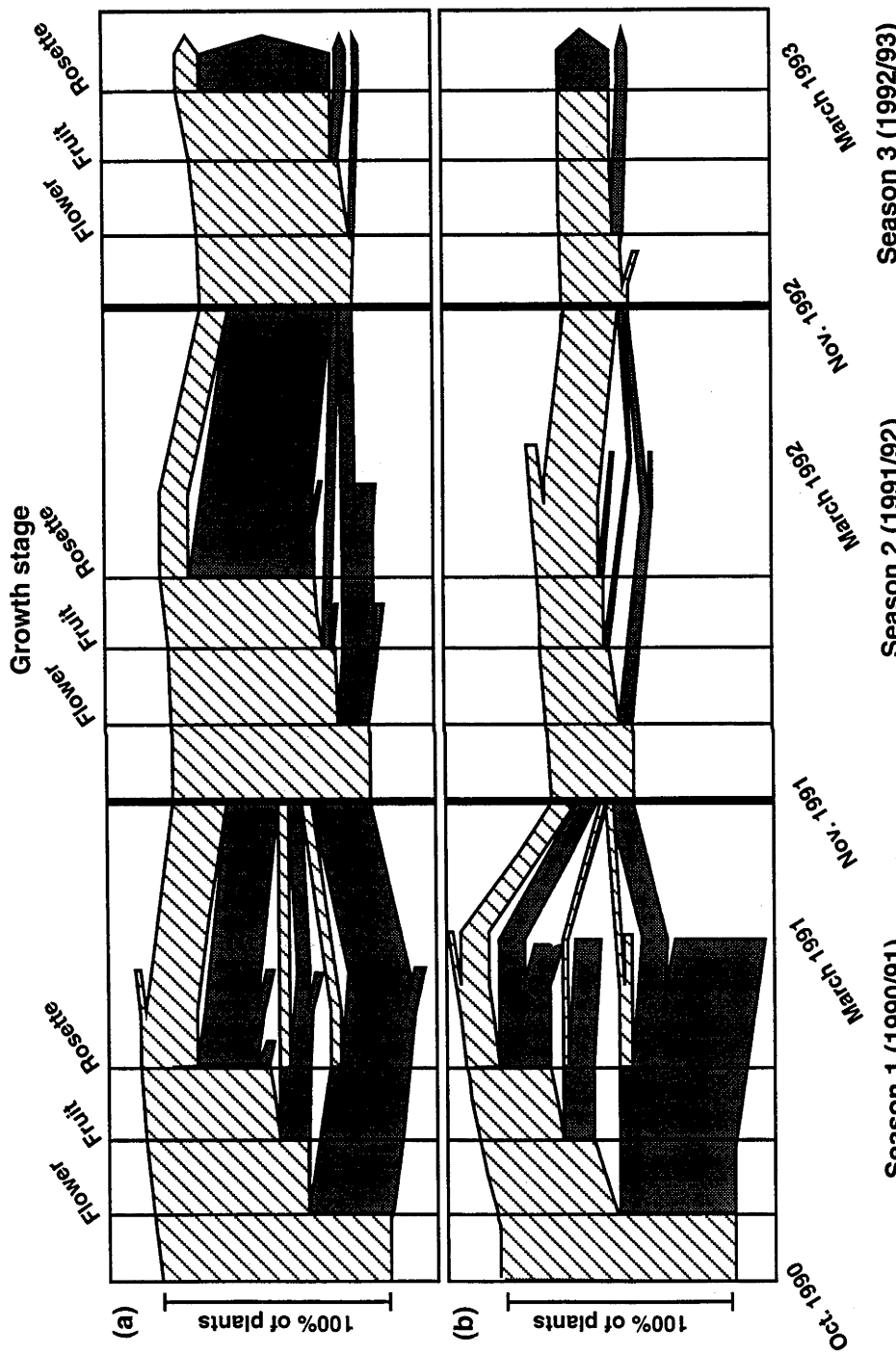


Fig. 3.3 Summary of adult *H. gramineum* demography over thee field sesons, illustrating the proportion of original plants surviving to the next growth stage (either flowering, fruiting or producing a rosette), season-by-season. The original population is indicated by the vertical height of the hatched area at the base of the 'tree' (far left, indicated by October 1990, season 1). The graph is read from left to right, passing through seasons of possible flowering, fruiting, or rosette production. Failure to flower, fruit or produce a rosette during any season is indicated by the respective shaded area, in proportion to the population affected. At the beginning of each subsequent season, the remaining population is drawn together and represented by the height of the new hatched area. Mortalities between growth stages or seasons are depicted as truncated branches.

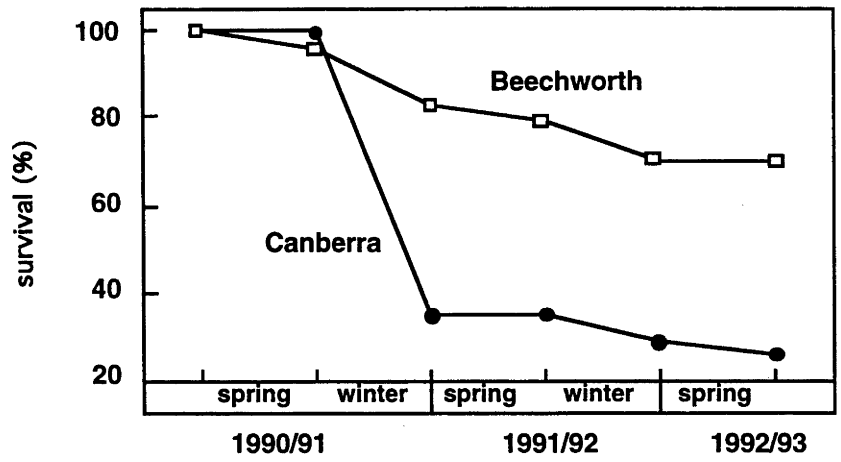


Fig. 3.4 Survival of the original plants, in populations of *H. gramineum* in Canberra and Beechworth, till March 1993.

3.4.2.2. Sites 2 and 3 - Canberra

(a) Seasonal demography

Demography of plants at the combined Canberra sites is summarised in figure 3.3b. Roughly half the plants failed to flower in the first season, and consequently produced no fruit. About 12% of these non-flowering individuals produced a rosette by March of 1991, of which equal proportions survived and died during winter. Of the flowering plants, including fruiting and non-fruiting individuals, slightly more than half (53%) survived winter. A majority (71%) of flowering plants failed to produce an over-wintering rosette. Fifty-seven per cent of these did not survive till the following spring. As at Beechworth, proportionally more plants flowered in subsequent years, though in contrast to the former population, almost all (94%) produced an over-wintering rosette. By the end of season 3 (March), there were no rosettes on any of the surveyed plants.

(b) Mortality

Except for season 1 in which 65% of plants in the Canberra populations died, mortality of *H. gramineum* was relatively constant (about 12%, Fig. 3.4), as at Beechworth. The high mortality rate during winter of season 1 probably relates to low rainfall and resultant low soil moisture (Fig. 3.5, climatic data provided by the

Australian Bureau of Meteorology: Canberra weather station located at the Canberra airport about 4 km from Mt. Ainslie and 9 km from Smith's Paddock; Beechworth station located 8 km from the study site). Illustrating the drought's severity, 88% of all Canberra mortalities occurred over the winter of season 1.

Trends in the demographic data at Beechworth and Canberra are summarised in table 3.1. Since small plants tended to move into larger size classes in later years, the data have not been split according to previous growth stage. Similar trends emerge from both regions. It is clear, for example, that the proportion of flowering and fruiting plants increases from season 1 to 3 at Beechworth and Canberra, with concomitant decreases in the percentage of non-flowering/fruiting plants. By contrast, the proportions of plants producing regrowth showed no consistent patterns, either between seasons within a region, or between regions.

Table 3.1 Summary table of proportions (%) of live *H. gramineum* plants at each growth stage. Note that values represent total proportions (%) and are not split according to the number of plants that achieved the preceding stage. B = Beechworth and C = Canberra.

Growth Stage	Season 1		Season 2		Season 3	
	B	C	B	C	B	C
Flower	66	49	85	93	94	86
Fruit	51	36	79	90	91	86
Rosette	34	20	12	83	20	0

3.5. Modelling a population of *H. gramineum*

3.5.1. Background

Demographic models enable the fate of populations under various conditions to be simulated, based on knowledge of key processes involved in maintenance of the populations. For plants, the most important demographic variables include recruitment from seeds, and possibly a seed bank, establishment and maturation, reproduction and dispersal of propagules. After empirically estimating these processes and incorporating them within a model, the parameters can be manipulated, enabling predictions to be made about the viability of the population under different circumstances.

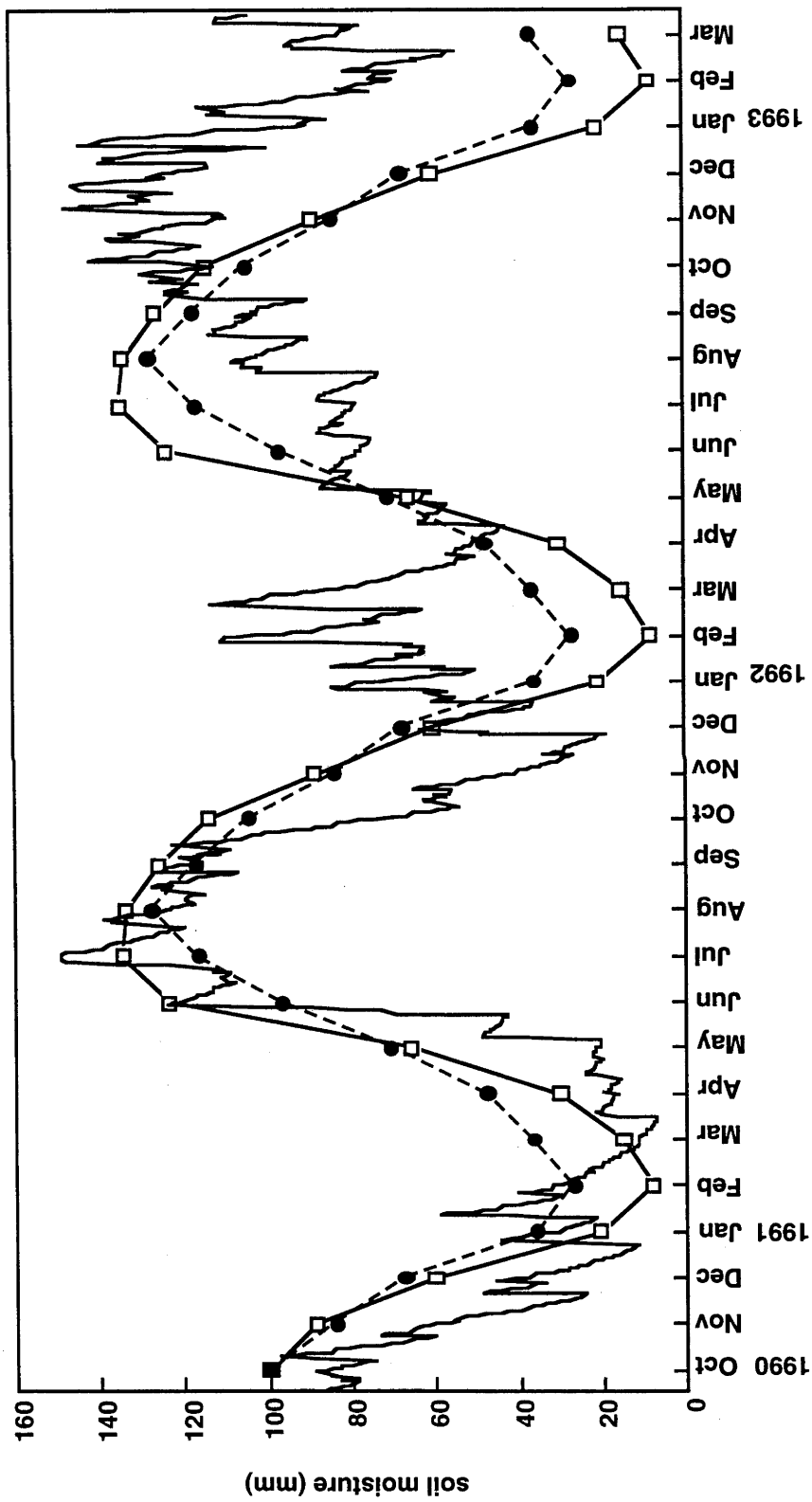


Fig. 3.5 Estimated mean monthly soil moisture at Canberra (- - -●- - -) and at Beechworth (——□——), and daily soil moisture at Canberra (-⋀⋀-), during the three-year survey, illustrating the drought in Canberra during season 1 and early in season 2. Daily estimates based on daily rainfall, 180 mm maximum soil moisture storage and evapo-transpiration rate, calculated as (pan A evaporation rate at Fairbairn Airport, Canberra x soil moisture prior to evaporation)/180. Mothly estimates are based on mean monthly rates.

3.5.2. Simulation of an H. gramineum *population - procedure and results*

Leslie projection matrices (see Pielou 1977) enable the projection of population size at a given time (year) for a developmental stage-structured population. In the following, the main findings from chapter 2 and the preceding results of this chapter have been integrated to model critical processes in populations of *H. gramineum*. Model parameters are then varied to simulate possible effects of *A. hyperici* on the population growth of *H. gramineum*.

Populations of *H. gramineum* can be considered to comprise newly produced seeds, a seed bank, seedlings, juveniles, small (single-stemmed) adult plants, larger (multi-stemmed) adults and vegetatively produced daughters. The following matrix (Table 3.2) aims to reflect this stage-structure, and summarises the probability of seeds entering the seed bank or germinating and passing through the seedling stage to establish as juveniles (including vegetative daughters), before maturing to subsequent growth stages. Multi-stemmed adults may regress to single stems, possibly as a consequence of stress or damage. Seed production and daughter ramets are included, the latter incorporated as 'juveniles' such that the overall population dynamics can be simulated. Nominally, the annual cycle runs from March to February, commencing immediately after seed dispersal. In setting up the projection matrix, it was decided that there were reasonable estimates of all parameters except juvenile survival. This parameter, indicated in table 3.2 by the italicised superscript numeral '*5*', was, therefore, adjusted to give a stable population. The following model represents favourable seasons, excluding drought years, and in reality, populations may expand under such conditions. The true value of juvenile survival is, thus, likely to be higher than that indicated. As a basis for examining the population projections, however, a stable population is considered a more convenient baseline. Other parameters within the model, as designated by superscript numerals (see Table 3.2), were estimated as follows:

1 - The probability of seed entering the seed bank: Estimated as 0.8 since few seeds germinate immediately, and up to about 20% may be removed by seed predators (chapter 2, section 2.5.1.).

2 - The probability of seeds germinating and establishing in their first year: Estimated as 0.0004, since about 0.4% of seeds germinate in the field (chapter 2) and it is estimated that approximately 10% of those survive their first year.

3 - The probability of seeds remaining in the seed bank: In chapter 2 (section 2.4.1.1. and Fig. 2.7), it was shown that about 37% of seeds remain viable after six months field exposure. This probability of viable seeds remaining in the bank was, therefore, estimated as 0.38.

4 - The probability of a seed from the seed bank germinating and establishing: In the seed burial experiment (chapter 2, section 2.4.1.2., fig. 2.9), it was shown that burial reduced germination/seedling emergence to 1% of the rates for seeds on the surface. A value of 0.000004 (1% of the value of parameter '2' x a nominal 1% seedling establishment estimate) was, therefore, adopted.

5 - As observed in the text, this parameter, juvenile growth and survival to adult stages, was adjusted to establish a stable population.

6 - Seed production of single-stemmed plants: This parameter, 740, was reduced in comparison to parameter *8* (see below), because small plants are likely to produce fewer fruits and seeds.

7 - The probability of a single-stemmed adult maturing to a multi-stemmed adult: The present chapter demonstrated that plants progress to the next growth stage each year. Given that annual mortality approximates 12% (section 3.4.2., this chapter), this parameter was estimated as 0.88, since no single-stemmed plants are expected to remain in this category.

8 - Seed number produced by a multi-stemmed adult: The number of seeds produced by a multi-stemmed adult was estimated as about 1300, since approximately 150 - 200 seeds are produced per fruit (chapter 2, section 2.3.), and on average, 7 - 8 fruit develop to maturity annually.

9 - The probability of a daughter being produced (included in the 'juvenile' stage): Each multi-stemmed adult produced about one vegetative daughter in the three-year field survey (this chapter). This parameter was, therefore, estimated as 0.3 daughters per plant per year.

10 and *11* - The probability of multi-stemmed plants surviving: Estimated as 12%, as above (parameter *7*). Some multi-stemmed plants regressed to a

single stem stage, roughly 33% of the total each year. As such, parameters *10* and *11* were estimated as 0.3 and 0.58, respectively.

Table 3.2 summarises parameters of the model. Figure 3.6 shows a simulation of an (arbitrary) initial population of 100 adult plants which achieves stability (natality = mortality) after seven years. At this point, a stable stage-structured distribution of 56 juveniles/daughters, 28 single-stemmed adults and 58 multi-stemmed adults is reached.

Table 3.2 Summary of the values and annual transition probabilities in the projection matrix used to model a stable population of *H. gramineum*. Estimates and probabilities derive from results in chapter 2, and the present chapter. Note that the Juvenile stage comprises vegetatively produced 'daughters' and established seedlings. Italicised superscript numerals refer to discussion in the text (section 3.5.2) of the estimation of that parameter.

Transitions (next year)	**Developmental Stage (this year)**				
	Seed	**Seed bank**	**Juvenile & daughter**	**Single-stemmed adult**	**Multi-stemmed adult**
Seed	0	0	0	740[6]	1300[8]
Seed bank	0.8[1]	0.38[3]	0	0	0
Juvenile & daughter	0.0004[2]	0.000004[4]	0	0	0.30[9]
Single-stemmed adult	0	0	0.18279[5]	0	0.30[10]
Multi-stemmed adult	0	0	0	0.88[7]	0.58[11]

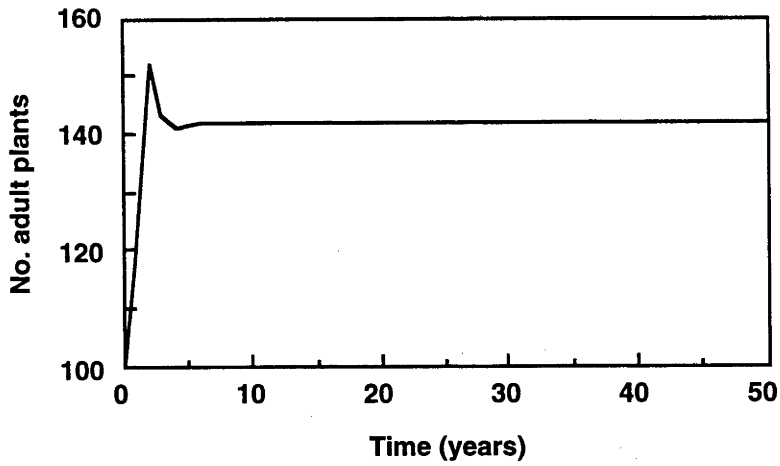


Fig. 3.6 A stable population of *H. gramineum*, as simulated by a Leslie projection matrix, starting with an initial population of 100 adult plants. See Table 3.2 for parameters within the model.

By altering the transition probabilities, the effect of environmental variables on the population such as weather or predators can be simulated. In the following, the potential effects of *A. hyperici* on populations of *H. gramineum* have been simulated by altering various parameters (see Table 3.3), which might be affected by *A. hyperici.* In all cases, it is assumed that the probability of a seed entering the seed bank remains constant (80%). Population trajectories are graphed as the $\log_e$ number of plants remaining over time (Fig. 3.7).

(a) Halving seed production: Potentially, *A. hyperici* could damage populations of *H. gramineum* by reducing seed production. Simulating a decrease in seed production of 50% (Table 3.3, treatment a), the rate of population decline is about 3.5% per year (Fig. 3.7, curve a).

(b) Halving the establishment of vegetative daughters: Halving the production of vegetative daughters (Table 3.3, treatment b) causes a less severe decline (1.4% per year) in population growth (Fig. 3.7, curve b).

Table 3.3 Summary of the values and probabilities of transition between growth stages in the projection matrix used to simulate the possible effect of herbivory on a population of *H. gramineum*. Parameters that have been manipulated (in treatments a - i) are indicated in bold; other parameters have not been varied from those used to model the 'stable population' (see table 3.2). Manipulation of the model parameters involved treatment a = halving the number of seeds; b = halving the production of daughters; c = halving both the number of seeds and the production of daughters; d = increasing the chance of germination by 20%; e = halving adult growth; f = halving adult growth while simultaneously increasing the chance of germination by 20%; g = doubling the mortality of adults; h = doubling the mortality of all plants (juveniles and adults), and i = doubling the mortality of all plants while simultaneously increasing the chance of germination by 20%.

Transitions	Treat-ment	Seed	Seed bank	Juvenile & daughters	Single-stemmed adult	Multi-stemmed adult
Seed	a	0	0	0	**370**	**650**
Seed bank		0.8	0.38	0	0	0
Juvenile & daughters		0.0004	0.000004	0	0.00	0.30
Single-stemmed adult		0	0	0.18279	0	0.30
Multi-stemmed adult		0	0	0	0.88	0.58
Seed	b	0	0	0	740	1300
Seed bank		0.8	0.38	0	0	0
Juvenile & daughters		0.0004	0.000004	0	0.00	**0.165**
Single-stemmed adult		0	0	0.18279	0	0.30
Multi-stemmed adult		0	0	0	0.88	0.58
Seed	c	0	0	0	**370**	**650**
Seed bank		0.8	0.38	0	0	0
Juvenile & daughters		0.0004	0.000004	0	0.00	**0.165**
Single-stemmed adult		0	0	0.18279	0	0.30
Multi-stemmed adult		0	0	0	0.88	0.58
Seed	d	0	0	0	740	1300
Seed bank		0.8	0.38	0	0	0
Juvenile & daughters		**0.00048**	0.000004	0	0.00	0.30
Single-stemmed adult		0	0	0.18279	0	0.30
Multi-stemmed adult		0	0	0	0.88	0.58
Seed	e	0	0	0	740	1300
Seed bank		0.8	0.38	0	0	0
Juvenile & daughters		0.0004	0.000004	0	0.00	0.30
Single-stemmed adult		0	0	0.18279	**0.44**	**0.59**
Multi-stemmed adult		0	0	0	**0.44**	**0.29**
Seed	f	0	0	0	740	1300
Seed bank		0.8	0.38	0	0	0
Juvenile & daughters		**0.00048**	0.000004	0	0.00	0.30
Single-stemmed adult		0	0	0.18279	**0.44**	**0.59**
Multi-stemmed adult		0	0	0	**0.44**	**0.29**
Seed	g	0	0	0	740	1300
Seed bank		0.8	0.38	0	0	0
Juvenile & daughters		0.0004	0.000004	0	0.00	0.30
Single-stemmed adult		0	0	0.18279	0	**0.25**
Multi-stemmed adult		0	0	0	**0.76**	**0.51**
Seed	h	0	0	0	740	1300
Seed bank		0.8	0.38	0	0	0
Juvenile & daughters		0.0004	0.000004	0	0.00	0.30
Single-stemmed adult		0	0	**0.091395**	0	**0.25**
Multi-stemmed adult		0	0	0	**0.76**	**0.51**
Seed	i	0	0	0	740	1300
Seed bank		0.8	0.38	0	0	0
Juvenile & daughters		**0.00048**	0.000004	0	0.00	0.30
Single-stemmed adult		0	0	**0.091395**	0	**0.25**
Multi-stemmed adult		0	0	0	**0.76**	**0.51**

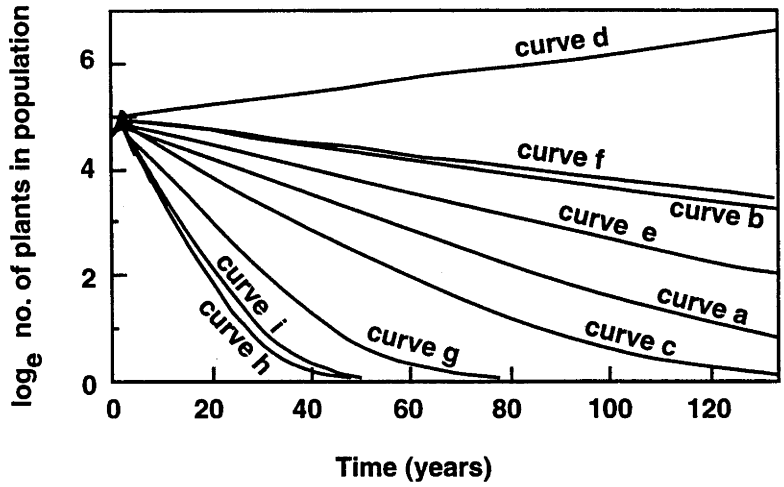


Fig. 3.7 Population change of *H. gramineum*, as simulated in the projection matrices summarised in Table 3.3. Curve a = halving the number of seeds; curve b = halving the production of daughters; curve c = halving both the number of seeds and the production of daughters; curve d = increasing the chance of germination by 20%; curve e = halving adult growth; curve f = halving adult growth while simultaneously increasing the chance of germination by 20%; curve g = doubling the mortality of adults; curve h = doubling the mortality of all plants (juveniles and adults), and curve i = doubling the mortality of all plants while simultaneously increasing the chance of germination by 20%.

(c) Halving seed production and vegetative daughters: The effect of halving the establishment of both seedlings and daughters (Table 3.3, treatment c), increases the rate of decline to about 3.1% per year (Fig. 3.7, curve c).

(d) Increasing seed germinability by 20%: In the absence of any other reductions in plant growth or productivity, increasing the germinability of seeds by 20% (Table 3.3, treatment d), as apparently occurred following herbivory of *H. gramineum* individuals in chapter 2, causes the modelled population to increase at a rate of about 1.3 % per year (Fig. 3.7, curve d).

(e) Halving adult growth: In treatment e, adult growth was halved by decreasing the probability of adults progressing to or remaining at the next growth stage by 50%. Maintaining mortality of adults constant at 12% per year but halving their growth

rate in the above manner increases the probability of a multi-stemmed adult regressing to the single-stemmed form. Varying parameters of the model in this way causes a constant decline in the population of 2.36% per year (Fig. 3.7, curve e).

(f) Halving adult growth and increasing seed germinability: The combined effect treatments d and e, a simultaneous 50% reduction in growth and an increase in seed germinability of 20% (Table 3.3, treatment f) lessens the rate of population decline, compared with halving growth alone, to about 1.2% per year (Fig. 3.7, curve f).

(g) and (h) Doubling the mortality of plants: Doubling the mortality of adult plants (Table 3.3, treatment g) causes an exponential decline in size of the modelled population (Fig. 3.8, curve g). Altering the model in this way causes the population to halve within about 10 years, a decline of approximately 9.9% per year. As might be expected, doubling the mortality of all plants (adults and juveniles; Table 3.3, treatment h) causes more rapid exponential decline in the population (Fig. 3.7, curve h): the half-life of the population is reduced to about 7 years, at which point the rate of decline in the population is about 15.8% per annum.

(i) Doubling the mortality of all plants and increasing seed germinability: When the mortality of all plants is doubled while increasing the germinability of seeds by 20% (Table 3.3, treatment i; Fig. 3.7, curve i), the trajectory of population decline is similar to that caused by treatment h (doubling the mortality of all plants), although the population's half life is extended to about 8 years (a decline of approximately 14.8% per year). The positive effects of enhanced seed germinability are, therefore, countered by the rapid decline in population numbers.

3.6. Discussion

3.6.1. Stage-structured models of plant demography

Werner (1975) and Werner and Caswell (1977) demonstrate the importance of plant growth stage, rather than plant age in affecting the population dynamics of Teasel (*Dipsacus* spp.). In the present study, the age of plants was unknown, so individuals were allocated to size classes as a possible reflection of growth stage, depending on the average shoot height on the first day of season 1. For several measures of growth, the size classes generally reflected the productivity of plants. 'Large' plants produced, for example, more and longer shoots. Nevertheless, size

class, as defined by shoot length, was ultimately a poor predictor of *H. gramineum* performance because of highly significant ($P < 0.001$) size x season interactions. The interaction for initial and final shoot lengths indicate that the length of 'small' plants at the end of all three seasons was not significantly different from that of either 'medium' or 'large' plants. Evidently, such interactions between size and season reflect transition from one size division to another with time: the demography of a single cohort of plants was followed and it appears that after the first season, no 'juveniles' remained because all had progressed into adult plants. Transition from seedling to juvenile stages might affect population growth rates, but was not examined in this survey for reasons outlined above.

Patterns of adult *H. gramineum* demography indicate that in most seasons, a majority of plants flower and mature at least one fruit. With time, increasing proportions of plants flower, reflecting transition to greater reproductive maturity of the populations. In trying to determine the effect that flowering of the spider orchid (*Ophrys sphegodes*) in one year has on its chance of flowering the next year, Hutchings (1987b) found little support for the hypothesis that flowering would be reduced. In *H. gramineum*, flowering in one season does not apparently reduce flowering the following year. Unless the plants died, those that flowered one year generally survived winter and flowered the next.

Seasonal and site variability in the proportion of plants that produced a rosette suggests that this growth 'stage' may be a poor indicator of plant demographic success. Plants that failed to regrow by the end of March, for example, may have produced such shoots later in the autumn. However, a survey of the Smith's Paddock site confirmed that there had been no change in the proportion of plants that developed rosettes between late March and July 1992. While significant proportions of plants do over-winter with a rosette, it seems that firstly, the production of such a structure does not guarantee winter survival, as rosette plants suffer winter mortality, and secondly, that rosettes do not seem to be an over-wintering requirement, because many non-rosette plants survive till spring.

The most obvious differences between Beechworth and Canberra populations of *H. gramineum* were adult mortality rates. In the former, the risk of death was fairly constant between seasons. Harper (1977) notes the need to distinguish between survival curves, in which the age of plants is known, and depletion curves, in which their age is unknown. The depletion curve for Beechworth indicates a fairly constant depletion rate of about 12% per annum though within seasons, mortality rates varied, and were highest over winter. Similarly, in Canberra populations of

H. gramineum, mortality was highest during winter. As noted, the high death rate during winter of season 1 accounted for most deaths and probably reflects a drought, locally prevalent during 1991/2. This drought is apparently the major cause of differences between Beechworth and Canberra populations (Figs. 3.3a and b). In other seasons, the mortality in Canberra populations are similar to the Beechworth population.

In a comparison of plant population stability at widely separated study sites, Kelly (1989) found that the dynamics of *Euphrasia* spp. varied widely between sites. It was concluded that populations of *Euphrasia* vary like 'shifting clouds', filling available site niches by responding to factors such as rainfall and the height of surrounding turf. Apparently rainfall and soil moisture have major impacts on the demography of *H. gramineum*, though competition with surrounding vegetation may also affect plant performance. In this survey, Canberra populations grew in more strongly competitive environments, as indicated by herbaceous biomass, than the Beechworth population, where mortality and site biomass was lower.

Finally, it should be recognised that in this study, the demographic description of *H. gramineum* has not given detailed consideration to the effect of vegetatively produced daughter plants on population stability. Producing daughters may, for example, affect adult growth and survival rates. Although the survival curves generally indicate a low parental death rate, the fate of daughter plants whose parents die has not been investigated. In addition, daughter ramets may have greater survival or growth than juveniles of similar size derived from seedlings. Data collected on these aspects of the population biology of *H. gramineum* are limited and do not warrant further detailed analyses. Future studies of *H. gramineum* populations should investigate such recruitment and mortalities. This is of added importance given the projection matrix which indicates the relative sensitivity of *H. gramineum* populations to halving the establishment rate of daughters.

3.6.2. Demographic projections following herbivory

As noted by Boyce (1992), simple projection matrices essentially offer two outcomes: perturbations cause the simulated population to either increase to infinity, or decline to extinction. Variation in vital processes alter the rate of these outcomes. A stable initial population is thus a convenient baseline from which to examine alteration of parameters.

As expected, trajectories of the simulated *H. gramineum* population declined when parameters were reduced, and increased when the processes were enhanced. In demonstrating differential rates of population growth/decline when parameters of the matrix were varied, simulations suggest that populations of *H. gramineum* are not particularly sensitive to reductions in plant growth. A decrease in growth, measured by transition to the next adult stage of 50% (a significant reduction in the productivity of most plants) results, for example, in population decline at about 2.4% per annum. Such decreases in productivity are countered, to some extent, by increases in the germinability of seeds matured on mite-infested plants, as indicated in chapter 2: A decline of about 1.2% per year. The importance of seed production to population stability is apparent in curve a of figure 3.7. Halving seed production leads to more rapid declines in the size of populations than halving adult growth. By comparison, populations appear less sensitive to halving production of daughters. Clearly, the effects of *A. hyperici* on seed germination warrant careful re-examination, particularly if production of fruit is negatively affected. This is because recruitment, especially from seeds, seems to be a sensitive stage in the population dynamics of *H. gramineum.*

If *A. hyperici* increases the mortality of *H. gramineum*, the trajectory of population decline is steeper than that merely following equivalent reductions in growth. If mites double the mortality of all plants, for example, the rate of decline is about 9.9% per year. The simulated impact of *A. hyperici* on plant growth, mortality and recruitment highlight the need to experimentally investigate the effect of *A. hyperici* on other demographic processes. Subsequent chapters in this thesis aim to study such phenomena.

3.7. Summary

Life history attributes and the population dynamics of *H. gramineum* were monitored for three years at two field sites in Canberra and one in Victoria. Classification of the selected plants into three size groups based on shoot height at the start of season 1 (October 1990) did not aid prediction of their reproductive success or the fate of established plants, since by the end of season 1, all plants were of similar height and remained so subsequently. The number of shoots changed less obviously between seasons and probably represents a more rigorous means of sub-dividing *H. gramineum* populations. Identifying three broad growth phases, viz. (1) flowering (2) fruiting and (3) production of an over-wintering 'rosette', the proportion of plants progressing to each stage was monitored and

indicated that, with time, increasing numbers of *H. gramineum* flowered and matured fruit, thereby suggesting increasing reproductive maturation of the study plants. A high proportion of non-rosette plants also survived. With the exception of populations at Canberra in the first season, in which a majority of all deaths occurred and were attributed to a local drought, mortality of *H. gramineum* was fairly constant between seasons, about 12% per annum. Within seasons, most deaths occurred over winter.

Drawing on the results from this study and those of chapter 2, a Leslie projection matrix was used to simulate changes in a population of *H. gramineum* under varying patterns of herbivory by *A. hyperici*. Doubling the mortality of all plants caused a rapid decline in the size of the modelled population, which then had a half life of seven years. Halving adult growth had relatively minor effects on decline of the population. This effect was partly countered by increasing the germinability of seeds, as might occur with *A. hyperici*. Halving seed and daughter production caused rapid declines in the population, indicating that recruitment of *H. gramineum* is a sensitive life-history stage.

SECTION C:

DISPERSAL AND HOST-PREFERENCE OF *ACULUS HYPERICI*

Section C comprises two chapters which investigate aspects of the biology of *A. hyperici*: Chapter 4 re-analyses the data collected during host-specificity screening of *A. hyperici*, in light of the infra-generic phylogeny of the tested species of *Hypericum*. Chapter 5 investigates the dispersal and host-selection of *A. hyperici* in field populations of *H. gramineum* and *H. perforatum*.

CHAPTER 4

DISPERSAL AND HOST-PLANT SELECTION OF *ACULUS HYPERICI*

4.1. Introduction

Dispersal of organisms from a given site may confer advantages associated with foraging, release from intraspecific competition, avoidance of natural enemies and other beneficial outcomes. Wind-dispersal is well documented for fruits, seeds, pollen, and fungal spores (see, for example, Pedgley 1982; Burdon 1987; Andersen 1993). Other wind-dispersed organisms, such as small arthropods, have received less attention. For herbivorous arthropods, the primarily passive process of wind-dispersal may be complicated by the necessity for dispersal to a specific host, and/or the active process of host-selection. Several studies have described down-wind dispersal of scale insect larvae (Greathead 1972; Willard 1973; Willard 1974; Willard 1976; Wainhouse 1980). With notable exceptions (Thresh 1966; Nault and Styer 1969; Johnson and Croft 1976), few studies have investigated wind-dispersal of mites, despite their importance as herbivores and as vectors of disease (Thresh 1966; Nault and Styer 1969; Krantz 1978).

The success or otherwise of weed biological control relies, in part, on the successful establishment of biological control agents in their new environment, and subsequent radiation of the agents from their point of release. Field observations in Europe indicate that most dispersal of *Aculus hyperici* occurs in summer, after mites migrate to the apices of host inflorescences, possibly thereby maximising their potential dispersal distance (Wainhouse 1980). This chapter aims to investigate field dispersal of *A. hyperici* and the associated process of host-plant selection. A series of mite releases into field populations of *Hypericum perforatum* and *H. gramineum* were used to establish the rate of dispersal and the distance covered. The chapter also aims to examine the frequency with which *A. hyperici* is dispersed to the non-target, *H. gramineum*, in comparison with its frequently co-occurring host, *H. perforatum*.

4.2. Materials and Methods

Two experiments were carried out to investigate the dispersal and host-plant selection behaviour of *A. hyperici*. In both experiments, *A. hyperici* was released onto over-wintering *H. perforatum* vegetative rosettes, in a small plot (3 x 3 m, virtually a point-source) within field population(s) of *H. perforatum* and/or *H. gramineum*, and its subsequent dispersal monitored.

4.2.1. Experiment 1 - Dispersal of A. hyperici

4.2.1.1. Site description

Dispersal of *A. hyperici* was monitored at a field population of *H. perforatum*, adjacent to Lake Eucumbene, near Adaminaby, south-eastern NSW, Australia (36° 00' S, 148° 40' E). The roughly rectangular site comprised a cleared area of 150 x 80 m, bordered on three sides by *Eucalyptus pauciflora* and on the fourth by a sealed road. The site was heavily infested with *H. perforatum*, though several relatively inconspicuous native grasses, forbs and small shrubs were also present. The site was chosen because of the large *H. perforatum* population, because of its isolation from other *A. hyperici* release sites (the nearest was about 80 km to the north), thereby minimising the likelihood of migration of mites into the site from other experiments and release areas, and because the cleared landscape facilitated short-distance (1 - ca. 80 m) wind dispersal of mites. Prevailing winds at Cooma, New South Wales, 41 km south-east of Adaminaby were from the north-west and south-west, depending on the season (20 year average, recorded at the Cooma Visitors Centre; Australian Bureau of Meteorology). This supports observations made on each visit, that the prevailing winds at the study site were from the west.

At the centre of the site, a 3 x 3 m mite-release plot was delimited at the corners by permanent wooden stakes. Permanent stakes were also placed at 1, 10, 30, 100 and 200 m to magnetic north, south, east and west of the perimeter of the release area (Fig. 4.1).

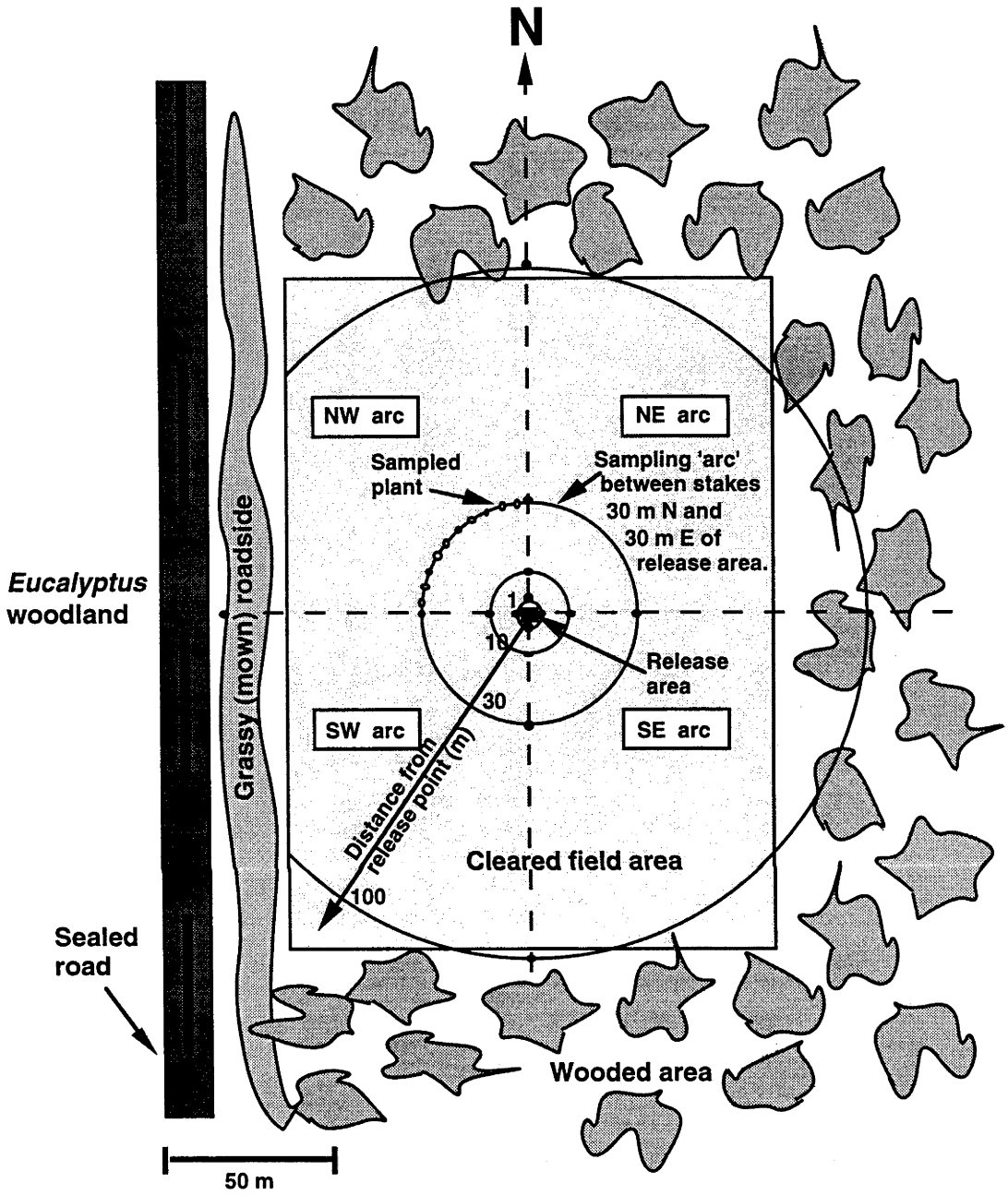


Fig. 4.1 Schematic diagram of the Adaminaby mite-release site, with dominant features of the area noted. Sampling arcs 1, 10, 30 and 100 m from the release area (central black square) are indicated north-east (NE), north-west (NW), south-east (SE) and south-west (SW) of the release point. Note that arcs at 200 m have been omitted from the diagram. Closed black circles on the sampling arcs north, south, east and west of the release area represent wooden stakes. Open circles along the 30 m arc (NW) indicate the 10 randomly selected plants for that distance and direction from the release area.

4.2.1.2. Release of *A. hyperici*

In winter (September) 1991, approximately 100 vegetative *H. perforatum* buds, each infested with at least 10 - 20 adult mites, were placed into the crown of all *H. perforatum* rosettes (about 60 rosettes) in the release plot.

4.2.1.3. Sampling of plants

The distance dispersed by *A. hyperici* was estimated 5, 9, 14, and 18 months after the initial release of mites. This was achieved by sampling *H. perforatum* individuals at designated distances (1, 10, 30, 100 and 200 m) from the release point. Sampled plants were randomly selected within an arc visually formed between stakes of adjacent compass points indicating the same distance from the point of release (Fig. 4.1). In addition to sampling in the cleared site, sampling at 100 m included plants that occurred in wooded areas, particularly in the north-eastern (NE) and south-eastern (SE) arcs. In the north-western (NW) and south-western (SW) arcs sampling at '100 m' was restricted to the fenceline bordering the western side of the field site. At 200 m from the release area, samples were taken from the NE and SE arcs only, since the population of *H. perforatum* did not extend west of the road. These sampling arcs occurred in the wooded area, out of the cleared field site. Within all arcs, 10 plants were selected, yielding a total of 40 plants per distance. Plants were sampled by collecting 3 - 5 floral buds (5 and 14 months after release, in spring/summer) or vegetative buds (9 and 18 months after release, in autumn/winter) per plant, placing buds from the same plant in separate plastic bags and storing all bags in an ice-cooled poly-styrene covered box while returning to the laboratory. Samples were then stored at -18°C before scoring for the presence/absence of mites. On some plants, live mites were not observed, though their characteristic cast skins were obvious. In such cases, mites were scored as present. Freezing the samples killed all mites, but facilitated more efficient scoring of plants. The process did not affect the appearance of mites, which were easily verified as *A. hyperici* under a dissecting microscope, or their cast skins, after thawing.

4.2.1.4. Analysis of Experiment 1

Experiment 1 was not replicated at other field sites because at commencement, it was not possible to locate additional sites suitable for mite release that were sufficiently isolated from other release sites to ensure that no 'cross-infestations' occurred. Owing to this lack of replication, results of the experiment do not warrant statistical modelling. The data are, therefore, analysed and described graphically.

4.2.2. Experiment 2 - Host-plant selection

Host-plant selection of *A. hyperici* was investigated at three field sites. Two of these were situated near Beechworth, NE Victoria (36° 23' S, 146° 44' E) beneath a *Eucalyptus polyanthemos* and/or *E. macrorhyncha* canopy, but located about 2 km from each other. The third site occurred in a clearing (about 100 x 100 m) adjacent to a *Pinus radiata* plantation at Pierce's Creek, ACT (35° 20' S, 148° 55' E). All sites had an understorey dominated by *H. perforatum*, interspersed with *H. gramineum* and several other native forbs. A survey prior to commencement of the experiment confirmed that *A. hyperici* was not present at any of the sites.

4.2.2.1. Procedure

At each site, approximately 100 *A. hyperici*-infested vegetative buds of *H. perforatum* were used to infest a marked 3 x 3 m release plot in winter (July) 1992, as in experiment 1, above. Within a radius of up to 30 m from the release area, 30 *H. gramineum* individuals were then randomly selected and paired with the nearest *H. perforatum* individual within 30 cm. If no *H. perforatum* occurred within 30 cm of the selected *H. gramineum*, that individual was discarded in favour of another. The distance from the centre of the release point to the pair of plants was then recorded.

Eight months later, in March 1993 after the main *A. hyperici* dispersal period during spring/summer, the pairs of plants were sampled to score for the presence/absence of mites. Sampling of *H. perforatum* involved removing 3 - 5 infructescences and 3 - 5 vegetative buds. Individual *H. gramineum* plants were sampled by removing 3 - 5 shoots comprising leaves and fruits, which frequently involved removing the whole plant. Sampled material was stored at -18°C, prior to

scoring the plants. Plants were rated as 'infested' if one or more mites were detected on any of the shoots. Occasionally, live mites were not detected, though their characteristic cast skins were present. In such cases, the plants were scored as mite-infested. In addition to the presence/absence of *A. hyperici*, the severity of mite-infestation on each plant was rated from 1 to 4 (no mites present to heavy infestation). Where live mites were not found, the infestation rating was based on the density of cast skins. In both measures, it was assumed that rating the infestation from zero to heavy reflected total mite abundance, and did not bias for plant size.

While sampling *Hypericum* pairs, the postulated generic specificity of *A. hyperici* was also investigated by screening 20 randomly chosen individuals of each of two other species prevalent within the sampling area at each site. Such plants were screened by sampling 3 - 5 vegetative shoots per plant, as above. At both Beechworth sites, the sampled species were *Brachyloma daphnoides* and *Hibbertia riparia*, shrubs of about the same height and size as flowering *Hypericum perforatum*. At Pierce's Creek, *Pomax umbellata*, a forb about the size of *H. gramineum*, and *Carex appressa*, a sedge about the size of *H. perforatum*, were screened.

4.2.2.2. Analysis of Experiment 2

Host-selection behaviour of *A. hyperici* was analysed by logistic regression (binary analysis: binomial errors with logit link, Genstat 5 (Lane *et al.* 1987; Digby *et al.* 1989), modelling the probability that *A. hyperici* would either infest, or not infest one or other of the paired *Hypericum* species. Non-*Hypericum* taxa were excluded from analyses, since mites were not found on any of the sampled plants.

In a separate analysis, the severity of *A. hyperici*-infestation of *H. gramineum* and *H. perforatum* was compared by two-way analysis of variance (ANOVA, Genstat 5) of the infestation ratings. Distance from the release area was fitted as a covariate.

4.3. Results

4.3.1. Experiment 1 - Dispersal of A. hyperici

Dispersal of *A. hyperici* from the release area is illustrated in figure 4.2. The general trend was for mites to be detected increasing distances from the point of

release, and for the proportion of mite-infested plants at these distances to increase, with time. However, at no time were any mites detected on plants greater than 100 m from the point of release. The proportion of infested plants in the release area was always relatively high (90 - 100%), although at 14 months, this proportion had dropped to 65%, before increasing in subsequent censuses.

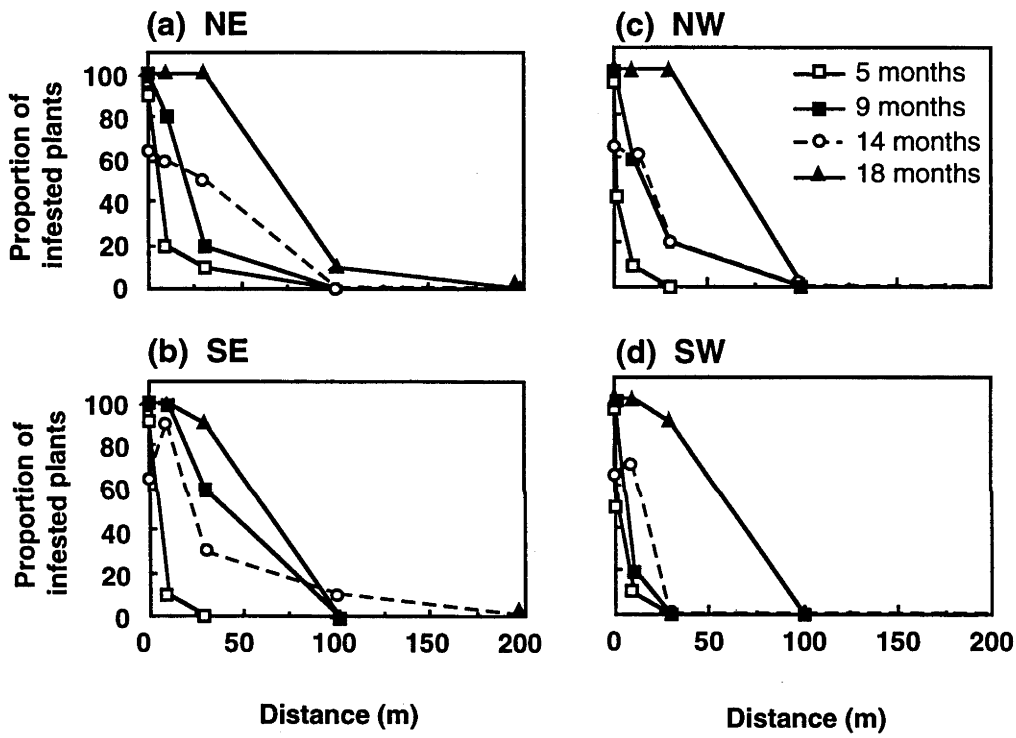


Fig. 4.2 Distance dispersed by *A. hyperici* in four compass directions (fig. a, north-east, NE; b, south-east, SE; c, north-west, NW; and d, south-west, SW) over time, as indicated by the proportion of infested plants, following release of mites in a 3 x 3 m release plot. The legend in figure c refers to all graphs.

Five months after inoculation, all plants in the release area were heavily infested. Moreover, it is clear that a majority (95 - 100%) of plants 1 m from the release area had also become infested (Fig. 4.2). After 9 months, mites had spread at least 30 m downwind of the prevailing westerly winds. The proportion of infested plants 30 m

from the release area had risen to 60% in the SE sampling arc and 20% in the NE and NW arcs, but had spread no further in the SW arc.

By 14 months, *A. hyperici* had dispersed at least 100 m from the initial point of release to the south east, and the proportion of infested plants at 10 m in the SE arc had risen to 90% (Fig. 4.2b). Mites were still not present 30 m from the release point in the SW arc, but the proportion of infested plants at 10 m had risen from 20 to 70% in this sector. Eighteen months after inoculating the release area, 90 - 100 % of plants at 30 m in all directions were infested. At this census, mites were detected 100 m from the release plot in the NE direction, but in none of the other sampling arcs. At no time were mites detected further than 100 m from the point-release area. Averaging dispersal in the four compass directions, the total distance dispersed by mites with time is summarised in figure 4.3. The roughly linear relationship on a log-log plot suggests that after 5 months, dispersal has a Weibull distribution from the point of release. Later, the point-source model no longer seems applicable at this scale, indicating a shift from primary point-source patterns to a complex secondary pattern, reflecting redispersal from secondary colonies.

4.3.2. Experiment 2 - Host-plant selection

In the analysis of the host-selectivity of *A. hyperici*, study site was an insignificant factor, while species was highly significant. The probability of *H. gramineum* becoming infested by *A. hyperici* was significantly less (31% chance of infestation) than the probability of *H. perforatum* becoming infested (51% chance, Fig. 4.4a). The observation that *A. hyperici* was not observed on other taxa at any study sites suggests that mites may actively select host-plants, and/or only establish populations on their selected hosts. This finding supports the expectation that the host-selection behaviour of *A. hyperici* is specific to *Hypericum*.

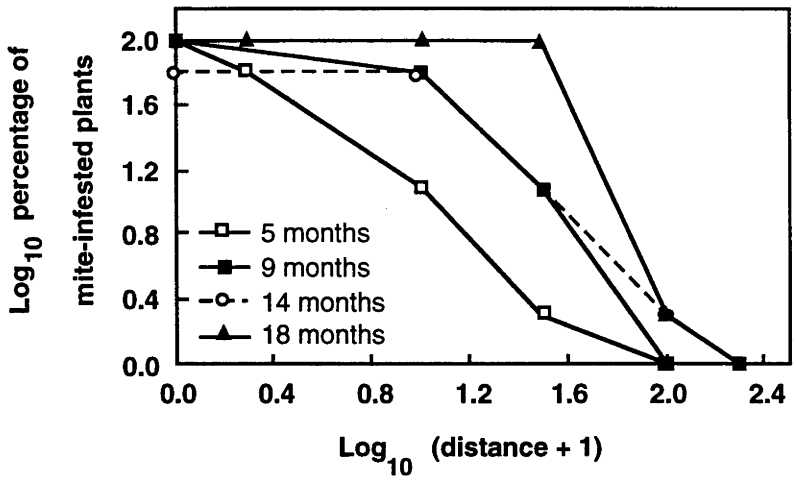


Fig. 4.3 Summary of the mean distance dispersed by *A. hyperici* over time, as indicated by the proportion of infested plants, averaged across all sampling directions.

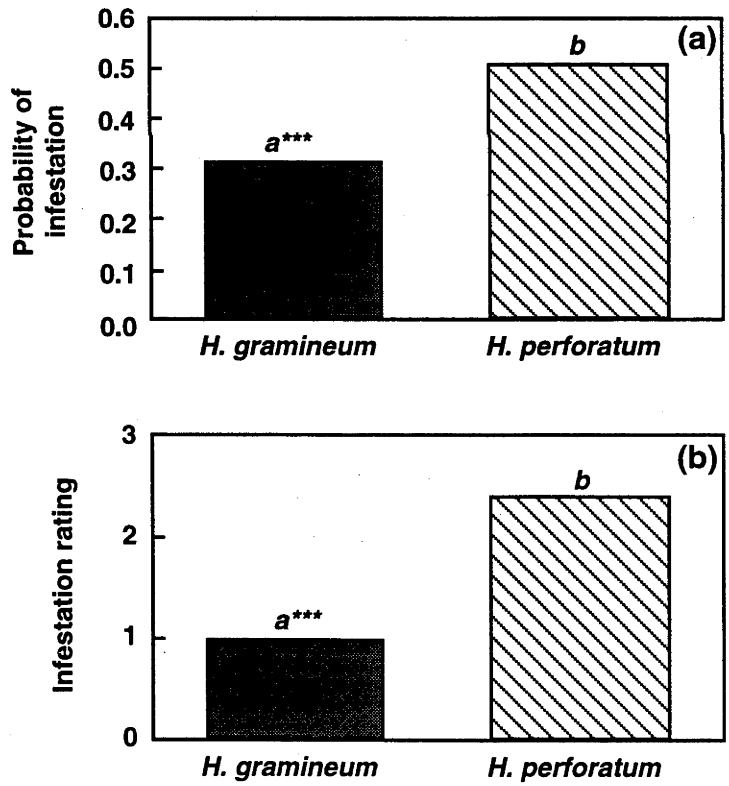


Fig. 4.4 (a) The probability of *H. gramineum* and *H. perforatum* being infested by *A. hyperici* and (b) the average level of infestation of each species. Columns with different letters differ significantly (***P < 0.001).

Comparison of *Aculus*-infestation levels on *H. gramineum* and *H. perforatum* suggests that either *H. perforatum* is a larger target for the mites and, therefore, more likely to intercept them, or that *H. perforatum* is a more suitable host for *A. hyperici* than *H. gramineum*. Distance from the area of release was not a significant covariate in the analysis. There was, however, a highly significant ($P < 0.001$) difference between the infestation ratings on *H. gramineum*, which was relatively weakly infested, and *H. perforatum*, which was heavily infested (Fig. 4.4b). There were no site x species interactions.

4.4. Discussion

4.4.1. Dispersal of A. hyperici

Wind is generally considered the principal vector for dispersal of small, terrestrial wingless arthropods (Washburn and Washburn 1984). In addition, herbivorous arthropods disperse locally by walking to adjacent plants, though wind dispersal is more effective over longer distances. In mites and the first instar crawlers of some scale insects, wind dispersal is often characterised by the adoption of a 'pre-dispersal posture', whereby the arthropods rear back onto their hind legs (or anal sucker, in the case of eriophyid mites) and raise their fore legs and/or antennae into the air such that the longitudinal axis of the body is 45° to 90° to the substrate (Nault and Styer 1969; Johnson and Croft 1976; Washburn and Washburn 1984). This position is thought to elevate arthropods above the relatively still boundary layer of their substrate, and maximise the drag, until the wind velocity is sufficient to tear their tarsi from the substrate (Washburn and Washburn 1984). In the laboratory, *A. hyperici* was frequently observed adopting similar dispersal postures.

Wind speeds required to generate sufficient drag vary between arthropod taxa. At constant wind-speeds generated by a fan, Johnson and Croft (1976) estimate that for the phytoseid mite, *Amblyseius fallacis*, most dispersal occurs at wind speeds between 10 and 22 km h^{-1}. At speeds significantly above or below this range, dispersal is minimal because mites do not adopt pre-dispersal postures. Winds are usually gusty, and do not constantly provide appropriate dispersal velocities. Nevertheless, the evidence suggests that aerially dispersed terrestrial arthropods avoid dispersal at low wind speeds and similarly, avoid adoption of a dispersal position at excessive speeds, thereby possibly reducing the chance of remaining air-borne too long and landing outside favourable habitat.

In a field survey using silicone grease-coated slides as sticky traps, Nault and Styer (1969) trapped the wheat curl mite, *Aceria tulipae*, up to 1.6 km from the nearest source, concluding that wind is the most important factor in eriophyid mite dispersal. Results of this chapter, notably that the furthest detected dispersals occurred in sampling arcs downwind of the prevailing winds, combined with observations of an apparent 'dispersal posture' suggest that *Aculus hyperici* is also wind-dispersed.

Willard (1974) indicates that although wind is the primary agent of dispersal for *Aonidiella aurantii* (Homoptera: Diaspididae) crawlers, birds and mammals may act as dispersal vectors. *Aculus hyperici* may be transported by aphids and chrysomelid beetles (pers. obs., see chapter 2), while anecdotal evidence suggests it may also be transported short distances by passing mammals such as kangaroos and domestic livestock. Gibson and Painter (1957) document the transportation of *Aceria tulipae* by aphids, but consider such dispersal of secondary importance. It is likely that animal-mediated dispersal of *A. hyperici* is also relatively rare. It provides, nevertheless, another means of long-distance dispersal. If *A. hyperici* employs arthropods such as biological control agents, native herbivores or pollinators that visit both *H. perforatum* and *H. gramineum* as dispersal vectors, the possibility of successful dispersal to un-infested populations of *H. gramineum* is also increased, since the vector actively selects the appropriate host.

A potential source of error in the dispersal survey involved the possible inadvertent dispersal of mites, perhaps on clothing or footwear, when sampling. Attempts were made to minimise the likelihood of such 'cross-contamination' by sampling from the outer distances inwards, though this was not always possible. Cross-contamination does not seem to have been a major problem in the time-span of this survey. Local severe cross-contamination is expected to disrupt the shape of the dispersal distribution leading, prematurely, to high proportions of infestation at greater distances, relative to the smooth distributions actually observed.

In aerially dispersed taxa, an inverse exponential decline characterises the number of arthropods trapped with increasing distance from the dispersal point (Thresh 1966; Greathead 1972; Andersen 1993; Portnoy and Willson 1993), typically, a Weibull distribution. This decline is a function of wind speed, turbulence, the settling velocity of the arthropod and the height from which the arthropod is released. Data from the Adaminaby experiment are, at 5 months, consistent with a simple model of primary mite dispersal, illustrated in figure 4.5. In essence, the

model estimates that a majority of dispersal occurs relatively locally (within metres), though there remains the small chance for a few, potentially important, long-distance (several hundred to several thousand metres) dispersals. The model can be summarised as,

$$P = d^{-k}$$

where P = the probability of dispersal, d = distance (m) and k = a constant which increases with the settling velocity of the dispersing agent.

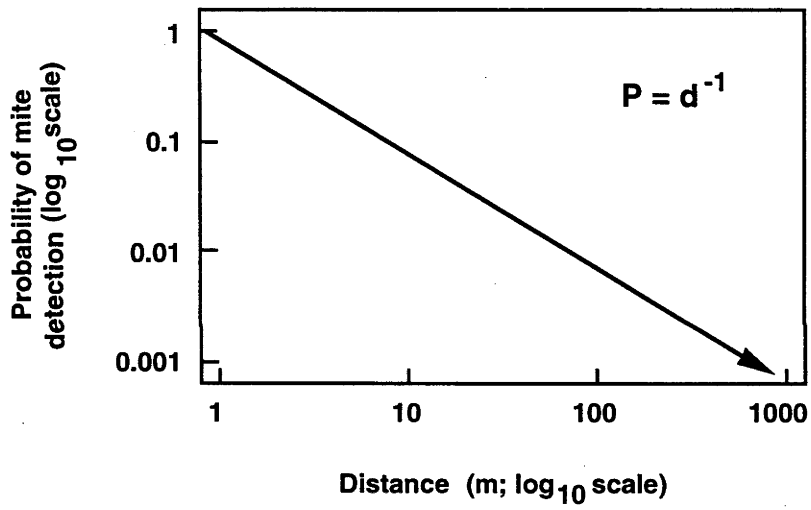


Fig. 4.5 Proposed simple model of dispersal by *A. hyperici*, at 5 months. The model estimates the probability (P) of detecting a mite with distance (d, metres).

Although not specifically incorporated in the survey, radiation from the release area did not appear to be influenced by seasons. Rather, after releasing mites in September (winter), censuses conducted the following February (summer), June, December and April, thereby spanning all seasons, imply that dispersal continues year round. With time, proportionally more plants at greater distances become infested. At several *A. hyperici* release sites administered by the CSIRO, mites dispersed at least 800 m from the release plot within 12 months (P. Jupp, pers.

comm.). The comparatively limited recorded annual dispersal of *A. hyperici* at Adaminaby may reflect the relatively high altitude of the site and a colder environment, resulting in relatively small (30 - 40 cm tall) host plants, which in turn may have affected dispersal distance. Greathead (1972), Willard (1974) and Wainhouse (1980) report that the distance dispersed by scale insect crawlers is a function of wind speed and plant height, the crawlers dispersing from the upper canopy of their hosts. The relatively limited dispersal of *A. hyperici* at Adaminaby may also stem from the eucalypt woodland that bordered the downwind sides of the otherwise cleared study site. Such barriers disrupt wind-flow and affect dispersal of wind-borne organisms (Pedgley 1982).

Sampling technique and the surrounding vegetation of the study site limit more detailed description of the dispersal of *A. hyperici.* While the sampling method was a time-efficient means of estimating dispersal distance, a more precise method might be to sample until a specified number of infested plants had been encountered. The sampling method that was employed may also have been affected by the woodland bordering much of the site. The density of the *H. perforatum* population within the woodland was reduced, which has consequences for secondary density-dependent host-selection. Moreover, the woodland, as noted, is likely to have affected wind flow and therefore, mite dispersal (Pedgley 1982).

4.4.2. Host-plant selection

After initiating wind-dispersal behaviour and becoming air-borne, the movement of small wingless arthropods becomes a passive process, with analogies to dispersal of pollen, small seeds, fern spores, and fungal spores (Pedgley 1982). Yet the clear host-specificity displayed by *A. hyperici* in establishing populations on *Hypericum* in favour of other taxa in the Australian environment complicates the outcome of wind dispersal in this arthropod.

The probability of detecting *A. hyperici* on *H. perforatum* is significantly ($P < 0.001$) greater than the likelihood of detecting it on *H. gramineum.* In the field, it is possible that *A. hyperici* does not actively select between these *Hypericum* species, but tries to establish populations on either, depending on that with which it collides during wind dispersal, or walks onto upon landing nearby. For this reason, *A. hyperici* may establish more frequently on the larger, more 'apparent' (*sensu* Feeney 1976) *H. perforatum*, which is likely to obstruct wind-flow and suffer more wind-borne particle impaction, than *H. gramineum.* It therefore appears that dispersal of *A. hyperici* involves interactions between active pre-dispersal

behaviour, passive air-borne trajectories and active generic selection. Within *Hypericum*, herbivory may also involve active selection behaviour, but it is likely to be strongly influenced by plant apparency.

Rausher (1983) classifies insect selection of host plants into pre- and post-alighting discrimination, whereby pre-alighting discrimination occurs when insects approach and alight on certain plants in a given habitat, while post-alighting discrimination, mediated through chemical and tactile stimuli, occurs after insects land on plants. Wind dispersal in *A. hyperici* suggests that this species employs post-alighting host discrimination, since pre-alighting volatile chemical stimuli or deterrent 'clues' are likely to be carried down wind, in a similar direction to the dispersing mites, as has been observed by Morrow (1989).

It has recently been argued that the role of plant chemistry in host selectivity of herbivorous arthropods has been overemphasised (Bernays and Graham 1988; Thompson 1988) and that other processes, such as the role of natural enemies and predators (Bernays and Graham 1988; Denno *et al.* 1990; Scriber *et al.* 1991), and the ecology of host plants, which may affect the foraging behaviour of herbivores (Karowe 1990; see discussion section of chapter 5) are equally important. The ability to learn which hosts are more suitable also effects host-selection in herbivorous arthropods (Jermy 1988, reviewed by Jaenike 1990). Previous experience, for instance, affects patterns of host acceptance in the apple maggot fly, *Rhagoletis pomonella*: oviposition is more likely on a fruit type with which the fly has had previous experience (Papaj and Prokopy 1988). The duration of time since last oviposition may also affect host acceptance in fecund herbivores. In reviewing the process, Jaenike (1990) notes that the probability of females ovipositing on lower-ranked hosts increases with time since the last egg was laid in *Battus philenor* (Lepidoptera: Papilionidae), *R. pomonella* (Diptera: Tephritidae) and *Euphydryus editha* (Lepidoptera: Nymphalidae). Similar processes may affect the host-selection of *A. hyperici*, but investigation of their significance was beyond the scope of this thesis.

4.4.3. A possible model of dispersal and host-selectivity of A. hyperici

Results from the above studies demonstrate that in the field, *A. hyperici* encounters and infests the non-target native species, *H. gramineum*, while dispersing from release sites centred in populations of *H. perforatum*. A model for the dispersal and host-selection of mites in the field is proposed in figure 4.6. The model summarises the decisions of *A. hyperici* leading to host selection for feeding and reproduction,

following passive wind dispersal. A combination of several factors suggest that despite potential utilisation of *H. gramineum*, *H. perforatum* will remain the primary host species. Essentially, these are the mite's demonstrated field specificity for *Hypericum* and the higher probability of establishing on *H. perforatum*, which is likely to be a function of plant size and abundance. Moreover, once established, larger populations are likely to develop on the target, than on the non-target species.

4.5. Summary

The dispersal and host-selection behaviour of *A. hyperici* was monitored in two field experiments. The proportion of mites dispersing from a point-source followed an inverse distribution with distance. The anticipated *Hypericum*-specificity of *A. hyperici* was confirmed: mites were only ever detected on *H. perforatum* and *H. gramineum* despite the possibility of dispersal to other genera. Within *Hypericum*, there was a greater probability that mites would be located on *H. perforatum* than on *H. gramineum*, and larger infestations developed on the target weed than on the indigenous species.

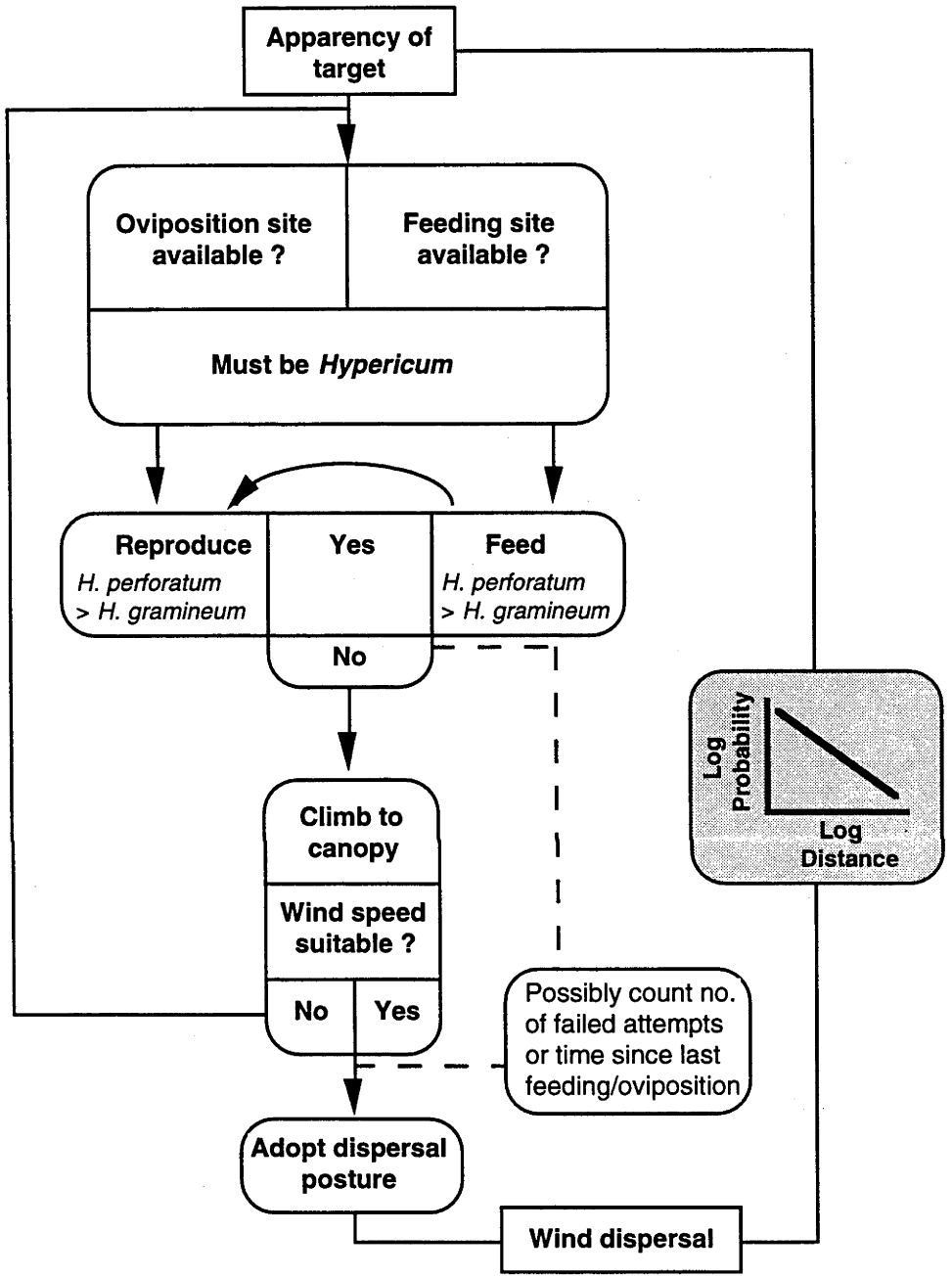


Fig. 4.6 Schematic model of the processes leading to dispersal and host-selection by *A. hyperici*

CHAPTER 5

OVIPOSITION AND LARVAL DEVELOPMENT OF *ACULUS HYPERICI* *

5.1. Introduction

Selection favouring the expansion of a herbivore's host-range to include novel species requires that fitness on the potential host(s) is at least commensurate with fitness on existing hosts (Karowe 1990). Alternatively, herbivores may feed on a new host, but require the original host to maintain viable populations. Both possibilities may occur under several circumstances including a lowering of competition, predation or parasitism (Bernays and Graham 1988; Fry 1989; Denno *et al.* 1990). The process has been studied most extensively in agricultural systems and recently been documented under more natural conditions (Berenbaum and Zangerl 1991; Bowers *et al.* 1992).

Prior to release of biological control agents into field populations of weedy species, extensive host-specificity screening occurs to eliminate from further investigation herbivorous arthropods displaying the potential for utilisation of important non-target taxa (Wapshere *et al.* 1989). Often, such non-targets include species foreign to the herbivore. Inclusion of these species in the diet of the herbivore requires expansion of their host-range and may also require ecological, behavioural and physiological adjustment.

Generally, host-specificity testing involves starvation and/or choice tests, in which the herbivores are placed on a plant, or given a choice of species and allowed to feed and/or oviposit, or die. Analysis of such experiments has traditionally involved techniques such as analysis of variance in which, for example, the numbers of eggs laid on different plant species are compared. Recent reports (see, for example, Felsenstein 1985; Pagel and Harvey 1988; Gittleman and Luh 1992) indicate that such multi-species comparisons may not be statistically rigorous, since

* Data collected in this chapter was kindly provided by J. Cullen and P. Jupp, CSIRO Division of Entomology, Canberra. It was included in an application to the Australian Plant Quarantine Service to release *A. hyperici* into the Australian environment. The data has not been submitted, or accepted for publication in any journal. In this chapter, I re-analyse and interpret the data. All such analyses, summaries of data and interpretations are original work.

all taxa form part of a structured, phylogenetic hierarchy. At various levels in the phylogeny, assumptions of statistical independence are, therefore, violated. One method of accounting for such structure is to analyse data by employing the phylogeny of the species as the basis for a nested analysis of variance (Clutton-Brock 1977, see Felsenstein 1985).

Detailed and/or reliable estimates of the phylogeny of many weed species are unavailable and preclude phylogenetically-based analyses, as recommended by Felsenstein (1985). By contrast, the infra-generic taxonomy of *Hypericum* has been well studied revealing clear evolutionary trends (Robson 1968; Robson 1977; Robson 1990), and rendering the host-specificity data collected on *Aculus hyperici* amenable to such analysis.

This chapter aims to re-analyse the *A. hyperici* host-specificity data with consideration given to the infra-generic classification of *Hypericum*, thereby minimising some of the difficulties highlighted by Felsenstein (1985) and others. Results of the analyses are then interpreted and discussed in light of the phylogeny of this genus.

5.2. Materials and Methods

Data on the oviposition and larval development of *A. hyperici*, collected during host-specificity trials of this mite by the CSIRO Division of Entomology, Canberra (see footnote on page 98), were re-analysed. Re-analysis included those *Hypericum* species for which the data were available and for which the infra-generic classification was known. In the first analysis, (analysis 1), the developmental rate of larval *A. hyperici* on various *Hypericum* species was investigated. In the second (analysis 2), the number of eggs oviposited by mites reared on different *Hypericum* species was analysed. Data used in both analyses were collected from an experiment conducted in a quarantine laboratory, with a 22°C day (12 hours) and an 18°C night.

5.2.1. Larval development

For each *Hypericum* species (see Table 5.1), ten leaves were placed onto absorbent cotton wool held within petri dishes and moistened with Hoagland's solution (Hoagland 1920; Hoagland and Arnon 1938). Five petri dishes (replicates) were prepared for each species. Three eggs of *A. hyperici* were then placed onto each leaf within the petri dishes. After the first egg on each leaf hatched, the remaining two eggs were removed. Petri dishes were then incorporated into a fully randomised experimental design and monitored daily, scoring for each mite (1) the number of days spent as a larval nymph (larval days), (2) the number of days spent as an adult (adult days), and (3) the total longevity (days) of the mite (larval days + adult days). The experiment was terminated when the experimental mite died, usually after 25 - 40 days, though in rare cases, mites survived less than 10 days, or greater than 45 days.

Table 5.1 Species of *Hypericum* included in re-analyses of the host-specificity of *A. hyperici*. The proposed centre of origin of each species, reflecting present distributions are also provided (Robson 1968, 1977, 1990).

Species	Proposed origin
H. gramineum Forst.	Australia
H. japonicum Thunb. ex Murray	Australasia
H. canariense L.	Canary Islands
H. reptans Hook. f. & Thompson ex Dyer	Eurasia
H. perforatum L.	Eurasia
H. tetrapterum Fries.	Eurasia
H. olympicum L.	South-eastern Europe, Asia
H. pulchrum L.	Central Europe

5.2.2. Oviposition

On reaching adulthood, some adult mites in the experiment outlined above subsequently oviposited onto their host leaf. The number of eggs oviposited onto each leaf and the number of resultant hatchings was also scored.

5.2.3. Analyses of data

5.2.3.1. Larval development

Scores of the larval days, adult days and the total longevity of mites were averaged across leaves within a petri dish. Data for each species were compared by a nested analysis of variance (ANOVA, Genstat 5; Lane *et al.* 1987; Digby *et al.* 1989) in order to reflect the *Hypericum* phylogeny depicted in Fig. 5.1a. This phylogeny is based on that of Robson (1977, 1990, pers. comm.). Square-root transformations of the data were necessary to meet assumptions of the ANOVA, though un-transformed data are presented for discussion.

Analysis was conducted employing four nesting levels, the highest of which (level 1) groups the taxa into two large clades; one including the Australian species *H. gramineum* and *H. japonicum*, the other including the remaining species. The lowest level of analysis (level 4) treats each species as a separate clade within the nested structure. Grouping the taxa in this way enables comparison between the clades of each level.

5.2.3.2. Oviposition

Within petri dishes, many leaves were not utilised for oviposition. As such, the proportion of leaves (within a dish) employed as oviposition sites was calculated and analysed, in addition to the total number of eggs laid, and the total number of hatchings.

Data transformations were not necessary in analysis 2. Analyses of these data includes the additional species, *H. canariense* and *H. olympicum*, the data for which were unavailable for analysis 1, above. Inclusion of these species alters the nesting structure of the ANOVA slightly, such that five nesting levels are incorporated. The resultant phylogeny (Robson 1977, 1990, pers. comm.) differs from that depicted in figure 5.1a only slightly (contrast Figs. 5.1a and b).

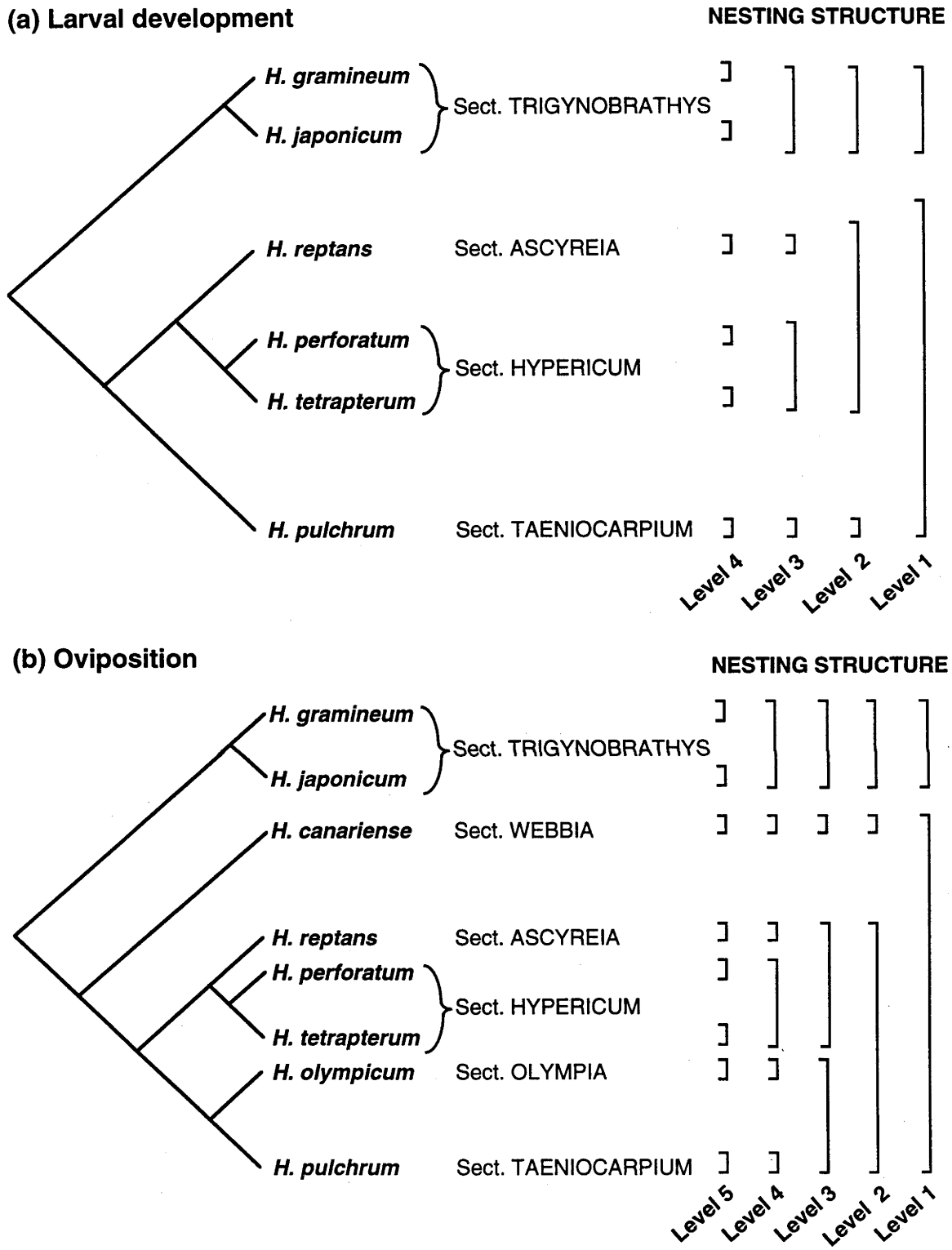


Fig. 5.1 The phylogeny of *Hypericum* species used in (a) analyses of larval growth and (b) oviposition of *A. hyperici*, based on the phylogenies produced by Robson (1977, 1990, pers. comm.). The nesting structure of ANOVAs derive from the hierarchical structure of the phylogeny, and enable comparison of groups of species (clades; indicated by square brackets) at various phylogenetic levels.

5.3. Results

5.3.1. Larval development

5.3.1.1. Comparison at species level (ANOVA level 4)

A comparison of the maturation of *A. hyperici* larvae and the longevity of mites reared on six species of *Hypericum* is presented in figure 5.2. Highly significant ($P < 0.001$) differences in the maturation time of mites (larval days) on different species were evident (Fig. 5.2a). Those feeding on leaves of *H. japonicum, H. reptans* and *H. tetrapterum* spent longer in the larval phases of the life cycle (6.6 - 6.7 days) than those that fed off *H. gramineum* or *H. perforatum* (5.3 - 5.5). Larvae feeding off *H. pulchrum* leaves matured faster (4.5 days) than those reared on the other species.

Differences in the rate of larval development on various species of *Hypericum* were not sustained in adulthood, since the time spent in the adult phase of the life cycle (adult days) did not vary significantly ($P = 0.650$) between mites raised on the different species (Fig. 5.2b). Nevertheless, the trend was for mites to survive as adults for slightly longer on *H. perforatum, H. reptans* and *H. gramineum* than on other species. Adding the duration of larval development to the time spent in adulthood also indicates minimal ($P = 0.930$) differences in the longevity of mites on different *Hypericum* species (Fig. 5.2c).

5.3.1.2. Comparisons at higher levels of classification (ANOVA levels 1 - 3)

Comparison of larval days at level 2 in the nested ANOVA (see Fig. 5.1a) indicates that mites in the clade comprising *H. pulchrum* spent less time as larvae ($P < 0.001$) than mites in clades comprising *H. gramineum* and *H. japonicum*, or those in the *H. reptans/ perforatum/tetrapterum* group (Table 5.2a). Significant differences in larval days between species groupings at level 3 ($P = 0.020$) and at level 4 ($P < 0.001$) were also apparent (Table 5.2a), but in comparison with these, the analysis at level 2 explained more variance (43% compared with 6% and 28% at levels 3 and 4 respectively). As in the inter-species comparisons, there were no significant differences ($P \geq 0.158$) in adult days or longevity of mites at higher levels of the analyses (Table 5.2b and c).

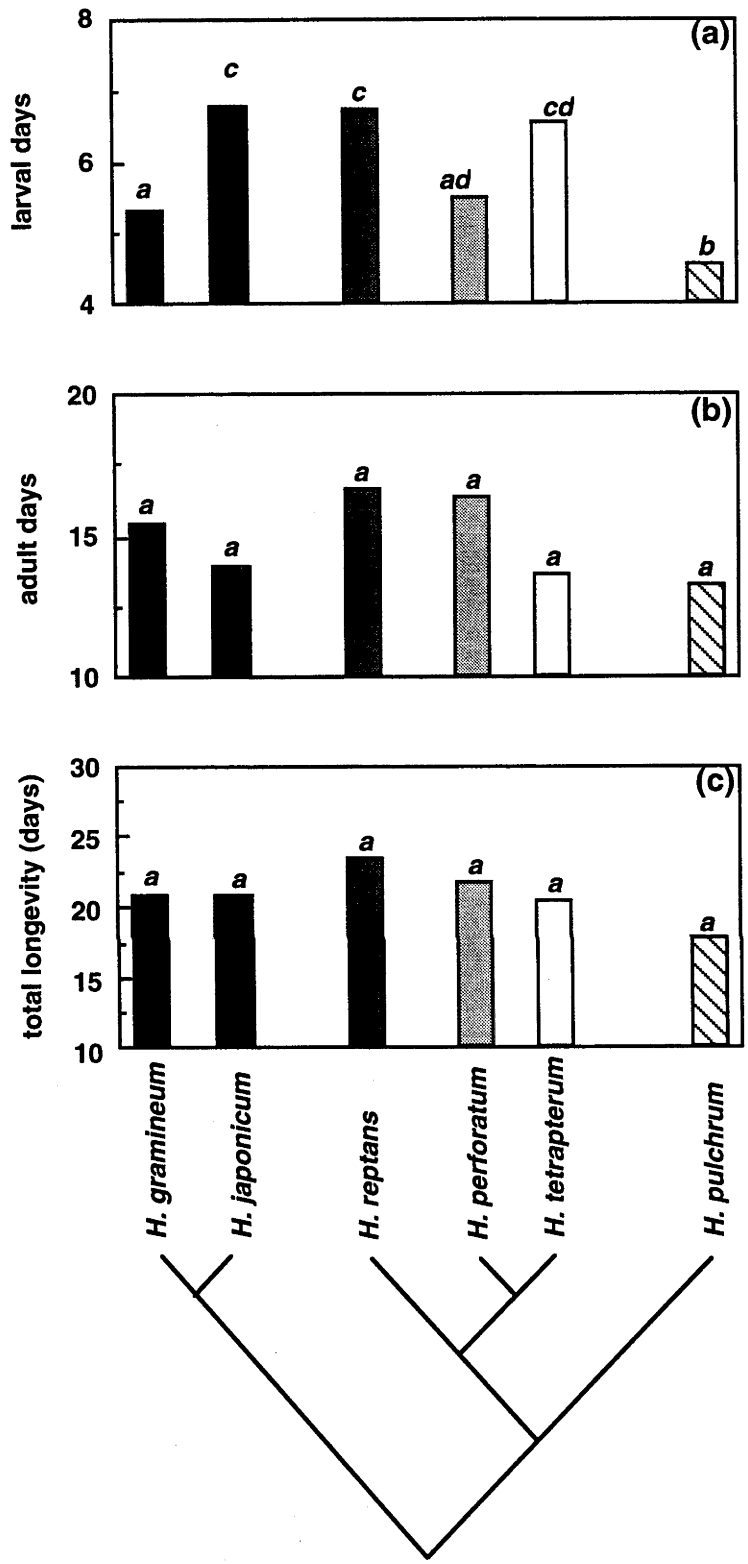


Fig. 5.2 Comparison of (a) larval development rate (a), adult survival (b), and total survival (c) of *A. hyperici* reared on different species of *Hypericum* in relation to the proposed phylogeny of the species. Columns with different lettering differ significantly ($P < 0.05$).

Table 5.2 Comparison at different nesting (phylogenetic) levels of (a) larval longevity (b) adult longevity and (c) total longevity of *A. hyperici* reared on different species of *Hypericum.* Within a nesting level, cells with different lettering differ significantly ($P \leq 0.020$), as indicated by ANOVA.

(a) larval longevity

	Nesting level			
	4	**3**	**2**	**1**
Probability (P)	<.001	0.020	<.001	0.254
H. gramineum	5.31 *a*	6.02 *a*	6.02 *a*	6.02 *a*
H. japonicum	*c* 6.77			
H. reptans	6.74 *c*	6.74 *c*	6.25 *a*	5.79 *a*
H. perforatum	5.48 *ad*	6.01 *a*		
H. tetrapterum	6.57 *cd*			
H. pulchrum	*b* 4.51	*b* 4.51	*b* 4.51	

(b) adult longevity

Nesting level			
4	**3**	**2**	**1**
0.650	0.583	0.414	0.904
15.45 *a*	14.67 *a*	14.67 *a*	14.67 *a*
a 13.91			
16.57 *a*	16.57 *a*	15.52 *a*	14.90 *a*
16.32 *a*	14.98 *a*		
13.62 *a*			
a 13.25	*a* 13.25	*a* 13.25	

(c) total longevity

Nesting level			
4	**3**	**2**	**1**
0.930	0.483	0.158	0.958
20.79 *a*	20.79 *a*	20.79 *a*	20.79 *a*
a 21.81			
23.33 *a*	23.33 *a*	21.90 *a*	20.89 *a*
21.81 *a*	21.16 *a*		
20.52 *a*			
a 17.89	*a* 17.89	*a* 17.89	

5.3.2. Oviposition

In contrast to the analyses of larval development, analyses of the oviposition of *A. hyperici* on leaves of different *Hypericum* hosts revealed consistent significant ($P \leq 0.008$) differences between species in the proportion of leaves that received eggs (Fig. 5.3a), the total number of laid eggs (Fig. 5.3b), and the number of resultant hatchings (Fig. 5.3c).

5.3.2.1. Comparison at species level (ANOVA level 5)

Mites oviposited onto a higher proportion of *H. perforatum* leaves than onto those of other species (Fig. 5.3a). Differences in this parameter were significant ($P < 0.05$) for all species, except *H. gramineum*. The proportion of *H. olympicum* leaves utilised as oviposition sites was highly significantly ($P = 0.005$) lower than those of *H. perforatum*.

Similar species-differences emerged in the total number of eggs laid ($P = 0.002$) and the number of mites hatching from such eggs ($P = 0.008$). In both such measures , *H. perforatum* was utilised significantly ($P < 0.05$) more than the other species.

5.3.2.2. Comparison at higher levels of classification (ANOVA levels 1 - 4)

At level 3, the group comprising *H. reptans/perforatum/tetrapterum* provided the highest index of oviposition, as measured by the proportion of leaves onto which eggs were laid, the total number of eggs laid and the number of hatchings (Table 5.3a, b and c). In the analysis of the proportion of leaves receiving oviposition (Table 5.3a), the *H. reptans/perforatum/tetrapterum* clade differs from the *H. olympicum/pulchrum* group ($P = 0.018$, Table 5.3a), but neither the *H. gramineum/japonicum* group nor the clade comprised of *H. canariense*. Similar trends occurred in comparisons of total ovipositions ($P = 0.015$) and total hatchings ($P = 0.011$, Table 5.3b and c).

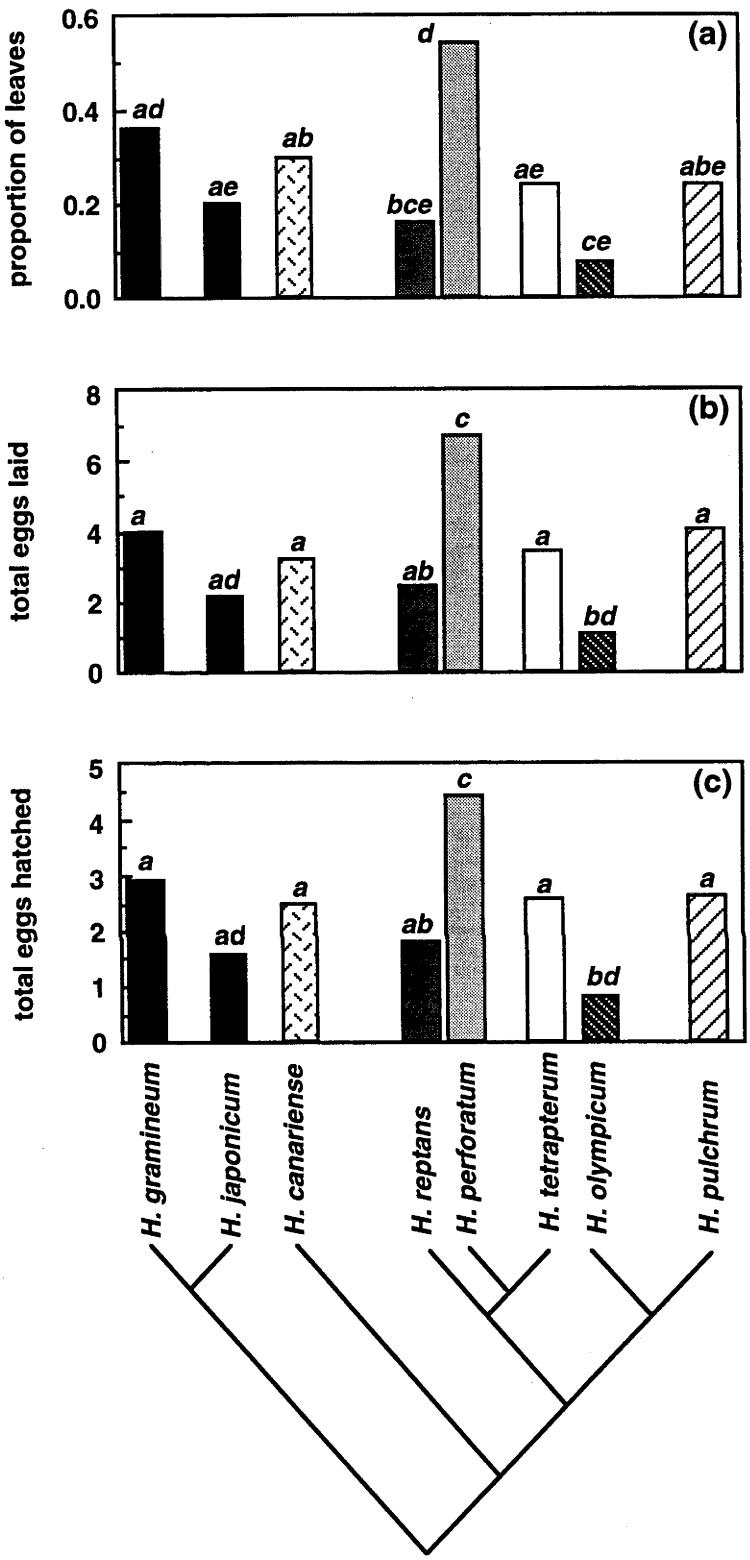
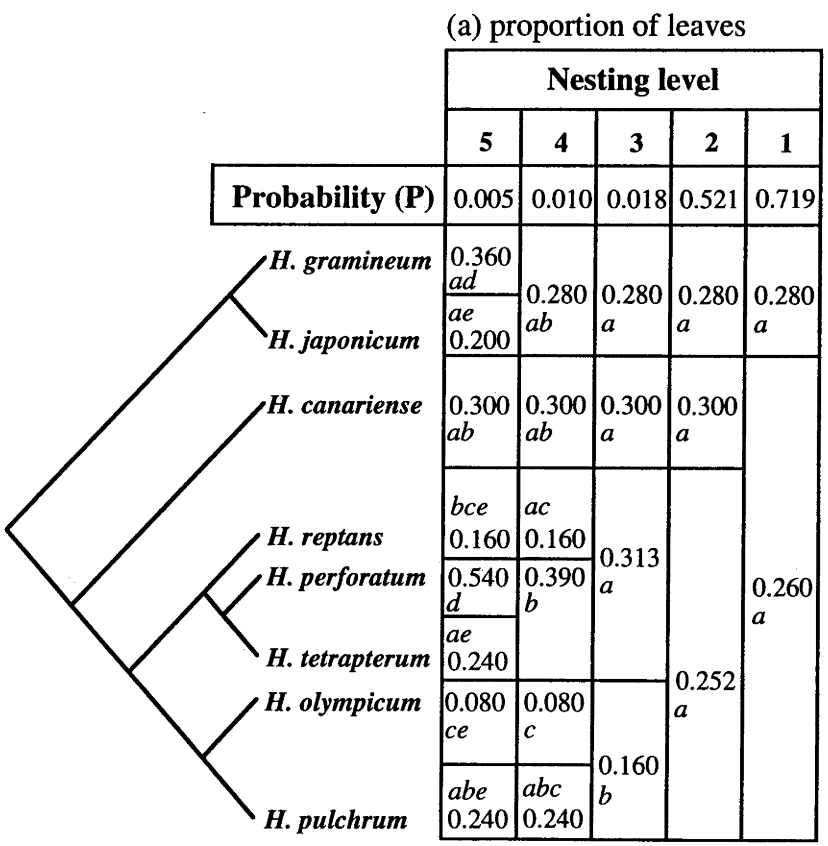


Fig. 5.3 Comparison of several measures of oviposition in *A. hyperici* reared on different species of *Hypericum* in relation to the proposed phylogeny of the species. Columns with different lettering differ significantly ($P < 0.05$).

Table. 5.3 Comparison at different nesting (phylogenetic) levels of (a) the proportion of leaves of a given species used for oviposition by *A. hyperici,* (b) the total number of eggs laid and (c) the number of resultant hatchings, from *A. hyperici* reared on different species of *Hypericum.* Within a nesting level, cells with different lettering differ significantly ($P \leq 0.018$), as indicated by ANOVA.



(a) proportion of leaves

	Nesting level				
	5	4	3	2	1
Probability (P)	0.005	0.010	0.018	0.521	0.719
H. gramineum	0.360 *ad*	0.280 *ab*	0.280 *a*	0.280 *a*	0.280 *a*
H. japonicum	*ae* 0.200				
H. canariense	0.300 *ab*	0.300 *ab*	0.300 *a*	0.300 *a*	0.260 *a*
H. reptans	*bce* 0.160	*ac* 0.160	0.313 *a*	0.252 *a*	
H. perforatum	0.540 *d*	0.390 *b*			
H. tetrapterum	*ae* 0.240				
H. olympicum	0.080 *ce*	0.080 *c*	0.160 *b*		
H. pulchrum	*abe* 0.240	*abc* 0.240			

(b) total eggs laid

	Nesting level				
	5	4	3	2	1
	0.002	<.001	0.015	0.671	0.492
	3.98 *a*	3.07 *a*	3.07 *ab*	3.07 *a*	3.07 *a*
	ad 2.16				
	3.19 *a*	3.19 *ac*	3.19 *ab*	3.19 *a*	3.46 *a*
	ab 2.42	*ac* 2.42	4.17 *b*	3.52 *a*	
	6.69 *c*	5.04 *b*			
	a 3.40				
	1.03 *bd*	1.03 *c*	2.54 *a*		
	a 4.05	*ab* 4.05			

(c) total eggs hatched

	Nesting level				
	5	4	3	2	1
	0.008	0.002	0.011	0.998	0.607
	2.91 *a*	2.24 *a*	2.24 *ab*	2.24 *a*	2.24 *a*
	ad 1.56				
	2.44 *a*	2.44 *ab*	2.44 *ab*	2.44 *a*	2.44 *a*
	ab 1.81	*ac* 1.81	2.92 *b*	2.44 *a*	
	4.40 *c*	3.47 *b*			
	a 2.54				
	0.83 *bd*	0.83 *c*	1.73 *a*		
	a 2.63	*ab* 2.63			

Despite significant ($P < 0.008$) differences between groups at level 3, analyses of species groupings at level 4 accounted for more variance (17% - 23%) than such analyses at level 3 (9% - 11%). At level 4, all above measures of oviposition were consistently highest in the *H. perforatum/tetrapterum* group (though not all differences were significant, $P \geq 0.05$) and lowest in the clade comprised of *H. olympicum* (Table 5.3a, b and c).

5.4. Discussion

5.4.1. Host-specificity of A. hyperici

Re-analysing data collected during the host-specificity trials of *A. hyperici* by structuring a nested analysis of variance to reflect the estimated phylogeny of the tested *Hypericum* species generally supports previous conclusions regarding the host-specificity of *A. hyperici* (CSIRO 1991). Principally, the above analyses indicate that while larval development and reproductive maturation are possible on several species of *Hypericum*, such indices of population growth are highest on *H. perforatum*. Of the Australian native species, mite growth and fecundity is highest on *H. gramineum*. Few obvious phylogenetic patterns emerged in the nested analyses, suggesting that mites do not respond strongly to phylogenetic differences between species. Rather, it appears that the mites show specificity to the genus and respond to the particular traits of individual species. Evidently, complex phylogenetic structuring of analyses are scarcely necessary to examine interactions between plant and herbivore in the *Hypericum* - *A. hyperici* system.

Hypericum is a relatively large genus comprising about 380 species (Robson 1977). As observed by the CSIRO and confirmed in the above analyses, there is clearly a range in the suitability of different species as hosts for *A. hyperici*. However, there is little indication that those that are phylogenetically closely related to *H. perforatum* necessarily serve as better hosts than more distantly related species. In comparisons of clades above the species level, the clade comprising *H. tetrapterum* and *H. perforatum*, members of the generic section *Hypericum*, usually differed from other clades at the same level of analysis. This, however, is likely to be a function of *H. perforatum* biasing the analysis and suggests that in future, similar comparisons should exclude the 'preferred' host. In its European native range, *A. hyperici* has been observed on *H. tetrapterum* (syn. *H. quadrangulum*) and *H. hirsutum*, in addition to *H. perforatum*. Given the above results, it seems

highly probable that the mite would also be found on other species of *Hypericum* occurring within the native range of *A. hyperici.*

Enhanced population development of mites on *H. perforatum* is primarily mediated through oviposition, where clear species differences emerge. Host-specificity during growth and development of hatched mites suggests that *H. perforatum* is a preferred host, since the time spent as a larval instar (larval days) was relatively low on *H. perforatum*, implying that the physiology and chemistry of this species does not retard maturation. Importantly, however, this measure of mite performance was similar on *H. gramineum* and *H. pulchrum*, highlighting the need for further investigation of the impact of *A. hyperici* on non-target species.

Once adulthood is achieved, *A. hyperici* does not display clear host preferences, measured in terms of the number of days spent in adulthood (adult days) or the total longevity of mites, since these parameters do not vary significantly between different *Hypericum* species. It therefore appears that host-specialisation is of prime importance during oviposition and larval growth. If mites successfully mature on lower-ranked species, however, their subsequent survival does not appear to be significantly affected. Several researchers (Roininen and Tahvanainen 1989; Horner and Abrahamson 1992; Hanks *et al.* 1993) report that oviposition preference does not necessarily reflect the success of larval development. In *A. hyperici,* oviposition preference and larval growth (as measured by developmental time) seem correlated, higher ranked hosts ensuring less developmental time. Oviposition preference and adult survival, by contrast, do not appear tightly linked, possibly because the phago-stimulants/inhibitors present in *Hypericum* diminish in importance as feeding cues, after mites mature.

5.4.2. Problems with host-specificity screening

Host-specificity trials, such as those above, involve starvation, or 'non-choice' testing of the herbivore's physiological host range, by presenting the arthropod with a species on which it may either feed and survive, or starve and die (Zwölfer and Harris 1971). Although such tests can be conducted under controlled conditions and may delimit a physiological host range (i.e., host plants on which arthropods are physiologically capable of development) allowing rejection of species irrelevant to further consideration (Cullen 1989), they have been criticised. Cullen (1989) notes, for example, that non-choice tests may provide results under unrealistic circumstances as they ignore the ecologies of the herbivore and (potential) hosts. Multiple-choice tests, in which herbivores are presented with a

feeding/oviposition choice between two or more plant species, address some of the short comings of starvation tests, ostensibly by reflecting the choices available to herbivores in the field, but remain limited by contrived laboratory conditions.

5.4.2.1. Herbivore ecology

One of the reasons that starvation and choice tests may not adequately represent the host-range of biological control arthropods such as *A. hyperici* is that under controlled conditions, the host-selection behaviour and ecology of herbivores may be affected. As noted in chapter 4, the ability to learn from previous experience, the time since last oviposition and the presence of predators, among other factors, may all affect host acceptance.

5.4.2.2. Plant ecology

The host's own ecology may interact with the ecology of herbivore host-plant location. Allopatric distributions of herbivores and some plants, for example, may limit the likelihood of biological control agents encountering physiologically suitable host plants. Similarly, seasonal differences in the life cycles of hosts and herbivores may restrict effective utilisation of potential hosts. Finally, the density of plants in the herbivore's environment may affect host selection. Courtney and Forsberg (1988), for instance, found that two pierid butterflies varied their selection of host plants for oviposition according to whether the preferred hosts were rare or locally abundant. After ranking host preferences, Courtney and Forsberg found that lower ranked hosts are selected if they are more abundant than higher ranked plants. Solomon (1981), working with moths, observed similar density-related host selection strategies, as did Morrow (1989), studying patch colonisation of a chrysomelid beetle. In the latter study, however, host-patch selection was complicated in high wind speeds, the author suggesting that such turbulence altered the dispersion of phagostimulatory/inhibitory odours.

5.4.3. Phylogenetic comparisons

Aside from the ecological problems associated with conducting starvation/choice tests, several recent publications (see, for example, Felsenstein 1985; Pagel and Harvey 1988; Gittleman and Luh 1992) highlight the need for consideration of the phylogeny of tested host-species when analysing such experiments. Felsenstein

(1985) observes that in multi-species comparisons (such as those typically required during host-specificity determination), species cannot, statistically, be regarded as independent units, since they form part of an hierarchically structured phylogeny. As such, employment of traditional comparative techniques such as one-way analyses of variance may be inappropriate, since the inherent assumption of independence in such models may over-estimate the significance of hypothesis tests (Felsenstein 1985). Techniques that are recommended to account for similarly structured data are largely designed for use in correlations of continuous variables (Felsenstein 1985; Pagel and Harvey 1988), and as such are inappropriate for analysis of designed experiments, such as those conducted above.

Confronted with a similar taxonomic/phylogenetic problem, Clutton-Brock and Harvey (1977, see Felsenstein 1985) used a nested analysis of variance, finding that the taxonomic level which accounted for most variance in their study was genus. They employed this taxon, rather than species, in subsequent analyses. As previously described, a nested analysis of variance was also used in the present re-analysis of the *A. hyperici* host-specificity data.

As in Clutton-Brock and Harvey's study, in some of the above analyses, taxonomic levels higher than species yielded significant differences that explained more variation than did comparison of species themselves. In this study, it emerged that taking phylogeny into account made only slight improvements in the proportion of explained variance, suggesting that the phylogenetic basis for mite impact is weak. Nevertheless, comparisons between infra-generic taxa in future screening of biological control agents may reveal host-preference patterns of some practical importance. In plant families comprising many weedy species, for instance, it may enable whole genera, or generic sections to be eliminated from further consideration, by testing representative species. Although accurate knowledge of the taxonomy and phylogeny of such groups is necessary, Wapshere's (1974) 'centrifugal phylogenetic testing method' employs this principle. Nevertheless, caution should still be exercised during host-specificity trials, particularly given findings that some butterflies use plants that are chemically similar, but taxonomically diverse (Jaenike 1990). Clearly, herbivore host-utilisation patterns are affected by a diversity of parameters including plant/herbivore ecologies, plant chemistry and evolutionary history, as well as the physiology of herbivores.

5.5. Summary

Data on the oviposition and larval development of *A. hyperici* reared on several species of *Hypericum* were re-analysed, employing a nested analysis of variance to account for non-independence of the closely related test species. Results support previous conclusions that mite growth and development is superior on the target species, *H. perforatum*, though non-target species, including the indigenous *H. gramineum*, may also support mite feeding, growth and reproduction. The pattern of mite utilisation of non-target *Hypericum* species did not strongly reflect the infra-generic phylogeny of the genus. Higher level, phylogenetic comparisons may reveal differences in the physiological suitability of whole taxa, such as sections or subsections, as hosts, but such comparisons were not practical on the limited data set.

Reliance on non-choice starvation tests as sole indicators of host range appear inappropriate, given the complex interactions between host and herbivore ecology, behaviour and physiology that ultimately determine host-selection. Nevertheless, there remain advantages in such controlled experiments which clearly indicate the taxa on which herbivores are physiologically incapable of surviving, thereby shortening the list of species requiring more thorough investigation.

SECTION D:

HERBIVORY OF *HYPERICUM* SPECIES BY *A. HYPERICI*

Section D comprises three chapters which examine the impact of *A. hyperici* on growth and productivity of *Hypericum* species. In chapter 6, the impact of mites on growth of *H. gramineum*, *H. perforatum*, *H. japonicum* and *H. tetrapterum* is compared in a glasshouse experiment. Chapter 7 examines growth of the former two species as affected by *A. hyperici* in combination with various environmental stresses in three other glasshouse experiments. In two field-based experiments, chapter 8 investigates the impact of mites on field grown *H. gramineum* and *H. perforatum*.

CHAPTER 6

THE IMPACT OF *ACULUS HYPERICI* ON GROWTH OF FOUR *HYPERICUM* SPECIES

6.1. Introduction

Andres (1981) and Turner (1985) note that in releasing an arthropod for biological control of weeds, there is a difference in the herbivores utilising the weed and/or non-target taxa and having a significant impact on the biology of such plants. Consequently, although some biological control herbivores may accept non-target species for oviposition, and their larvae may develop and complete their life cycles on such novel taxa, the agents may be released into field populations of weeds because it is considered that the impact of the agent on the biology of non-target taxa is likely to be minimal, or at least readily managed (Cullen 1989).

Host specificity screening of potential biological control herbivores follows guidelines recently summarised by Wapshere *et al.* (1989). These essentially involve a series of choice or 'no-choice' tests (refer chapter 5) which investigate the feeding and oviposition preferences of the potential biological control herbivores. Rarely, however, does such preliminary screening of herbivores examine the impact that agents may have on plant growth.

Several authors note that within-plant variation in leaf age and leaf size, and inter-plant variation in plant size, age and nutrition, may affect host oviposition preferences, and/or larval feeding and development of herbivorous arthropods (Ives 1978; Coley 1980; Rhoades 1983; Kearsley and Whitham 1989; Aide and Zimmerman 1990; Minkenberg and Ottenheim 1990; Bowers and Stamp 1993). If herbivores display a distinct host size or age preference, they may affect the population structure of their hosts (Crawley 1983, 1989). Seedling recruitment of oak trees (*Quercus robur*, see Crawley 1983) and the shrub *Gutierrezia microcephala* (Parker and Salzman 1985) were affected by herbivores in this way. The gall-forming aphid *Pemphigus betae* was 70 times as common on mature *Populus angustifolia* than on juveniles (Kearsley and Whitham 1989).

Plant developmental stage, and size (as measured, for example, by height or mass) are factors that correlate highly with chronological age (Harper 1977; Kearsley and Whitham 1989). Preferences for hosts of a particular age, size or developmental stage may be due to variation in the plant tissue nutrients required by herbivores, and/or plant defensive chemistry (Coley 1980; Minkenberg and Ottenheim 1990; Reavey 1991; Bowers and Stamp 1993). Coley (1980) argues, for example, that younger leaves invest in metabolically less expensive defensive compounds, relying primarily on escaping herbivory through rapid growth. She hypothesises that older tissues are richer in effective, but metabolically expensive, digestibility-reducing defences and that consequently immature tissues suffer relatively higher levels of herbivory than mature tissues. Moreover, during senescence of older leaves, their nitrogenous compounds may be transported out to juvenile leaves and other points of active plant growth (Mattson and Haack 1987b), thereby rendering younger tissues more nutritious to herbivores.

As noted in chapter 1, *Aculus hyperici* was released into field populations of *Hypericum perforatum* in May 1991. To date, the impact of this eriophyid herbivore on plant growth has not been quantified on the target weed or any other species of *Hypericum.*

This chapter aims to investigate the impact of *A. hyperici* on growth of four *Hypericum* species: the target weed *H. perforatum*, the non-target native forb *H. gramineum*, identified as the native species potentially at most risk from *A. hyperici* (chapters 4 and 5; CSIRO 1991), the second indigenous *Hypericum* species, *H. japonicum*, and *H. tetrapterum*, a naturalised species weedy in some parts of Victoria (Lamp and Collet 1989). In addition, the impact of *A. hyperici* on seedlings and adult plants was investigated, because of the possibility that *A. hyperici* may differentially affect plant growth according to the age of its host.

6.2. Materials and Methods

Two experiments were conducted. In the first (experiment 1), the impact of *A. hyperici* on growth of *H. gramineum*, *H. perforatum*, *H. japonicum* and *H. tetrapterum* was investigated. This was supplemented by an examination of the potential differential effects of herbivory by *A. hyperici* on *H. gramineum* and *H. perforatum* of different ages (experiment 2). In experiments 1 and 2 (and those in subsequent chapters, as noted), designated plants were kept free of infestation by *A. hyperici* with four- to six-weekly applications of Omite® miticide, as recommended by the manufacturer. The effect of spraying Omite on the growth and tissue nutrients of *H. gramineum*, *H. perforatum*, *H. japonicum* and *H. tetrapterum* was investigated in a separate experiment (experiment 3).

Unless noted otherwise, all experiments were terminated after 24 weeks, at which point, plants were harvested and scored for several indices of growth (see section 6.2.6., below).

6.2.1. *Experimental designs and treatments*

Experiments 1 and 2 comprised randomised split plots, with 'mite-free' sub-plots separated from 'mite-infested' sub-plots, within larger plots. Experiment 3 was of randomised block design. All possible treatment combinations were applied to each species of *Hypericum*.

6.2.1.1. Experiment 1 - Impact of *A. hyperici* on four species of *Hypericum*

The experiment consisted of ten replicate blocks comprising eight treatment combinations :

(1) Mite herbivory - two levels of mite herbivory: plants either infested with *A. hyperici* or free of infestation.

(2) Species - four species of *Hypericum* were investigated: *H. gramineum*, *H. perforatum*, *H. japonicum* and *H. tetrapterum*.

(3) Harvest - plants were harvested either 12 weeks after beginning the experiment or at termination of the experiments, after 24 weeks (Harvests 1 and 2, respectively).

6.2.1.2. Experiment 2 - Impact of *A. hyperici* on plants of different age and size

This experiment comprised eight blocks with four treatment combinations applied to *H. gramineum* and *H. perforatum.*

(1) Mite herbivory - two levels of mite herbivory: plants either infested with *A. hyperici* or free of infestation.

(2) Plant age - plants of two ages were compared: six-week old seedlings, 4 - 6 cm in height, and 12 month old adult plants, 20 - 25 cm in height.

6.2.1.3. Experiment 3 - The effect of 'Omite' on *Hypericum* spp.

Experiment 3 incorporated five blocks with six treatment combinations:

(1) Omite application - plants were either regularly sprayed with Omite (treated), or sprayed with tap-water (untreated).

(2) Species - four species of *Hypericum* were compared: *H. gramineum*, *H. perforatum*, *H. japonicum* and *H. tetrapterum.*

6.2.2. Cultivation and preparation of plant material

H. gramineum plants used in the experiments were germinated from locally collected seeds as previously described (chapter 2). *Hypericum perforatum* individuals were also grown from seeds collected in the Canberra region of south eastern Australia (35° S, 149° E). These were germinated by washing overnight in running tap water, air-drying, sowing onto potting mixture and placing under a misting spray in a glasshouse with a daytime temperature of 20° - 28°C. Individuals of *H. japonicum* and *H. tetrapterum* were established from cuttings of glasshouse-grown specimens originally collected near Smokers Gap, ACT (35° 32' S, 148° 55'

E, *H. japonicum*), and provided by the Adelaide Botanical Gardens, South Australia (*H. tetrapterum*).

Once seeds had germinated and cuttings had become well-rooted, plants were washed free of soil and transplanted to 12 cm diameter pots of 1 L capacity containing a 2:1 mixture of sand to clay. They were then grown in a shade house for about 6 weeks till commencement of the experiments. Adult plants in the plant age experiment (experiment 2), were established 12 months before-hand in the above manner, but were transplanted to 20 cm diameter pots of about 8 L capacity after germinating, thereby minimising the potential for plants becoming pot-bound between transplantation and beginning the experiment, 12 months later.

On the first day of experiments, the date of seedling transplantation, 10 plants of each species were sub-sampled to estimate initial root and shoot dry mass. In experiment 2, adult plants were also sub-sampled. These were taken from a previously selected sample of adults of similar height. The remaining adults in this selected group were used for experimentation. All experimental plants were then randomly assigned to an experiment and subsequently, to a treatment within the given experiment.

6.2.3. Infestation of plants with mites

Mites were introduced to '+mite' treatments by placing infested vegetative *H. perforatum* buds into the crown of seedlings. Three buds, each infested with 5-10 mites were introduced. Plants were re-infested in the same manner after 6 and 8 weeks.

6.2.4. Estimating leaf area

Automated planimeter measures of leaf area proved unreliable because of the small size of *Hypericum* leaves. Instead, total plant leaf area (TPLA) was estimated from a simple linear regression of log total plant leaf area on log total plant shoot dry weight. Leaf area and shoot weight values for the regression were obtained from a sample of 7 plants of each of *H. gramineum*, *H. perforatum*, *H. japonicum* and *H. tetrapterum* grown in the same soil mixture and under similar glasshouse conditions. Leaf areas for plants used in the model were estimated by assigning all green leaves to size class categories, as follows (<3 mm^2, 3 - 5 mm^2, 6 - 10 mm^2,

11 - 18 mm^2, 19 - 27 mm^2, 28 - 33 mm^2, and 34 - 58 mm^2, 59 - 71 mm^2, 72 - 99 mm^2, 100 - 200 mm^2, 201 - 275 mm^2 and > 275 mm^2). The area of each leaf was scored as the mid-point for the size class to which it had been allocated (2 mm^2, 4 mm^2, 8 mm^2, 15 mm^2, 23 mm^2, 31 mm^2, 46 mm^2, 65 mm^2, 86 mm^2, 150 mm^2, 238 mm^2 and > 275 mm^2 respectively). Total plant leaf areas were estimated, by tallying the total of the means for each size class, and used to generate the resultant regression model (Table 6.1, Fig. 6.1). Since regression coefficients for the four species did not differ significantly, the data were pooled.

Table 6.1 Linear regression models for $\log_{10}$ total plant leaf area (A; mm^2) as a function of $\log_{10}$ shoot weight (B; g) for *H. gramineum*, *H. perforatum*, *H. japonicum*, *H. tetrapterum* and a pooled model comprising all species. Models are of the form $A = b_0 + b_1B$ with coefficients of determination (r^2) and degrees of freedom (d.f.) indicated for each. 95% lower and upper confidence limits respectively, are indicated in brackets.

Species	r^2	d.f.	b_0	b_1
H. gramineum	0.98	6	4.28 (4.09, 4.48)	1.13 (0.94, 1.32)
H. perforatum	0.96	6	4.38 (4.23, 4.53)	1.10 (0.84, 1.36)
H. japonicum	0.94	5	4.54 (4.35, 4.73)	1.03 (0.67, 1.40)
H. tetrapterum	0.98	6	4.41 (4.31, 4.51)	0.94 (0.77, 1.11)
Pooled model	0.95	26	4.41 (4.33, 4.49)	1.11 (0.99, 1.22)

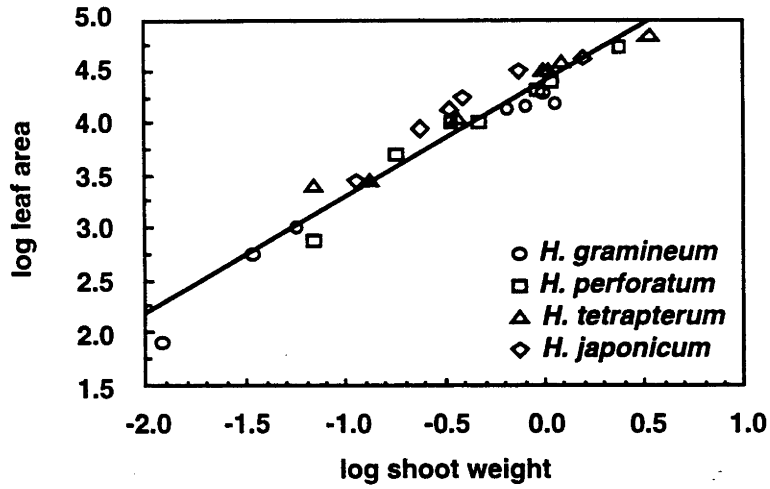


Fig. 6.1 Plot for the pooled simple linear regression model (refer Table 6.1) of log leaf area (mm^2) on log shoot weight (g). The equation for the regression model is:
Log_{10} leaf area (A) = 4.41 + 1.11 x log_{10} shoot wt (B);
$r^2 = 0.95$

6.2.5. *Nutrient analyses*

Shoot and root samples were assayed for total nitrogen and phosphorus content. Results are reported as parts per million (ppm) of tissue dry weight. Up to 0.3 g of oven-dried tissue was digested by a micro Kjeldahl technique, before analysing with a Technicon Auto-analyser II®.

6.2.6. *Measured growth parameters and analysis of data*

At completion of the experiment plants were harvested and the following growth and nutrient parameters measured: root weight (g), shoot weight (g) and total plant weight (TPW, g); root:shoot ratio (g g^{-1}); shoot length (cm); the number of shoots; total plant leaf area (TPLA, mm^2); the number of fruit; root, shoot and total plant relative growth rate (RGR, g g^{-1} $week^{-1}$); and root and shoot nitrogen and phosphorus content (ppm). Note that,

$$RGR = (\log_{10} M_i - \log_{10} M_f)/T_i - T_f,$$

where M_i = initial mass, M_f = final mass, T_i = initial time and T_f = final time, and

root:shoot (R:S) ratio = root mass/shoot mass

Most data were logarithmically (or log + 1) transformed to better satisfy assumptions of the statistical models, though raw, un-fitted means are presented to simplify comparisons. As a consequence of the minor phylogenetic effects on *A. hyperici* herbivory of *Hypericum* species revealed in chapter 5, nested analysis of variance (ANOVA) were deemed unnecessary to compare the impact of mites on growth of these four species. Simple ANOVAs, structured according to the experimental design, have, therefore, been used in analyses of the present experiments, and those of subsequent chapters.

Analyses of variance were performed for each growth parameter using Genstat 5 algorithms (Lane *et al.* 1987; Digby *et al.* 1989) and linear regression models using Statview 4 (Abacus Concepts, Statview 1992). At 5% significance, it is expected that approximately 5% of the main effects and interactions will be significant due to chance alone (Type 1 errors). Considering analyses of the three experiments, approximately 240 tests of significance (each with 17 factors and interactions x 14 - 15 growth and nutrient parameters) were performed. Accordingly, a more conservative 1% significance level has been adopted in this chapter, since probabilities in the range P = 0.01 - 0.05 are considered too tentative to warrant detailed discussion.

6.3. Results

6.3.1. Experiment 1 - Impact of A. hyperici *on four species of* Hypericum

A summary of the probabilities ($P \leq 0.05$) for factors and interactions in the 3-way ANOVA of experiment 1 is presented in Table 6.2. In general, main factors within the experiment were highly significant ($P < 0.001$), while the 2 and 3-factor interactions were generally not significant ($P > 0.05$).

Table 6.2 Probability table of each factor and interaction ($P \leq 0.05$) in experiment 1 (impact of *A. hyperici* on four species of *Hypericum*) using 3-way ANOVA. TPW = total plant weight; R:S ratio = root:shoot ratio; TPLA = total plant leaf area; RGR = relative growth rate; N/A = not applicable; N = nitrogen; P = phosphorus. See text for the units of each variable.

Factors & Interactions	**P - values**													
	root weight	shoot weight	TPW	R:S ratio	shoot length	no. shoots	TPLA	fruit	root RGR	shoot RGR	N - shoot	N - root	P - shoot	P - root
Species	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	N/A	<0.001	<0.001		<0.001	<0.001	<0.001
Mites	0.007	0.004	0.003	0.026			<0.001		<0.001	<0.001				
Harvest	0.001	<0.001	<0.001		0.001	0.041	<0.001		0.031	<0.001	0.002	0.026	0.001	
Species x Mites								N/A				0.035		
Species x Harvest	<0.001		<0.001				0.003	N/A	0.002	<0.001		<0.001		<0.001
Mites x Harvest									0.012	0.001				
Species x Mites x Harvest	0.039							N/A						

6.3.1.1. Differences between species

Highlighting ecological and physiological differences between the *Hypericum* species examined in the experiment, the species consistently differed significantly ($P \leq 0.001$) in all measures of plant growth (Table 6.2, Fig. 6.2). The relatively small indigenous forbs, *H. gramineum* and *H. japonicum*, always had significantly lower measures of growth than either of the naturalised taxa. In the latter, the trend was for *H. tetrapterum* to produce larger measures of absolute growth in the root and shoot systems compared with *H. perforatum*. The relative growth rate of *H. perforatum*, however, was usually higher than that of *H. tetrapterum*.

6.3.1.2. The effect of mites on plant growth

Overall, the presence of mites caused reductions ($P \leq 0.026$) in all indices of plant growth except shoot length and the number of shoots produced. These latter parameters remained largely unaffected by *A. hyperici* ($P \geq 0.179$). Measures of root growth were more adversely affected by herbivory than measures of shoot growth. On average, root mass was reduced by 25% and the relative growth rate of roots was reduced by 13%, compared with 21% and 8% decreases respectively, for the same indices of shoot growth. Mites caused a slight, though insignificant ($P = 0.539$) reduction in the number of fruit produced by *H. gramineum*.

6.3.1.3. The effect of harvest time on plant growth

As expected, plants harvested after 12 weeks (harvest 1) produced less biomass than those allowed to continue growing a further 12 weeks (harvest 2). That the difference in the number of fruit produced by *H. gramineum* at the two harvests did not differ significantly ($P = 0.196$, Fig. 6.2h) suggests that under the prevailing glasshouse conditions this species is able to flower and fruit within 12 weeks; the additional 12 weeks does not lead to higher fruit productivity. The root and shoot relative growth rates of all species were higher ($P \geq 0.031$) at harvest 1 than at harvest 2 (Fig. 6.2i and j), indicating that with time the relative growth rate decreases.

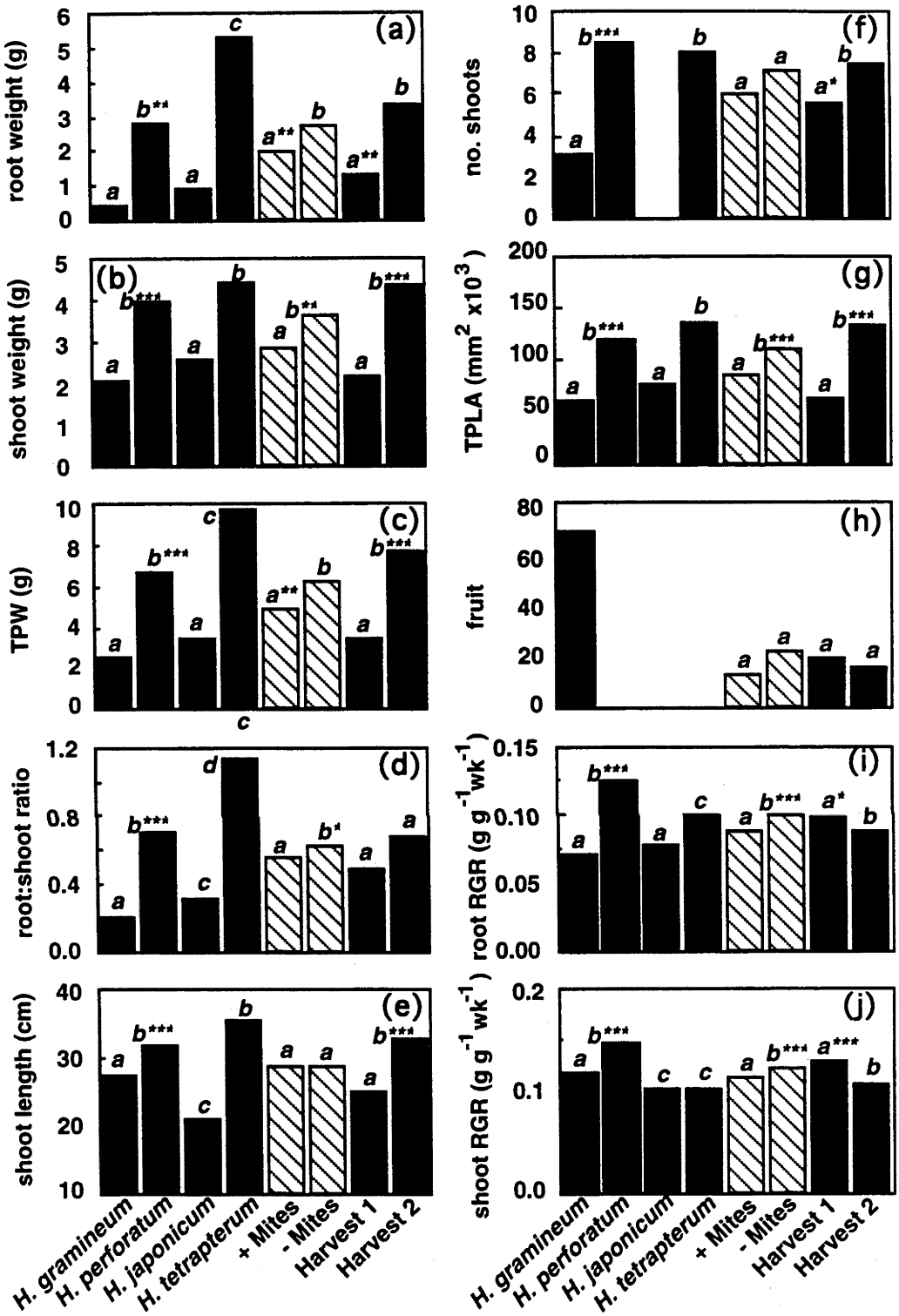


Fig. 6.2 Mean values for several indices of growth in experiment 1 (impact of *A. hyperici* on four species of *Hypericum*). Significant differences ($P \leq 0.05$) between treatments of the main factors species (■), mites (▨) and harvest (■) are indicated by columns with different lettering. The significance of F-tests between treatments of the main factors are also indicated ($P \leq 0.05$*, $P \leq 0.01$**, and $P \leq 0.001$***). Note that shoot number was not recorded for *H. japonicum* (f) and that fruit number (h) applies to *H. gramineum* only. TPW = total plant weight; TPLA = total plant leaf area; RGR = relative growth rate.

6.3.1.4. Factor interactions

Few of the potential 2 and 3-factor interactions yielded significant ($P \leq 0.05$) results, although the species x harvest interaction was significant ($P \leq 0.003$) for root weight, total plant weight (TPW) and total leaf area (TPLA; Table 6.2, Fig. 6.3). As above, all such estimates of growth were higher at harvest 2 than at harvest 1. Growth of *H. tetrapterum* was usually much higher after harvest 2 relative to that of the other species and appears to be a major cause of the significant interaction terms (see, for example, Fig. 6.3a). Species x harvest was also significant ($P \leq 0.002$) for the root and shoot relative growth rates (RGR; Table 6.2, Fig. 6.3e and f). As noted, these parameters were lower at harvest 2 than harvest 1. The relative growth rate of *H. perforatum* roots and shoots at harvest 1 was much greater than at harvest 2 and exceeded that of the other species at harvests 1 and 2, causing the significant ($P \leq 0.002$) interaction between factors.

Shoot relative growth rate was also significantly ($P < 0.001$) affected by the interaction between mites and harvest (Fig. 6.3f). At both harvests, mites limited the growth rate, though the reduction was significant for harvest 1 only. This implies that the effect of mite-herbivory may be strongest when plants are younger, and rapidly growing.

Interaction between species and mites would indicate that mites had a differential effect on growth of the four *Hypericum* species, affecting at least one of the species more than they affected any of the others. Table 6.3 summarises the growth parameters of each species in the presence and absence of *A. hyperici*. While mites cause marginal decreases in most indices of growth relative to controls, the species x mites interaction is not significant ($P \geq 0.056$) for any measure of growth. This suggests that the reductions in growth caused by mites are similar for *H. gramineum*, *H. perforatum*, *H. japonicum* and *H. tetrapterum*: Differences in the relative growth of mite-free and mite-infested individuals of each species are only marginal.

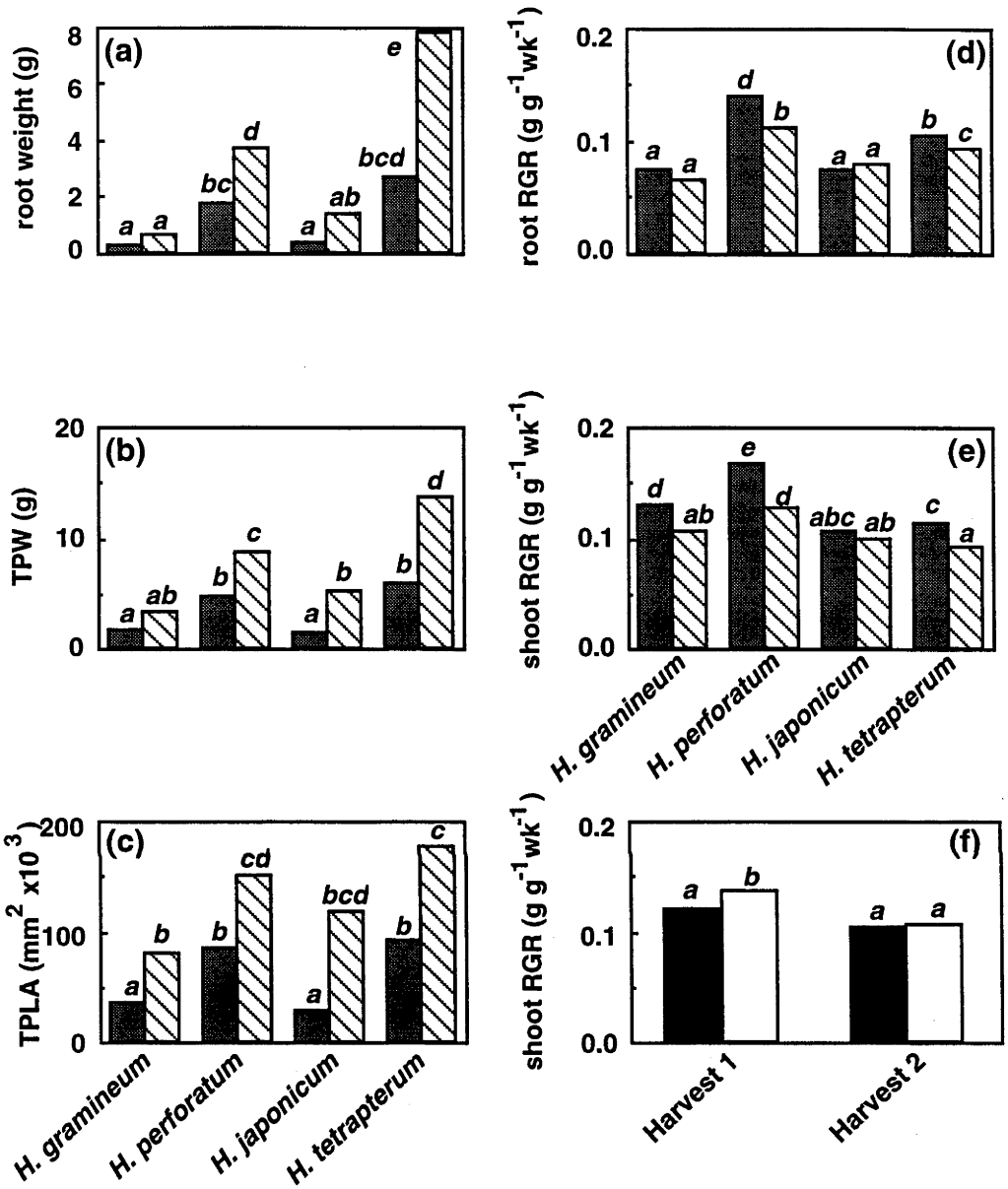


Fig. 6.3 Histograms for significant ($P \leq 0.01$) interactions between species and harvest (figs. a-e ■ = harvest 1 and ◪ = harvest 2) and between mites and harvest (fig. f ■ = +mites and □ = - mites) in experiment 1 (impact of mites on four species of *Hypericum*). Significant differences ($P \leq 0.05$) between treatments are indicated by columns with different lettering. TPW = total plant weight; TPLA = total plant leaf area; RGR = relative growth rate.

Table 6.3 Measures of growth in four *Hypericum* species after infestation with *A. hyperici* (+ Mites) compared with mite-free controls (- Mites) with standard errors of the arithmetic mean indicated. Within a species, none of the growth parameters varied significantly ($P \geq 0.056$) between '+mite' and '-mite' treatments. Note that the number of shoots produced by *H. japonicum* (shoot no.) was not scored. TPW = total plant weight; R:S = root:shoot ratio; TPLA = total plant leaf area; RGR relative growth rate.

Growth parameter	*H. gramineum*		*H. perforatum*		*H. japonicum*		*H. tetrapterum*	
	+ Mites	- Mites	+ Mites	- Mites	+ Mites	- Mites	+ Mites	- Mites
root weight (g)	0.22 ± 0.04	0.60 ± 0.14	2.63 ± 0.46	2.85 ± 0.28	0.79 ± 0.27	0.91 ± 0.22	4.29 ± 0.88	6.22 ± 1.33
shoot weight (g)	1.5 ± 0.34	2.63 ± 0.46	3.62 ± 0.52	4.2 ± 0.31	2.11 ± 0.56	2.93 ± 0.55	4.19 ± 0.48	4.59 ± 0.49
TPW (g)	1.71 ± 0.37	3.23 ± 0.58	6.24 ± 0.94	7.05 ± 0.55	2.89 ± 0.81	3.84 ± 0.74	8.48 ± 1.22	10.82 ± 1.75
R:S ratio ($g\ g^{-1}$)	0.18 ± 0.04	0.22 ± 0.02	0.71 ± 0.08	0.67 ± 0.04	0.32 ± 0.04	0.30 ± 0.03	0.99 ± 0.16	1.26 ± 0.20
shoot length (cm)	27.8 ± 3.0	26.9 ± 2.9	31.0 ± 1.4	32.2 ± 1.9	20.0 ± 2.4	21.0 ± 0.5	35.6 ± 2.1	34.7 ± 2.4
no. shoots	1.6 ± 0.31	4.4 ± 1.42	8.3 ± 0.50	8.6 ± 0.50	-	-	7.9 ± 0.85	8.1 ± 0.82
TPLA (mm^2)	41507 ± 10125	76653 ± 14706	108733 ± 17229	127493 ± 10523	61351 ± 17421	86699 ± 17742	127595 ± 15990	141150 ± 16634
no. fruit	51.4 ± 20.7	85.4 ± 32.9	0	0	0.5 ± 0.3	2.8 ± 1.7	0	0
root RGR ($g\ g^{-1}\ wk^{-1}$)	0.060 ± 0.004	0.082 ± 0.004	0.122 ± 0.005	0.128 ± 0.006	0.069 ± 0.006	0.084 ± 0.005	0.095 ± 0.005	0.103 ± 0.003
shoot RGR ($g\ g^{-1}\ wk^{-1}$)	0.109 ± 0.006	0.126 ± 0.126	0.143 ± 0.006	0.149 ± 0.008	0.094 ± 0.002	0.108 ± 0.005	0.101 ± 0.004	0.104 ± 0.004

6.3.1.5. Tissue nutrients

The concentration of total nitrogen and phosphorus in the roots and shoots of plants generally varied with species and harvest, but was not significantly ($P \geq 0.373$) affected by the presence of mites (Fig. 6.4). Shoot phosphorus was significantly ($P < 0.001$, Fig. 6.4) higher in *H. gramineum* and *H. japonicum* than in either of the naturalised species, while shoot nitrogen did not vary greatly ($P = 0.609$) between the four taxa. Root phosphorus was highest ($P < 0.001$) in *H. japonicum* and lowest in *H. tetrapterum*. Concentrations of root nitrogen showed a similar trend to that of shoot phosphorus, with the indigenous species containing higher levels than the naturalised species.

Overall, shoot nutrients were significantly ($P \leq 0.002$, Table 6.2) higher at harvest 1 compared to harvest 2. Root nutrients, by contrast, were higher at harvest 2 than at harvest 1, although the differences were only marginal ($P = 0.054$; root phosphorus, and $P = 0.026$, root nitrogen).

Species x harvest provided the only two significant ($P \leq 0.01$) interactions, affecting the concentration of root nitrogen and phosphorus (Fig. 6.4e and f). While the four *Hypericum* species did not differ significantly ($P > 0.05$) in root nitrogen at harvest 1, by harvest 2 the native forbs had significantly higher concentrations than were recorded for these species at harvest 1, and higher levels than either *H. perforatum* or *H. tetrapterum*, both of which had lower concentrations at harvest 2 than at harvest 1. The broad pattern was similar for root phosphorus, with concentrations in the natives higher at harvest 2 than at harvest 1 and greater than those of the introduced species, although the inter-specific differences varied slightly (contrast Fig. 6.4e and f).

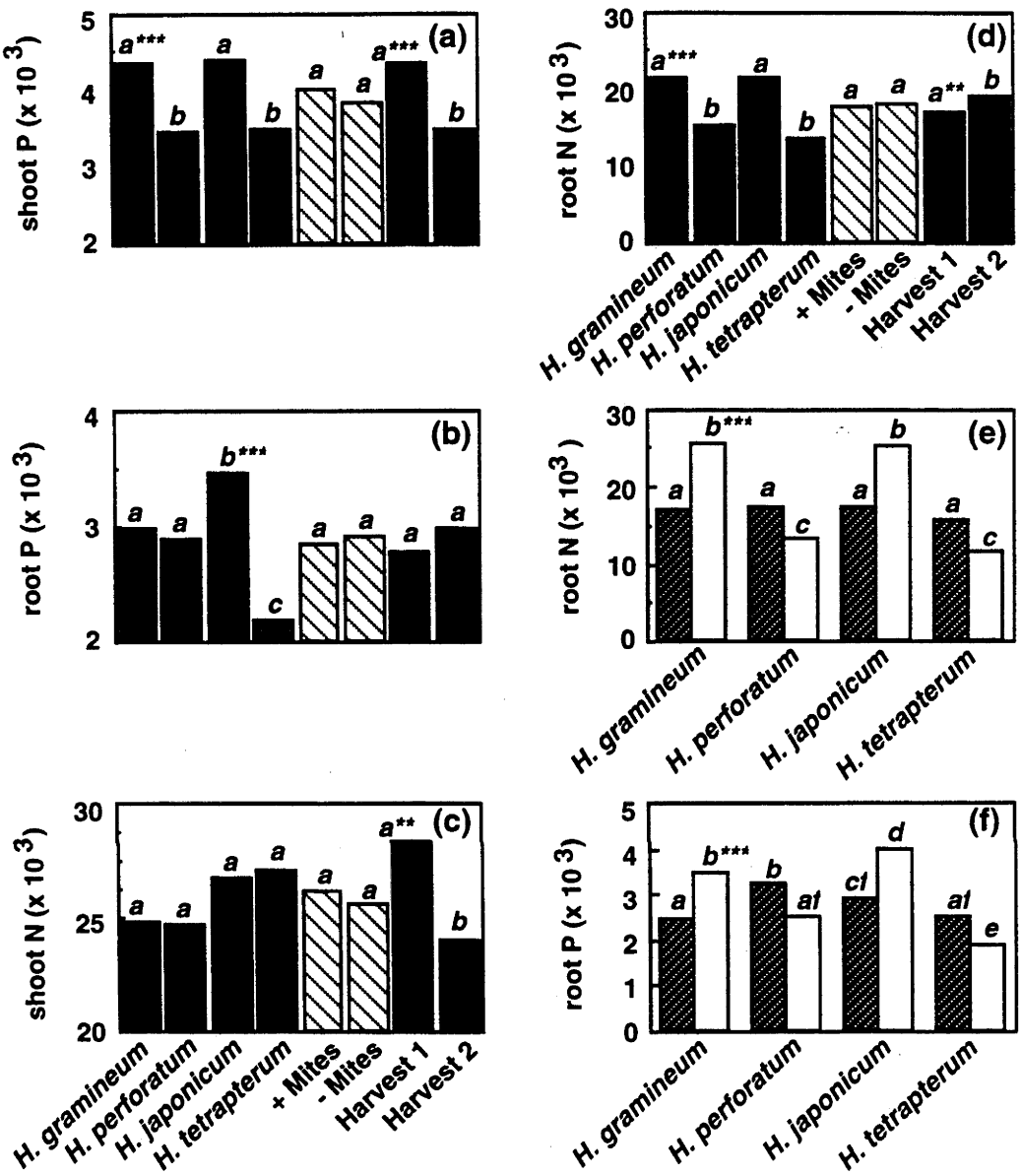


Fig 6.4 (a-d) Mean tissue nutrient concentration (N = nitrogen, P = phosphorus; ppm) in four *Hypericum* species after experimental treatments in experiment 1 (impact on four species of *Hypericum*). Significant differences ($P \leq 0.05$) between treatments of the main factors species (■), mites (▨) and harvest (■) are indicated by columns with different lettering. The significance of F-tests between treatments of the main factors is also indicated ($P \leq 0.05$*, $P \leq 0.01$** or $P \leq 0.001$***); (e-f) interaction in the significant ($P < 0.001$) species x harvest interaction (▩ = harvest 1, and □ = harvest 2) with significant ($P \leq 0.05$) differences between treatments indicated by different lettering.

6.3.2. Experiment 2 - Impact of A. hyperici *on plants of different age and size*

6.3.2.1. Plant growth

Table 6.4 summarises the probabilities ($P < 0.05$) for the main effects and interactions examined in the ANOVA of experiment 2. As in experiment 1, consistent differences in the effect of species, underlines inter-specific eco-physiological differences between *H. gramineum* and *H. perforatum*. The effect of mites on plant growth was only slight ($P \geq 0.175$, Table 6.4). Monitoring of plants every 4-weeks indicated that despite re-infestation, population development of *A. hyperici* in experiment 2 was poor, and may explain the lack of significant growth reductions attributable to the mite. Nevertheless, a consistent trend towards decreased productivity when infested by *A. hyperici* is evident (Table 6.5).

Plant age was also significant ($P \leq 0.003$) for most indices of plant growth. As expected, adult plants, which were larger than seedlings at the start of the experiment, had higher root, shoot and total weights ($P < 0.001$). They also possessed longer shoots ($P = 0.009$), had higher leaf areas ($P < 0.001$), and adult *H. gramineum* individuals produced more fruit than seedlings, although the difference was not significant ($P = 0.214$; Tables 6.4 and 6.5).

Comparisons of such measures of absolute growth are confounded by plant size, since young plants (seedlings) were much smaller than older plants (adults) at the start of the experiment. More relevant contrasts can be drawn from measures of growth relative to initial plant size. Root, shoot and total plant relative growth rates (RGR) provide such comparisons (Fig. 6.5). Inter-specific differences in relative growth rates were highly significant ($P < 0.001$) in this study. In all such indices, *H. gramineum* accumulated biomass at a lower rate than *H. perforatum*. As above, *A. hyperici* had no significant ($P > 0.332$) impact on the relative growth rate of *H. gramineum* or *H. perforatum*, possibly because of poor population development. The relative growth rate of seedlings of *H. gramineum* and *H. perforatum* was significantly ($P < 0.001$) higher than that of adult plants of both species (Fig. 6.5).

Table 6.4 Probability table of each factor and interaction ($P \leq 0.05$) in experiment 2 (impact of *A. hyperici* on plants of different age and size) using 3-way ANOVA. TPW = total plant weight; R:S ratio = root:shoot ratio; TPLA = total plant leaf area; RGR = relative growth rate; N/A = not applicable; N = nitrogen; P = phosphorus. See text for the units of each variable.

Factors & Interactions	**P - values**														
	root weight	shoot weight	TPW	R:S ratio	shoot length	no. shoots	TPLA	fruit	root RGR	shoot RGR	total RGR	N - shoot	N - root	P - shoot	P - root
Species	<0.001	<0.001	<0.001	<0.001	0.007	<0.001	<0.001	N/A	<0.001	<0.001	<0.001			<0.001	<0.001
Mites															
Age	<0.001	<0.001	<0.001		0.009	0.003	<0.001		<0.001	<0.001	<0.001				
Species x Mites								N/A							
Species x Age	<0.001	<0.001	<0.001		0.008		<0.001	N/A					0.012	0.029	
Mites x Age															
Species x Mites x Age								N/A							

Table 6.5 Arithmetic means of several measures of absolute growth for plants of differerent age/size following infestation with *A. hyperici*. Significant differences ($P \leq 0.01^b$ and $P \leq 0.001^a$; $P > 0.05$, not significant = ns) between treatments within the main effects (species, mites and age) of a 3-way ANOVA are shown. Note that comparisons of fruit set refer to *H. gramineum* only, since *H. perforatum* did not flower, or set fruit during the experiment. TPW = total plant weight; R:S = root:shoot ratio; TPLA = total plant leaf area.

Growth parameter	Species		Mites		Age	
	H. gramineum	*H. perforatum*	Present	Absent	Adults	Seedlings
root weight (g)	0.41 ± 0.05^a	2.27 ± 0.18	1.17 ± 0.19^{ns}	1.51 ± 0.23	1.65 ± 0.26^a	1.03 ± 0.13
shoot weight (g)	1.86 ± 0.16^a	4.56 ± 0.32	2.91 ± 0.33^{ns}	3.51 ± 0.37	3.97 ± 0.42^a	2.44 ± 0.18
TPW (g)	2.27 ± 0.20^a	6.83 ± 0.48	4.08 ± 0.51^{ns}	5.01 ± 0.58	5.62 ± 0.66^a	3.47 ± 0.30
R:S ratio ($g\ g^{-1}$)	0.22 ± 0.01^a	0.50 ± 0.02	0.35 ± 0.03^{ns}	0.38 ± 0.03	0.34 ± 0.03^{ns}	0.38 ± 0.03
shoot length (cm)	29.8 ± 1.1^b	33.7 ± 1.2	31.9 ± 1.2^{ns}	31.5 ± 1.3	33.8 ± 1.4^b	29.6 ± 1.0
no. shoots	3.3 ± 0.3^a	8.3 ± 0.4	5.8 ± 0.6^{ns}	5.9 ± 0.6	6.6 ± 0.7^b	5.8 ± 0.5
TPLA (mm^2)	45788 ± 4552^a	126661 ± 10050	77179 ± 9836^{ns}	95270 ± 11190	110344 ± 12785^a	62105 ± 5122
no. fruit	65.4 ± 11.0	N/A	73.0 ± 18.2^{ns}	57.8 ± 12.6	82.7 ± 19.6^{ns}	48.1 ± 8.6

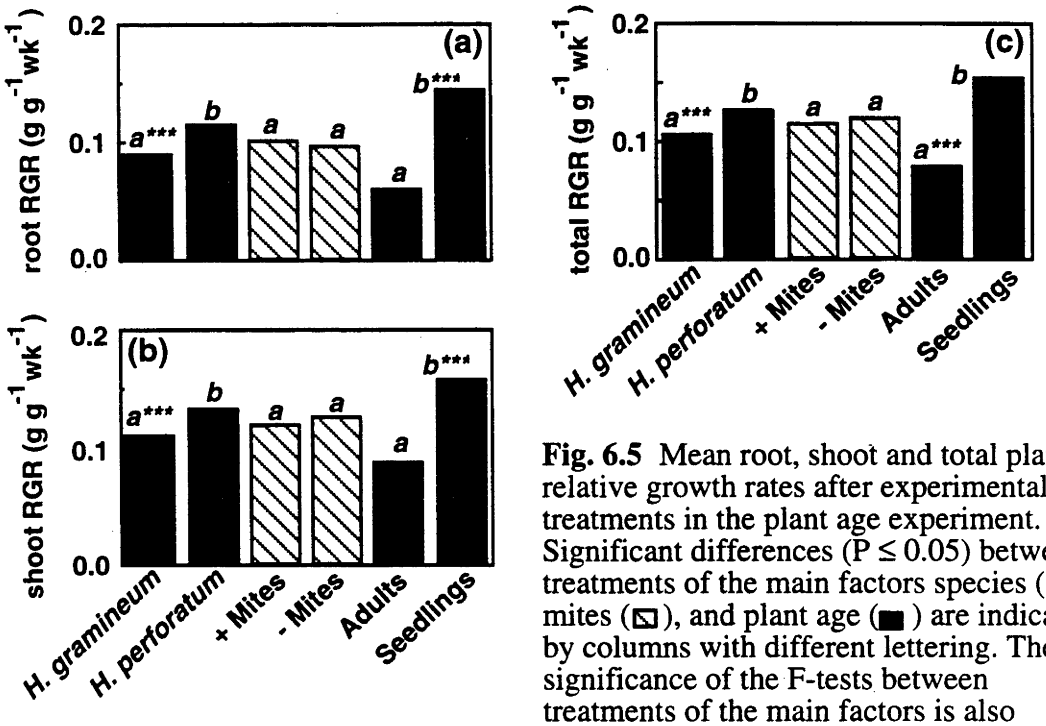


Fig. 6.5 Mean root, shoot and total plant relative growth rates after experimental treatments in the plant age experiment. Significant differences ($P \leq 0.05$) between treatments of the main factors species (■), mites (▨), and plant age (■) are indicated by columns with different lettering. The significance of the F-tests between treatments of the main factors is also indicated ($P \leq 0.05$*, $P \leq 0.01$**, and $P \leq 0.001$***).

Unlike absolute measures of plant growth which yielded significant ($P \leq 0.008$) interactions between species and age for root, shoot and total weight, as well as shoot length and leaf area (in all cases adult *H. perforatum* consistently had higher measures of growth than either *H. gramineum* or con-specific seedlings, Fig. 6.6), no factor interactions were significant ($P > 0.067$, Table 6.4) for relative estimates of growth.

6.3.2.2. Tissue nutrients

Root and shoot nutrients were not generally affected by experimental treatments in the plant age experiment (Fig. 6.7). Shoot phosphorus was significantly ($P < 0.001$) higher in *H. gramineum* than in *H. perforatum*, while root phosphorus was significantly higher in *H. perforatum* than in the native. Tissue nitrogen levels varied little between treatments ($P \geq 0.066$), and there were no significant ($P < 0.01$) factor interactions.

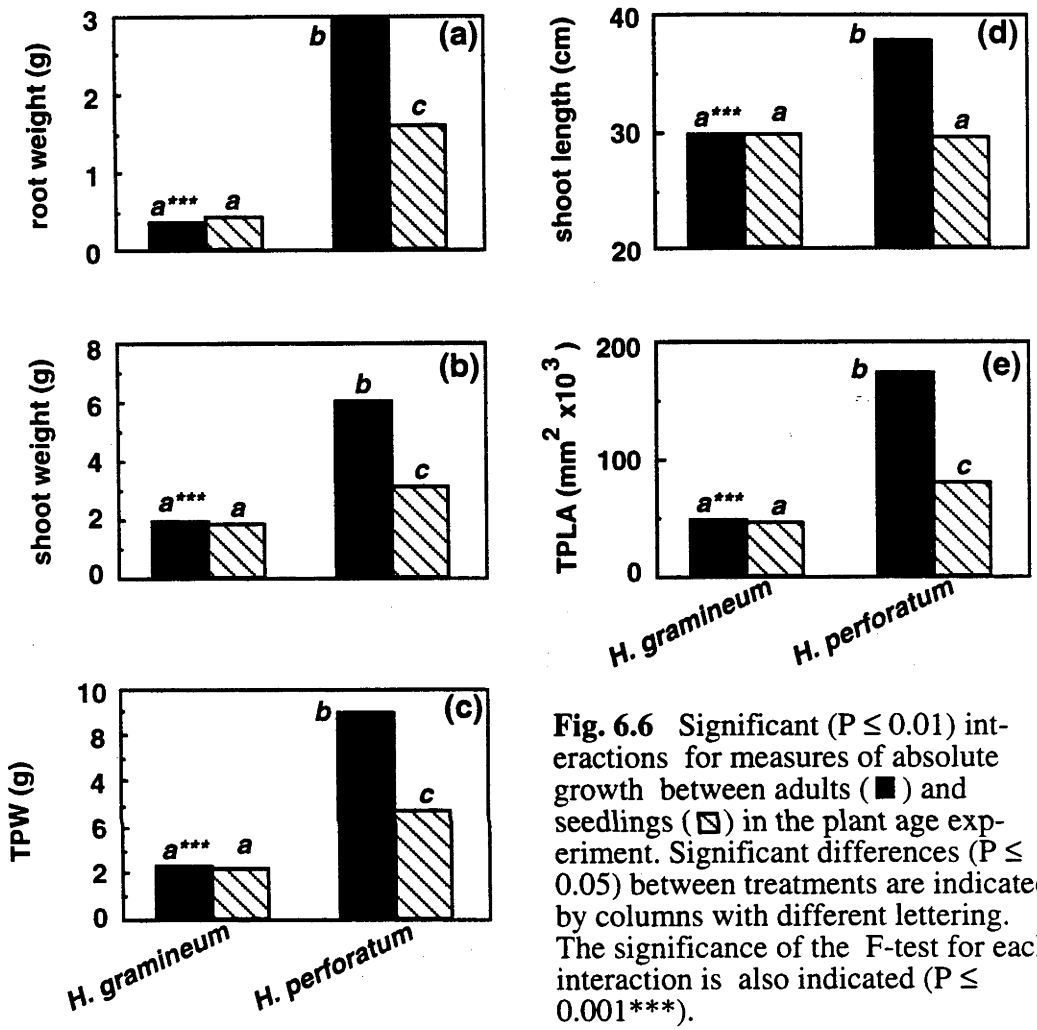


Fig. 6.6 Significant ($P \leq 0.01$) interactions for measures of absolute growth between adults (■) and seedlings (▨) in the plant age experiment. Significant differences ($P \leq 0.05$) between treatments are indicated by columns with different lettering. The significance of the F-test for each interaction is also indicated ($P \leq 0.001$***).

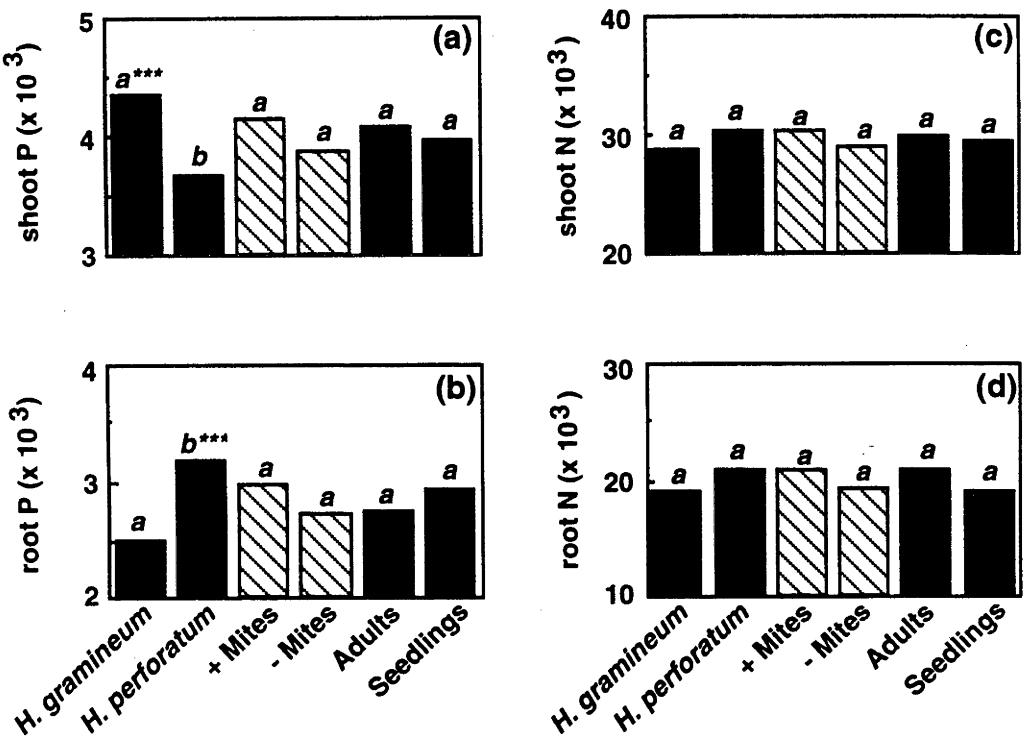


Fig 6.7 Mean tissue nutrient concentration (N = nitrogen, P = phosphorus; ppm) in *Hypericum* species of different age and size after experimental treatments in the plant age/size experiment. Significant differences ($P \leq 0.05$) between treatments within the main factors of species (■), mites (□) and plant age (■) are indicated by columns with different lettering. The significance of the F-tests between the treatments of main factors is also indicated ($P \leq 0.05$*, $P \leq 0.01$** or $P \leq 0.001$***).

6.3.3. Experiment 3 - The effect of 'Omite' on Hypericum *spp.*

Applications of Omite miticide had negligible effects on plant growth and tissue nutrients (Table 6.6). Differences between treated (sprayed) and untreated (control) plants were minimal ($P \geq 0.141$), and there were no significant ($P \geq 0.141$) interactions between species and spraying for any estimates of growth or tissue nutrient concentration.

Table 6.6 Means and standard errors for several measures of plant growth and tissue nutrients after spraying plants with the miticide, Omite. In a 2-way ANOVA comparing the effect of Omite on four species of *Hypericum*, there were no significant differences ($P \geq 0.141$) between sprayed (treated) and unsprayed (untreated) plants, nor in the interaction between the main effects of spraying and species ($P \geq 0.141$). Note that comparisons of fruit number refer to *H. gramineum* only, since other *Hypericum* species did not flower, or set fruit during the experiment. TPW = total plant weight; R:S = root:shoot ratio; TPLA = total plant leaf area; RGR = relative growth rate; N = nitrogen; P = phosphorus.

Growth/nutrient index	Omite-sprayed	Omite-free
root weight (g)	1.40 ± 0.34	1.14 ± 0.27
shoot weight (g)	2.62 ± 0.26	2.33 ± 0.28
TPW (g)	4.02 ± 0.50	3.47 ± 0.51
R:S ratio ($g\ g^{-1}$)	0.67 ± 0.20	0.41 ± 0.07
shoot length (cm)	25.6 ± 1.7	25.1 ±1.4
no. shoots	8.0 ± 0.9	7.0 ± 0.9
TPLA (mm^2)	76287 ± 8048	67396 ± 8690
no. fruit	31.5 ± 15.6	33.1 ± 15.1
root RGR ($g\ g^{-1}\ week^{-1}$)	0.103 ± 0.006	0.092 ± 0.007
shoot RGR ($g\ g^{-1}\ week^{-1}$)	0.136 ± 0.005	0.132 ± 0.006
shoot N (ppm)	20986 ± 1337	23661 ± 1415
root N (ppm)	14850± 1916	13114 ± 1110
shoot P (ppm)	2846 ± 185	3433 ± 317
root P (ppm)	2169 ± 207	2168 ± 140

6.4. Discussion

The miticide 'Omite', used to prevent establishment of *A. hyperici* populations in '-mite' treatments, had negligible effects on plant growth and tissue nutrients (total nitrogen and phosphorus). In further discussions, differences in such parameters are, therefore, assumed to be attributable to experimental treatments.

In experiments 1 and 2 above, apparent visual differences in the size and growth of different *Hypericum* species were confirmed through analysis of variance. While the smaller indigenous species, *H. gramineum* and *H. japonicum*, tended to produce lower measures of absolute growth, their relative growth rates were usually higher than in the larger introduced taxa. In all species, there were differences in root and shoot relative growth rates depending on the time of harvest. These suggested that plants harvested early (younger plants) have higher relative

growth rates than those harvested later (older plants). This observation was supported in the plant age experiment (experiment 2) in which the relative growth rate of seedlings was up to twice that of adults.

6.4.1. Effects of A. hyperici *on absolute measures of plant growth*

Growth and development of mite and other arthropod herbivores are widely believed to be limited by the availability of utilisable nitrogen (Rodriguez 1951; Mattson 1980; Jackson and Hunter 1983; White 1984; Wermelinger *et al.* 1985; Mattson and Haack 1987a,b; Casotti and Bradley 1991). Inter-specific differences in shoot nitrogen were not significant in experiment 1 (impact of *A. hyperici* on four *Hypericum* species) or experiment 2 (the effect of plant age on herbivory) and may explain the broadly similar host utilisation patterns of *A. hyperici* in the present chapter. Although the presence/absence of other phago-stimulatory or -inhibitory cues cannot be ignored, their potential effect on *A. hyperici* does not appear to have been strong in the above experiments. Had such cues elicited a stronger response from mites, significant species x mites interactions for plant growth parameters might have been observed. In the absence of such interactions, it appears that under the prevailing experimental conditions, herbivory by *A. hyperici* caused similar reductions in the absolute growth estimates of *H. gramineum*, *H. perforatum*, *H. japonicum* and *H. tetrapterum*.

Despite statistically significant reductions in *Hypericum* species attributable to *A. hyperici*, it should be noted that, as in experiment 2 (plant age), development of mite populations in experiment 1 (impact on four species) appears to have been limited. In a pilot experiment involving *H. gramineum*, *H. perforatum*, *H. japonicum*, and *H. tetrapterum*, difficulties were also encountered infesting plants. Such difficulties may reflect a general inability of *A. hyperici* to become well established on glasshouse-grown plants, possibly because such individuals 'out-grow' their mite infestation. This raises the need to examine whether environmentally stressed plants are less tolerant of herbivory by *A. hyperici* than healthy, vigorously growing conspecifics. Alternatively, the difficulties in establishing populations of *A. hyperici* in the current experiments might indicate the importance of other variables during the experiments. Although the trials were conducted in a glasshouse, these factors may include season, temperature, humidity or biotic factors such as predators and pathogens. If mites had established and grown more successfully, it is possible that different host-utilisation patterns may have emerged, and the anticipated species x mites interactions may have been much clearer.

6.4.2. Effects of A. hyperici *on relative measures of plant growth*

6.4.2.1. Relative growth rate (RGR)

The significant ($P < 0.001$) mites x harvest interaction for shoot relative growth rate (RGR) in experiment 1 (the impact of herbivory on four species; Fig. 6.3f) suggests that mites cause greater reductions in shoot relative growth rate in younger plants (harvest 1) than older plants (harvest 2). The trend was similar, though marginal ($P = 0.012$), for root relative growth rate. Such patterns were not evident in the plant age experiment (experiment 2), where shoot, root and total plant root relative growth rates varied only slightly ($P \geq 0.770$) between mite-infested and mite-free seedlings and adults. As noted, however, this may be because mites failed to become well-established in this trial, and were thus unable to exert significant ($P \leq 0.05$) influence.

6.4.2.2. Root:Shoot ratio

The experiments above generally indicate that the root:shoot ratio of *H. tetrapterum* and *H. perforatum* is higher than that of *H. gramineum* and *H. japonicum* and that herbivory by *A. hyperici* decreased the ratio of root:shoot biomass. Herbivory of plant shoots has been associated with reductions in the root:shoot ratio by other researchers (Wilson 1988a; Christiansen *et al.* 1989; Vranjic and Gullan 1990; Karban and Strauss 1993).

Several models of root:shoot ratio control have been proposed, but source-sink models, based on carbon and nitrogen uptake and transport, such as that proposed by Thornley (1972) are commonly accepted. One mechanism by which mite-herbivory may cause reductions in *Hypericum* growth is by acting as a 'sink', causing re-allocation of assimilates from the roots to the shoots, resulting overall decreases in plant growth, and manifested in the decreased root:shoot ratio.

6.4.2.3. Conclusions

In conclusion, it should be stressed that the above findings represent a preliminary examination of plant growth in the face of herbivory by *A. hyperici*. This is mainly because despite repeated infestation with the arthropod, few mites were found on plants at termination of the experiments. This may be because conditions were unfavourable for mites and/or plants during the experiment, though if this was the case, the reasons remain unknown. Mite establishment was particularly poor in the plant age experiment (experiment 2) which limits conclusions regarding the impact of *A. hyperici* on seedlings as compared with adult plants. From investigations in experiment 1, it appears that herbivory by *A. hyperici* may cause significant reductions in absolute and relative measures of growth of the target weed, *H. perforatum*. Significantly, the impact of *A. hyperici* appears largely similar on other *Hypericum* species including the native Australian forbs, *H. gramineum* and *H. japonicum*. This tentative finding clearly requires further glasshouse and field research and may reveal different host utilisation patterns.

6.5. Summary

The impact of *A. hyperici* on the growth of *H. gramineum* , *H. perforatum*, *H. japonicum* and *H. tetrapterum* was investigated (experiment 1). In light of the literature indicating that young plants may suffer higher levels of herbivory than older plants, the potential differential effects of mites on the growth of seedlings and adults of *H. gramineum* and *H. perforatum* was also examined (experiment 2). In experiment 1, mites reduced most measures of plant growth including root, shoot and total plant weights by 20 - 25% and their relative growth rates by 8 - 13%. Roots were the most adversely affected organs, apparently because herbivory of shoots induced re-allocation of root resources to the shoots, consistent with other shoot herbivores and 'source-sink' models of root:shoot ratio control. A lack of significant interactions between species and mites implied that mites caused similar growth reductions in all species. In experiment 2, seedlings displayed higher relative growth rates than adults. It was not possible to assess the impact of mites on plant growth in experiment 2, since the establishment and development of *A. hyperici* populations were severely limited. It is concluded that repetition of the experiments is desirable, perhaps under different conditions, and may reveal different host utilisation patterns.

CHAPTER 7

COMBINATIONS OF PLANT STRESS AND HERBIVORY BY *ACULUS HYPERICI* ON GROWTH OF *HYPERICUM GRAMINEUM* AND *H. PERFORATUM*

7.1. Introduction

There are many records of reductions in plant growth and productivity caused by herbivores (e.g. Crawley 1983; Sacchi *et al.* 1988; Crawley 1989; Fay and Hartnett 1991; Larson and Whitham 1991; Meyer and Whitlow 1992; Belsky *et al.* 1993; Karban and Strauss 1993; Kouki 1993; Trumble *et al.* 1993). Similarly, reductions in plant growth caused by abiotic stresses such as nutrient or water limitation, and other biotic stresses such as plant competition have been well documented (Kozlowski 1979; Cockfield and Potter 1986; Larsson 1989; Price 1991; Fox and Morrow 1992; Louda and Collinge 1992; Bozsa and Oliver 1993; Iason and Hester 1993; Turkington *et al.* 1993; Wilson and Tilman 1993).

Despite several hypotheses explaining the nature of herbivore-plant interactions, including those of Rhoades and Cates (1976), White (1984) and Coley *et al.* (1985), which have focused, to varying degrees, on the importance of tissue nutrients and/or chemistry as either phagostimulants or deterrents, few studies examine the combined effects of plant stress and herbivory on host plant growth. The scarcity of such investigations is surprising given Price's (1991) 'plant vigour hypothesis' that herbivores feed preferentially on vigorously growing plants, the number of studies reporting that stressed plants have higher tissue nitrogen concentrations than unstressed plants (e.g. Mitchell and Chandler 1939; Piene 1978; Stewart and Lahrer 1980; White 1984; Mattson and Haack 1987a,b; Larsson 1989; Louda and Collinge 1992; Waring and Cobb 1992), and that elevated nitrogen concentrations may lead to higher levels of herbivory, since plant herbivores are generally considered nitrogen limited (Mattson 1980).

In an extensive literature survey of herbivory, nutrient limitation, plant competition, and water stress, few studies were found that quantified the combined impact of biotic and abiotic stresses on plant growth and productivity.

7.1.1. Nutrient limitation and herbivory

Kaakeh *et al.* (1992) observed increased aphid density on fertilised apple trees (*Malus* sp.), and lower biomass of shoots, roots leaves and trunks of aphid-infested trees relative to aphid-free trees, although interactions between herbivory and nutrient enhancement were not examined. Clancy *et al.* (1993) observed that Douglas-fir trees (*Pseudotsuga menziesii*) resistant to the western spruce budworm (*Choristoneura occidentalis*) had lower levels of foliar nutrients but produced more radial trunk growth than susceptible trees. Waring and Price (1988) concluded that sawfly (*Euura lasiolepis*) damage of willow (*Salix lasiolepis*) is greater on rapidly growing, vigorous plants. The latter studies were conducted on tree species and none quantified, nor specifically investigated the manner in which combinations of herbivory and nutrient limitation/enhancement affect plant growth.

7.1.2. Plant competition and herbivory

Investigation of the combined effects of plant competition and herbivory has received more attention than combinations of nutrient limitation and herbivory. Cottam *et al.* (1986), for example, reported a synergism between plant competition and invertebrate herbivory of *Rumex obtusifolius*, in which beetle grazing reduced plant growth in competing plants, but not in non-competing plants. By contrast, Weiner (1993) found no synergism (statistical interaction) between snail herbivory and plant density on above-ground biomass of *Hypochaeris radicata*. Wiener concluded that increased snail density caused simple subtractive reductions on shoot biomass. Parker and Salzman (1985) found no multiplicative effects between herbivory and plant competitor removal on growth of the shrub *Gutierrezia microcephala*, despite a statistically significant interaction between these treatments on its survival.

Studies of competition and herbivory often reveal similar patterns to those examining interactions between fungal pathogenic attack and plant competition. Such studies generally show declines in plant productivity caused by the stresses in combination and individually (Groves and Williams 1975; Burdon *et al.* 1984; Paul 1989). The manner in which the stresses interact is investigated less frequently. However, Groves and Williams (1975) observed slightly greater than proportional reductions in plant growth following pathogenic infection of competitively stressed *Chondrilla juncea*.

7.1.3. Water stress and herbivory

Many studies describe the effects of water stressed plant tissues on herbivore growth, development and reproduction (Wearing 1967; Wearing and van Emden 1967; Bernays and Lewis 1986; English-Loeb 1989, 1990; Wagner and Frantz 1990; Waring and Price 1990; Louda and Collinge 1992; Mopper and Whitham 1992), but rarely consider potential interactions between the stresses. Cockfield and Potter (1986), however, observed a synergism between scale infestation and water stress in which combinations of the biotic and abiotic stresses caused greater than additive increases in the rate of leaf abscission in *Euonymus fortunei*. Louda and Collinge (1992) found that nitrogen and soluble carbohydrate concentrations were elevated in environmentally stressed bittercress (*Cardamine cordifolia*), leading to increased herbivory by chewing and leaf-mining insects and decreased plant size.

7.1.4. Experimental aims

Where biological control agents may survive and reproduce on non-target species in host-specificity trials, albeit at significantly lower rates, once released into the field the potential exists for these herbivores to inflict damage on non-target species. The possibility of damage is heightened if plant tissue quality for herbivores is enhanced by the increased availability of nitrogenous compounds during stress. In environmentally stressed plants, release of herbivores from nitrogen-limited growth may lead to synergistic reductions in plant growth. If stress also reduces the ability of the plants to tolerate herbivory, interactions between herbivores and environmental stress may lead to further decreases in plant growth. Clearly, a potential exists for biological control agents to have a significant impact on non-target species and/or to exert more effective biological control of weeds if host plants are stressed. This study aims to examine the hypothesis that stressed plants are more susceptible to herbivory than healthy plants. In so doing, three alternative hypotheses are tested. These are that (a) combinations of stresses may have simple additive (or subtractive) effects on plant growth, (b) that combinations of stresses interact in a simple multiplicative way, causing proportional reductions in plant growth, and (c) that stresses might interact in more complex, synergistic ways.

Combinations of herbivory and nutrient limitation, plant competition and water stress are investigated in three experiments. In the last, a 3-way interaction between two herbivores, *Aculus hyperici* and another *Hypericum perforatum* biological

control agent, *Aphis chloris* (see chapter 1), and water stress is investigated. To date, such a complex stress-interaction experiment has not been reported.

7.2. Materials and Methods

Three experiments were conducted to examine combinations of herbivory and plant stress on growth of *H. gramineum* and *H. perforatum*. In the first (experiment 1), combinations of plant nutrient limitation and herbivory by *A. hyperici* on plant growth were investigated. Experiment 2 examined the combined effects of plant competition and *Aculus*-herbivory on growth, while experiment 3 investigated combinations of water stress, and herbivory by *Aculus hyperici* and *Aphis chloris*. The experiments comprised randomised factorial blocks with all possible treatment combinations applied to *H. gramineum* and *H. perforatum*.

7.2.1. Experimental designs and treatments

7.2.1.1 Experiment 1 - Nutrient limitation

The experiment comprised seven replicate blocks, each containing four treatment combinations for each *Hypericum* species:

(1) Mite herbivory - two levels of mite herbivory: plants either infested with *A. hyperici* or free of infestation.

(2) Nutrient levels - two nutrient levels achieved by watering daily either with 250 mL of 100% Hoagland's solution (Hoagland 1920; Hoagland and Arnon 1938; 'High' nutrient regime), or with 250 mL of tap water ('Low' nutrient regime).

7.2.1.2 Experiment 2 - Plant competition

This experiment consisted of five replicate blocks, each comprising eight treatment combinations for each *Hypericum* species:

(1) Mite herbivory - two levels of mite herbivory: plants either infested with *A. hyperici* or free of infestation.

(2) Plant competition - four levels of plant competition were achieved by growing *Hypericum* free of competition and in combinations of root and/or shoot competition with the native Australian grass, *Themeda triandra*, as summarised diagrammatically in figure 7.1, and below:

(i) No root competition and no shoot competition (-R-S): pots containing an individual *Hypericum* plant.

(ii) No root competition, but shoot competition (-R+S): pairs of pots, one containing three *T. triandra* plants and the other containing a single *Hypericum* plant such that the roots of the grasses and the *Hypericum* seedling were separated, but the shoots of all plants were together. Where *Hypericum* shoots were not obviously shaded by *T. triandra*, they were manually inter-twined among the grass shoots.

(iii) Root competition, but no shoot competition (+R-S): pots containing three *T. triandra* plants and a single *Hypericum* plant, with shoots of the former separated from those of the latter by a clear plastic screen (20 x 30 cm).

(iv) Both root and shoot competition (+R+S): pots containing three *T. triandra* plants and a single *Hypericum* plant with shoots of the latter growing amongst those of the grasses, and where failing to do so, manually inter-twined, as above.

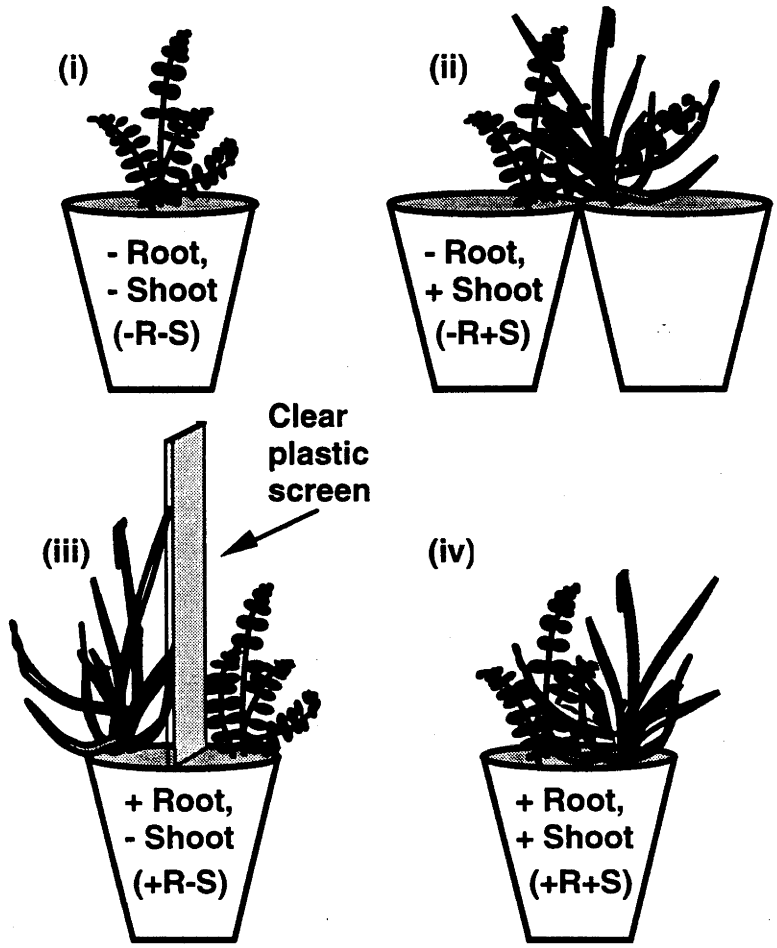


Fig. 7.1 Summary of competition treatments achieved with combinations of either *H. gramineum* or *H. perforatum* grown in pots in various combinations of root and/or shoot competition with the grass *T. triandra*. All treatments were applied to both *Hypericum* species in either the presence or absence of *A. hyperici*. Roman numerals refer to competition treatments, outlined above.

7.2.1.3 Experiment 3 - Water stress and aphid herbivory*

The experiment comprised six replicate blocks, each containing six treatment combinations of each *Hypericum* species:

(1) Water stress - Two watering levels: a high watering regime in which soils were watered to field capacity every 1-2 days, and a low regime in which soils were maintained between 10% and 14% field capacity.

(2) Aphid herbivory - Two levels of aphid herbivory: either plants were infested with *A. chloris* or free of infestation.

(3) Mite herbivory - Two levels of mite herbivory: either plants were infested with *A. hyperici* or free of infestation.

In experiments 1 and 2 (nutrient limitation and plant competition), mite-free treatments were sprayed every 4 - 6 weeks with Omite® miticide. Its negligible effect on growth, and plant tissue nitrogen and phosphorus content was established in a separate experiment (see chapter 6). Spraying with Omite was not possible in experiment 3 (water stress and aphid herbivory) as the miticide's effect on *A. chloris* was unknown. All pots in this experiment were therefore enclosed in fine, gauze-like paper to contain mites and/or aphids on plants. The photosynthetically active radiation in the bags ranged from 240 - 700 $\mu m\ m^{-2}\ s^{-1}$, while adjacent to the pots, but outside the bags, it ranged from 500 - 1100 $\mu m\ m^{-2}\ s^{-1}$. Based on four non-covered *H. gramineum* and five non-covered *H. perforatum* controls growing alongside the covered experimental plants, there was no indication of light-limited growth or changes in leaf morphology of bagged plants during the experiment.

7.2.2. *Cultivation and preparation of plant material*

All plants in the experiments were grown from locally collected seeds and germinated as previously described (chapters 2 and 6). Once germinated, all seedlings were transplanted to pots and grown in a shade house for about 6 weeks. After this time, 10 of each species were sub-sampled, as described in chapter 6, to estimate initial root and shoot dry weights. The remaining seedlings were randomly assigned a position and treatment in the experiments.

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* A paper based on this experiment has been published in *Oecologia* (1993), vol. 96(4) 517-525.

7.2.3. Preparation of pots

7.2.3.1 Experiments 1 & 2 - Nutrient limitation & Plant competition

In the nutrient limitation (experiment 1) and plant competition (experiment 2) experiments, seedlings were transplanted into 12 cm diameter pots of approximately 1 L capacity, containing a 2:1 mixture of coarse sand to clay. Pots in experiment 2 were surface-fertilised at the beginning of the experiment with approximately 2.5 g of Osmocote® (N:P:K - 16:3.5:10) slow-release fertiliser and watered daily to capacity with tap water. In the nutrient limitation experiment, plants were watered with either nutrient solution or tap water, as outlined above. Both trials were terminated 24 weeks after transplantation.

7.2.3.2 Experiment 3 - Water stress and aphid herbivory

Results from a pilot experiment indicated that *H. gramineum* and *H. perforatum* seedlings grown in a 3:1 mixture of clay to coarse sand and maintained just above wilting point (about 10% soil dry weight water content) by daily addition of water produce significantly ($P < 0.01$) less root and shoot biomass than plants maintained near field capacity. The same soil mixture and watering regimes were used in this study.

To determine the weight of all experimental pots near field capacity, the soil mixture was oven-dried at approximately 95° C for 30 h before filling pots, which had been lined with filter paper to prevent leakage of soil. Soil-filled pots were weighed and their soil water content recorded as 0%. All pots were then watered, allowed to drain for 30 mins, watered again, and allowed to drain for 60 mins before determining their gain in weight (recorded as field capacity). These measures were used to determine the total weight that pots should attain to achieve the pre-determined soil water content. Individual pots were maintained at their required soil water contents, according to the watering regime.

Approximately 3 weeks prior to commencement of the experimental treatments seedlings were transplanted to pots of 1 L capacity, as above, and fertilised with a water-soluble fertiliser (N:P:K approx. 23:4:18). On the first day of the experiment, all pots were also surface-fertilised with 2.5 g of Osmocote®. The

required soil water contents were achieved after 7 days, and mites and aphids were introduced after 17 and 30 days respectively. The experiment ran for 100 days from the date seedlings were transplanted.

7.2.4. Infestation of plants with herbivores

Mites were introduced to '+mite' treatments using the same technique described in chapter 6 (section 6.2.3.). Aphids were introduced to experiment 3 in the same manner, though vegetative buds were infested with approximately 30 adults. Where establishment of either herbivore failed, the inoculation was repeated after 4 weeks.

7.2.5. Estimating leaf areas

The mean individual leaf area (ILA) and the total plant leaf area (TPLA) were estimated by simple linear regression, as described in chapter 6 (section 6.2.4.). A pooled regression model, combining leaf areas and shoot weights from *H. gramineum* and *H. perforatum* was employed, since the regression coefficients for the two species did not differ significantly (see Table 6.1, chapter 6). The model for these two species (excluding *H. japonicum* and *H. tetrapterum*, which are included in the pooled model of chapter 6) can be summarised as,

$$\log_{10} \text{leaf area (TPLA)} = 4.34 + 1.14 \times \log_{10} \text{shoot weight}; \; r^2 = 0.97$$

7.2.6. Estimating root growth in experiment 2 (plant competition)

It was not possible to separate the roots of experimental *Hypericum* plants from those of their *T. triandra* competitors in '+ root competition' treatments of experiment 2. In order to estimate the effects of experimental treatments on the roots of such replicates, root mass was estimated based on the results of a separate experiment (experiment 2a, Plant density; see below). This experiment examined the root:shoot ratio of *H. gramineum* and *H. perforatum* grown under intra-specific competition at various densities. At the conclusion of experiment 2a, the average root and shoot weight of plants grown in each pot at each density were estimated.

A simple linear regression of $\log_{10}$ mean root:shoot ratio on $\log_{10}$ mean shoot weight from the data collected in the plant density experiment was used to predict the root:shoot ratio of '+ root competition' plants in experiment 2 (plant

competition). This estimate assumes that for plants of similar shoot weight, the root:shoot ratio will be the same whether competition is intra-specific or due to *T. triandra*. Knowing the shoot weight and having an estimate of the root:shoot ratio of such plants, it was then possible to estimate their root mass from the root:shoot ratio in the '- root competition' treatments. As a result of the indirect means of estimating root:shoot ratios and subsequently root mass, these variables have not been analysed formally, although trends in the data have been compared with the results of '- root competition' replicates.

7.2.6.1 Experiment 2a - Plant density

(a) Experimental design and cultivation of plants

The experiment was a randomised block design, comprising six blocks. Each block contained one replicate of each of *H. gramineum* or *H. perforatum* planted at 1, 3, 9 or 27 plants per pot.

Seedlings of *H. gramineum* and *H. perforatum* were germinated from locally collected seeds, as described in chapters 2 and 6. At the two-leaf stage, seedlings were transplanted to 1 L capacity pots containing a 2:1 mixture of coarse sand to clay. After 4 weeks, when the *H. gramineum* seedlings were about 1 cm in height and the *H. perforatum* seedlings were about 2 cm in height, the pots were thinned to the required plant density. All pots were then fertilised with Aquasol® liquid fertiliser (N:P:K - 23:4:18) and subsequently, 2.5 g of Osmocote® slow-release fertiliser (N:P:K - 16:3.5:10). The pots were allowed to stand for a week, before randomly assigning treatments to experimental blocks. Pots were watered daily for the duration of the experiment, till its termination, 12 weeks after thinning. Upon termination, the shoots of each pot were harvested, and the roots from each, washed free of soil, before oven-drying at 60°C for 5 days.

(b) Analysis of experiment 2a - Plant density

As noted above, the mean root:shoot ratio of an individual plant from each pot was calculated. This variate was then analysed by 2-way analysis of variance, after logarithmic data transformations. For each species, separate simple linear regressions of $\log_{10}$ root:shoot ratio on $\log_{10}$ shoot weight were then used to

estimate the root weight in '+ root competition' replicates of experiment 2 (plant competition).

The accuracy of predictions based on these regressions was tested by 'predicting' the root weight of plants in '- root competition' treatments, and comparing the predictions with the empirically derived values using ANOVA. Main factors within the ANOVA were 'derivation' - with the variables of root weight and root:shoot ratio either predicted or empirically derived, 'species' - either *H. gramineum* or *H. perforatum*, and ' competition treatment' - either -R-S or -R+S. Logarithmic data transformations were required prior to this analysis.

7.2.7. Nutrient analyses

Shoot and root samples from all experimental plants were assayed for total nitrogen and phosphorus content. Results are reported as percentage (%) and parts per million (ppm) of tissue dry weight respectively. Up to 0.3 g of oven-dried tissue was digested by a micro Kjeldahl technique, before analysing with a Technicon Auto-analyser II®.

7.2.8. Measured growth parameters and analyses of data

At completion of the experiment, plants were harvested and several growth and nutrient parameters measured. The parameters that were measured and analysed in chapter 6 (see chapter 6, section 6.2.6.) were also estimated and analysed for the present three experiments. For all experiments in the current chapter, the units of measurement are the same as for those in chapter 6: root, shoot and total mass (g), root:shoot ratio (g g^{-1}), shoot length (cm), total plant leaf area (TPLA, mm^2) and relative growth rate (RGR, g g^{-1} $week^{-1}$). In addition, the specific leaf area (SLA, mm^2 g^{-1}), an indication of leaf toughness, was measured in experiment 1 (nutrient limitation), as was the individual leaf area (ILA, mm^2), calculated as the mean of the scored leaf area, according to the size class category to which it had been allocated (see chapter 6, section 6.2.4.). Also, the total plant relative growth rate (total RGR, see chapter 6) was measured in experiment 2 (plant competition), though ILA was not. Although most data were logarithmically transformed prior to analysis, the observed, arithmetic means are presented in the results to simplify comparisons.

Analyses of variance (ANOVA) were performed for each growth parameter, as outlined in chapter 6. Considering analyses of all three stress experiments in the present chapter, approximately 400 tests of significance (each with 7 - 14 factors and interactions x 14 - 15 growth and nutrient parameters) were performed, which should yield about 20 type 1 errors at $P \leq 0.05$. As in chapter 6, this is considered misleading. Accordingly, a 1% significance level has also been adopted in this chapter, since probabilities in the range $P = 0.01 - 0.05$ are considered too tentative to warrant more than cursory consideration.

A consequence of logarithmic data transformations is that simple additive/subtractive effects in an ANOVA represent multiplicative/divisive effects on a linear (untransformed) scale. Factor interactions in an ANOVA of log-transformed data represent other, more complex relationships, including simple additive effects. Hypotheses (a) and (c) above, that a combination of stresses lead to additive/subtractive, or more complex reductions on plant growth respectively, would be supported by statistically significant interactions between factors in an ANOVA. Hypothesis (b), that combinations of stresses lead to multiplicative (proportional) reductions in growth would be supported by significant main factor effects and no significant interactions in an analysis of log-transformed data.

Results of the three experiments are presented in turn. Consistent trends and discrepancies are then noted in the discussion section (section 7.6.).

7.3. Results of Experiment 1 - Nutrient limitation

Probabilities ($P < 0.05$) for all 101 main effects and interactions examined in the ANOVA are summarised in Table 7.1. The majority of significant ($P \leq 0.01$) effects were generated by the main effects: species, mites and nutrients. For all growth parameters, significant ($P \leq 0.01$) main effects resulted from the 'high stress' treatments (+mites and low nutrients) reducing growth. That species was usually a significant ($P \leq 0.01$) factor highlights the distinct physiology of *H. gramineum* and *H. perforatum* and underlines their ecological differences.

Table 7.1. Summary table of ANOVA for each factor and interaction ($P \leq 0.05$) in the nutrient limitation experiment. TPW = total plant weight; R:S ratio = root:shoot ratio; ILA = individual leaf area; TPLA = total plant leaf area; SLA = specific leaf area; RGR = relative growth rate; N/A = not applicable; N = nitrogen; P = phosphorus. See text for the units of each variable.

Factors & Interactions	**P - values**													
	root weight	shoot weight	TPW	R:S ratio	shoot length	ILA	TPLA	SLA	fruit	root RGR	shoot RGR	N - shoot	N - root	P - shoot	P - root
Species	<0.001		0.013	<0.001	<0.001	<0.001		<0.001	N/A	<0.001		<0.001		0.009	
Mites	<0.001									0.029		<0.001		<0.001	
Nutrients	0.005	<0.001	<0.001	0.002	<0.001	0.003	<0.001		0.005		<0.001	<0.001	<0.001	<0.001	
Species x Mites	<0.001			0.047				0.011	N/A	0.027				0.006	
Species x Nutrients					0.021				N/A						
Mites x Nutrients													0.028		
Species x Mites x Nutrients									N/A						

Of 57 possible 2- and 3-factor interactions in the ANOVA, only 8 (14%) showed any clear trends ($P \leq 0.05$; see Table 7.1). All but one of these (mites x nutrients for the root nitrogen content) included species and consistently indicated that growth of *H. perforatum* is more severely retarded by mites than *H. gramineum*. Of relevance to the biological control of the former species are the species x mites interactions in which the root relative growth rate (root RGR; $P = 0.027$) and root:shoot ratio ($P = 0.047$) of *H. perforatum* are significantly decreased. Reductions in the same growth parameters of *H. gramineum* were negligible.

7.3.1. Nitrogen and phosphorus content (experiment 1)

Shoot nitrogen and phosphorus concentration were significantly ($P \leq 0.01$) affected by the nutrient regime and the presence of mites, but not by factor interactions (Fig. 7.2a-d, Table 7.1). Differences in nutrient concentration were usually apparent in the shoots, not the roots, in which nitrogen and phosphorus differences were generally minor ($P > 0.05$). Where significant ($P \leq 0.01$) differences were detected, such as in the effect of high nutrients on root nitrogen content, *H. perforatum* had higher nitrogen and phosphorus concentrations than *H. gramineum*. In the only significant factor interaction (species x mites, $P = 0.006$), the presence of mites was associated with a higher concentration of phosphorus in *H. perforatum* shoots. The trend was the same in *H. gramineum* shoots, though insignificant ($P > 0.05$; Fig. 7.2e).

Calculation of the root:shoot nitrogen ratio (total root nitrogen content/total shoot nitrogen content, where total contents are determined by multiplying the nitrogen concentration [ppm/g] by the root and shoot dry weights, respectively) suggests that mites cause re-allocation of nitrogen from the roots to the shoots, since the ratio is decreased (Fig. 7.2f). Similar changes in the root phosphorus:shoot phosphorus ratios were evident in mite-infested plants (Fig. 7.2f).

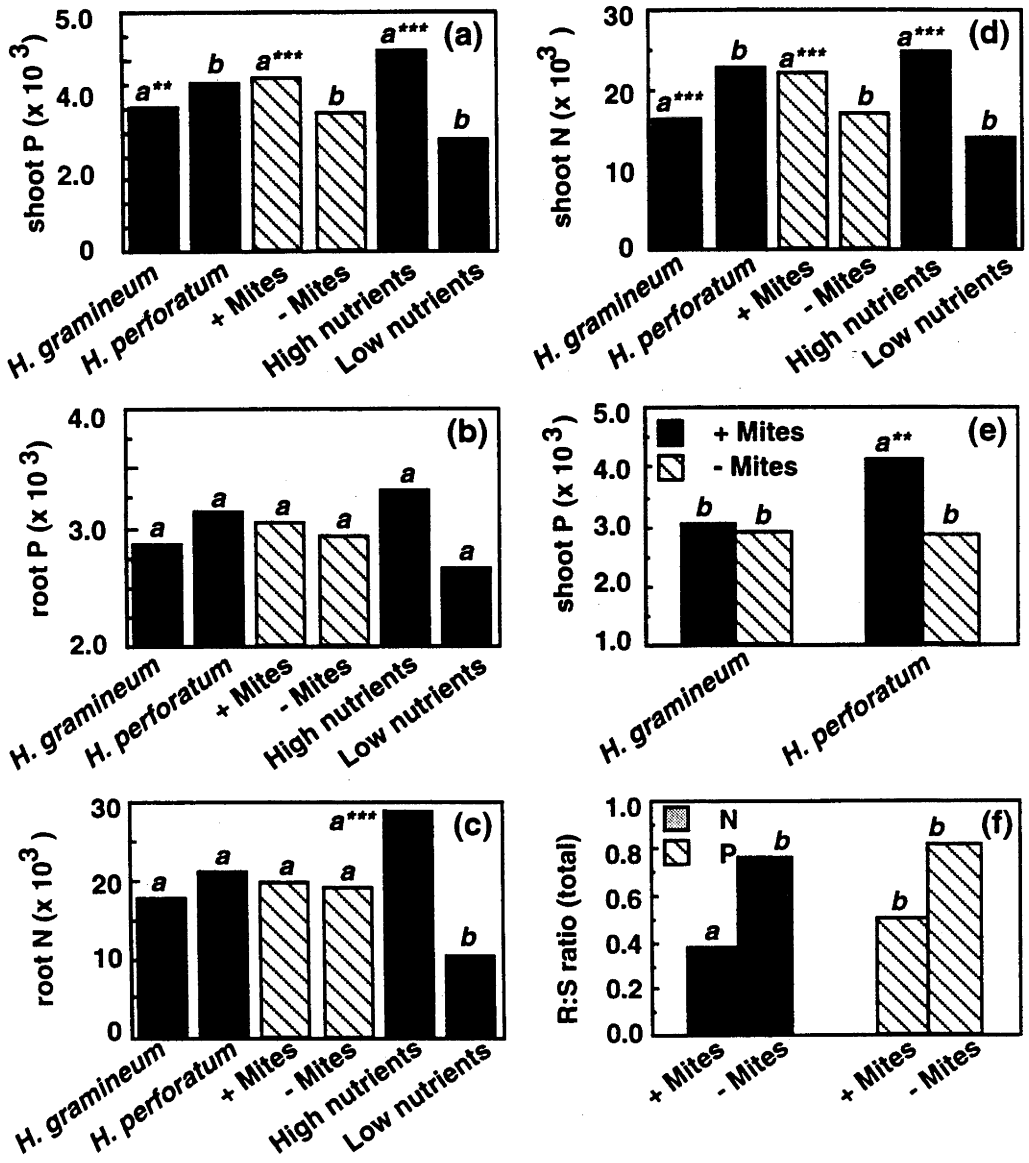

Fig. 7.2 (a-d) Mean tissue nutrient concentration (N = nitrogen, P = phosphorus; ppm) after experimental treatments in the nutrient limitation experiment. Significant differences ($P \leq 0.05$) between treatments of the main factors species (■), mites (▨) and nutrients (■) are indicated by columns with different lettering. The significance of F-tests for treatments within the main factors is also indicated ($P \leq 0.05$*, $P \leq 0.01$**, and $P \leq 0.001$***); (e) mites x species interaction on shoot phosphorus concentration (ppm); (f) root:shoot total nutrient ratios.

7.3.2. Root system (experiment 1)

H. perforatum produced significantly ($P < 0.001$) more root mass than *H. gramineum* during the experiment (Fig. 7.3a). Overall, both low nutrients and the presence of mites severely ($P \leq 0.005$) reduced root mass. On average root mass was reduced by 37% in mite-infested treatments, and the root relative growth rate was reduced by 7%. However, the highly significant ($P < 0.001$) species x mites interaction indicates that *H. perforatum* root mass was dramatically decreased (reduced by 46%), while the decrease in *H. gramineum* was minimal (6%, $P > 0.05$; Fig. 7.4). As noted, the species x mites interaction was also more adverse for *H. perforatum*'s root relative growth rate and root:shoot ratio than for that of *H. gramineum*.

7.3.3. Shoot system (experiment 1)

Shoot dry weight did not differ significantly between species or mite treatments ($P = 0.787$, species; $P = 0.512$, mites) or treatment interactions ($P \geq 0.463$) during the experiment, although when stressed, a trend in both species towards decreased shoot mass was evident. Low nutrients produced the only significant ($P < 0.001$) result, reducing shoot weight (Table 7.1, Fig. 7.3b). Although marginal, the average reduction in the shoot mass of *H. gramineum* and *H. perforatum* attributable to mites was about 27% and 28% respectively. In *H. gramineum*, mites reduced the shoot relative growth rate by approximately 4%, although they caused no change in that of *H. perforatum*. In contrast to the root system, mites had no significant ($P \leq 0.01$) effect on the shoots of either *H. gramineum* or *H. perforatum*, as estimated by shoot length, the individual leaf area (ILA), total plant leaf area (TPLA), the specific leaf area (SLA), the number of fruits produced, or the shoot relative growth rate (shoot RGR; Table 7.1, Fig. 7.3a-k). Low nutrients, by comparison, severely decreased ($P \leq 0.01$) each of these measures of growth. No significant ($P \leq 0.01$) factor interactions were detected for any of the shoot growth parameters.

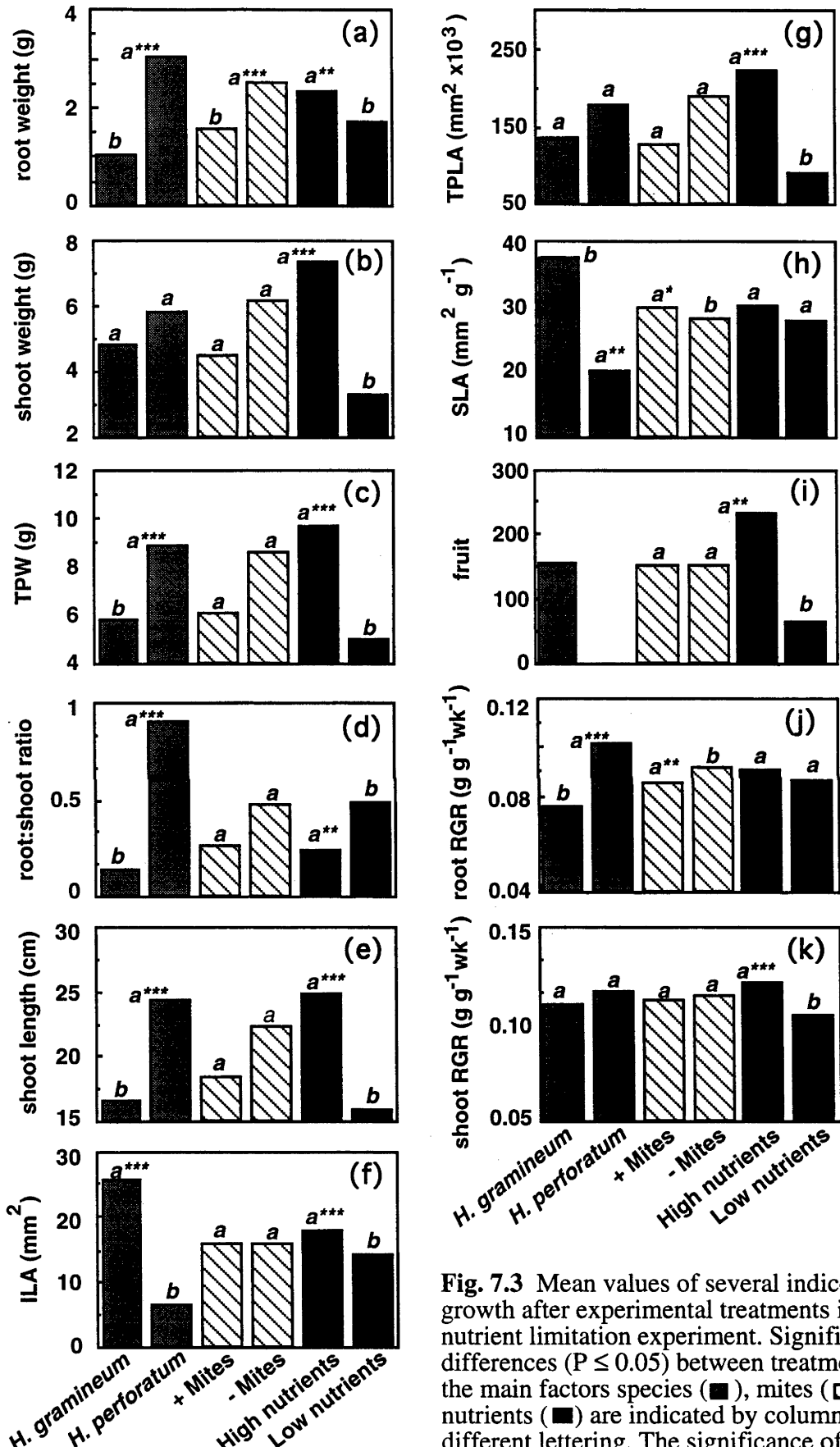

Fig. 7.3 Mean values of several indices of growth after experimental treatments in the nutrient limitation experiment. Significant differences ($P \leq 0.05$) between treatments of the main factors species (■), mites (▨) and nutrients (■) are indicated by columns with different lettering. The significance of F-tests between treatments of the main factors is also indicated ($P \leq 0.05$*, $P \leq 0.01$**, and $P \leq 0.001$***). Note that (i) applies to *H. gramineum* only.

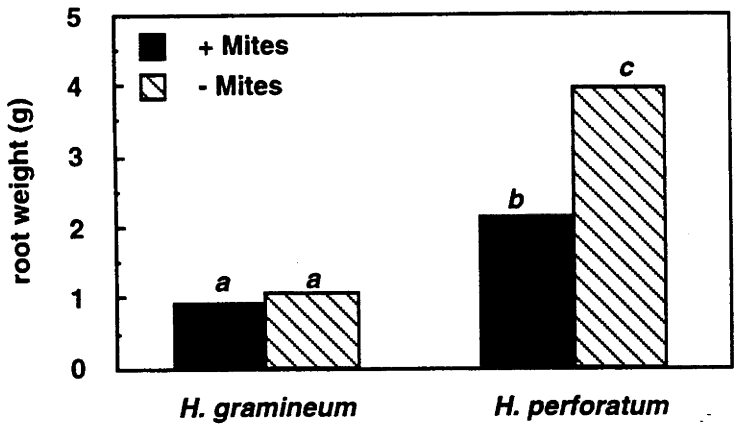

Fig. 7.4 Histogram of the mites x species interaction on *Hypericum* root mass (g). Significant differences ($P \leq 0.05$) are indicated by columns with different letters. The overall F-test was highly significant ($P < 0.001$).

7.3.4. Whole plant effects (experiment 1)

Total plant weight (TPW) was significantly ($P \leq 0.01$) reduced under the low nutrient regime; species differences in total plant weight were relatively marginal ($P = 0.013$, Table 7.1). The root:shoot ratio increased slightly ($P = 0.002$) under low nutrients and was marginally decreased by mites. No other main factors or interactions were significant ($P > 0.05$) for either parameter.

Table 7.2 summarises the combined effects of individual stresses (low nutrients and +mites) on *Hypericum* growth. The combination of low nutrients and the presence of mites leads to simple proportional reductions in plant mass relative to the unstressed control plants (high nutrients and -mites), close to the levels predicted from a model of proportional growth under combinations of stress (calculated as 100x the proportional growth under nutrient limitation x the proportional growth after *Aculus*-herbivory). Taking the high nutrient and mite-free specimens to represent 100% growth, nutrient limitation in the presence of herbivory reduced *Hypericum* total plant weights to 34% of controls, very close to the expected value of 35%. A potential synergism between stresses leading to greater than proportional decreases in growth is not obvious, as is consistent with the general lack of significant ($P \leq 0.05$) interaction terms in the ANOVA.

Table 7.2. Relative (%) plant growth (total plant weight) of *Hypericum* in relation to various stress combinations, taking the unstressed plants to represent 100%, for the three experiments in chapter 7. Expected values (%) for the hypothesis that combined stresses yield the product of the proportional growth under each separate stress are presented in round brackets. Weights of the unstressed controls are presented in square brackets. Note that data for *H. gramineum* and *H. perforatum* have been pooled since species differences were only marginally significant ($P \geq 0.013$).

Experiment	Stress Combinations				
	Mites	High nutrients		Low nutrients	
Nutrient limitation	- Mites	100 [11.6 g]		49	
	+ Mites	71		34 (35)	
		No competition, -R-S		Root & Shoot Competition, +R+S	
Plant competition	- Mites	100 [11.5 g]		55	
	+ Mites	89		49 (49)	
		High water		Low water	
		- Aphids	+ Aphids	- Aphids	+ Aphids
Water stress and aphid herbivory	- Mites	100 [2.4 g]	80	33	22
	+ Mites	55	48	18	16 (14)

7.4. Results of Experiment 2 - Plant competition

7.4.1. Experiment 2a - Plant density

The main factors species and plant density had highly significant ($P < 0.001$) effects on the average root:shoot ratio, as did the species x density interaction ($P = 0.001$). Overall, the root:shoot ratio of *H. perforatum* was greater than that of *H. gramineum* (Table 7.3). With increasing plant density, the average root:shoot ratio of both species tended to increase, although the difference was marginal ($P > 0.05$) for *H. perforatum*.

Table 7.3 Average root:shoot ratios for *H. gramineum* and *H. perforatum* seedlings grown at various plant densities. The F-test for the species x density interaction was highly significant (P = 0.001). Differences (P ≤ 0.05) between treatments are indicated by differing superscript letters.

Plant density (plants pot^{-1})	Root:Shoot ratio	
	H. gramineum	*H. perforatum*
1	0.51^{a}	0.88^{c}
3	0.40^{a}	1.12^{c}
9	0.57^{ab}	1.24^{c}
27	0.73^{b}	1.25^{c}

7.4.2. Regression of root:shoot ratio on shoot mass

Separate linear regressions of the $\log_{10}$ mean root:shoot ratio on the $\log_{10}$ mean shoot mass were modelled for *H. gramineum* and *H. perforatum* (Fig. 7.5). These models were used to estimate indices of root growth for '+ root competition' treatments in experiment 2 (plant competition).

7.4.3. Accuracy of regression predictions

Table 7.4 summarises the mean empirical values for root mass and the root:shoot ratio of '- root competition' replicates and those of the 'predictions', derived from the linear regression employed in experiment 2a. Predictions of root growth and the root:shoot ratio significantly (P < 0.001) underestimate the observed values. Main factors of the ANOVA (species, derivation and competition treatment) all differed significantly (P ≤ 0.011) for both root weight and root:shoot ratio. Overall, the predictions for the root mass of *H. gramineum* and *H. perforatum* were underestimated by 36% and 46% respectively, while the predictions underestimated the root:shoot ratio of these species by 26% and 41% respectively. That there were no significant (P ≥ 0.195) interactions in the ANOVA between the main factors indicates that the underestimation was similar for both *H. gramineum* and *H. perforatum*, and for both -R-S and -R+S treatments. Relatively, therefore, the magnitude of differences between treatments remains the same. In order to compare root mass (and associated indices) under different experimental treatments, the data presented for roots derive from the regression predictions. Where observed values for '-R' treatments differ from 'predictions' for the same treatments, the difference is also indicated.

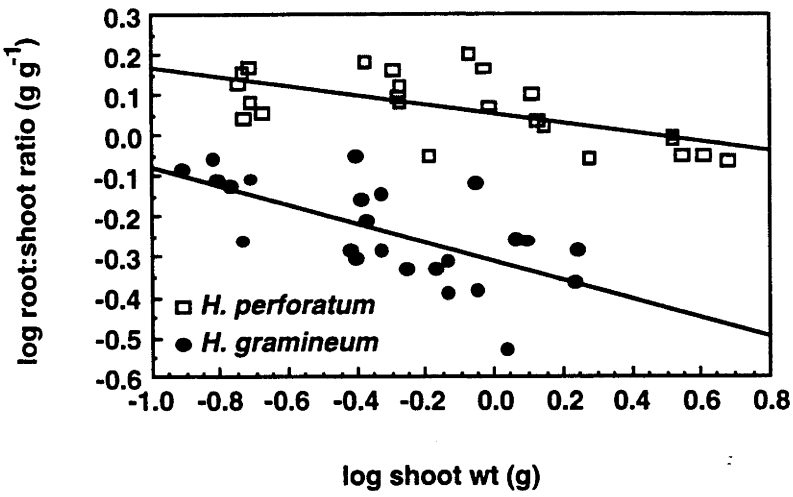

Fig. 7.5 Plot of the regression models based on data collected in experiment 2a (plant density), used to predict the root:shoot ratios (R:S) and which enabled estimation of root weight in '+ root competition' treatments of experiment 2 (plant competition). Separate regression models were used for each species. Both regressions were significant ($P \leq 0.007$). Equations for the models are as follows, with 95% confidence limits indicated,

H. gramineum: Log_{10} R:S = -0.33 ± 0.05 - 0.24 ± 0.12 x log_{10} shoot wt; $r^2 = 0.42$,

H. perforatum: Log_{10} R:S = -0.05 ± 0.03 -0.12 ± 0.06 x log_{10} shoot wt; $r^2 = 0.42$.

Table 7.4 Arithmetic means of the empirically derived values for root mass and root:shoot ratio in the plant competition experiment (experiment 2), and their respective means, as predicted by the simple linear regression employed in the plant density experiment (experiment 2a). Overall, the empirical and the predicted estimates differed significantly ($P < 0.001$), though no 2-way or 3-way factor interactions were significant ($P \geq 0.105$).

Growth index	Species	Competition Treatment			
		-R-S		-R+S	
		Empirical	Predicted	Empirical	Predicted
Root wt (g)	*H. gramineum*	5.98 ± 0.91	2.43 ± 0.12	2.25 ± 0.38	1.92 ± 0.19
	H. perforatum	13.18 ± 1.11	5.06 ± 0.29	8.51 ± 1.21	3.85 ± 0.40
Root:shoot	*H. gramineum*	0.67 ± 0.09	0.28 ± 0.01	0.33 ± 0.04	0.31 ± 0.01
ratio (g g^{-1})	*H. perforatum*	2.39 ± 0.17	0.91 ± 0.01	2.12 ± 0.17	0.95 ± 0.01

7.4.4. Experiment 2 - Plant competition

Probabilities ($P < 0.05$) for all statistically analysed factors and interactions in the plant competition experiment (experiment 2) are summarised in Table 7.5. It is again evident that the majority of significant ($P \leq 0.01$) effects resulted from the main factors, particularly species and treatment, indicating that plants within the experiment were competitively stressed. Mites consistently, though marginally ($P > 0.01$), reduced most measures of growth.

In general, factor interactions were not significant ($P > 0.05$, Table 7.5) in the ANOVA. The interaction between species and mites was marginal for all measures of growth. Nevertheless, a consistent trend towards decreased growth in the presence of mites was more severe for *H. perforatum* than for *H. gramineum*. This trend emphasises the host-specificity of *A. hyperici*.

7.4.4.1 Nitrogen and phosphorus content (experiment 2)

Results suggest that *H. gramineum* generally has higher concentrations of root and shoot nitrogen and phosphorus than *H. perforatum* (Fig. 7.6a-d). Differences between the species were highly significant for all measures of tissue nutrients except root phosphorus ($P < 0.001$ for shoot nitrogen and phosphorus , $P = 0.006$ for root nitrogen, and $P = 0.212$ for root phosphorus).

It was not possible to determine the nutrient concentration in roots of plants subjected to '+ root competition' treatments. However, the concentration of nitrogen within roots of '- root competition' treatments increased with competition between shoots ($P = 0.003$). In such treatments, shoot competition had no effect on root phosphorus content ($P = 0.988$, Fig. 7.6). Competition between grass and *Hypericum* roots decreased ($P < 0.001$) the nitrogen and phosphorus concentration of shoots, although differences between the root competition treatments (+R-S and +R+S) were insignificant ($P > 0.05$). By contrast, the trend for shoot competition was to increase shoot nutrient concentration, but the effect was only slight ($P > 0.05$). In general, mites caused increases in shoot nutrients, and concomitant decreases in those of the roots, although differences between mite-infested and mite-free plants were slight ($P \geq 0.568$).

Table 7.5. Summary table of ANOVA for each factor and interaction ($P \leq 0.05$) in the plant competition experiment. TPLA = total plant leaf area; RGR = relative growth rate; N/A = not applicable; N = nitrogen; P = phosphorus. See text for units of variables.

Factors & Interactions	**P - values**								
	shoot weight	shoot length	TPLA	fruit	shoot RGR	N - shoot	N - root	P - shoot	P - root
Mites									
Species	0.003	<0.001	0.003	N/A		<0.001	0.006	<0.001	
Treatment	<0.001	0.008	<0.001	0.031	<0.001	<0.001	0.003	<0.001	
Species x Mites		0.029		N/A					
Species x Treatment				N/A			0.012		
Mites x Treatment						0.011		0.028	
Species x Mites x Treatment				N/A					

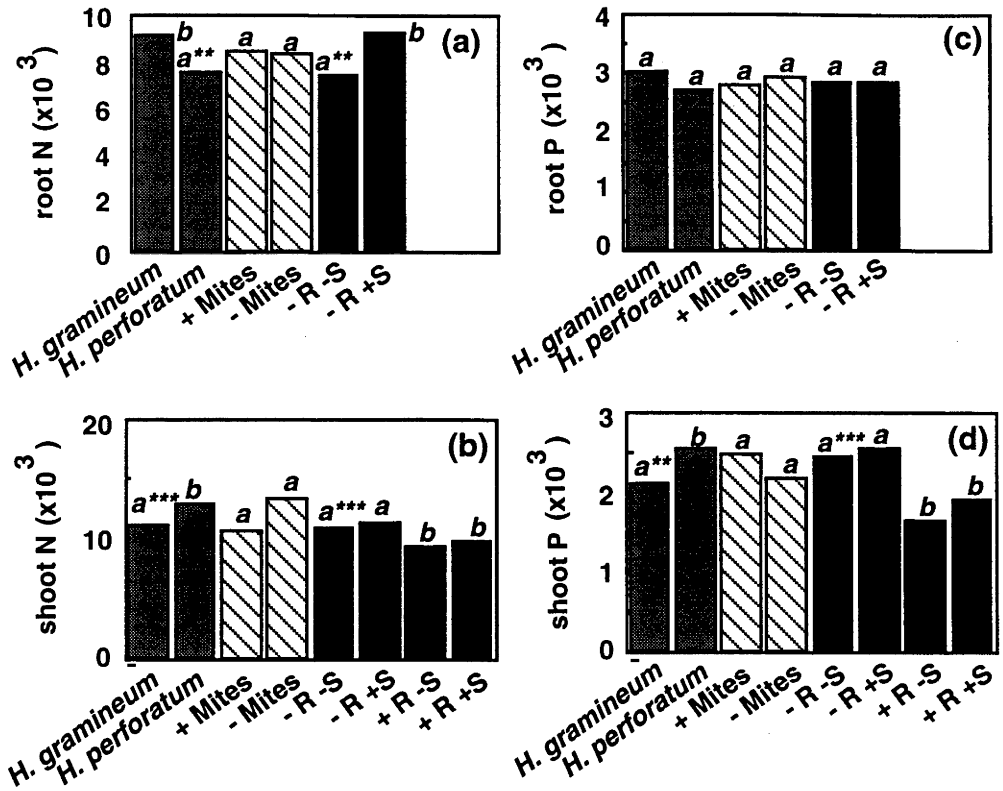

Fig. 7.6 Mean tissue nutrient concentration (N = nitrogen, P = phosphorus; ppm) after experimental treatments in the plant competition experiment. Significant differences ($P \leq 0.05$) between treatments of the main factors species (■), mites (□) and competition treatment (■) are indicated by columns with different lettering. The significance of F-tests between treatments of the main factors is also indicated ($P \leq 0.05$*, $P \leq 0.01$**, and $P \leq 0.001$***).

7.4.4.2 Root system (experiment 2)

As observed above (section 7.4.3.), estimates of root growth in '+ root competition' treatments of the plant competition experiment are likely to be underestimated by up to about 46%, although the relative differences between experimental treatments remains similar. Mites caused a clear trend towards decreased root growth, as measured by both absolute mass, and the relative growth rate. Overall, mites reduced root growth by about 16%, and decreased the relative growth rate of roots by about 3%. (Fig 7.7a and d). The effects appeared more pronounced on *H. perforatum* than on *H. gramineum*: Root mass and relative growth rate were reduced by about 20% and 4% respectively, in the target weed,

and by approximately 3% and 1% in the non-target native species. Competition also reduced root growth and relative growth rates. Root competition appeared more adverse to root growth than did shoot competition (Fig. 7.7).

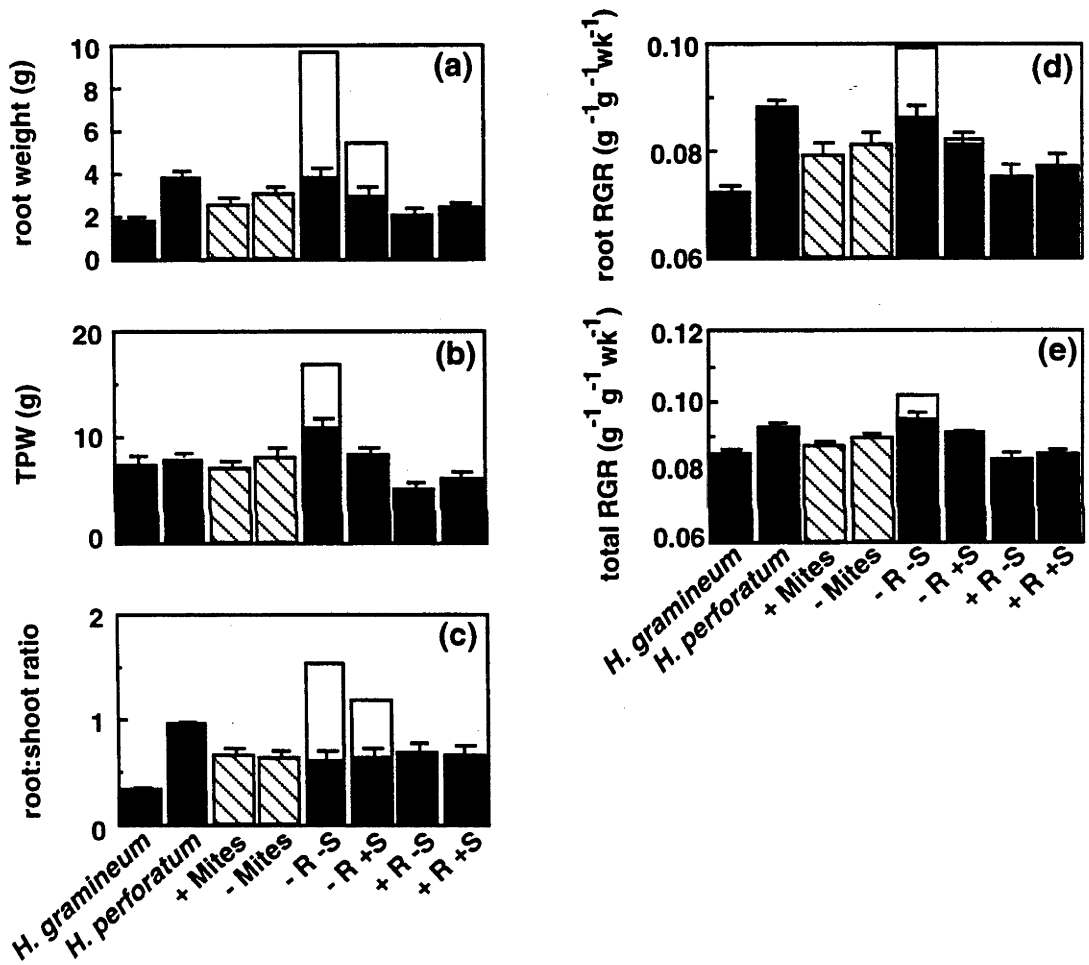

Fig. 7.7 Estimates of several indices of root growth and associated indices in the plant competition experiment, based on the results of the density experiment (experiment 2a), with standard errors of the arithmetic mean indicated. Predictions underestimate the observed values, as indicated in '-R' treatments of the main factor, competition (■).:The white proportion of such columns indicates the difference between the 'predicted' and observed values for those treatments. Treatments within the main factors of species (■) and mites (▨) are also indicated.

7.4.4.3 Shoot system (experiment 2)

The effect of mites on shoot growth was generally insignificant ($P \geq 0.05$), though they consistently reduced such indices (Fig. 7.8a - e). Overall, shoot weight, for example, was reduced by about 13%, while shoot relative growth rate was decreased by 2%. Mites caused proportionally greater reductions in growth of *H. perforatum* shoots than growth of *H. gramineum* shoots. Shoot weight was reduced by 23% in the *H. perforatum*, for instance, and by only 4% in the native non-target.

All measures of shoot growth, including shoot weight, shoot length total leaf area (TPLA) and shoot relative growth rate (shoot RGR) were highly significantly reduced by plant competition ($P \leq 0.008$, Fig. 7.8). The number of fruit produced was the only growth index to be only marginally affected ($P = 0.031$, Fig. 7.8d). As anticipated, growth in the root and shoot competition-free controls (-R-S) was consistently significantly higher than in any of the other treatments. Plants without root competition, but with shoot competition (-R+S) were the next most productive, their shoots always growing more than plants subjected to either of the root competition combinations (+R-S, or +R+S). The +R-S and the +R+S treatments never differed significantly ($P < 0.05$) suggesting that competition for soil resources (root competition) has a greater affect on *Hypericum* shoot growth than competition for light (shoot competition). No indices of shoot growth were affected by significant ($P \leq 0.01$) factor interactions.

7.4.4.4 Whole plant effects (experiment 2)

On average, mites reduced total plant weight (TPW, Fig. 7.7b) by about 14%, though the effect appeared more severe on *H. perforatum* (decreased by about 22%) than on *H. gramineum* (decreased by approximately 4%). Overall, mites also reduced the total plant relative growth rate (total RGR, Fig. 7.7e), with the decreases again less severe on *H. gramineum* (1% reduction) than on *H. perforatum* (3% decrease).

Based on the results of experiment 2a, mites caused a slight (2%) increase in the root:shoot ratio, although the difference between mite-free and mite-infested plants in this parameter appeared marginal (Fig. 7.7c). The root:shoot ratios of both *H. gramineum* and *H. perforatum* were similarly affected.

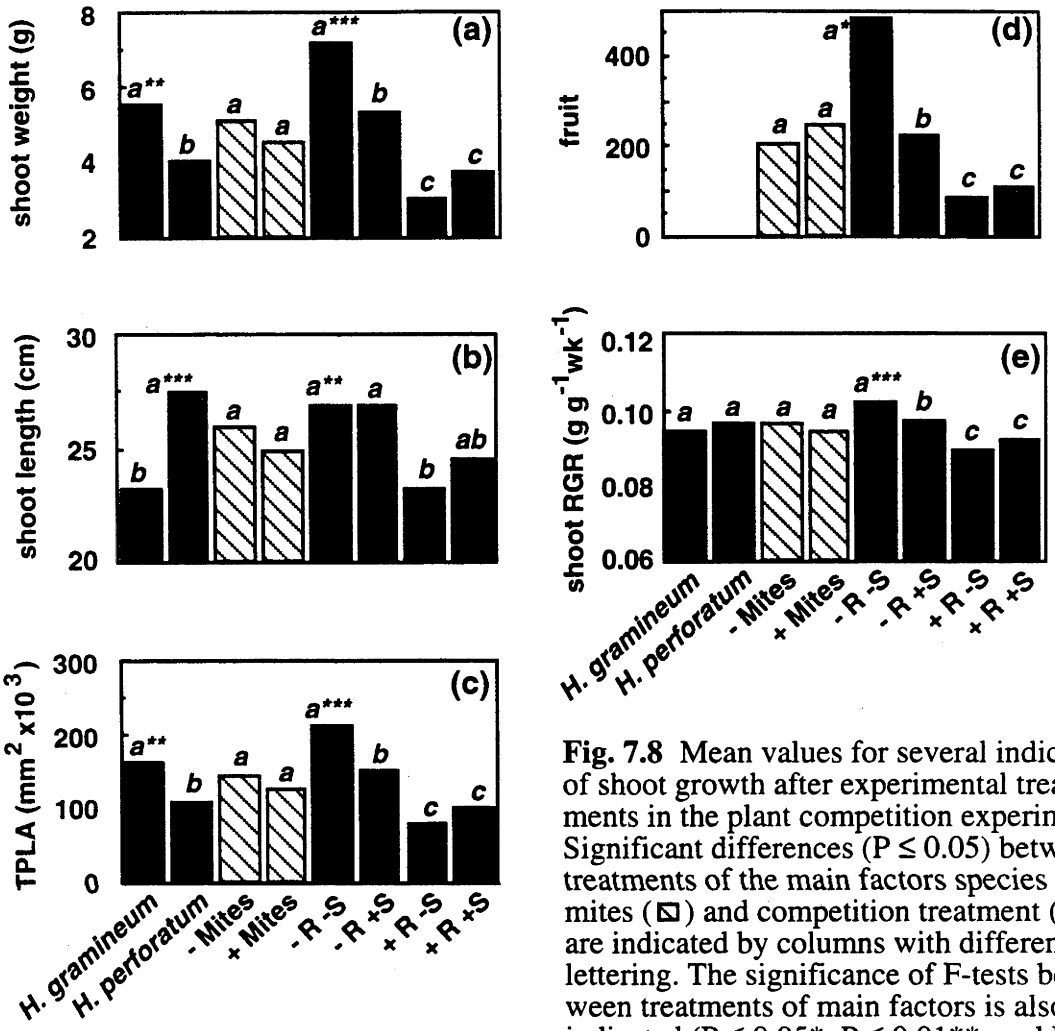

Fig. 7.8 Mean values for several indices of shoot growth after experimental treatments in the plant competition experiment. Significant differences ($P \leq 0.05$) between treatments of the main factors species (■), mites (▨) and competition treatment (■) are indicated by columns with different lettering. The significance of F-tests between treatments of main factors is also indicated ($P \leq 0.05$*, $P \leq 0.01$**, and $P \leq 0.001$***).

Competition had negative effects on growth of both species. As with the individual root and shoot systems, the effect of root competition on whole plants, as indicated by total leaf area, total mass, total relative growth rate and the root:shoot ratio, appeared to exert greater reductions on the growth of plants than shoot competition.

In both *H. gramineum* and *H. perforatum*, combinations of herbivory and root and shoot competition caused proportional decreases in plant biomass, relative to the competition- and mite-free combinations (Table 7.2). In combination, competition and herbivory reduced plant growth to 49% of the stress-free controls; the same value predicted by the model of proportional growth under several stresses.

7.5. Results of Experiment 3 - Water stress and aphid herbivory

Figure 7.9 illustrates the distinct and maintained differences in mean soil water content of pots subjected to high and low watering regimes during the experiment.

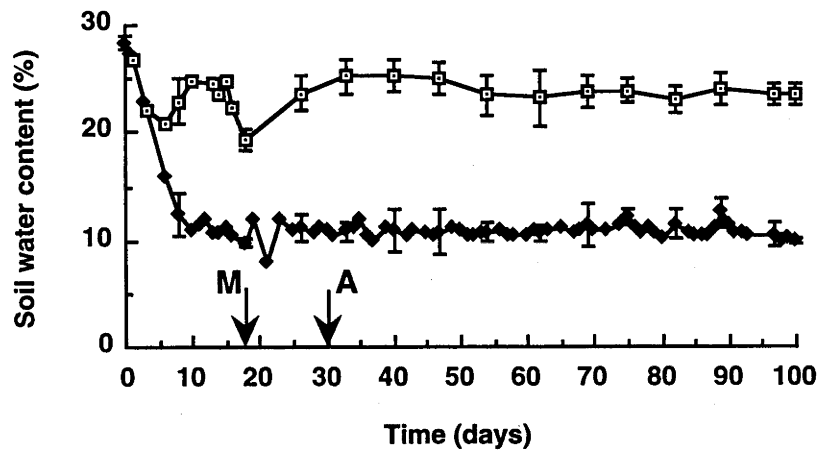

Fig. 7.9 Mean percentage soil water content over the 100 days during the water stress and aphid herbivory experiment for high and low watering regimes, with standard deviations marked at regular intervals. M = time of mite introduction; A = time of aphid introduction.

⊡ High water treatment; ● Low water treatment.

Analyses of variance were calculated for 14 measures of seedling growth including the nitrogen and phosphorus contents of the shoot and root systems. Main effects were generally highly significant ($P < 0.001$, Table 7.6), though there were few significant interactions.

Table 7.6 Probability table of each factor and interaction ($P \leq 0.05$) in the water stress and aphid herbivory experiment using 3-way ANOVAs. TPW = total plant weight; R:S ratio = root:shoot ratio; ILA = individual leaf area; TPLA = total plant leaf area; RGR = relative growth rate; N/A = not applicable; N = nitrogen; P = phosphorus. Note that the 4-way interaction term (Mites x Species x Water x Aphids) is omitted from the table as the P - values for all parameters were > 0.05. See text for the units of each variable.

Factors & Interactions	P - values													
	root weight	shoot weight	TPW	R:S ratio	shoot length	ILA	TPLA	fruit	root RGR	shoot RGR	N - shoot	N - root	P - shoot	P - root
Mites	<0.001	<0.001	<0.001		<0.001	0.009	<0.001		<0.001	<0.001	0.043			
Species	0.001		0.016	<0.001	0.002	<0.001	0.010	N/A		<0.001				
Water	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.031	<0.001	<0.001	<0.001			0.007
Aphids	<0.001	<0.001	<0.001	0.028	<0.001	<0.001	<0.001		<0.001	<0.001	<0.001			
Species x Mites						<0.001		N/A						
Mites x Water														
Species x Water				<0.001	0.048	0.009		N/A						
Mites x Aphids								0.024			0.032			
Species x Aphids				0.013	0.031	<0.001		N/A						
Water x Aphids											0.029		0.026	
Species x Mites x Water		0.045	0.038		0.019			N/A			0.027			
Species x Mites x Aphids		0.008	0.012				0.023	N/A		0.020				
Mites x Water x Aphids		0.050												
Species x Water x Aphids								N/A						0.012

7.5.1. Nitrogen and phosphorus content (experiment 3)

Shoot nitrogen contents were slightly higher in *H. gramineum* than in *H. perforatum*. Each high stress treatment (+mites, low water, +aphids) caused increases for both taxa, though only low water and +aphids were significantly higher ($P < 0.01$; Table 7.6, Fig. 7.10a). The nitrogen content of roots showed the opposite trends in response to treatments, though none was significant (Fig. 7.10b). Phosphorus concentrations generally showed the opposite trends to those of nitrogen, but none was significant (Table 7.6, Figs. 7.10c and d).

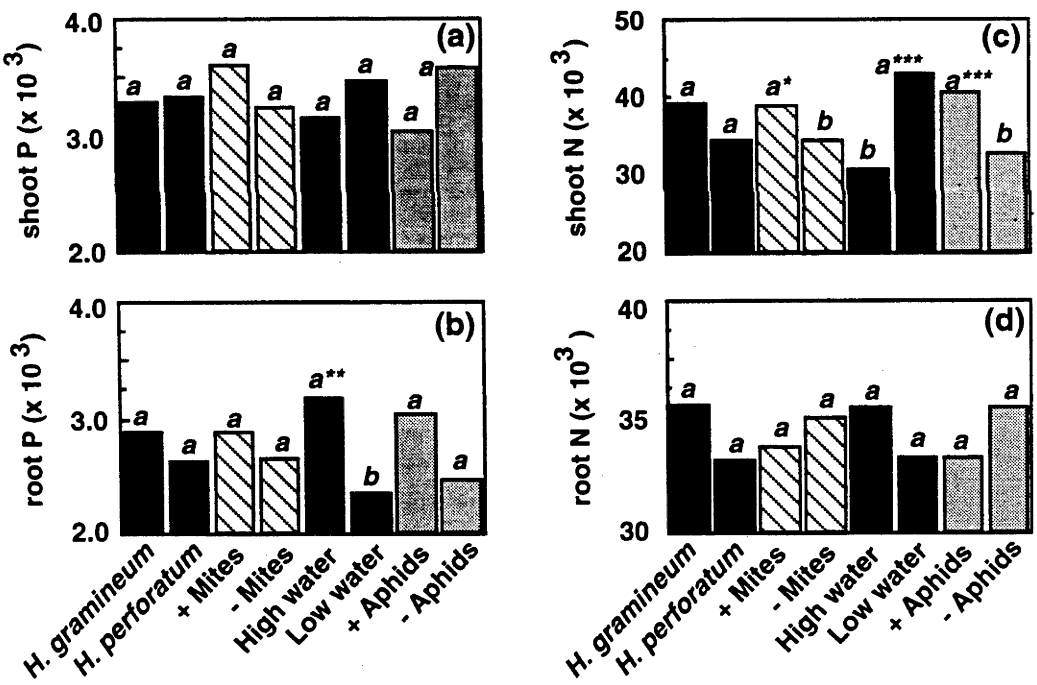

Fig. 7.10 Mean tissue nutrient concentration (N = nitrogen, P = phosphorus; ppm) after experimental treatments in the water stress and aphid herbivory experiment. Significant differences ($P \leq 0.05$) between treatments of the main factors species (■), mites (▨), watering regime (■) and aphids (▨) are indicated by columns with different lettering. The significance of F-tests between treatments of the main factors is also indicated ($P \leq 0.05$*, $P \leq 0.01$**, and $P \leq 0.001$***).

7.5.2. Root system (experiment 3)

Root weight and root relative growth rate were reduced ($P < 0.001$, Fig. 7.11a and i) by each of the stresses (+mites, low water and +aphids). The root mass of *H. perforatum* was greater ($P = 0.001$) than that of *H. gramineum*, but the relative growth rate of the latter species was marginally ($P = 0.234$) higher.

7.5.3. Shoot system (experiment 3)

Main factors (species, mites, water and aphids) had highly significant ($P \leq 0.009$) effects on shoot weight, shoot length, individual leaf area (ILA), total leaf area (TPLA) and shoot relative growth rate (Table 7.6, Fig. 7.11). Where such differences were found, high stresses (low water, +mites and +aphids) reduced the magnitude of growth. Fruit production was marginally ($P = 0.031$) reduced by water stress, but was not significantly affected by the other factors (Fig. 7.11h).

7.5.4. Significant factor interactions

The 4-way interaction between mites, species, water and aphids was not significant ($P > 0.05$) for any of the measured parameters and has, therefore, been omitted from further discussion. Of the remaining 10 possible 2- and 3-factor interactions from the 14 growth and nutrient parameters examined, only five were significant ($P \leq 0.01$), with a further 16 in the range $0.05 \geq P > 0.01$. Of the five significant interactions, all included the species term.

H. perforatum was usually more severely affected than *H. gramineum*, highlighting the hypothesised host-specificity of the biological control agents. In the only significant ($P = 0.008$) 3-factor interaction (species x mites x aphids), the combination of +mites and +aphids caused a severe reduction in *H. gramineum* shoot weight, while in *H. perforatum* the combined effects of both herbivores are the same as either alone (Fig. 7.12a). Nevertheless, the high stress (+mites, +aphids) treatment combinations caused proportionally greater biomass reductions in *H. perforatum* than in *H. gramineum*, relative to the low stress (-mites, -aphids) combination. This interaction requires a complex explanation which suggests that further research is required to confirm the trend.

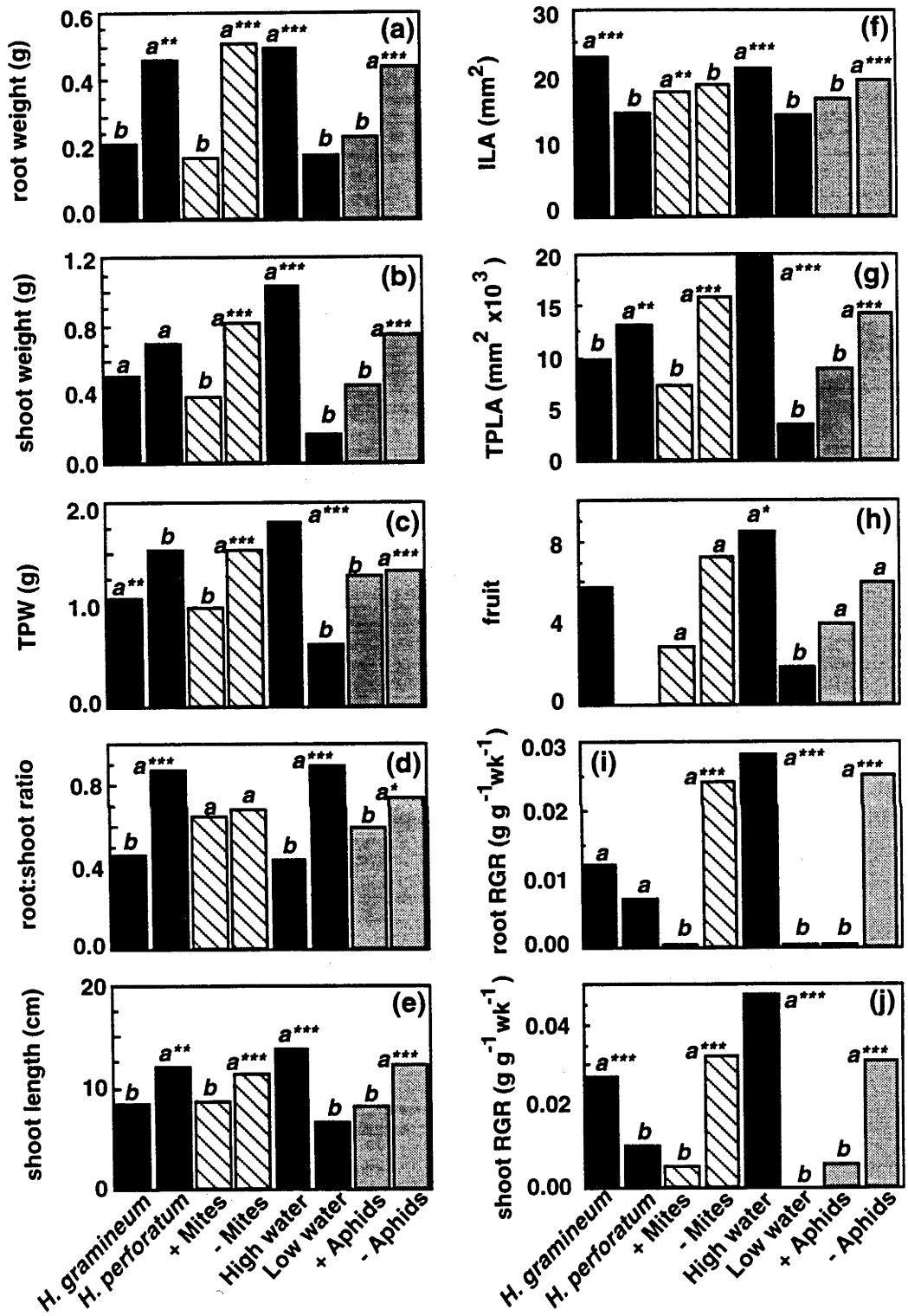

Fig. 7.11 Mean values of several indices of growth after experimental treatments in the water stress and aphid herbivory experiment. Significant differences ($P \leq 0.05$) between treatments of the main factors species (■), mites (▨), watering regime (■) and aphids (▩) are indicated by columns with different lettering. The significance of F-tests between treatments of the main factors is also indicated ($P \leq 0.05$*, $P \leq 0.01$**, and $P \leq 0.001$***). Note that (h) applies to *H. gramineum* only.

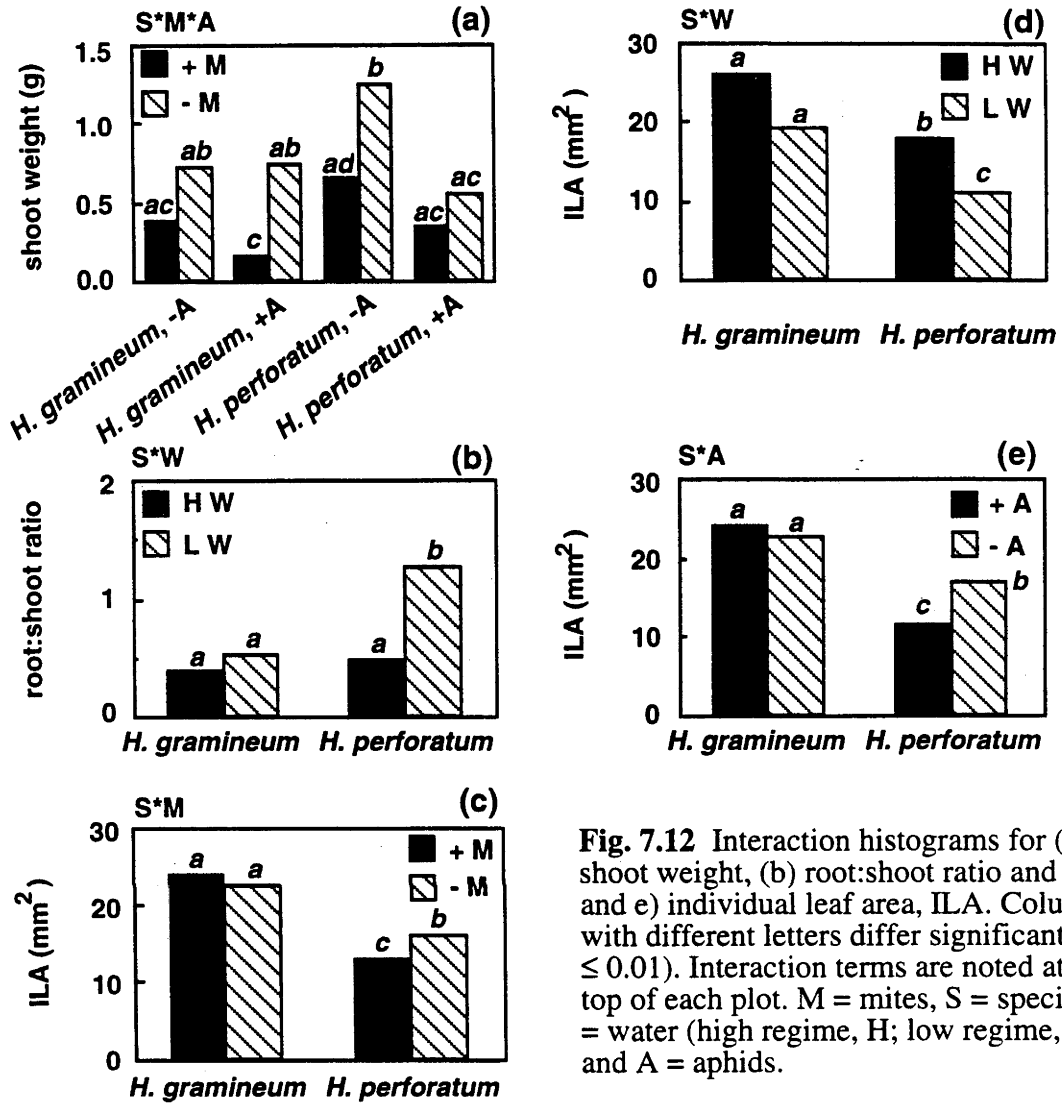

Fig. 7.12 Interaction histograms for (a) shoot weight, (b) root:shoot ratio and (c, d and e) individual leaf area, ILA. Columns with different letters differ significantly ($P \leq 0.01$). Interaction terms are noted at the top of each plot. M = mites, S = species, W = water (high regime, H; low regime, L) and A = aphids.

Species and water interacted significantly ($P < 0.001$) on the root:shoot ratio (Fig. 7.12b). Under the low watering regime, *H. perforatum* produced significantly more root tissue relative to shoot tissue when compared with well-watered controls. *H. gramineum* did not, though it shared a similar trend.

Mean leaf area (ILA) had three significant ($P \leq 0.01$) 2-factor interactions. *H. perforatum* showed relatively greater reductions in ILA than *H. gramineum* when subjected to mites, aphids or water stress (Figs. 7.12c-e).

7.5.5. Whole plant effects (experiment 3)

Total plant weight (TPW) was reduced ($P < 0.001$) by mites, low water and aphids (Fig. 7.11c). Overall, the species also differed ($P = 0.016$) in total mass, *H. gramineum* weighing slightly less than *H. perforatum* (1.04 g ± 0.17 s.e. and 1.51 g ± 0.21 s.e., respectively).

Although the mites x aphids interaction was non-significant ($P \geq 0.851$) for both root weight and shoot weight, mites reduced the former parameter by 66%, and the latter by 53%, while aphids caused 47% and 40% reductions, respectively. The slightly greater decreases in root mass failed, nevertheless, to significantly alter the root:shoot ratio of infested plants relative to herbivore-free plants (Fig. 7.11d). By contrast, the low watering regime caused a large increase in the root:shoot ratio.

In this experiment, the combined effect of individual stress factors on total plant growth was also roughly multiplicative. The combination of mites, aphids and water stress was, therefore, expected to yield a total plant weight approximately 14% of the unstressed weight of *Hypericum*. In fact, the combined stresses resulted in plant mass being reduced to 16% of controls.

7.6. Discussion

A consistent finding in all experiments presented in this chapter is that the total mass of *H. perforatum* exceeds that of *H. gramineum*, reflecting the observed differences in the height of these plants in field populations. The total mass of *H. perforatum* exceeded that of *H. gramineum* by 7% (plant competition experiment) to 35% (nutrient limitation experiment). Similarly, the root:shoot ratio of the *H. perforatum* was consistently much higher than that of *H. gramineum*, exceeding that of the native species by 47% (water stress and aphid herbivory experiment) to 77% (nutrient limitation). The relatively high root:shoot ratio of *H. perforatum* may confer advantages on this species under abiotic stresses such as water, or nutrient limitation, or under biotic stresses such as herbivores or root competition. With a relatively larger root system, the potential to allocate resources to below-ground storage seems comparatively greater, and may, therefore, enable greater tolerance of the stress.

7.6.1. Effects of water, nutrients and competitive stresses

This study demonstrates that growth of nutrient limited and competitively- and water- stressed *Hypericum* seedlings may be severely retarded relative to unstressed controls, consistent with other plant growth/stress experiments. In general the stresses reduced measures of growth such as shoot biomass and total biomass, their relative growth rates, shoot length and the number of shoots. Stress tended to increase absolute measures of root growth, consistent with Thornley's (1972) model of root:shoot ratio control (see below), but decreased the relative growth rate of roots. Kozlowski (1979) and Larsson (1989) indicate that general phenomena associated with plant stress include reductions in cell growth and elongation leading to smaller leaves, fruit and buds as well as reductions in their growth rates.

In the above experiments, water stress caused a significant increase in shoot tissue nitrogen concentrations. Competition between shoots in all combinations of root and shoot competition also caused slight increases in plant nutrients, but the effect was complicated by decreases in concentrations of nitrogen and phosphorus in shoots of plants suffering root competition. It was not possible to determine the changes in nutrient status of roots from '+ root competition' replicates. Other researchers report similar changes in tissue nutrient concentration following stress (Waring and Cobb 1992, and references therein; Mitchell and Chandler 1939; Piene 1978; Steigner and Muller-Scharer 1992).

7.6.2. Effects of mite herbivory

Herbivory by mites (and aphids; experiment 3) caused slight increases in shoot nutrient concentration, at the expense of roots, though the effect was greater in *H. perforatum* than in *H. gramineum*. A possible explanation for increases in shoot nutrient content caused by herbivores is that they act as nutrient sinks, causing re-allocation of nutrients from roots to their feeding sites in the shoots. Data from the root:shoot nitrogen and phosphorus ratios in experiment 1 (nutrient limitation) support this notion, suggesting that proportionally more plant nutrients are directed towards the shoots than the roots in mite-infested plants. While differences in total nutrients may reflect differences in root and shoot biomass, the decreasing ratio of root:shoot nutrient concentration suggests mite herbivory induces re-allocation of nutrients, particularly given that nitrogen and phosphorus removed from shoots by *A. hyperici* was not included in the calculation. That the effect was strongest in *H.*

perforatum highlights the greater utilisation of *H. perforatum* as a host for *A. hyperici*. Other studies have reported similar trends in nutrient re-allocation (e.g. Vranjic 1993).

Aculus hyperici also caused reductions in most indices of plant growth, though the effects were more severe on *H. perforatum* than on *H. gramineum*. Moreover, measures of root growth such as root weight and relative growth rate, as well as other parameters involving roots such as total plant weight and total relative growth rate, were generally more adversely affected than shoot growth parameters.

The presence of significant ($P \leq 0.01$) interactions between mites and species for several indices of growth demonstrates that populations of *A. hyperici* grow and reproduce more effectively on *H. perforatum* than on *H. gramineum*. Such interactions in the probability range $P = 0.01 - 0.05$ further support this suggestion.

7.6.3. Root: shoot ratios

Herbivory by *A. hyperici* was generally manifested in greater reductions in both absolute and relative estimates of root growth than shoot growth. Consequently, herbivory by mites (and *Aphis chloris*) tended to decrease the ratio of root:shoot biomass. Abiotic stresses such as nutrient limitation and water stress increased this ratio. There is also some evidence from experiments 2 and 2a (plant competition and density) that root competition also increases the root:shoot ratio. This, however, is not obvious in the former experiment, because root growth was underestimated by up to 46%. Nevertheless, differences between observed and predicted values were roughly equal in all treatments of all main experimental factors. The underlying trend seems to support, therefore, the patterns of the other stress experiments. Differences in the root:shoot ratio between experiments 2 and 2a may be a consequence of intra-specific competition in the former, and inter-specific competition in the latter. The two forms of competitive stress may differ in severity, thereby altering the nature of the response.

As noted in chapter 6, herbivory of plant shoots commonly decreases the root:shoot ratio. Accepting Thornley's (1972) 'source-sink' model of root:shoot ratio control (see discussion section of chapter 6), the higher root:shoot ratios evident in nutrient limited, water stressed and competitively stressed *Hypericum* plants are

explicable, because when soil resources are in short supply, assimilates are directed towards root growth, thereby increasing the plant's ability to acquire the limiting resource.

One mechanism by which herbivory combined with other stresses may interact to cause reductions in plant growth is by decreasing root growth, thereby constraining the plant's ability to access a limited resource. The experiments above generally indicate that *H. perforatum*'s root mass is severely reduced by *A. hyperici*, and that the biological interaction of herbivory and environmental stress causes roughly proportional reductions in growth. The effects are similar, but less severe for *H. gramineum*.

7.6.4. 'Plant vigour' hypothesis

The 'plant vigour' model of herbivore-plant interactions proposed by Price (1991) argues that vigorously growing plants are a preferable food source for herbivores, and consequently that herbivore populations increase on such vigorous plants. Implicit in the model, and many studies of herbivore-plant interactions, is that larger herbivore populations lead to greater levels of plant herbivory. An applied illustration of the hypothesis is in the biological control of *Salvinia molesta*, in which nitrogen fertilisation of the weed led to better control by increasing the plant's growth and foliar nitrogen content, thereby releasing a leaf-chewing weevil and moth herbivores from nitrogen-limited growth (Room *et al.* 1989).

Fertilising *Hypericum* plants raised tissue nitrogen levels. Similarly, shoot nitrogen increased following water and competitive stress, but mites were unable to capitalise on the increases and cause greater than proportional growth reductions. This may be because *A. hyperici* has a threshold for nitrogen, beyond which, mite growth and population expansion plateau. Other studies report that arthropod herbivores can respond non-linearly to plant stress, whereby their populations achieve greatest densities on moderately stressed plants, while non-stressed and severely stressed plants support poor population development (White 1984; Mattson and Haack 1987a,b; English-Loeb 1989; Larsson 1989; English-Loeb 1990; Louda and Collinge 1992; Mopper and Whitham 1992).

7.6.5. Non-linearity of herbivore impacts

Non-linear impacts of herbivore density on plants may also occur as populations of the herbivores vary through inter-specific competition. Competition between herbivores for feeding sites and plant nutritional resources may also account for the weak effects observed in the water stress and aphid herbivory experiment. Crawley and Pattrasudhi (1988) noted that there are few published accounts of inter-specific competition between herbivores. However, their study of arthropod competition for flower heads of ragwort (*Senecio jacobaea* L.) confirmed the expectation that such competition exists and that it may favour one herbivore over another. By contrast, Morris (1992) found that removal of flea beetles had insignificant effects on aphid population survival. These herbivores represent different feeding guilds: the aphids are phloem sap-suckers and the flea beetles are leaf- and stem-chewers, so they may not have directly competed for resources. In the water stress and aphid herbivory study, *A. chloris* is a phloem sap-sucker, while *A. hyperici* consumes the cell contents of epidermal cells whose walls it penetrates with cheliceral stylets (Jeppson *et al.* 1975; see chapter 1). Typically, *A. chloris* is found on stems or leaf abaxial surfaces, while *A. hyperici* generally feeds on immature leaves of developing apical buds. As such, they are unlikely to experience direct competition for feeding sites. Their main interaction is therefore likely to be through resource competition. Conceivably, retardations in growth caused by either *A. hyperici* or *A. chloris* could limit the relative abundance of feeding sites for the other. Investigation of such interactions was beyond the scope of this thesis.

In the above three experiments, populations of *A. hyperici* and their interaction with *A. chloris* were not monitored, except to confirm their presence at the end of the experiments. Nevertheless, it appears that well-fertilised, well-watered, vigorous *Hypericum* plants are able to 'out-grow' the negative effects of *A. hyperici* herbivory. Price's hypothesis (1991) may hold true under some circumstances, but it is not clearly reflected in greater herbivory of plants (as measured by reductions in plant growth), in the present plant-herbivore system. The observation that growth of environmentally stressed plants is more severely retarded than in the vigorous plants supports Harris's (1980) argument that biological control of some weeds may be more effective if agents are released onto stressed plants (Harris 1980). However, these results do not suggest a synergism. Evidently, different models of herbivore plant interactions may apply under different circumstances (Larsson 1989; Price 1991).

7.6.6. Combinations of stresses

Combinations of herbivory by *A. hyperici* and other biotic or abiotic stresses caused few significant interactions (on a logarithmic scale), lending little support to the hypothesis that combinations of stress and herbivory cause synergistic reductions in plant growth. Rather, results suggest that combinations of stresses cause multiplicative (proportional) reductions in growth, approximately equivalent to the product of the proportional growth under individual stresses (Table 7.2).

In a study of the combined effect of the fungal pathogen *Puccinia chondrillina* on growth of skeleton weed (*Chondrilla juncea*) competitively stressed by clover (*Trifolium subterraneum*), Groves and Williams (1975) found slightly greater than proportional reductions in growth. As noted, Cottam *et al.* (1986) reported synergistic reductions in plant growth following competition and invertebrate herbivory. Re-examination of their data, however, suggests that they too observed simple proportional reductions. The model of simple proportional reductions outlined above predicts that in their system, herbivory and competition would decrease root and rosette dry weights to about 13% and 24% of ungrazed, non-competing controls. In fact, these parameters were decreased to 12.4% and 21.5% of the controls, respectively, approximating proportional reductions.

James *et al.* (1992) investigated the combined effects of moth and beetle herbivory on density and shoot biomass of field-grown ragwort (*Senecio jacobaea*). Examination of their data also indicates that the proportional growth model is applicable to several indices of plant growth including the number of small plants per study plot, the total above-ground plot biomass and the above-ground biomass of large plants. Although predictions of the proportional growth model do not reflect the empirical data as closely as other examined studies, the predictions fall very close to or within the range of the empirical data, which were highly variable. It is possible that the model does not reflect the data of James *et al.* more closely, or indeed several of their other indices, because of the high variability of their system. Moreover, in their experiment, herbivory by moth larvae was simulated by hand-defoliation and hand-defloration, and may not have accurately mimicked the extent of herbivory caused by natural populations of the larvae. Baldwin (1990) notes that mechanical simulations of herbivory may poorly reflect natural herbivory for a

variety of reasons. It would be profitable to repeat the experiment conducted by James *et al.* using actual larvae in combination with the beetles to further test the proportional growth model on the ragwort-herbivore system.

Confusion about interactions between factors and possible synergistic effects on plant growth stems from interpretation of the term 'interaction' and, to a large extent, whether the data being compared have been logarithmically transformed. As in this study, Parker and Salzman (1985) use the term 'interaction' as the statistical non-additivity of effects, most usually arising when plants respond non-linearly to combinations of treatments. They found no such interaction between herbivory and plant competition on growth in their plant-herbivore system. In a study of the combination of slug-herbivory and competition on plant growth, Rees and Brown (1992) found no interactions concluding that, on the logarithmic scale, the factors were additive. Importantly, however, they note that on a linear scale, the combined effect of such biotic stresses is multiplicative (proportional) and conclude that it is more sensible to view combinations of herbivory and competition in this way. Results of this study and those of Groves and Williams (1975), Cottam *et al.* (1986) and Rees and Brown (1992) imply that proportional (multiplicative), or slightly greater than proportional reductions in plant growth may characterise plant stress-herbivore interactions. If generally true, this is an important and simple way to predict effects of stress in complex systems and suggests that complex factorial experiments may not be essential.

7.6.7. Glasshouse versus field experiments

Waring and Cobb (1992) demonstrate the risks associated with extrapolating results from glasshouse-based studies of water stress and herbivory to field studies, indicating that the results of each may be contradictory. In biological control of weeds, it is obvious that careful glasshouse experimentation must precede field release of biological control agents. The above experiments imply that *A. hyperici* will have severe negative impacts on growth of *H. perforatum* and relatively small effects on the native non-target, *H. gramineum*; an ideal biological control scenario. With the results of Waring and Cobb's (1992) survey in mind, however, the post-release field behaviour and impact of *A. hyperici* needs to be carefully studied on both target and non-target species in the field. In particular, it would be of value to examine plant growth following combinations of herbivory and environmental stress to assess the efficiency of biological control agents on target species, and their impact on non-targets. This is because combinations of stresses may achieve

better biological control by causing proportional growth reductions and may inflict greater damage on non-targets than individual stress factors.

7.7. Summary

To estimate the impact of *A. hyperici* on growth of the non-target native species, *H. gramineum*, and its efficiency as a control agent for *H. perforatum*, the combined effects of herbivory and nutrient limitation, plant competition, and water stress on growth *H. gramineum* and *H. perforatum* were measured. The alternative hypotheses that, in combination, herbivory and environmental stress(es) would cause (a) additive (or subtractive) reductions in plant growth, (b) simple multiplicative (proportional) reductions, or (c) exert more complex synergistic reductions on growth were examined in three glasshouse experiments. Individually, stresses reduced measures of plant growth. In combination, herbivory by *A. hyperici* and environmental stress caused reductions in growth approximately equivalent to the product of the proportional growth following either herbivory or stress(es) alone. *A. hyperici* had relatively minor effects on growth of the non-target native, but caused severe reductions in several measures of *H. perforatum* growth. Results are discussed in relation to the biological control of *H. perforatum* relative to growth of its indigenous congener, *H. gramineum*.

CHAPTER 8

THE IMPACT OF *ACULUS HYPERICI* ON FIELD-GROWN *HYPERICUM GRAMINEUM* AND *H. PERFORATUM*

8.1. Introduction

There are no published accounts of the effect of weed biological control agents on growth of non-target native species, either in natural populations, or manipulated field experiments. Even when agents are capable of survival to reproductive maturity on non-target species, most attention has focused on the behaviour, development and general population dynamics of the herbivores (see, for example, Rees 1977; Peschken 1984; Andres 1985).

Chapter 7 demonstrated that in glasshouse studies, combinations of environmental stress and herbivory by *Aculus hyperici* cause proportional reductions in growth of the Australian native, *Hypericum gramineum* and the weed, *H. perforatum.* Since results of glasshouse stress experiments may not be extrapolated to the field with certainty (Waring and Cobb 1992), the need for field experimentation to investigate the hypothesis of proportional growth was highlighted, and might provide a more accurate picture of *A. hyperici*'s impact on growth of *H. gramineum* and *H. perforatum* under natural conditions.

This chapter aims, therefore, to assess the impact of *A. hyperici* on growth of *H. gramineum* in the field. The hypothesis that combinations of herbivory and plant stress cause proportional reductions in growth, as detailed in chapter 7, will be investigated in a field experiment examining competition and herbivory, following up a similar experiment in the previous chapter. To date, combinations of herbivory and plant competition have rarely been studied in the field, and have never compared interactions between a biological control arthropod and competitive stress on the growth of a non-target species and a target weed.

8.2. Materials and Methods

Two field experiments were undertaken: The first assessed the impact of herbivory by *A. hyperici* on above-ground growth of *H. gramineum* (experiment 1). The second investigated the combined effects of herbivory and competitive stress on field-grown *H. gramineum* and *H. perforatum* (experiment 2).

8.2.1. Experimental designs and treatments

8.2.1.1. Experiment 1 - Field Impact of *A. hyperici* on *H. gramineum*

In winter (July) 1992 at each of three field sites (Table 8.1), 40 naturally occurring *H. gramineum* plants comprising four shoots not longer than 2 cm, were randomly selected. Half of the plants were allocated for infestation with *A. hyperici.* Infestation was achieved by placing four vegetative *H. perforatum* buds, each infested with 5 - 10 mites into the crown of plants. At the time of infestation, each shoot was in direct contact with at least one of the infested buds. Over the following 3 weeks, few buds moved, thereby ensuring the potential for transfer of mites to shoots. The remaining 20 plants were left as controls, free of infestation.

Table 8.1 Location of study sites used in the field impact and competition experiments.

Experiment	Habitat	Site	Site latitude & longitude
Experiment 1: Field impact	Woodland	Mt. Ainslie W2	35° 17' S, 149° 10' E
	Grassland	Pierce's Creek	35° 20' S, 148° 55' E
	Woodland	Smith's Paddock W1	35° 17' S, 149° 05' E
Experiment 2: Field competition	Grassland	Border West, ACT	35° 10' S, 149° 09' E
		Horsepark, ACT	35° 10' S, 149° 08' E
		Radio CY, ACT	35° 13' S, 149° 02' E
	Woodland	Border North, ACT	35° 10' S, 149° 09' E
		Honeysuckle Creek, ACT	35° 35' S, 149° 00' E
		Kowen Forest, NSW	35° 16' S, 149° 15' E

In early autumn (March) the following year after a full season's growth, plants were relocated and the number of flowering shoots, the total number of shoots, the mean shoot height (cm), and the number of fruits scored. In addition, plants were ranked from 1 - 5 for damage caused by grazing herbivores (1 = no apparent damage; 5 = severe damage). A random sub-sample of 7 - 10 plants at each site indicated that about 97% of '+mite' treatments remained infested at completion of the experiment. All '-mite' replicates remained un-infested. Above-ground shoots were then harvested at the soil surface, placed in paper bags and oven-dried at 60°C for 5 days in the laboratory, before determining the shoot mass (dry weight, g).

8.2.1.2. Experiment 2 - Effects of *A. hyperici* and Competition on *Hypericum*

Seedlings of *H. gramineum* and *H. perforatum* were grown from locally collected seeds. Prior to transplanting into field study sites, seedlings were sub-sampled to allow estimation of relative growth rates, as previously described (chapters 6 and 7). In July of 1992 when the majority of seedlings had grown to 4 - 6 cm in height, they were transplanted to field sites (Table 8.1) and treatments within a factorial design experiment, comprising 8 treatment combinations (2 habitats x 2 levels of mite infestation x 4 competition treatments). All combinations were applied to *H. gramineum* and *H. perforatum*, as follows:

(1) Habitat - seedlings transplanted into either *Themeda triandra*-dominated grassland, or *Eucalyptus* woodland with an understorey of native forbs.

(2) Mite herbivory - two levels of mite herbivory: plants either infested with *A. hyperici* or free of infestation. Mite-free treatments were sprayed every 4 weeks with Omite®, having determined that the miticide had negligible effects on plant growth and tissue nutrients (chapter 6).

(3) Plant competition - four levels of plant competition were achieved by growing plants in combinations of root and/or shoot competition with the surrounding flora, as summarised below:

(i) No root competition and no shoot competition (-R-S).

(ii) No root competition, but shoot competition (-R+S).

(iii) Root competition, but no shoot competition (+R-S).

(iv) Both root and shoot competition (+R+S).

The experiment was commenced in December of 1991 by digging all of the holes into which seedlings would be transplanted. At each site, the soil from holes was homogenised and sifted through 1 cm^2 mesh to remove roots. PVC tubing (12 cm diameter x 15 cm long) was placed into holes that had randomly been assigned to root competition-free treatments (-R). All holes were then refilled with the sifted soil and left to subside for 7 months, till July 1992. It was assumed that within this time, roots from surrounding plants would re-grow into the soil of holes without tubing, ensuring root competition (+R treatments) after seedling transplantation.

Shoot competition-free treatments (-S) were achieved by attaching a 100 x 100 cm piece of nylon shading cloth (75% light transmittance) to the ground with tent pegs. The cloth was slit in the centre to allow *Hypericum* seedlings to grow, unimpeded by surrounding plants, through the slit. This technique pushed aside surrounding shoots, but enabled their continued root growth. In shoot competition treatments (+S) the shoots grew among the foliage of surrounding grasses and forbs.

The effect of PVC tubing on plant growth was examined by comparing root and shoot competition-free treatments (-R-S) with growth of *H. gramineum* and *H. perforatum* seedlings grown at the same field sites, but with vegetation in a 0.5 m radius around the plants trimmed to ground level and sprayed with Roundup® (glyphosate) herbicide. The herbicide was applied twice: in December 1991 when the field sites were prepared, and one month before transplantation of seedlings to the field, in June, 1992. In this way, root and shoot competition was eliminated from such seedlings.

The experiment incorporated the spring/summer growth period of 1992/93, and was terminated in March 1993 after 8 months. At completion of the experiment, plants were scored for the number of flowering shoots, the total number of shoots, the mean shoot height (cm) the number of fruits and a ranking for grazing damage (as above). The shoot system was also harvested as above to determine shoot dry weight (g). Root mass (g) in root competition-free treatments (-R) was measured by removing root balls from tubes, washing away any soil, oven-drying and then

weighing the samples. Calculation of the root relative growth rate (RGR, g g^{-1} $week^{-1}$), the total plant relative growth rate, total plant weight (TPW, g), and the root:shoot ratio (g g^{-1}) was then possible for '-R' replicates.

Indices of root growth in root competition, '+R', replicates were estimated using the same regression procedure and model outlined in the plant competition and plant density experiments (experiments 2 and 2a) of chapter 7 and as such, the magnitude of root growth and associated indices are likely to be underestimated. Nevertheless, because all predictions were similarly affected by the regression model, the relative differences remain the same, and therefore, comparable. As in chapter 7, in order to compare root growth and associated indices under different experimental treatments, the data presented for all treatments derive from the regression predictions. Where observed values for '-R' treatments differ from 'predictions' for the same treatments, the difference is also indicated. As in chapter 7, these measures of growth were not formally analysed owing to the potential for error. Rather, the estimates are presented graphically, and the apparent trends discussed. Estimation of root growth by regression in the above manner assumes that the root:shoot ratio of field grown plants is similar to those grown in the glasshouse. As noted, it is not always practical to extrapolate from glasshouses to the field. However, empirically derived measures of root growth and the root:shoot ratios for the '- R' treatments in the field competition experiment support this assumption.

8.2.2. Nutrient analyses

The nitrogen and phosphorus concentration (ppm) of roots from '-R' treatments, and of all shoots were estimated by a micro Kjeldahl technique, as previously described (chapters 6 and 7).

8.2.3. Analyses of data

Despite intensive searching, location of all replicates at the termination of experiment 1 (field impact on *H. gramineum* shoots) was not possible because abundant growth of grasses in the preceding season obscured many of the plants. Consequently, the experiment became unbalanced. The technique of restricted maximum likelihood estimation (REML, Genstat 5 algorithms Lane *et al* 1987; Digby *et al* 1989) was, therefore, used to analyse the data. Scores for damage caused by grazing herbivores were used as a covariate within the analysis.

The field competition experiment (experiment 2) was analysed by 4-way analysis of variance (ANOVA, Genstat 5) and also used the damage score as a covariate. Data from both experiments were logarithmically transformed prior to analysis to meet assumptions of normality. Back-transformed data including adjustment for the covariate are presented in the figures.

8.3. Results

8.3.1. Experiment 1 (Field impact of A. hyperici *on* H. gramineum)

Mites had no significant ($P \geq 0.064$) effect on any measure of *H. gramineum* shoot growth. Although *A. hyperici* caused marginal reductions in the total number of shoots, a slight trend towards increased productivity was evident in the mite's presence (Table 8.2).

Table 8.2 The effect of *A. hyperici* on *H. gramineum* shoot growth in the field impact experiment (experiment 1). Mites had no significant ($P \geq 0.064$) effect on any index of growth. Note that values are observed arithmetic means and as such, have not been adjusted for the 'grazing damage' covariate. In '+mite' treatments, n = 40; in '-mite' treatments, n = 43. Standard errors are indicated.

Growth parameter	+ Mites	- Mites
total no. shoots	3.1 ± 0.2	3.4 ± 0.4
no. flowering shoots	2.0 ± 0.1	2.0 ± 0.2
shoot length (cm)	24.2 ± 1.2	25.1 ± 1.4
fruit	29.1 ± 5.4	24.5 ± 4.2
shoot weight (g)	0.32 ± 0.05	0.31 ± 0.06

8.3.2. Experiment 2 (Effects of A. hyperici *and competition on* Hypericum)

8.3.2.1. Effect of PVC tubes on plant growth

Compared with the competition-free treatments (-R-S), shoot growth and tissue nutrients were not affected ($P \geq 0.05$) by the presence of the PVC tubes that enabled maintenance of competition-free root growth (-R), as summarised in (Table 8.3). Tubes also had minimal effects on estimates of root and whole plant growth.

Table 8.3 The effect of PVC tubing on indices of plant growth, as indicated by a comparison of root and shoot competition-free (-R-S) treatments with 'Roundup' treatments, in the field competition experiment. Note that values are observed arithmetic means and as such, have not been adjusted for the 'grazing damage' covariate. None of the comparisons differ significantly ($P > 0.05$). In 'PVC tube' treatments, n = 20; in 'Roundup' treatments, n = 24. Standard errors are indicated.

Growth/nutrient index	PVC Tube (-R-S)	Roundup
root weight (g)	0.40 ± 0.08	0.47 ± 0.16
shoot weight (g)	0.87 ± 0.37	0.56 ± 0.17
TPW (g)	0.91 ± 0.18	1.03 ± 0.32
root:shoot ratio ($g\ g^{-1}$)	0.94 ± 0.09	0.99 ± 0.08
shoot length (cm)	18.7 ± 2.2	24.7 ± 2.7
no. flowering shoots	2.4 ± 0.4	2.3 ± 0.3
total no. shoots	8.4 ± 1.1	6.7 ± 0.8
TPLA (mm^2)	21696 ± 11080	12576 ± 4543
fruit	24.9 ± 9.4	20.6 ± 6.8
root RGR ($g\ g^{-1}\ week^{-1}$)	0.036 ± 0.002	0.034 ± 0.003
shoot RGR ($g\ g^{-1}\ week^{-1}$)	0.044 ± 0.003	0.040 ± 0.003
total RGR ($g\ g^{-1}\ week^{-1}$)	0.039 ± 0.002	0.037 ± 0.003
shoot N (ppm)	13657 ± 1514	10158 ± 925
shoot P (ppm)	1898 ± 276	1613 ± 198

Table 8.4 summarises the probabilities ($P < 0.05$) for all factors and interactions in the field competition experiment. For each analysis, the significance of the grazing covariate is also indicated, although in general, it did not explain a significant ($P > 0.05$) amount of the variance. As in previous chapters, to reduce the possibility of type 1 errors, a 1% level of significance ($P \leq 0.01$) has been adopted for discussion, since > 200 tests of significance have been performed. It is clear that the majority of significant ($P \leq 0.05$) results occurred in the main factors. Species and competition treatment were consistently significant ($P \leq 0.05$) for most measures. By contrast, habitat and mites were not significant ($P \geq 0.302$ and $P \geq 0.174$, respectively) for any measured parameter.

Interactions between main factors were not significant ($P > 0.05$) for any estimates of plant growth, except for a marginal interaction between habitat and mites for fruit production ($P = 0.026$), in which the presence of mites was more severe in grassland than woodland. However, a species x mites interaction was highly significant ($P = 0.007$) for root nitrogen concentration (see below).

8.3.2.2. Nitrogen and phosphorus content

Shoot nutrients differed significantly ($P \leq 0.002$) between species, with concentrations of nitrogen and phosphorus higher in *H. perforatum* (Fig. 8.1). The reverse was true for root nutrients: nitrogen and phosphorus were more concentrated in *H. gramineum*, but the difference was significant ($P = 0.004$) for only root nitrogen.

Mites and habitat had insignificant effects on tissue nutrients, though a consistent trend to decreased nitrogen and phosphorus in woodlands may reflect poorer soils at such sites. Competition also generally affected tissue nutrients only marginally. A significant decrease in root nitrogen of plants experiencing shoot competition (Fig. 8.1a), however, highlights a similar trend for shoot nitrogen and phosphorus (Fig. 8.1b and d). Root phosphorus, by contrast, increased marginally under shoot competition (Fig 8.1c). As noted, the species x mites interaction was significant ($P = 0.007$) for root nitrogen. The presence of mites caused a significant increase in *H. perforatum* root nitrogen, but a decrease in that of *H. gramineum* (Fig. 8.2). A similar, though marginal ($P = 0.026$) trend was evident for shoot nitrogen.

Table 8.4 Probability table for each factor and interaction ($P \leq 0.05$) in the field competition experiment using 4-way ANOVAs. The significance of the grazing damage covariate is also indicated. Note that the 4-way interaction term (habitat x species x mites x competition treatment) is omitted from the table as the P - values for all parameters were > 0.05. TPLA = total plant leaf area; RGR = relative growth rate; N/A = not applicable; N = nitrogen; P = phosphorus. See text for units of variables.

Factors & Interactions	**P - values**										
	shoot weight	shoot length	TPLA	total shoots	flowering shoots	fruit	shoot RGR	N - shoot	N - root	P - shoot	P - root
Species		0.002		0.033	<0.001	<0.001		<0.001	0.004	0.002	
Mites											
Habitat											
Treatment	0.010	0.013	0.010	0.042	0.033	0.050	0.021		0.016		
Habitat x Species											
Habitat x Mites											
Habitat x Treatment											
Species x Mites								0.026	0.007		
Species x Treatment											
Mites x Treatment											
Habitat x Species x Mites											
Habitat x Species x Treatment											
Habitat x Mites x Treatment											
Species x Mites x Treatment									0.030		
Covariate	≥0.096	≥0.019	0.963	≥0.285	≥0.181	≥0.182	≥0.078	≥0.067	≥0.377	≥0.343	≥0.113

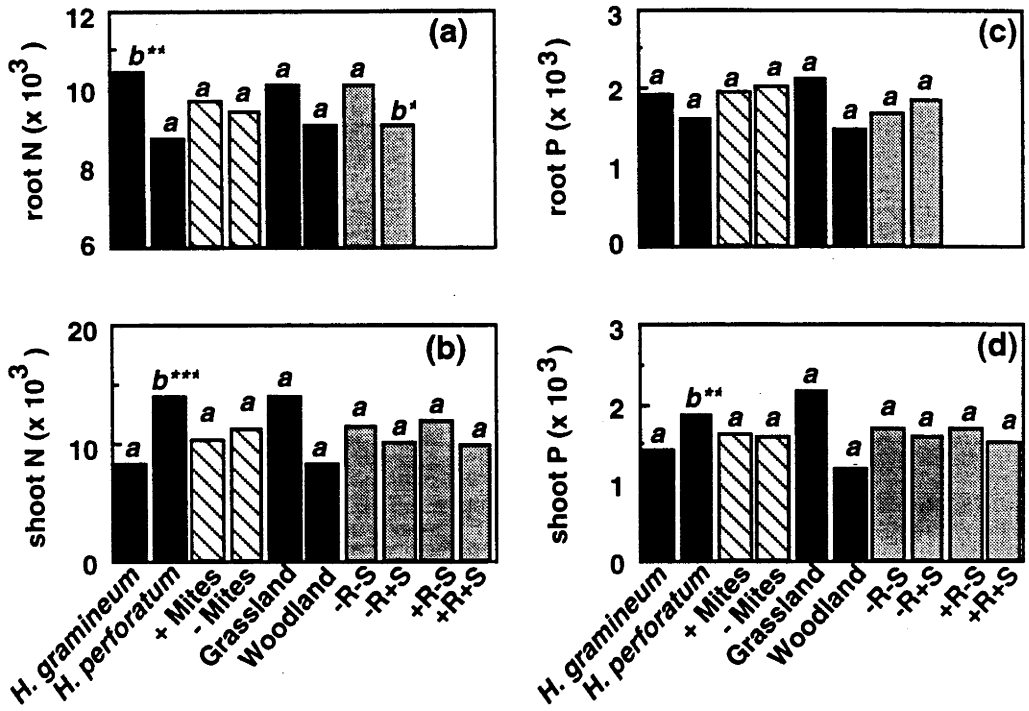

Fig. 8.1 Mean tissue nutrient concentration (N = nitrogen, P = phosphorus; ppm) after experimental treatments in the field competition experiment. Significant differences ($P \leq 0.05$) between treatments within the main factors of species (■), mites (▨), habitat (■) and competition treatment (▤) are indicated by columns with different lettering. The significance of F-tests for treatments within the main factors is also indicated ($P \leq 0.05$*, $P \leq 0.01$**, $P \leq 0.001$).

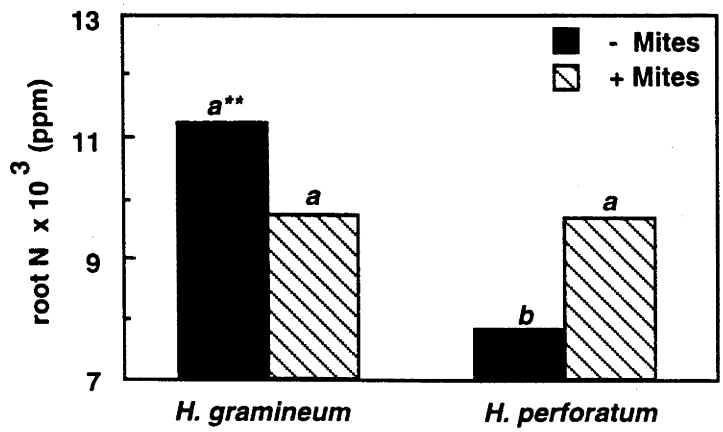

Fig. 8.2 Histogram of the significant (P** = 0.007) species x mites interaction on root nitrogen concentration. Significant differences ($P \leq 0.05$) between the main factors of species and mites are indicated by columns with different lettering.

8.3.2.3. Root system

All measures of root growth including root mass, root relative growth rate and the root:shoot ratio were higher in *H. perforatum* than in *H. gramineum* (Fig. 8.3a-c) Root and shoot competition markedly reduced the former two parameters, but had marginal effects on the latter. Competition between roots appeared to decrease growth more than did shoot competition. In combination, root and shoot competition reduced growth by more than did either form of competition alone. Neither root nor shoot competition had any major impact on the root:shoot ratio.

Differences in root growth between the grassland and woodland habitats were only slight, although the former was higher than the latter for absolute measures of growth, while the reverse was true for relative growth rates. Overall, mites caused a slight increase, about 10%, in root mass. There was some indication of a species x mites interaction for root mass and root relative growth rate, since mites were associated with 1% and 5% decreases in these respective measures of *H. perforatum* growth. By comparison, mite-infestation was associated with a 20% increase in the root mass of *H. gramineum*, and caused no change in the relative growth rate of the roots of this species.

8.3.2.4. Shoot system

Competition had significant ($P \leq 0.05$) negative effects on all measures of shoot growth including shoot weight, shoot length, total plant leaf area (TPLA), total number of shoots, the number of flowering shoots, the number of fruit and the shoot relative growth rate (Fig. 8.4). Competition between surrounding plants and *Hypericum* for light (shoot competition) resulted in obvious shoot elongation (Fig. 8.4c). Shoot length in the presence of shoot-competition was the only index of growth that exceeded that of the controls (-R-S). Interestingly, the generally smaller *H. gramineum* was characterised by marginally higher shoot mass than its weedy congener. Although the species x mites interaction was not significant ($P = 0.343$), it is probable that the higher mass of *H. gramineum* is partly a consequence of mites causing a slight (12%) increase in the shoot weight of the native species, but a 26% decrease in the shoot mass of *H. perforatum*. Root competition caused the most severe reductions in measures of shoot growth, and while shoot competition also caused decreases, its effects were generally more severe in combination with root competition (Fig. 8.4).

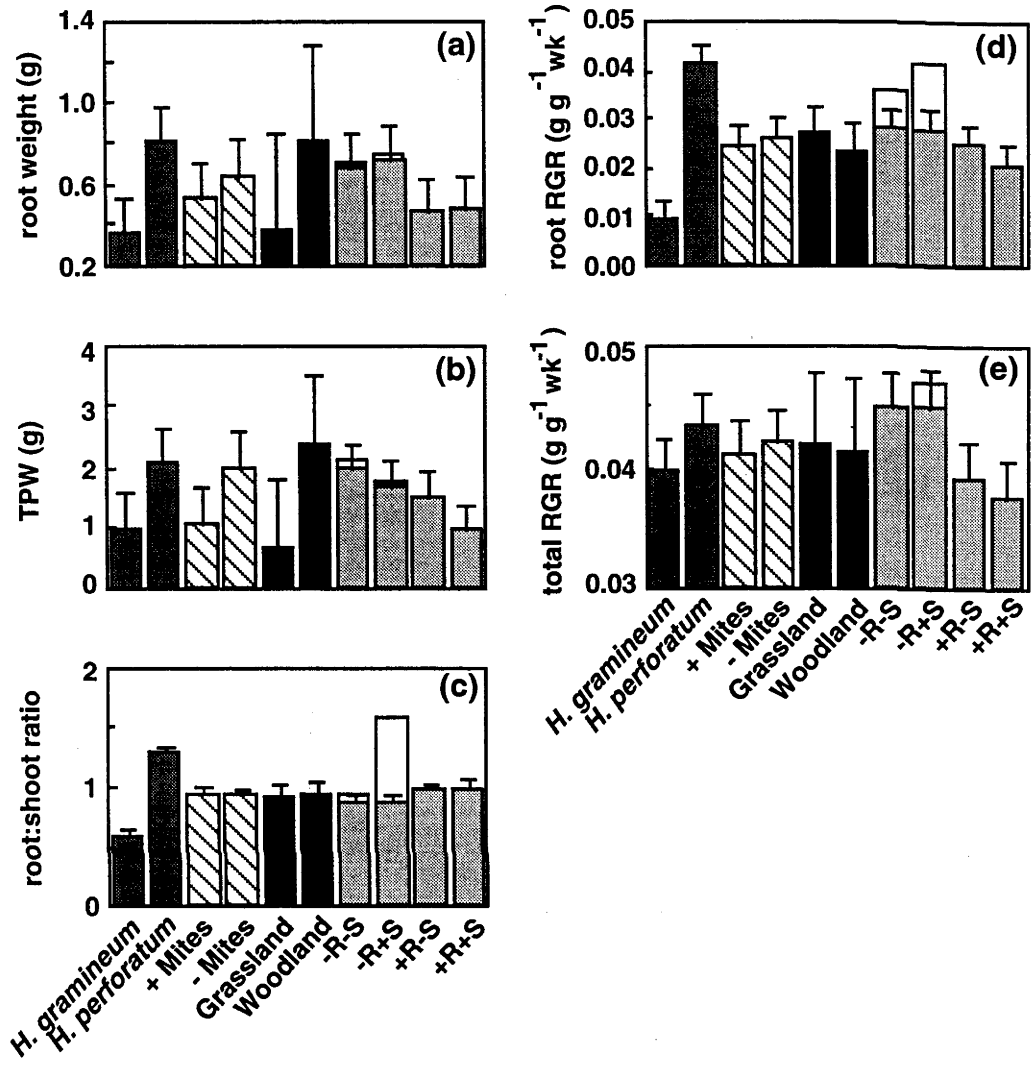

Fig. 8.3 Estimates of several indices of root growth and associated parameters in the field competition experiment. To simplify comparisons, values are adjusted for the 'grazing damage' covariate, with standard errors of the model indicated. Estimates derive from predictions made in the regression of root:shoot ratio on shoot weight in the plant density experiment (experiment 2a, chapter 7). Predictions underestimate the observed values, as indicated in '-R' treatments of the main factor, competition (▣): The white proportion of such columns indicates the difference between the 'predicted' and observed values for those treatments. Treatments within the main factors of species (■), mites (▣) and habitat (■) are also indicated.

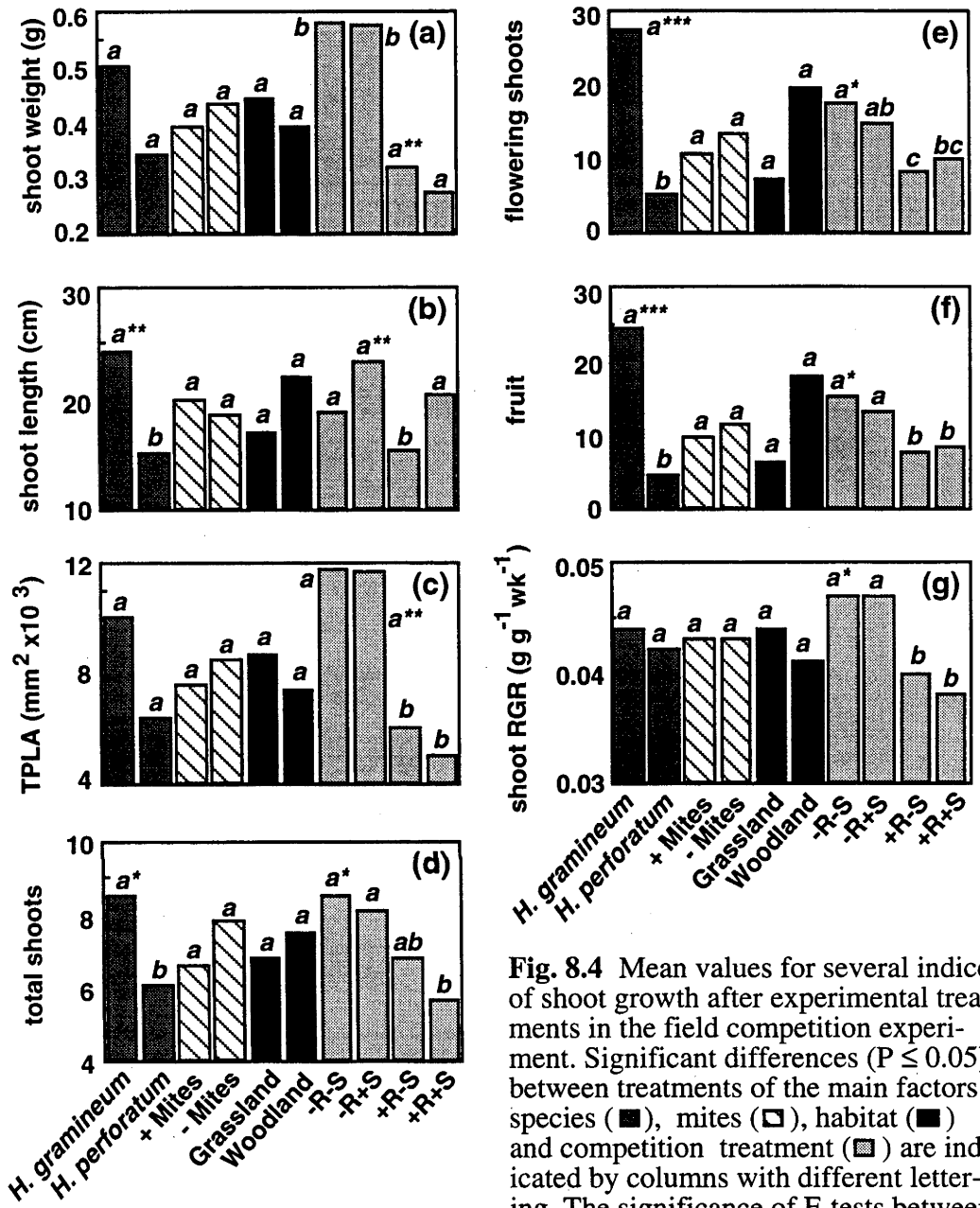

Fig. 8.4 Mean values for several indices of shoot growth after experimental treatments in the field competition experiment. Significant differences ($P \leq 0.05$) between treatments of the main factors species (■), mites (□), habitat (■) and competition treatment (▨) are indicated by columns with different lettering. The significance of F-tests between treatments of the main factors is also indicated ($P \leq 0.05$*, $P \leq 0.01$**, and $P \leq 0.001$***).

8.3.2.5. Whole plant effects

The root:shoot ratio of *Hypericum* remained largely unaffected by root and shoot competition. Total plant weight and the total plant relative growth rates, by contrast, were reduced by root competition and suffered slight reductions in the presence of

shoot competition. Despite the higher shoot mass of *H. gramineum*, *H. perforatum* had a slightly higher total plant weight, probably because of its extensive root system, which was almost twice the mass of the native species.

On average, mites reduced the total plant weight and the total relative growth rate by about 8% and 0.5%, respectively. The effect of mites was more severe on *H. perforatum* (total weight and total relative growth rate reduced by 22% and 2%, respectively) than on *H. gramineum*, in which both measures increased by approximately 8% and 5% respectively. Mites were associated with a decline of about 2% in the root:shoot ratio of both the non-target native and the target weed.

8.4. Discussion

8.4.1. Effects of A. hyperici *on plant growth*

In both the field impact (experiment 1) and the field competition experiments (experiment 2) *A. hyperici* had insignificant effects on growth of *H. gramineum* shoots ($P \geq 0.065$ and 0.256 for experiments 1 and 2, respectively). In the field competition experiment, growth of *H. perforatum* shoots was also reduced only marginally, but in all cases, the magnitude of reduction was greater for the weed than the native species. In fact, some measures of growth in *H. gramineum* actually increased, albeit insignificantly, following herbivory by *A. hyperici*. Mites were associated with reductions in other indices of shoot growth including shoot length, the number of flowering shoots, and the relative growth rate of shoots. Supporting notions of the host-specificity of *A. hyperici* (CSIRO 1991; chapter 5), the magnitude of the reductions was consistently higher for *H. perforatum*.

Vranjic (1993), Karban and Strauss (1993) and others (Christiansen *et al.* 1989) report that shoot herbivory also causes reductions in root growth. Supporting the above studies and data discussed in chapters 6 and 7, the field competition experiment detected consistent reductions in measures of root growth, though the effects were generally marginal, and insufficient to alter root:shoot ratios of either *Hypericum* species.

In the field competition experiment, the general lack of significant mite effects may indicate that *A. hyperici* failed to establish on '+mite' treatments. Sub-sampling of plants at the end of the experiment, however, confirmed the presence of *A. hyperici* on about 97% of replicates, indicating that it did establish and persist, but that its

effects were minor. Under field conditions, the negative effect of *A. hyperici* on plant growth appears somewhat ameliorated relative to its effect in glasshouse studies (see chapter 7), possibly because the field environment is less favourable in terms of temperature, and has more predators and general environmental hazards, which affect arthropod population development.

8.4.2. Effects of competition on plant growth

Consistent with the glasshouse competition experiment reported in chapter 7 and similar field research by Wilson and Tilman (1993) Rees and Brown (1992) and Bergelson (1990), plant competition generally reduced indices of *H. gramineum* and *H. perforatum* growth. In this study, like that of Wilson (1988b), root competition (+R) exerted the greatest influences on plant growth, and generally amplified the relatively marginal effects of competition for light. Plant growth in root competition-free treatments (-R) was not inhibited by the root exclosures for surrounding plants, suggesting that results in '-R' treatments were not artefacts of growth within a confined space.

In a glasshouse experiment, Moore *et al.* (1989) indicated that *H. perforatum* is out-competed by *Trifolium subterraneum*, which suppresses shoot growth and can lead to mortality of the former species. Although the root system of competitively stressed *Hypericum* replicates were not examined in their study, they suggested that the negative effects of *T. subterraneum* competitors on growth of *H. perforatum* were mediated through competition for light (shoot competition), with the former out-competing the latter. The present data suggest that root competition has a greater affect on *H. perforatum* and *H. gramineum* growth than competition for light. Such observations may contrast with those of Moore *et al.* (1989) because the canopy of *T. subterraneum* seems denser than that of competing grasses and forbs at field sites in this study, thereby affording stronger shoot competition. Without separating the effects of root competition from shoot competition, however, it is difficult to determine the relative importance of each (Wilson 1988b). Until the experiment conducted by Moore *et al.* is repeated to distinguish the effects root and shoot competition on plant growth, their conclusions must be treated with caution.

8.4.3. Confounding effects of herbivory and competition

Reader (1992) recently observed that in field studies of plant competition the results may be confounded by herbivory, since removing a plant's neighbours may also reduce food and shelter available to herbivores. It was demonstrated that in removing neighbours from target plants, increased plant survival following release from competition was confounded by declines in slug-herbivory, because the availability of shelter to herbivores, afforded by surrounding vegetation, was reduced. Connell (1983, 1990) notes the same effect, describing the result as 'apparent competition'.

In the present study 'apparent competition' by grazing herbivores may also have confounded interpretation of the data. If so, such effects were measured by including the damage covariate in analyses. Because the covariate did not explain significant variation in the data of either experiment 1 or 2, 'apparent competition' was evidently not of importance in the field impact or competition experiments.

8.4.4. Combinations of A. hyperici *and plant competition on* Hypericum *growth*

As in the glasshouse study of competition and *Aculus*-herbivory, few interactions were significant in the field competition experiment. Indeed, the sole significant ($P \leq 0.01$) interaction was between species and mites on root nitrogen. There were three additional interactions in the range $P = 0.05 - 0.01$. Excepting the habitat x mites interaction for fruit productivity, they too, were for tissue nutrients.

Rather than statistical interactions between factors, which would indicate complex, synergistic effects on plant growth, growth under competitive stress and herbivory is best explained by the proportional growth model, defined in chapter 7 and discussed by Rees and Brown (1992). Taking back-transformations of the data adjusted for the covariate of grazing damage, and pooling *H. gramineum* and *H. perforatum* since their respective total weights were similar, this model predicts that total plant mass under the two biotic stresses would be about 48% of the mite- and competition-free replicates. In fact, total mass was approximately 52% of unstressed replicates, approximating the model's prediction. The above field data

therefore support the model of proportional growth proposed to explain plant growth under combinations of stress, as in the glasshouse experiments of chapter 7.

8.4.5. The impact of A. hyperici *on* H. gramineum *and the biological control of* H. perforatum

Field studies detailed in this chapter suggest that the post-release feeding behaviour of *A. hyperici* is unlikely to have appreciable negative impacts on growth and productivity of the non-target native species, *H. gramineum*. Whether stressed or not by root and shoot competition with neighbouring grasses and forbs, *A. hyperici* had only slight effects on growth of *H. gramineum*. This desirable biological control scenario should, however, be interpreted in the light of the generally slight growth reductions also experienced by the target weed, *H. perforatum*, in the presence of *A. hyperici*. These experiments were conducted over a single growth season of about 8 months. It is possible that the effects of *A. hyperici* may be cumulative, successively reducing plant growth over a number of years, but such a hypothesis requires longer term research, which was not within the scope of this project. Stress combinations may, of course, also impact negatively on the growth of *H. gramineum*. Further monitoring of infested *H. perforatum* and *H. gramineum* is therefore desirable to determine the long term effects of *A. hyperici* on growth of these species.

8.5. Summary

The effect of *A. hyperici* on field-grown *H. gramineum* was assessed in two experiments. In the first, mites introduced onto winter rosettes of established *H. gramineum* field populations had no effect on five measures of shoot growth and productivity. In the second, the impact of *A. hyperici* on field transplants of competitively stressed *H. gramineum* was compared with its effect on similarly treated *H. perforatum*. This experiment confirms that root competition, and to a lesser extent shoot competition, reduces most estimates of *Hypericum* growth. Herbivory by *A. hyperici* caused marginal decreases in growth of *H. perforatum*, and had almost no effect on growth of *H. gramineum*. In combination, the two stresses cause proportional reductions in growth, though the magnitude of the decreases are greater for *H. perforatum* than for *H. gramineum*.

SECTION E:

GENERAL DISCUSSION

The single chapter in this section synthesises the major findings of the study. Details of experimental results, which were discussed in earlier chapters, are not reiterated. A brief discussion of the effectiveness of *Aculus hyperici* as a biological control agent for *Hypericum* perforatum is followed by consideration of the impacts the mite is likely to have on growth and the populations dynamics of *H. gramineum.* Finally, conclusions for the whole thesis are provided.

CHAPTER 9

GENERAL DISCUSSION

9.1. Introduction

A primary aim of this thesis has been to determine the impact of the biological control mite, *Aculus hyperici*, on growth of the non-target Australian native forb, *Hypericum gramineum*. More generally, the thesis addresses questions about the impact of herbivores, especially in relation to other environmental factors, on plant growth and population stability. In the absence of any previous studies of the ecology of *H. gramineum*, this aim has been addressed by, firstly, outlining the population ecology of the forb (Section B), providing a basis from which to assess the impact of mites on its population dynamics. Secondly, finding that *A. hyperici* dispersed to, and selected *H. gramineum* as a host plant (Section C), the impact of mites on growth and productivity of individual *H. gramineum* plants was quantified and compared to its impact on growth of the target weed, *H. perforatum*. Detailed interpretation of results from experiments designed to test specific hypotheses associated with the aims of the thesis, with reference to the literature, are provided in chapters 2 - 7. This chapter integrates the major results from preceding chapters, providing conclusions for the broad aim of the study.

9.2. Biological control of *H. perforatum* by *A. hyperici*

Aculus hyperici has the potential to exert significant reductions in growth of *H. perforatum*. Among other reasons, this arises from its capacity to reduce growth of this species in a variety of habitats, its short generation time which enhances its ability to rapidly infest target plants, and its capacity to be widely dispersed in a relatively short period of time (Wapshere 1984; CSIRO 1991). What then is the potential for this mite to control *H. perforatum*?

Chapters 6 to 8 of this thesis indicate that in both glasshouse and field studies, *A. hyperici* is able to reduce shoot growth and the reproductive potential of *H. perforatum*, satisfying a predicted requirement for successful control of this weed, that both plant growth and seed production be limited (Groves 1989). Root growth

is more adversely affected by the mites than shoot growth. Chapters 6 to 8 demonstrated, for example, that on average, mites usually retard shoot growth of *H. perforatum* by about 27%, and decrease that of roots by approximately 30%. In the water-stress experiment of chapter 7, reductions in growth of both roots and shoots were much higher: shoot growth was reduced by about 44%, while root growth was reduced by 75%. Similarly, reductions in the relative growth rate is higher for roots than for shoots. However, in the field competition experiment (chapter 8), decreases in growth of *H. perforatum* were not generally significant ($P > 0.05$). This implies that alone, but under optimal environmental conditions, *A. hyperici* is, in the short term, unable to significantly reduce plant productivity in *H. perforatum* individuals. This, in turn, suggests that in the absence of other stresses, *A. hyperici* will be unable to reduce the density of weedy infestations. Biological control of *H. perforatum* with *A. hyperici* may be more successful if the plants are environmentally stressed by, for example, water-limitation, and/or combinations of other herbivores such as the previously released and widely dispersed *Aphis chloris* and *Chrysolina quadrigemina*. Harris (1980) predicts that biological control of weeds is more likely to be successful if the control agents are released onto stressed plants that are less able to tolerate herbivory. These predictions have recently been supported by James *et al.* (1992). In sustaining herbivore-induced stress of ragwort (*Senecio jacobaea*) with combinations of biological control agents active in different seasons and targeted at different plant tissues, James *et al.* observed greater decreases in plant productivity than either herbivore achieved alone.

The field experiment on combinations of plant competition and *A. hyperici* reported above was conducted over 8 months and did not test the potential for cumulative effects of *A. hyperici*-herbivory on *H. perforatum*. Cumulative effects of herbivory could further decrease plant growth and fitness, since *A. hyperici* may mediate more significant reductions in growth of the target weed with time. For instance, the high root:shoot ratio of *H. perforatum*, may, under such sustained herbivory, be further reduced than experiments in the preceding chapters indicated, thereby impairing the ability of plants to regenerate during stress, or in subsequent seasons. Indeed, in populations of St. John's Wort in northern New South Wales into which some of the initial introductions of *A. hyperici* into Australia were made, marked reductions in plant growth and population density are only now becoming apparent, after 2.5 - 3 years of *Aculus*-herbivory (P. Jupp, pers. comm.). A controlled experiment to investigate the longer term impacts of *A. hyperici* on the growth and population dynamics of *H. perforatum* at these sites has recently been established (initiated in October 1993) by the CSIRO and local weed control officers.

Finally, since the non-target native species, *H. gramineum*, becomes infested by *A. hyperici*, a potentially beneficial outcome of this otherwise undesirable interaction is that when population densities of the target weed fall, *H. gramineum* may serve as a 'reservoir' to maintain mite populations. In this way, *H. perforatum* may be rapidly re-infested by mites from the non-target, when the weeds begin to re-establish. Rees (1977) observed similar potential for non-target Canadian thistles, utilised by the weevil, *Rhinocyllus conicus*, to serve as a reservoir for reinfestation of thistle populations, noting that such a process could minimise the normal fluctuation associated with host-parasite relationships. Infestation of *H. gramineum* by *A. hyperici* may also minimise such fluctuations in control of *H. perforatum*, which have characterised biological control of this weed in Australia, and facilitate the sustained suppression of this noxious species.

9.3. The impact of *A. hyperici* on *H. gramineum*

By introducing an organism into a new environment to biologically control weed populations, an unavoidable hazard is that populations of non-target species may also be affected. The likelihood of this depends on herbivore dispersal to the non-target taxa and utilisation of those species. To reduce the abundance of non-target species, biological control agents must exert a detrimental impact on the biology of their novel host (Andres 1981; Turner 1985).

9.3.1. Dispersal of A. hyperici

Advantages of wind dispersal are that no biological vectors are required and relatively large distances can be covered in short periods of time. This enables dispersal to healthy hosts which may allow rapid growth and freedom from predators or parasites. This vector enabled rapid dispersal of mites during the present study. Chapter 4 demonstrated that following infestation and establishment of *A. hyperici* in a given population, mites spread from the initial point of introduction, and rapidly established on a majority of neighbouring plants. Within the short term, about six months, the probability of establishment over greater distances declined linearly, on a log-log scale. After six months the pattern of distribution shifted, reflecting the establishment of secondary mite colonies within the plant population. This was indicated in the area surrounding the initial infestation point, in which all plants eventually became infested. At other field release sites of *A. hyperici* in south-eastern Australia, mites were observed at least 800 m from the point of introduction within one year (P. Jupp, pers. comm.).

Dodd (1929, 1936, 1940, see Hill and Stone 1985) reported that within 12 months, *Tetranychus desertorum* (Acari: Tetranychidae) was located 8 km from its point of introduction to Australia. Long distance wind dispersal appears typical of phytophagous mite species (Thresh 1966; Nault and Styer 1969). These patterns of frequent local infestation and rare long-distance establishment are typical of wind dispersal and, though hard to define, may be characterised by Weibull distributions. This has implications for the dynamics of infestation locally, within populations and on a regional scale, between populations.

In Australia, individual *H. gramineum* plants do not form the dense populations that typify many infestations of *H. perforatum*. The native species is widespread but rarely very common, while the weed is locally dominant at scales of 0.1 - 10 ha, but absent in many areas. With *H. gramineum* only covering about 1% of the ground at sites where the species grows (3 - 5 plants m^{-2}), only about 1% of mites are likely to directly reach *H. gramineum* hosts, following wind dispersal. In comparison, the denser populations of *H. perforatum*, in which cover may average 10 - 20% and locally exceed 50%, are likely to suffer proportionally higher rates of infestation. This order of magnitude of difference in abundance, and also in plant size, makes *H. perforatum* a more 'apparent' species. Whatever the actual pattern of dispersal and search behaviour employed by the mite, differences in apparency between the native non-target species and the target weed seem likely to cause major differences in infestation of the species. *Aculus hyperici* has a demonstrated specificity for different species of *Hypericum*, developing more rapidly and producing more fecund females on *H. perforatum* than on other taxa (CSIRO 1991; chapter 5). In combination with the greater apparency of *H. perforatum*, the relatively rapid development of mite populations on the target weed suggests that this species is likely to be utilised by, and sustain greater populations of *A. hyperici*, than is *H. gramineum*. It is probable that these aspects of mite behaviour will drive the dynamics of the infestation of populations of both the target and non-target species.

The probability that mites will disperse to and establish on the native non-target is, in sympatric populations of *H. gramineum* and *H. perforatum*, significantly less than the probability of establishing on the target weed, (chapter 4). As noted above, this process is likely to be affected by the local density of potential host species. Such density-related host-selection has been noted for several other herbivorous arthropods (Courtney and Forsberg 1988; Jaenike 1990; Bowers *et al.* 1992). Faced with the choice of remaining and risking reduced growth and reproduction, or dispersing and risking failure to locate another host, desiccation, predation or

other biotic and abiotic dangers, it is apparent that some *A. hyperici* individuals remain and infest *H. gramineum.*

9.3.2. The effect of A. hyperici *on growth of* H. gramineum

The interaction between *A. hyperici* and *H. gramineum* is schematically summarised in figure 9.1. Growth of *H. gramineum* infested with *A. hyperici*, as measured by several indices including root and shoot mass, and relative growth rate , is reduced relative to un-infested plants, although in general, the reductions are marginal and not significant ($P > 0.05$). Nevertheless, as with *H. perforatum*, where herbivory by *A. hyperici* causes reductions in growth, roots appear to be more adversely affected than shoots. The effect of mites on root mass, for instance, ranged increases of approximately 19% in the field competition experiment (chapter 8), to reductions of up to 67% in the water stress experiment of chapter 7. Decreases in shoot mass, by comparison ranged from slight (12%) increases in the field competition experiment to 44% reductions in the water stress experiment. As expected, such reductions in root and shoot growth were associated with concomitant decreases in absolute and relative measures of whole plant growth.

In common with other shoot herbivores (Christiansen *et al.* 1989; Vranjic and Gullan 1990; Karban and Strauss 1993; Vranjic 1993), *A. hyperici* feeding on *Hypericum* shoots seem to act as a 'sink', drawing nutrients and photosynthate from the roots, where they might otherwise be allocated and stored. As discussed below, mites remove these resources from the plant, leading to slight, but consistent shifts in the ratio of root mass to shoot mass and the ratio of nutrients within the roots to the shoots.

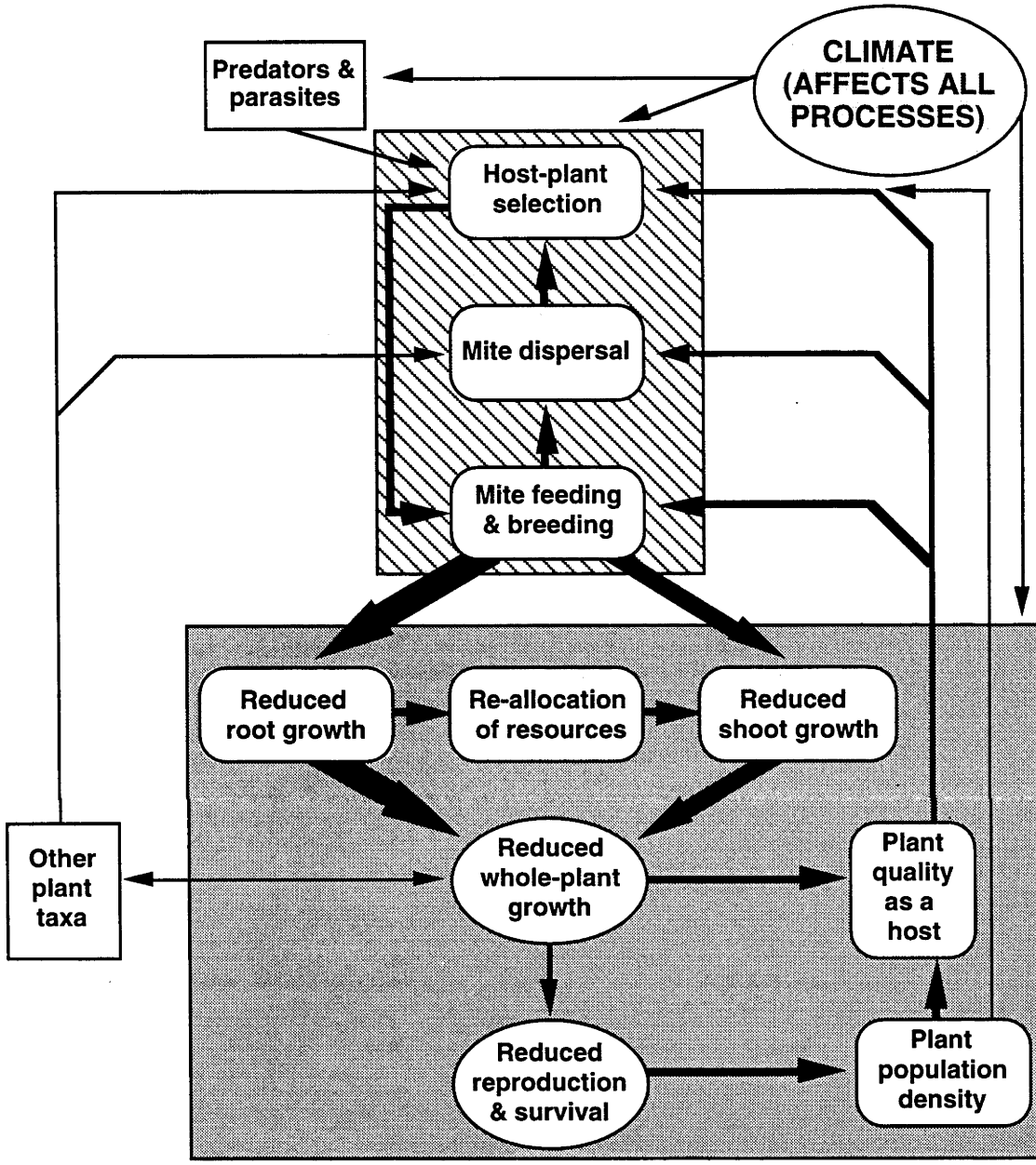

Fig. 9.1 Proposed sequence of events leading to selection and utilisation of *H. gramineum* by *A. hyperici*. Processes affecting mite behaviour are enclosed in the hatched box. Those affecting individual plant and population growth are enclosed within the shaded box. The thickness of flow lines represents the relative importance of that process.

The effect of *A. hyperici* on fruit production was highly variable, but consistently insignificant ($P \geq 0.263$). Examining the impact of mites on fruit production in *H. gramineum*, chapter 6 (experiment 1) indicated that infested plants suffer reductions of about 58%. The decrease was more marginal in the field competition experiment (approximately 10%; chapter 8). In the glasshouse-based competition experiment (chapter 7), mites increased fruit production by 19%. Mites also increased fruit production by 19% in the field impact experiment (chapter 8). The lack of any clear patterns in fruit production following infestation by *A. hyperici* implies that mites have minimal impact on this measure of plant productivity, and that the non-significant changes in the growth index reflect natural variability in fruit production. The absence of any obvious mite impact is unusual, since herbivory usually decreases fruit productivity (Crawley 1983, references therein). In combination, the increase in germinability of seeds matured on mite-infested plants (chapter 2), and the variable fruit production of infested individuals suggests that further investigation is required into the effects of mites on plant fecundity. The impact of mites on vegetative spread of *H. gramineum* was not examined in this study but is also likely to affect the dynamics of *H. gramineum* populations. Clearly, this aspect of the reproductive ecology of the native species also requires examination.

9.3.3. Comparison of H. gramineum *and* H. perforatum

Prior to release of *A. hyperici* into the Australian environment, it was anticipated that the effects of the mite on *H. gramineum* would be minimal, particularly in comparison with the negative impact mites were expected to have on growth of the target weed. To date, the expectation of minor effects on the native has been realised. By contrast, *A. hyperici* has not yet achieved its hypothesised potential on the target weed.

In table 9.1, the percentage change in total plant weight (TPW), and root:shoot ratio (R:S) caused by mites, relative to mite-free replicates, are summarised from all experiments that investigated the effects of mites on total *Hypericum* growth. The table was compiled by calculating the average of these growth indices across all other experimental treatments. It is clear that the impact of mites on both species is variable, though usually negative, and that generally, differences between *H. gramineum* and *H. perforatum* are marginal ($P > 0.05$). Usually, however, *H. perforatum* suffers proportionally higher decreases in growth than *H. gramineum.*

Table 9.1 Summary table of the changes in total plant weight (TPW) and the root:shoot ratio (R:S) of *H. gramineum* and *H. perforatum* caused by *A. hyperici* in various plant growth experiments. Decreases are calculated from the observed (arithmetic) means of results from mite-infested and mite-free treatments, across all other experimental manipulations. Calculation of the decreases in the field competition experiment (chapter 8) are taken from back-transformations of the log-transformed data, following adjustment for the covariate of damage caused by grazing herbivores. Decreases in the growth indices caused by mites were non-significant for all trials with *H. gramineum* and in all except that marked by an asterisk ($^{*}P \leq 0.05$), for *H. perforatum*. Values prefixed by '+' increased following *A. hyperici*-infestation.

		Change attributable to *A. hyperici* (%)			
Chapter	**Experiment**	***H. gramineum***		***H. perforatum***	
		TPW	R:S	TPW	R:S
6	Expt. 1: Impact on four species	-47.1	-18.3	-11.5	+4.9
6	Expt. 2: Impact on different sizes	-21.7	-2.3	-17.5	-9.5
7	Expt. 1: Nutrient limitation	-23.3	+10.6	-34.2	-55.4*
7	Expt. 2: Glasshouse competition	-4.0	+0.9	-21.7	+2.9
7	Expt. 3: Water & Aphid stress	-37.8	-9.2	-36.3	-12.4
8	Expt. 2: Field competition	7.4	0.1	-22.2	2.3
	MEAN	**-21.1**	**-3.0**	**-23.9**	**-11.2**

A consistent pattern to emerge in this thesis is that the root:shoot ratio of *H. gramineum* is much less than that of *H. perforatum*. This suggests that the ability of *H. perforatum* to store resources in its roots may be greater than that of the native species and consequently, that *H. perforatum* has a greater potential to tolerate certain abiotic and biotic stresses.

Figure 9.2 plots the root:shoot ratio (R:S) of plants under various stresses against total mass of the same plant, as a percentage of the unstressed control. In figure 9.2a, the consistently lower root:shoot ratio of *H. gramineum*, compared with that of *H. perforatum*, is clear. There is some indication that as stress decreases (i.e, increase along the horizontal axis), so too does the root:shoot ratio. Across both species, the trend is weak, though clearer for *H. gramineum* than for *H. perforatum*.

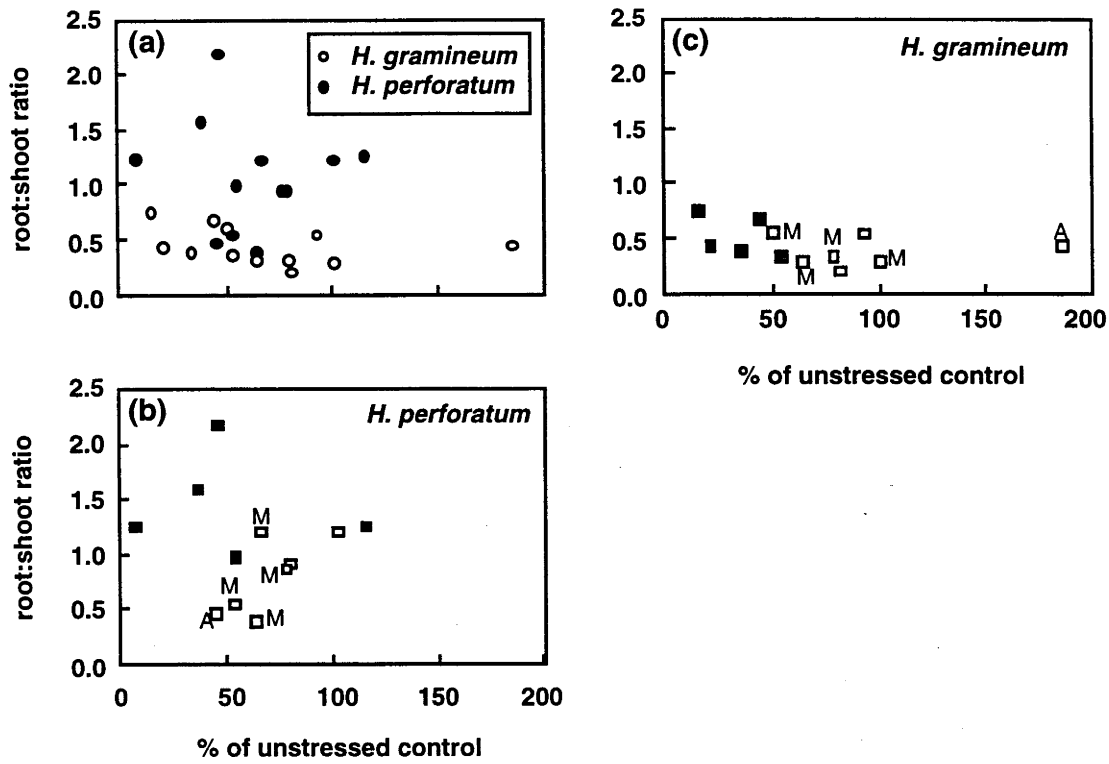

Fig. 9.2 (a) The root:shoot ratio of *H. gramineum* and *H. perforatum* observed in the stress experiments of chapters 7 and 8, plotted against growth of that plant, as a percentage of their unstressed control. Split by species, the same data are plotted classifying stress according to its effect on the root system (closed squares) and the shoot system (open squares). Shoot stress imposed by mite-infestation (M) and aphid-infestation (A) is indicated for *H. perforatum* (b) and *H. gramineum* (c).

By classifying stress according to its primary effects on roots or shoots, it appears that root stresses (nutrient limitation, root competition and water limitation) generally increase the root:shoot ratio of *H. perforatum*, while shoot stresses, particularly herbivory by mites or aphids, but to a lesser extent shoot competition, decrease the ratio (Fig. 9.2b). The trend is relatively weak in *H. gramineum* (Fig. 9.2c). These patterns of response are consistent with 'source-sink' models of root:shoot ratio control, as described in previous chapters: resource allocation shifts in favour of the stressed system. The herbivores studied in this thesis elicit a relatively stronger, though usually non-significant, change in the root:shoot ratio of *H. perforatum* than *H. gramineum*. This pattern probably reflects more rapid population development of these 'host-specific' biological control agents on their target weed species, in comparison with the non-target native.

9.3.4. Variation between experiments

Variation between experiments in the effect of mites on all measures of *H. gramineum* and *H. perforatum* growth were common in the present study. Differences between the glasshouse experiments and those conducted in the field were anticipated given that it is often difficult to extrapolate results obtained in glasshouse trials to those conducted under field conditions (Waring and Cobb 1992). This is because the physical and biotic environment experienced by plants and herbivores in glasshouse studies differ markedly from those experienced in the field.

A possible cause of the differences between the field and glasshouse studies may relate to the plant growth medium. In glasshouse-grown potted plants, the volume of substrate and the water available for plant growth is restricted relative to that in the field, where roots may grow deep into the soil profile, largely avoiding physical constraints. The soil of pot-grown plants occurs in a shallow layer, with the pot's base breaking the continuity present in field soil profiles. In pots, this often leads to a 'perched' water table and prevents free drainage (Bunt 1988). Moreover, in glasshouse experiments plants may adapt to regular watering regimes. In the field, by comparison, adequate supplies of water for plant growth may be less predictable. Another possible reason for differences in the field and glasshouse experiments is that air temperatures fluctuate widely in field experiments, but are relatively well controlled in glasshouses. The mean temperature minima and maxima of glasshouses utilised in the present study were 20° and 35°C, respectively, in summer and 12° and 20°C, respectively, in winter. During the field experiments of chapter 8, by comparison, the air temperature ranged from -5.6° to 36.5°C. Clearly, such temperature variation may have affected host-plant growth, and probably mite developmental rates.

Variation in results between glasshouse trials may partly reflect variations in the level of mite-infestation. To minimise such variation, experimental plants were of the same developmental stage when infested and received similar numbers of mites. This latter variable was more difficult to standardise because of difficulties in manipulating the small (50 µm long) arthropods. Because of their size, the only practical means of assessing populations of *A. hyperici* is to destructively sample plants. This sampling technique would have affected growth of the experimental treatments. Consequently, mite population development was not monitored in the above experiments except to confirm their presence at the termination of the

experimental period. The +mite treatments may therefore include a range of mite infestations from slight to heavy, and this variation may mask clear statistical differences between +mite and -mite treatments. Thus, the trends in growth may be more indicative than the simple statistical tests imply.

Differences between the glasshouse experiments may also reflect variation between soils. In the water stress experiment, that which caused the most severe limitations on plant growth, a heavy clay soil was selected to reduce the rate of drainage, thereby allowing control of the soil moisture content. In the high watering regimes, it is likely that this slow-draining soil led to a perched water table, as noted above, in comparison with the more freely draining soils of other glasshouse experiments. In the low watering regime, by contrast, the soil became hard and difficult to penetrate, probably physically limiting root growth as well as providing water-limited growth.

Finally, differences between the results obtained in the various glasshouse trials may reflect differences in the air temperature and humidity of the glasshouses. As noted above, there was variation in air temperatures between the seasons. Since the glasshouse experiments were conducted at various times of the year, this variation in temperature may have affected plant growth and probably mite developmental rates.

9.4. The impact of *A. hyperici* on populations of *H. gramineum*

Mite-induced reductions in growth of field grown *H. gramineum* (chapter 8) are likely to affect populations of the native forb. Although decreases in the growth of individual plants are generally statistically insignificant, any reductions in plant productivity is likely to translate into some impact on the population. Within an infested population of *H. gramineum*, it is probable that the proportion of affected plants will be a function of the density of the plant population: low density populations will be at relatively low risk, as the probability of a mite encountering a plant is small, while higher density populations are at higher risk. The likelihood of mite establishment with distance, discussed in chapter 4, suggests that mite infestation will vary with the square-root of population density. Such density-dependence regulates many plant-herbivore interactions.

9.4.1. Factors influencing the A. hyperici-H. gramineum *interaction*

This thesis has focused on the interaction between *A. hyperici* and *H. gramineum* assuming that if mites alight on the native species, this is followed by removal of the novel host's resources. Many other factors may influence the likelihood, and impact of *Aculus* herbivory of *H. gramineum*. These were not central issues in this thesis, but deserve some consideration.

9.4.1.1. Climate, predators and pathogens

Aculus hyperici does not form the galls or erinea that protect many other eriophyids from climatic extremes, and which help maintain the microclimate of eriophyid feeding and reproductive sites (Jeppson *et al.* 1975). In comparison with *H. perforatum*, this lack of protection may be exacerbated if *H. gramineum* is employed as a host. This is because the architecture of *H. gramineum* provides relatively few vegetative buds which, on the target weed, are numerous and among which, the mites are typically found. Such sites probably provide more protection for mites on *H. perforatum* than on *H. gramineum*. Architectural differences between *H. gramineum* and *H. perforatum* are therefore likely to favour mite population development on the target weed.

In addition to buffering mites from climatic extremes, galls also protect herbivores from predators. Exposed mites such as *A. hyperici* may, therefore, suffer relatively high rates of predation, reducing their impact on target and non-target species. Predation of biological weed control herbivores has been reported for *H. perforatum*. Wilson (1960) indicated that attempts to introduce *Anaitis plagiata* (Lepidoptera: Geometridae) into Australia for biological control of this weed may not have been successful because predators, particularly ants, reduced their populations. However, Harris *et al.* (1969) suggested that a viral disease accidentally imported with the larvae may have contributed to the failure of these moths to become established.

Competition between phytophagous arthropods may also reduce the impact of herbivory by one or more of the species on plant growth, although their combined impact may be high. While it is now common biological weed control practice to introduce several herbivores targeted at different tissue resources in specific seasons (see, for example James *et al.* 1992), it is possible that if the control agents compete for similar resources, populations of one may suffer at the expense of the

other. Similarly, competition between herbivores may result in the competitive exclusion of native arthropods from their indigenous hosts or conversely, native arthropods may limit the establishment of the introduced control agents on non-target native species (Turner 1985).

9.4.1.2. Host-plant condition

The condition of host plants plays a major role in the biology of herbivores and has been the subject of debate in the recent ecological literature. Some authors such as Price (1991), argue that rapidly growing healthy plants offer better conditions for herbivore growth than stressed plants because they provide better quality resources to the herbivores, while others (e.g. White 1984; Mattson and Haack 1987a,b) indicate that because utilisable nitrogen levels often increase in moderately stressed plants, herbivore populations limited by nitrogen availability, perform better on stressed hosts. These hypotheses generally assume that increases in the herbivore population lead to decreases in plant productivity, but rarely test the assumption.

Tightly linked to the condition of the host and its impact on the herbivore is the ability of plants to tolerate herbivory. Smith (1989) defines plant 'tolerance' as the inherent ability of plants to recover from herbivore damage. He contrasts 'tolerance' with 'plant-mediated antibiosis', in which the biology of the herbivore is adversely affected by, for example, plant toxins. Further, he suggests that 'tolerance' differs from 'antixenosis', in which the plant acts as a poor host, providing, for instance, tissues of low nutritive value. Frequently, there is overlap in these three modes of plant resistance to phytophagous arthropods (Smith 1989). Current hypotheses debate the level of plant resistance to herbivory conferred by environmentally-mediated host-plant condition.

As Larsson (1989) notes, different guilds of herbivores may respond differently to plant stress, associated with their various feeding modes. It seems likely that, as in many ecological systems, there exists a continuum of responses to host condition, whereby some herbivores perform better on stressed individuals, while others perform better on healthy individuals (Waring and Price 1990; Price 1991), although the condition of both plant and herbivore affects the interaction (Vranjic 1993).

In this study, it was not possible to determine the effect of host-plant condition on *A. hyperici* because monitoring the mite required destructive sampling. The effect of stress on host plants was, however, more apparent. Alone, stresses reduced

plant growth, and in combination, plant growth was roughly the product of the proportional growth under the stresses individually. 'Tolerance' of *Hypericum* to herbivory by *A. hyperici* did not decrease with increasing stress, since stress combinations did not induce synergistic reductions in plant growth, as might be expected if tolerance were reduced. This was despite increases in the tissue nitrogen concentration of stressed plants, which often limits herbivore population expansion. Similarly, herbivory of *Hypericum* did not increase, as reflected by more damage to host plants, on the relatively vigorously growing unstressed controls. The results of chapter 7, in which establishment of mites was known with certainty, support neither the stress nor the vigour hypotheses of plant-herbivore interaction. These hypotheses may describe certain plant-herbivore systems, but the *Aculus-Hypericum* system seems to lie between the two relatively extreme models. In the *Aculus-Hypericum* system, it appears that there is a simple linear proportional relationship between mite populations and the availability of host plant tissue.

9.4.1.3. Size and age of host-plants

An advantage of studying herbivory of herbaceous plants is that its impact can be monitored on all plant organs (i.e, root, shoot, and reproductive systems) relatively easily, enabling predictions to be made regarding the effect of herbivory on total plant growth. The ability to study plants at different developmental stages in a controlled environment is also easier in forbs, and may be important if herbivory varies between plants of different age and size. Vigorously growing seedlings for example, may be more tolerant of herbivory than older plants, being able to 'out-grow' herbivore infestations. By contrast, they may represent a poorly defended and easily digested food resource, of high nutritive value (Coley *et al.* 1985).

In the water and aphid stress experiment of chapter 7, there was some indication that smaller, water-stressed plants were more severely affected by *A. hyperici* than larger plants. Although the treatment effects imply that this was a result of water stress, the possibility that it was actually a size effect deserves consideration. The plant size/age experiment described in chapter 6 was initiated to examine the possibility that seedlings and adults of both *H. gramineum* and *H, perforatum* are differentially affected by *A. hyperici*-herbivory. Unfortunately, populations of mites established irregularly in this trial. Repetition of the experiment may shed further light on the influence of host-plant size/age on herbivore growth, and its impact on plant survival.

The size and age of host-plants also affects their stored reserves and hence the capacity of infested plants to tolerate herbivory. Large plants with extensive root systems may be able to tolerate continued herbivory over several seasons. Smaller plants and seedlings have less capacity to store resources and may suffer relatively severe damage over shorter periods of time. Such possibilities can only be investigated with further experimentation with plants of different age and size.

9.4.1.4. Cumulative effects of herbivory

On larger plants with well developed storage capacities, the cumulative effects of herbivory may eventually decrease plant growth, whereas in the short term herbivore-mediated reductions in plant productivity may be less apparent. Comparatively little work has been conducted to examine this aspect of herbivore-plant interactions. Strauss (1991) noted that in a three year study of chrysomelid grazing, beetles had direct negative impacts on plant growth, as well as cumulative effects; the longer plants were exposed to chrysomelid herbivory, the more likely they were to die. Karban and Strauss (1993) observed cumulative effects of spittlebugs (*Philaenus spumarius*) on *Erigeron glaucus*. This insect reduced flower and seed production but had no significant impact on vegetative growth. In addition, the negative effect of spittlebugs on the reproductive output of their hosts was evident the following year, after the herbivores had been removed. This implies that reductions in stored reserves mediated by insects in one year can have negative effects on fitness and growth in subsequent years. To this extent, herbivores resemble some abiotic stresses. In limiting plant growth and storage in one season, stress limits the capacity of plants to regenerate in subsequent seasons.

The variable success of *C. quadrigemina* in controlling *H. perforatum* in Australia has been attributed, in part, to the plant's ability to regenerate from stored reserves (Groves 1989). Briese (1989) indicated that a major reason for introducing *Aphis chloris* into Australia was to sustain the stress imposed on *H. perforatum* by *C. quadrigemina* during periods or seasons when the beetles were inactive. Consistent with other phloem-feeding herbivores (see, for example, Vranjic and Gullan 1990), *A. chloris* has the potential to reduce the ability of *H. perforatum* to regenerate from stored root reserves. Plant growth following herbivory by combinations of these herbivores is likely to approximate the product of the proportional growth following herbivory by each of these agents individually, as outlined in chapter 7. The cumulative effects of herbivory by combinations of *C. quadrigemina*, *Aculus hyperici* and *Aphis chloris* may offer a better chance to control *H. perforatum*, by reducing the plant's high root:shoot ratio and thereby, its capacity to regenerate

from a well developed root system. The ability of these arthropods to feed and reproduce on *H. gramineum* may also heighten the risk of damage to the native non-target species, whose root system is not so well developed, and has a relatively limited capacity for storage.

9.4.1.5. Host range expansion

Several examples of host-range expansion by biological control herbivores onto non-target native species were cited in chapter 1. The process has also been demonstrated in this thesis. The question remains as to whether the host range of *A. hyperici* is likely to expand further, possibly evolving to encompass other non-target native flora.

It has recently been argued that the role of predators and parasites in the evolution of host specificity has been underestimated (Bernays and Graham 1988; Denno *et al.* 1990). If these agents are able to limit the host range of *A. hyperici* in its native European habitat, there are contrasting possible outcomes following its establishment in Australia: either, the diet of the herbivore will extend to include other novel species, or, alternatively, indigenous predators, parasites and competitors will limit extension of the herbivore's host range. In Europe, predators and pathogens do not appear to restrict the range of *A. hyperici* to *Hypericum* species or indeed other plant taxa, although no studies have been undertaken to investigate this possibility. Nor in Australia, have such predators been found, despite a survey conducted by the CSIRO soon after the initial mite introductions. In the apparent absence of predators and a diet clearly limited to *Hypericum* species, it seems that other factors restrict the host range of *A. hyperici.* It is possible that these include the morphology, anatomy or plant chemistry of *Hypericum.* Regarding the latter possibility, perhaps the secondary metabolite, hypericin, which is apparently unique to *Hypericum*, is involved in host recognition. If this is the case, extension of *A. hyperici*'s diet to include other genera is unlikely. Moreover, the chance of this occurring in Australia seems no greater than the chance of its occurrence in Europe.

Several conceptual models of host plant suitability for *A. hyperici* are proposed in figure 9.3. These comprise various combinations of host 'quality', which may be affected by the physical and biotic environment of the plant, and/or the host's phylogeny. Model 1 is based strictly on host phylogeny. If the host is a member of an appropriate taxon, mites accept the species, regardless of its quality. In Model 2, plant quality, in addition to phylogeny, affects host selection: while plants may be

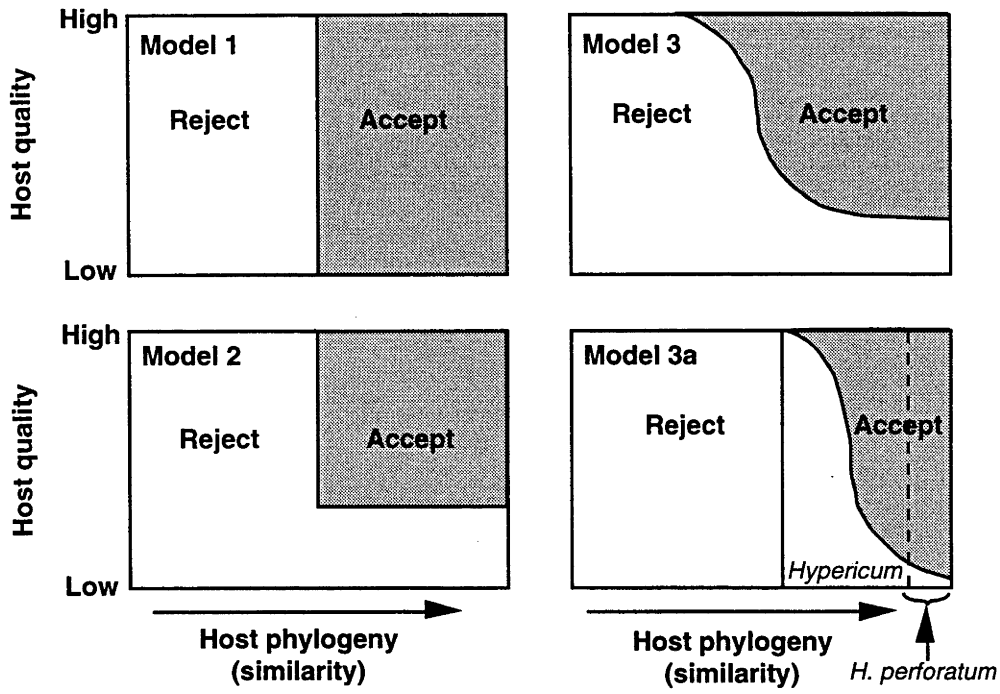

Fig. 9.3 Possible models of host-selection by *A. hyperici*, as affected by various combinations of host phylogeny and quality, with the taxon either accepted or rejected as a host, as indicated. Model 1 reflects host selection based purely on phylogeny. Model 2 combines phylogeny and quality, as does Model 3, though in the latter, the decision to either accept or reject a host is not absolute. Model 3a reflects host selection by *A. hyperici*: Phylogeny is important, but within the favoured taxon (genus), host quality may vary. *H. perforatum* seems to be the highest quality host for this herbivore.

of the appropriate taxon, utilisation of this taxon for feeding and oviposition depends on plant quality, above or below critical levels of which, mites respectively, will, or will not select the plant. Model 3 is similar to Model 2 in combining host quality and phylogeny as cues to host selection, although the boundaries are less precise. If host quality is high enough, the taxon is likely to be employed as a host irrespective of its phylogeny. By contrast, if host quality is low, the plant will not be utilised, even if, ordinarily, it represents a suitable taxon. Although restricted to *Hypericum*, within the genus there is clearly a range of host suitability for development and establishment of *A. hyperici* populations (CSIRO 1991; chapter 5). Apparently, a form of Model 3 (Model 3a) reflects host utilisation by *A. hyperici*: Restricted by phylogeny to *Hypericum*, the frequency with which

mites utilise different species varies with increasing quality. In supporting the most rapid population growth of *A. hyperici*, *H. perforatum* seems to be the species of highest quality. There appear to be distinct varietal forms of *H. perforatum* in Australia (M. Campbell, pers. comm.). It would be interesting to examine different varieties of this species to determine whether *H. perforatum* quality varies, intra-specifically, as a host. Such research is currently being undertaken by staff at the CSIRO.

The above factors, predators, pathogens and host quality, combine to determine the level of herbivore damage sustained by plants. Under different conditions, the importance of each in the interaction between herbivore and plant varies. So too does the impact of herbivory on plant growth, productivity and fitness.

9.5. Modelling the effect of *A. hyperici* on populations of *H. gramineum*

In chapter 3, a projection matrix was used to summarise the dynamics of a typical population of *H. gramineum*. Assuming that *A. hyperici* might decrease the probabilities of transition from one growth stage to another, it was predicted that if mites doubled the mortality of all plants, there would be a rapid decline, almost 16% per year, in the size of the population. As noted in chapter 3, reductions in any parameters of these simplistic models inevitably result in population decline, since the simulations maintain the decrease for the duration of the population's viability. As such, the transition matrix used to simulate an *Aculus*-infested *H. gramineum* population in chapter 3 assumed that no other factors affected the herbivore, plant growth, or the interaction between mite and plant. Such assumptions are inappropriate for several reasons, as considered below. Nevertheless, with quantification, and knowledge of the manner in which *A. hyperici* affects *H. gramineum*, projection matrices are useful to predict likely trends in the response of *H. gramineum* populations to mite-infestations.

Decreases in growth of *H. gramineum* attributable to *A. hyperici*, obtained from results in Section D of the thesis, and particularly chapter 8 (Field-impact of *A. hyperici* herbivory), have been used to estimate the decreases in the probability of transition between growth stages in the matrix described in chapter 3. The revised model assumes that the probability of a seed entering the seed bank remains the same (0.8). Seeds produced by *A. hyperici*-infested adults appear to germinate at a higher rate (20%) than those from un-infested plants (see chapter 2) and

consequently, the probability of germinating from the seed bank in the revised matrix is increased by 20% to 0.00048. In the absence of firm data on the effect of mites on growth and survival of juvenile and 'daughter' plants, their associated transitions have not been varied. By contrast, the probabilities of single-stemmed and multi-stemmed adults progressing to each of the next stages is reduced by 10% in the revised model. Larger reductions were observed in other experiments, but employing the results of the field trial is preferred to those of the glasshouse, because of variation between results obtained in the two. Although fruit production increased by about 20% in the field impact experiment, this measure of growth was highly variable, decreasing by about 60% in chapter 6 (see experiment 1, impact of mites on four *Hypericum* species). The average decrease observed across all experiments of this study was 11%. Since other studies generally report declines in fruit, and therefore, probably, seed production, this parameter was also reduced by 10% in the revised matrix. All modelled parameters are included in table 9.2, which summarises the original transition probabilities and the proportions by which these parameters are multiplied to achieve the respective increases/decreases in the modelled transition values.

Studies of plant-herbivore interactions suggest that the effects of *A. hyperici* on populations of *H. gramineum* are probably density-dependent, with populations at higher density suffering relatively higher rates of mite-infestation than lower density populations. Such relationships contrast those simulated in the projection matrices of chapter 3, which assumed that the effects of herbivores on plant populations are independent of population density or size.

To simulate this density-dependent effect, the probability (P) of plants becoming infested by mites each year was estimated as,

$$P = \frac{\sqrt{\text{Plant population density}}}{\sqrt{\text{Plant population density}} + 1}$$

where the plant population density is defined as the number of adults m^{-2}. This relationship is based on the inverse relationship of mite establishment with the source-plant distances observed in chapter 4, estimating interplant distance as the square-root of population density. The probability of remaining un-infested (Q) is,

$$Q = 1 - P.$$

Table 9.2 Transition probabilities used to model a stable population of *H. gramineum* (original model). Density-dependent herbivory by *A. hyperici*, modelled in a revised matrix (see text) multiplies the transition probabilities by the values indicated (Mites). The same model was also used to examine the impact of drought on population growth (Drought), alone, and in combination with mites, by multiplying all values.

Manip-ulation	Transitions	Seed	Seed bank	Juveniles and daughters	Single-stemmed adult	Multi-stemmed adult
Original Model	Seed	0	0	0	740	1300
	Seed bank	0.8	0.38	0	0	0
	Juveniles & daughters	0.0004	0.000004	0	0	0.3
	Single-stemmed adult	0	0	0.18279	0	0.3
	Multi-stemmed adult	0	0	0	0.88	0.58
Mites	Seed	1	1	1	0.9	0.9
	Seed bank	1	1	1	1	1
	Juveniles & daughters	1.2	1	1	1	0.9
	Single-stemmed adult	1	1	1	1	0.9
	Multi-stemmed adult	1	1	1	0.9	0.9
Drought	Seed	1	1	1	0.5	0.5
	Seed bank	1	1	1	1	1
	Juveniles & daughters	0.1	0.1	1	1	0.4
	Single-stemmed adult	1	1	0.25	1	1.5
	Multi-stemmed adult	1	1	1	0.6	0.7

To calculate infestation rates for individuals of different ages, the probability of infestation was increased with stage in the life cycle. In calculating the proportions of mite-free and mite-infested individuals, the stages of the vegetative population are varied by multiplying:

(a) the number of mite-infested juveniles and daughters by 1 - Q,
(b) the number of mite-free juveniles and daughters by Q,

(c) the number of mite-infested single-stemmed adults by $1 - Q^2$,
(d) the number of mite-free single-stemmed adults by Q^2,

(e) the number of mite-infested multi-stemmed adults by 1 - Q^3, and
(f) the number of mite-free multi-stemmed adults by Q^3.

Starting with a stable stage-structured population (56 juveniles/daughters, 28 single-stemmed adults and 58 multi-stemmed adults), changes in the population following *A. hyperici* herbivory were simulated using the density-dependent model. Simulations of three initial plant densities (3.6, 0.36 and 0.036 plants m^{-2}) indicate a much greater rate of decline, the higher the density (Fig. 9.4a). The modelled densities represent the range from typical to sparse populations of *H. gramineum.*

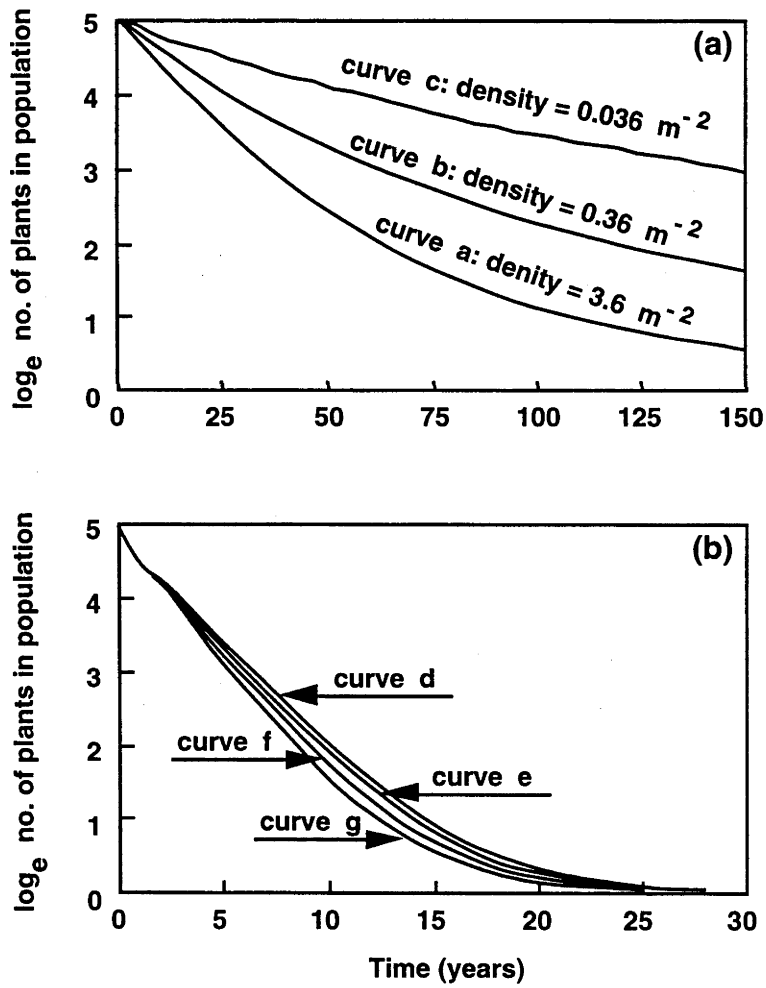

Fig. 9.4 Simulated density-dependent changes in a stable stage-structured population of *H. gramineum* following infestation of the population by *A. hyperici* (a), as summarised in table 9.2 and text, and (b) a drought-stressed population without mites (curve d) and with mites at an initial plant density of 3.6 plants m^{-2} (curve e), 0.36 plants m^{-2} (curve f) and 0.036 plants m^{-2} (curve g).

Simulating reduction in plant growth and survival resulting from a sustained drought, figure 9.4b, curve d, illustrates a rapid decline in the population of 3.6 plants m^{-2} in the absence of mites. This drought reduces germination and establishment of seedlings by 90%, growth and survival of juveniles by 75%, seed production by 50%, and growth and survival of single and multi-stemmed adults by 40 and 30% respectively. In addition, the probability of multi-stemmed adults regressing to the single-stemmed form is increased by 50%, as summarised in table 9.2. A drought of this severity has drastic consequences for the modelled *H. gramineum* population. At five years the rate of population decline approximates 22.7% per annum. A drought of this severity sustained for 16 years seems unlikely.

Chapters 7 and 8 described a series of experiments which investigated combinations of plant stress and herbivory by *A. hyperici*. Growth under combinations of stress was characterised as the product of the proportional growth under the stresses alone. Multiplying the effects of mites on plant growth with those of the drought simulated to achieve such proportional decreases in growth causes drastic and rapid reductions in the plant population. At natural field densities of 3.6 m^{-2} , the rate of population decline is about 27% each year (Fig. 9.4b, curve e). As expected in a density-dependent system, the rate of decline is less when the population density is reduced to 0.36 m^{-2}, though the decrease is slight (Fig. 9.4b, curve f). Even at the lowest population density simulated above, 0.036 plants m^{-2}, the rate of population decline is about 24.1% after five years (Fig. 9.4b, curve g).

The model of proportional plant growth under combinations of stress, as summarised above and discussed in chapter 7, also explains combinations of stress in the simulated population. As tabulated below (Table 9.3), the 'proportional growth model' predicts that under combinations of mite and drought stress, the simulated *H. gramineum* population would be reduced to about 1.8% of the unstressed, stable population after 10 years, assuming an initial population density of 3.6 plants m^{-2}. At the lower density of 0.036 plants m^{-2}, the model predicts that the population would be 3% of the unstressed population. In fact, the simulations project the populations to be about 1.8% and 2.9% respectively, at each of the densities. Predictions of the proportional growth model are very close to the simulated population response. Clearly, mite herbivory in combination with a severe plant stress such as drought leads to a decline in population size, more rapid than for either the drought or the effects of mites alone. The size of such a stressed

population is apparently roughly equivalent to the product of the proportional size of the population following drought and mite stress individually.

Table 9.3 Simulated *H. gramineum* population size after 10 years of various combinations of mite-stress and drought stress, taking the initial unstressed population to represent 100%. Expected values (%) for the hypothesis that combined stresses yield the product of the proportional population size under each separate stress are presented in round brackets. The initial unstressed population comprised 142 plants.

Initial population density	**Stress Combinations**		
	Mites	Drought-free	Drought-stressed
3.6 plants m^{-2}	- Mites	100 [142.3]	3.4
	+ Mites	58.8	1.8 (2.0)
0.036 plants m^{-2}	- Mites	100 [142.3]	3.4
	+ Mites	84.1	2.9 (3.0)

Any reductions in modelled parameters of projection matrices such as above lead to population decline unless offset by increases in other transition probabilities. Increases in seed germinability, for example, ameliorated the effects of reduced growth (see original simulation model, chapter 3). Although the density-dependent model probably simulates the nature of the *Hypericum-Aculus* interaction more accurately than does the model employed in chapter 3, the above projections, still based on uncertainties about the impact of mites on, for example, plant survival, remain hypothetical. Variation in the transition probabilities, the severity of drought or other stresses, and the impact of mites on plant growth alter the projected population trajectories clearly affect the rate of population change. Nevertheless, the simulations represent likely trends following mite infestation: *Aculus hyperici* will probably reduce the size of *H. gramineum* populations and do so more rapidly in denser populations of the species. In the absence of other stresses, the annual rate at which this occurs seems very low. Moreover, such mite-induced population decline relies on sustained levels of herbivory over one or more years. In the presence of other stresses, the rate of population decline increases, again, assuming

sustained growth reduction. The precise impact of *A. hyperici* on populations of *H. gramineum* will stem from interactions between environmental variables and the density of host populations. Combinations of such factors also drive the dynamics of the impact of this agent on populations of the target weed.

9.6. The impact of *A. hyperici* on other species of *Hypericum*

Host-specificity screening of *A. hyperici* prior to its field release indicated the potential for this mite to damage non-target *Hypericum* species other than *H. gramineum*. The Australian native, *H. japonicum* and several species of horticultural/ornamental importance were among such taxa. Results presented in chapter 6 support this conclusion, principally because the mites affected growth of the target weed, *H. perforatum*, and the non-target species, *H. gramineum*, *H. japonicum*, and *H. tetrapterum* almost equally. This implies that these species all have the potential to serve as hosts for *A. hyperici*. As noted, this finding must be treated with caution, given that populations of mites failed to establish at similar levels on *H. perforatum* and *H. gramineum* as they did in other glasshouse experiments.

With regard to the potentially damaging effect of *A. hyperici* on *H. japonicum*, it should be noted that in contrast to *H. gramineum*, populations of *H. japonicum* rarely co-occur with those of the target weed. Such allopatric separation of *H. perforatum* and *H. japonicum* might serve as a barrier to damage by the mite. Turner (1985) hypothesised that potential non-target species may be utilised by introduced herbivores if other non-target species are able to bridge the allopatric distribution of the target plant and the potential non-target host of concern. This presents a mechanism by which *A. hyperici* could contact populations of *H. japonicum* or *H. tetrapterum*, via *H. gramineum*.

Lamp and Collet (1989) regard *H. tetrapterum* as a weed in some parts of Victoria, and as such, reduction in its population density by *A. hyperici* may be viewed as a bonus (CSIRO 1991). Indeed, the mite has been deliberately introduced into some infestations of this 'non-target' species by the Keith Turnbull Institute of Victoria in collaboration with the CSIRO, although to date, it has not had any noticeable impacts on plant growth or population density (P. Jupp, pers. comm.). As observed in previous chapters of this thesis, there is a continuum from herbivores utilising a novel host to affecting the growth of that plant. Although *A. hyperici* may be able to utilise non-targets such as *H. tetrapterum*, it may not affect their

abundance dramatically, or perhaps, only at critical densities, which might vary according to the suitability of the species as a host.

9.6.1. Adequacy of current screening techniques

The marginal effects on growth of *H. gramineum* reported in this thesis were anticipated prior to release of *A. hyperici*. By contrast, it was expected that mites would induce more significant reductions in growth of *H. perforatum* than were revealed in this study. These results suggest that current screening techniques should be expanded to investigate the impact of control agents on plant growth, in addition to the host-specificity of agents. Clearly, it is impractical to experimentally compare the growth of all test species in the presence and absence of potential agents. However, the impact of agents on growth of target species and their impact on growth of non-target species that are clearly at risk of utilisation should, at least, be examined. Quantification of the likely effects of control agents on the target species would enable more informed decisions to be made as to whether the advantages in releasing biological control agents outweigh the risk posed to non-target taxa.

Crawley (1989) suggests that deliberate introductions of herbivores into new environments for biological control of weeds represent large-scale 'field experiments', albeit usually unreplicated, capable of providing information on the interaction between plants and herbivores. In participating in 'the experiment', this study has been of some practical importance in quantifying the impact of *A. hyperici* on *Hypericum* and particularly, on the growth and population dynamics of *H. gramineum*. In addition, this plant-herbivore system has provided a means of investigating theoretical aspects of the interaction between phytophagous arthropods and their hosts. Continued study of *H. perforatum*, *A. hyperici* and *H. gramineum* may reveal interesting patterns in this plant herbivore system of further practical importance to biological control of St. John's Wort, and the implications of such control for populations of *H. gramineum* and other non-target species. Moreover, this large scale 'field experiment' may help clarify some of the theoretical issues concerning interactions between plants and herbivores. Such theories have been the focus of much research in the ecological literature, and continue to be vigorously discussed. Progress in these debates will benefit from continued research and further, affect management of introduced species and indigenous flora alike.

9.7. Future research

Despite attempting to quantify the most important variables in the *A. hyperici-H. gramineum-H. perforatum* system, there remain many unanswered questions. As noted above, investigation of some of these unresolved issues may be of practical value to the successful control of *H. perforatum* in Australia, and contribute to some of the continuing biological debates.

The aim of this thesis has been to examine growth and population dynamics of *H. gramineum* as affected by mite-herbivory. Only the most basic features of the biology of this mite, and indeed most other acarine species (Jeppson *et al.* 1975), are understood. A primary goal for future research is therefore to investigate the population dynamics of *A. hyperici* as affected by the condition of host plants. Clarification of the impact of mites on vigorously growing seedlings in comparison with their effect on older plants may also be fruitful, especially as a similar experiment in this thesis (plant age experiment, chapter 6) was largely unsuccessful. Further research into the population dynamics of mites as affected by host-plant condition offers practical benefits in terms of more efficient biological weed control, and may help clarify the continuing debate over the effect of plant condition on herbivore population development.

Plant-herbivore growth experiments are difficult to perform and are therefore usually conducted in controlled glasshouses. As has been demonstrated in this thesis and many other publications, direct extrapolation of glasshouse-derived results to the field is inappropriate. Further research should, therefore, explore the interaction between *A. hyperici* and host-plants in the field. In particular, the impact of this mite on *H. gramineum* and *H. japonicum*, the two Australian indigenous species of *Hypericum*, warrants careful monitoring, particularly in light of the inconclusive experiment investigating growth of the latter, as affected by *A. hyperici* (chapter 6). Future experiments and surveys should include examination of the long term, cumulative effects of herbivory by *A. hyperici* on the population dynamics of these non-target species. Similarly, long-term monitoring of *A. hyperici*-infested populations of *H. perforatum* should occur to monitor the effect of this agent on the population dynamics of the weed, either alone, or in combination with other stresses, and determine the environmental and economic results of its introduction. As noted by Howarth (1983), the success of a biological control agent such as *A. hyperici* may then be judged on its detrimental impact on the target weed, in combination with its effect on the non-target native flora.

9.8. Conclusions

Examination of the interaction between *A. hyperici*, *H. gramineum* and other taxa under various field and glasshouse conditions using surveys, experiments and projections matrices suggests that:

- *Aculus hyperici* is likely to encounter and infest field populations of *H. gramineum.*

- *A. hyperici* either rejects or fails to establish on taxa other than *Hypericum.* This generic specificity may be in response to the presence of a secondary metabolite, hypericin.

- The probability of mites infesting populations of *H. gramineum* and the target weed, *H. perforatum*, depends on plant separation and possibly plant size, with high density populations at greater risk of infestation than low density populations. To this extent, the larger *H. perforatum* is more likely to be infested than *H. gramineum.* This observation supports Feeney's (1976) model of plant apparency.

- Mite populations are likely to develop more rapidly on *H. perforatum* than on *H. gramineum* or other *Hypericum* species, suggesting that interactions between plant chemistry and host quality affect the dynamics of mite population growth.

- There is little difference between *H. gramineum* and *H. perforatum* in mite-induced growth reductions, relative to their respective mite-free controls. Nevertheless, mite-induced decreases in growth were consistently, albeit non-significantly, greater on the target weed species, than on the non-target native.

- Despite *A. hyperici* feeding on shoots, the root growth of infested plants is likely to be more severely affected by *A. hyperici* than the shoot growth. This is a widespread phenomenon among shoot herbivores and is consistent with 'source - sink' models of root:shoot ratio control, as proposed by Thornley (1972).

- Combinations of environmental stress and mite herbivory appear to cause reductions in growth (of all affected plant parts) roughly equivalent to the product of proportional growth under the stresses and herbivory individually. This observation supports neither the 'stress' nor the 'vigour' hypotheses of plant-herbivore interaction. The *A. hyperici-Hypericum* system may lie between these

hypotheses, which reflect opposite extremes in a continuum of possible herbivore responses to plant quality.

- Simulations of the impact of *A. hyperici* on populations of *H. gramineum* indicate a slow decrease in the decline of typical populations under sustained herbivory over several years. The rate of population decline is likely to decrease with time. This is because as the density of populations decrease, so too does the probability of mite infestation of plants within the population. Such density-dependence is characteristic of many plant-herbivore systems. Combinations of stress are likely to affect populations of *H. gramineum* in a proportional manner, similar to that indicated for stress combinations on plant growth.

- Long term monitoring of field populations of *H. perforatum*, *H. gramineum* and other non-target species, such as *H. japonicum*, is required to investigate whether the effects of *A. hyperici* may be cumulative.

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