Evidence for resistance in *Hypericum perforatum* to a biological control agent, the eriophyid mite *Aculus hyperici*

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Abstract

The eriophyid mite, *Aculus hyperici*, was released in Australia in 1991 for the biological control of St. John's wort, *Hypericum perforatum*. Establishment was variable, with some sites showing rapid increases in mite populations to levels at which plant damage was observed after two years, while others failed completely. Differences could not all be explained in terms of climate or release site conditions. Two laboratory experiments were set up to determine whether different forms of the target weed might show differences in susceptibility to attack by *A. hyperici* to measure the impact of the mites on these forms. Overall, there was a negative correlation between mite density and both host-plant root and shoot biomass, demonstrating the impact of *A. hyperici*. However, populations of the mite remained very low on plants collected from three of the eight regions tested, including sites where establishment failed in the field. It was concluded, that variable host-plant susceptibility may influence the impact of this highly promising agent, and needs to be monitored carefully.

Introduction

Variation in weed populations can have far reaching consequences for the practice of biological control. This was dramatically illustrated by the control of the narrow leaf form (one of three forms) of skeleton weed in Australia by *Puccinia chondrillina* . The gradual replacement of the controlled narrow leaf form of this weed by the intermediate and broad leaf forms shows how important the genetic variation of a weed can become (Burdon *et al.* 1981). St. John's wort (*Hypericum perforatum*) is a weed that has significant variation in morphology and in biochemistry (hypericin content) (Campbell *et al.* 1997). Until recently this variation has not had serious ramifications for the biological control of the weed. The eriophyid mite, *Aculus hyperici* was first released in 1991 at Pierce's Creek near Canberra in the Australian Capital Territory. While early establishment was successful in most areas, a significant number of the releases around Mudgee and Coolah in New South Wales failed to establish despite repeated inoculations and with no obvious influence of release site conditions (Jupp 1993). Two laboratory experiments were performed to determine, firstly, whether different forms of the target weed might show differences in susceptibility to attack by *A. hyperici* and, secondly, the effect of any variability in attack level on the subsequent impact on the root and shoot system of different forms of St. John's wort.

Methods

Experiment 1. Levels of susceptibility of four known forms of St. John's wort

Seed stocks of four morphologically different forms of St. John's wort, Mudgee Intermediate, Duntry Broad, Mudgee Tall and Narrow and Tuena Narrow, were washed for 24 hours in a muslin bag overnight and then germinated on a bed of 50/50 vermiculite and perlite. Fifteen seedlings of each form were pricked out into 145 mm pots and allowed to grow for two weeks into small rosettes. Five of these rosettes were kept as controls while ten were inoculated with five mite infested buds from existing mite cultures. Infected plants were placed at random in a room at 20°C and a 12 hour light period. The uninfected plants were placed in a second room under identical conditions. At two weekly intervals five buds were examined on each plant for mites and rated using the following rating scale:

0 = no mites
1 = <10 mites per bud
2 = 10–25 mites per bud
3 = 25–50 mites per bud
4 = >50 mites per bud

The plants were examined for a total of fourteen weeks until plants began to die due to mite activity.

Experiment 2. Effect of Aculus hyperici on St. John's wort from sites with known mite performance

Seeds of St. John's wort were collected from six sites of known release history. The location of these sites and their establishment history are summarized in Table 1. These seeds were washed and germinated as described for Experiment 1. Twenty seedlings of the same age and size from the different sites were pricked out into 45 cm pots in a sand/peat soil mix, fertilized with a standard measure of Osmocote® and allowed to grow for a further week. In addition to seed from sites with known establishment histories, seed of the Mudgee Intermediate and Tuena narrow forms were also prepared in the same way, as the results from Experiment 1 suggested that the former has lower susceptibility and the latter was highly susceptible. Five of each set of twenty plants of St. John's wort were washed, dried and weighed to determine plant variability. A further ten plants of each set were inoculated with five mite infested buds from an established mite culture. The last five plants were routinely sprayed with Omite® to prevent mite infestations, as this chemical does not affect the growth of St. John's wort plants (Willis 1994). The mite infested plants were sprayed with water as a control for any water effects. The positions of sprayed and unsprayed plants were assigned randomly over ten benches in a glasshouse. Plants were sprayed outside and allowed to dry before being placed back into the glasshouse. All plants were examined every two weeks for mites over a 14 week period using the rating system described above. At the end of 14 weeks all plants were harvested and their roots washed and dried in a drying oven for 48 hours. Both the root and shoot systems were then weighed.

Table 1. Sources of seed from release sites with known establishment histories.

Release site	Location	Release history	Present status
Mudgee, NSW	32º 36'E × 149º 35'S	6/1991, 4/1992	No establishment at present
Tuena, NSW	34º 01'E × 149º 25'S	6/1991, 4/1992, 10/1992	Establishment confirmed 5/1994
Coolah, NSW	31º 45'E × 149º 52'S	6/1991, 4/1992, 1/1992	No establishment at present
Beechworth, Victoria	36º 22'E × 146º 21'S	6/1991, 12/1992	Establishment confirmed 4/1992
Wyangle Station, NSW	35º 18'E × 148º 13'S	7/1991	Establishment confirmed 5/1992
Talmalmo, NSW	35º 55'E × 147º 24'S	6/1991	Establishment confirmed 3/1992

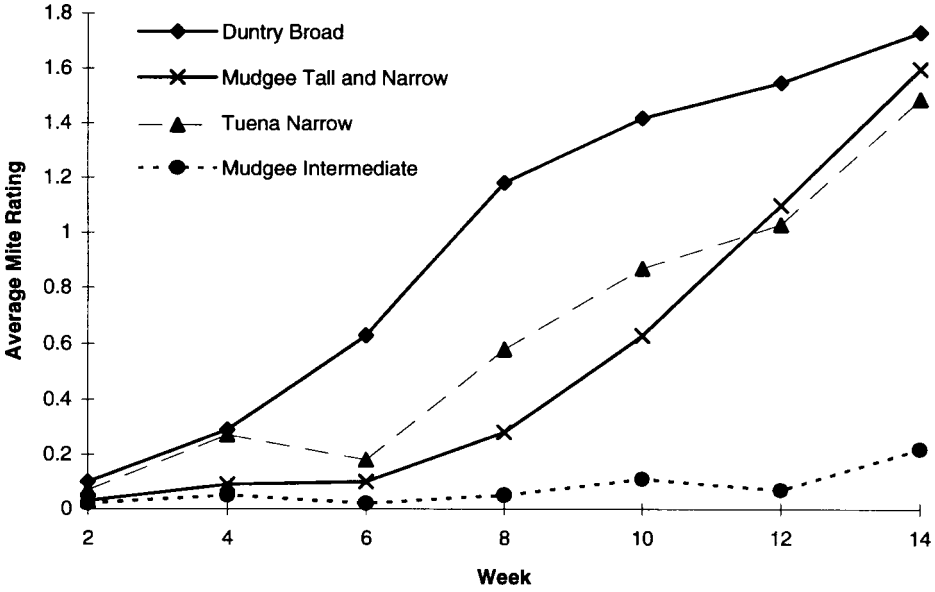

Figure 1. Average rating of *Aculus hyperici* per bud over a 14 week period on populations of St. John's wort with varying phenotypes.

Results

Experiment 1. Levels of susceptibility of four known forms of St. John's wort

Figure 1 shows the average mite rating over the course of the experiment for the four forms of St. John's wort tested. While mite colonies did survive on all test plants, the results show a much lower level of mite activity on the Mudgee Intermediate form relative to the three other forms, Duntry broad, Tuena narrow and Mudgee narrow. In the three susceptible forms, there was a steady increase in the average mite rating per bud over the course of the experiment with an accumulated rating for the 14 weeks much higher than the Mudgee Intermediate form (Table 2). Moreover, all plants of the Mudgee Intermediate form were alive after 16 weeks, whereas all of the Duntry broad and Mudgee Tall and narrow forms, and six out of ten plants of the Tuena narrow plants were dead due to the activity of *A. hyperici*.

Table 2. Accumulated mite ratings over 14 weeks on plant populations with differing phenotypes. (See text for explanation).

Plant population	Accumulated ratings over seven rating periods
Mudgee Intermediate	0.54
Mudgee Tall and Narrow	3.83
Tuena Narrow	4.49
Duntry Broad	6.90

Experiment 2. Effect of Aculus hyperici root and shoot systems of plants from sites with known mite performance.

As for Experiment 1, there was a steady build-up in mite densities on plants of the Tuena narrow form but little build up on the Mudgee Intermediate form, confirming their relative susceptibilities to *A. hyperici*. Plants originating from some field populations (Tuena, Wyangle Station, Beechworth and Talmalmo) also showed a build-up of mite populations, while others did not (Coolah and Mudgee). This corresponds with the history of establishment of mites in the field (Table 1). In plant populations which sustained a build-up of mite populations there were significant reductions in both root and shoot growth (Table 3). The overall mite density and feeding pressure placed on plants, as estimated by adding mite ratings over the 14 week period, is shown in Table 4. These estimates indicate that two of the populations (Tuena and Tuena narrow) sustained an intermediate level of mite population build-up. The root and shoot growth, relative to untreated control plants, showed a significant negative relationship with the estimates of mite activity (Figure 2), demonstrating a degree of impact that paralleled the level of mite colonization.

Table 3. Average root and shoot weight of St. John's wort plants after 14 weeks of mite infestation as compared with sprayed control plants.

Site	Treatment	Average weight of roots (g ± SE)		Average weight of shoots (g ± SE)	
Coolah	Control	7.16 ± 0.82		6.03 ± 0.29	
	+ mite	7.62 ± 1.33		5.65 ± 0.27	
Mudgee	Control	7.10 ± 0.25		5.18 ± 0.5	
	+ mite	6.52 ± 0.90		5.70 ± 0.39	
Mudgee Intermediate	Control	5.27 ± 0.67		6.54 ± 0.53	
	+ mite	4.49 ± 1.10		4.64 ± 0.91	
Tuena	Control	5.60 ± 0.38		6.80 ± 0.63	*
	+ mite	4.28 ± 0.58		4.60 ± 0.32	
Tuena Narrow	Control	3.23 ± 0.59	*[A]	5.40 ± 0.50	**
	+ mite	1.64 ± 0.31		3.22 ± 0.27	
Wyangle Station	Control	2.90 ± 1.29		3.78 ± 1.53	
	+ mite	1.56 ± 0.76		1.47 ± 0.59	
Beechworth	Control	5.07 ± 0.43	**	5.21 ± 0.44	**
	+ mite	2.01 ± 0.16		2.84 ± 0.37	
Talmalmo	Control	4.46 ± 0.32	**	6.27 ± 0.76	**
	+ mite	2.78 ± 0.36		3.68 ± 0.23	

[A] * indicates significant difference at the P=0.05 level and ** indicates significant difference at the P=0.01 level.

Discussion

The results presented here strongly support the hypothesis that the failure of the herbivorous mite, *A. hyperici* to establish on *H. perforatum* in certain localities was due to differences in the susceptibility of plants to mite attack. Plants from the field populations in which the mite had failed to establish both failed to support significant build-up of mites, while plants from all four populations in which the mites established well sustained large increases in mite population and suffered a corresponding reduction in growth and vigour.

The restricted host-range of eriophyid mites and their potential as biological control agents has long been recognised (Caresche and Wapshere 1974, Cromroy 1977, Cullen *et al.* 1982). The host ranges of particular populations of several species is limited to plant forms within a species, e.g. *Eriophyes cynodoniensis* on Bermuda grass (Cromroy 1977) and *Aceria chondrillae* on skeleton weed (Caresche and Wapshere 1974, Cullen and Moore 1983). The *A. hyperici-H. perforatum* interaction is interesting because it suggests that host-plant restriction may occur at sub-specific levels, yet evidence from host-specificity tests has shown that the mite can develop to some degree on other *Hypericum* species (CSIRO 1990, Willis 1994). These and the present data, moreover, suggest that

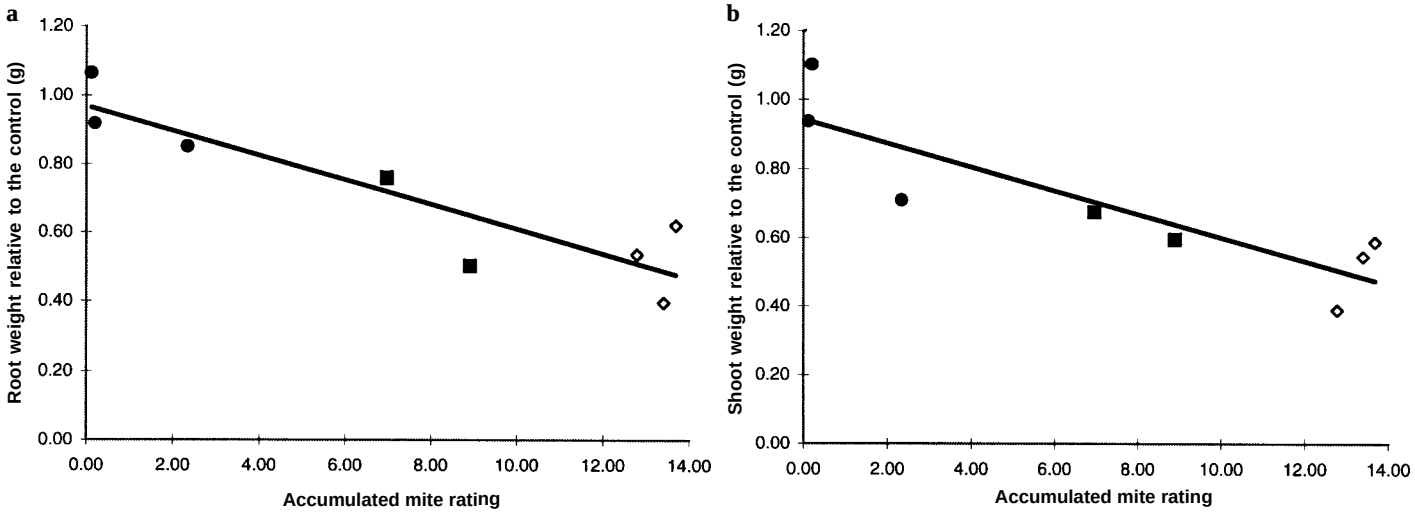

Figure 2. Relationship between accumulated mite rating with a) root weight (y=-0.0358x + 0.9688, R^2=0.8226) and b) shoot weight (y=-0.0342x + 0.9427, R^2=0.7697) of plants grown from seed stocks collected at various sites with varying susceptibility (◇ high, ■ medium, ● low) to *Aculus hyperici*.

there may be a range of variability in susceptibility to the mite, rather than a plant being either resistant or susceptible. Clearly for this anomaly to be resolved it is necessary to understand the plant-mite interaction that determines successful population build-up.

Despite early views that only one form of the weed was present in Australia, St. John's wort has been demonstrated to be quite variable in both morphology and plant chemistry (hypericin content) (Campbell 1997), susceptibility to anthracnose fungus (Shepherd 1995) and now response to attack by *A. hyperici*. In its native range in Europe, St. John's wort also forms variable populations with differences observed between populations of plants from the same habitats, from different habitats and between these and the populations introduced into Australia, when grown under the same conditions (Pritchard 1960). Pritchard suggests that there may have been genetic changes in the Australian populations since the introduction. *H. perforatum* is a facultative pseudogamous apomict (Noack 1939, sited in Pritchard 1960). In Europe, only 3% of seed were formed from sexual crossing (Robson 1968), but this value is not known for Australia. Given the observed variability in Australia and European plants, it is important to determine the levels of outcrossing under field conditions in Australia. Should this occur to a significant degree, this could hasten the spread of resistant forms through recombination and natural selection. If reproduction by seed is mainly asexual, St. John's wort plants would probably exist mainly as genetically isolated populations (given the high degree of vegetative reproduction (Briese these proceedings). In this case, the main risk for spreading resistance would be the transportation of seed from resistant populations to new areas, and such spread might be managed through quarantine.

Harris and Gill (1997) suggest that St. John's wort has been introduced on several occasions into Australia, with Mudgee being one of the first foci of introduction last century. Interestingly, the presumed source of the more recent Coolah infestation is contaminated fodder from the Mudgee area (R. Arnott personal communication). The lack of establishment of *A. hyperici* is, to date, limited to the Mudgee and Coolah regions (Mahr *et al.* 1997). It thus seems possible that the resistant form of St. John's wort resulted from a separate introduction to the Mudgee area and was further spread to Coolah. A detailed survey of the distribution of different forms of St. John's wort and their susceptibility to the mite is needed to determine the full extent of 'resistance' in Australia. Techniques being developed by Mayo and Roush (1997) to identify 'resistant' populations of the weed through their DNA profiles will be critical to the success of such a survey.

In summary, there is an inherent risk that resistance to *A. hyperici* might jeopardize the long-term impact of this highly promising biological control agent. The main tasks needed to reduce that risk are:

- the identification of 'resistant' plants and mapping of their distribution,
- determining the extent of outcrossing by *H. perforatum* in the field and its implications for the selection of resistance at a population level,
- determining the mechanism of resistance by the plants to the mite build-up and its mode of inheritance,
- testing the feasibility of finding other populations of the mite in Europe to which the 'resistant' plants are susceptible.

Such knowledge should help develop a strategy to manage resistance, should it threaten the effectiveness of biological control.

Table 4. Susceptibility of plants from different sites as indicated by accumulated mite ratings.

Susceptibility	Site	Sum of ratings over six periods
Low	Coolah	0.1
	Mudgee	0.2
	Mudgee Intermediate	2.3
Medium	Tuena	7.0
	Tuena Narrow	8.9
High	Wyangle Station	12.8
	Beechworth	13.4
	Talmalmo	13.7

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Genetic variability of *Hypericum perforatum* L. (Clusiaceae) and the detection of resistance to the biological control agent *Aculus hyperici* Liro (Eriophyidae)

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Summary

At least one form of *Hypericum perforatum* (St. John's wort) appears to be resistant to the most recently introduced biological control agent released in Australia, the eriophyid mite *Aculus hyperici*. A project has commenced that will investigate susceptibility of forms of *H. perforatum* to *A. hyperici*. Bioassays for plant susceptibility will be made on plants from a range of localities, including those sites at which the mite has so far failed to establish. Molecular markers for genomic DNA will be developed to enable differentiation between resistant and susceptible *H. perforatum*. The breeding system of *H. perforatum* in Australia will be investigated by cross-breeding experiments and, ultimately, the genetic control of resistance by linkage analysis of resistance markers. Information will be used to interpret field observations and further aid the search for more effective agents in Europe and elsewhere.

Introduction

The herbaceous perennial *Hypericum perforatum* L. or St. John's wort contains the toxin hypericin. Ingestion of this weed by stock causes photosensitization, resulting in skin irritation in areas exposed to light, followed by severe depression and loss of condition if animals are not removed from the source (Campbell *et al.* 1995). Management involving herbicides and improved pastures has controlled the weed on much pastoral land. Despite this, in New South Wales (NSW), Victoria and the south-east of South Australia St. John's wort is still a problem in non-arable land and poorly managed pasture, natural ecosystems, forestry reserves, water catchments and roadsides (Shepherd 1983, Campbell *et al.* 1995).

Fifteen biological control agents have been introduced into Australia, six of which have established (Briese 1986, 1989 and 1997, Campbell *et al.* 1995, Jupp and Cullen 1996). In May 1991, the eriophyid mite *Aculus hyperici* was released by CSIRO at 120 sites across NSW and Victoria (Jupp 1996). Of these, repeated releases at one site at Cassilis, and at another suspected five sites in the Liverpool Ranges of NSW, met with failure of the mite to establish in the field. Preliminary glasshouse trials with suspected non-susceptible and susceptible St. John's wort collections have supported field observations, and indicate that a form from Mudgee may also be resistant to the mite (Jupp *et al.* 1997).

It has been shown on the basis of plant morphology and biology that at least two forms of *H. perforatum* exist in Australia, although their taxonomic status remains confused (Campbell *et al.* 1992). The breeding system of *H. perforatum* is also unclear. The plant is considered to be a facultative pseudogamous apomict in Europe (Robson 1968). Given the variability of the weed and observed differences between Australian and European forms (Pritchard 1960) it is essential to determine what proportion of sexual seed is produced by cross-pollination in Australia.

This project aims to determine which forms of *H. perforatum* in Australia are resistant to *A. hyperici*, and to develop molecular markers for resistant types. Initial bioassays to determine plant susceptibility will involve the release of *A. hyperici* onto plants from localities across Australia, and two illegal imports from Canada, which are either suspected to be resistant, known to be susceptible or belong to an unknown category. Resistance will be correlated with morphological