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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)

G.M. Mayo and **R.T. Roush,** Department of Crop Protection and Co-operative Research Centre for Weed Management Systems, Waite Campus, University of Adelaide, PMB 1, Glen Osmond, South Australia 5064, Australia.

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

Table 1. Localities and collectors of *Hypericum perforatum* seed grown in the study.

Locality	State	Collector
Adelong	NSW	Malcolm Campbell
Bemboka	NSW	Malcolm Campbell
Brockman	WA	John Scott
Canada A	Canada	illegal import
Canada B	Canada	illegal import
Captain's Flat	NSW	Malcolm Campbell
Cassilis	NSW	Paul Lutschini
Coolah 1	NSW	Malcolm Campbell
Coolah 2	NSW	Paul Jupp
Coolah Creek	NSW	Paul Lutschini
Cowra	NSW	Malcolm Campbell
Daniel's Road	WA	Sandy Lloyd
Karridale	WA	John Scott
Mudgee Tall	NSW	Malcolm Campbell
Mudgee Intermediate	NSW	Malcolm Campbell
Mudgee	NSW	Paul Lutschini
Mudgee F1	NSW parent, bred ACT	Paul Jupp
Orange 1	NSW	Malcolm Campbell
Orange 2	NSW	Rick Roush
Scott Creek	SA	Gwen Mayo
Tuena 1	NSW	Malcolm Campbell
Tuena 2	NSW	Paul Jupp
Turon	NSW	Paul Lutschini
Wyangle	NSW	Malcolm Campbell

variation where possible, and molecular markers for genomic DNA will be developed to enable differentiation between resistant and susceptible *H. perforatum*, to enable the future collection of mites suitable for the control of resistant plant types.

Screening markers in F1 progeny should help to clarify the breeding system of *H. perforatum* in Australia. Herbivore-plant interaction studies have concentrated on single resistance genes in crop plants, and therefore it is of theoretical interest to investigate the genetic control of resistance in a natural ecosystem. Ultimately, the genetic control of resistance will be investigated by linkage analysis of resistance markers in segregating F2 progeny, which together with the gradation of susceptibility seen in the bioassay, will give an indication of whether resistance is under single or multiple gene control.

Significance

The well documented case of biological control of only one of three forms of *Chondrilla juncea* (skeleton weed) by the rust *Puccinia chondrillina* (Cullen and Groves 1981), emphasises the importance of recognising the effect that genetic variation within weed populations can have on the success of control programs. Subsequent to the spectacular control of form A by the rust, skeleton weed forms B and C invaded areas where only the former was originally present (Burdon *et al.* 1981). There is substantial justification in predicting a parallel in the case of mite-resistant St. John's wort, and given the enormous potential of resistant forms to spread from Liverpool Ranges and Mudgee areas, high priority should be given to obtaining mite populations that are capable of attacking resistant plants. It would be advantageous to have a marker system by which resistant plants (and therefore mites adapted to such plants) can be identified in Europe.

Also of importance to workers is the need to investigate the genetic variation within Australian St. John's wort populations, and how this relates to its breeding system. An understanding of the level of subspecific variation will contribute to modelling studies, and affect decisions on chemical, cultural and biological control programs and assessments of the distribution and impact of the weed.

Materials and methods

Plant propagation

St. John's wort plants were propagated by seed from 22 localities in Australia, and from two samples of medicinal herb seed illegally imported from Canada (Table 1).

Approximately 30 seeds from each of 25 localities were germinated as follows. Seed was washed 24–48 hours, surface sterilized and germinated on water agar, at 16h L / 8h D, 22 / 18°C, which resulted in 80–100% germination.

Individual 4–5 month old plants were propagated by naturally layered stem segments, or cuttings treated with 4 g L^{-1} indole acetic acid, to enable the use of identical plant material in susceptibility bioassays and molecular studies, and to safeguard against losing characterized plant material.

Notes are being made on morphological characters of glasshouse-grown plants. A more detailed morphological comparison will be made if resistance appears to correlate to morphology.

Mite culture

Mites are currently being cultured on susceptible plants in growth rooms at 16h L / 8h D, 18 / 15°C and approximately 50% humidity.

Bioassay

Glasshouse bioassays will involve the release of *A. hyperici* onto the rosette stage of suspected resistant, known susceptible and undetermined plants from localities across Australia, followed by plant harvest and analysis of mite numbers to determine plant susceptibility. To determine the minimum number of mites to transfer to experimental plants, the age structure of the mite population will be examined. Mites will be released onto plants by removing individual colony leaves, counting mite number, and placing the whole leaf onto the experimental plant. Approximately 100 mites per plant will be released onto vigorously growing six month old plants. Each pot will have a polypropylene collar and cover to prevent mites from blowing off in air currents, and to prevent contamination of plants. At the end of the bioassay, whole plants will be harvested, sonicated in ethanol to dislodge mites (Gibson 1975), and a subsample counted in a Doncaster nematode counting dish (Doncaster 1962). Glasshouse bioassays will be replaced in future with simpler, more efficient tissue culture assays if the latter are found to be a feasible alternative. Tissue culture assays will involve the culture of plant tips on nutrient agar, the release of a set number of mites onto those tips, followed by harvest of total plant material after two or three weeks, and counting as for the glasshouse assay. Initially both types of assay will be made concurrently.

The experimental design is a randomized complete block design, with 20 pots per block, and eight to ten replicates or blocks. Within a block, each pot will be from a different locality; treatment therefore is equivalent to locality. Five or six no mite control pots of susceptible plants will be placed randomly within the block to determine the level of cross-contamination.

Molecular markers

DNA will be extracted by a CTAB (cetyl trimethyl ammonium bromide) protocol (Doyle and Doyle 1990). RAPD markers for genomic DNA will be developed to distinguish between susceptible and resistant phenotypes. RAPDs may be converted to RFLPs markers by cloning RAPD fragments, and using them as probes for

RFLPs, to produce a method that is more reliable and transferrable between laboratories.

Cross pollination of resistant and susceptible strains will be undertaken in the glasshouse by emasculation of the 'female' recipient flower, followed by manual pollen transfer from the 'male' donor. Reciprocal crosses will be made. A pilot cross pollination study, including appropriate controls, will investigate stigma receptivity, and time and type of emasculation. Willis (1994) has shown that in the glasshouse virtually no seed is set by wind borne pollination in *H. perforatum*. Even though the risk of this or insect pollination should be minimal in the glasshouse, crosses will be bagged.

The analysis of polymorphic markers in the F1 of the cross described above, (or of any cross in which parental markers are polymorphic), will allow an approximation of the percentage of progeny produced by apomixis, and should help clarify the mode of reproduction of St. John's wort in Australia.

A bulked segregant analysis of RAPD markers in the F2 (Michelmore 1991) could approximately locate a single gene for resistance, by determining which markers co-segregate with resistance and are therefore more closely linked to the gene.

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