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Prospects for biological control of *Hieracium pilosella* with the rust *Puccinia hieracii* var. *piloselloidarum* in New Zealand

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Abstract. The rust *Puccinia hieracii* var. *piloselloidarum* has been studied in Europe with the intention of introducing it as a classical biological control agent for *Hieracium pilosella* in New Zealand. In a pilot study conducted in Europe, we demonstrated that plants grown from 14 different seed collections from New Zealand differed considerably in their response to a rust isolate from Pitlochry, Scotland. Urediniospores from this isolate germinated over a wide range of temperatures, on water agar. Highest germination rates were recorded at about 21°C when urediniospores were exposed to a short period of light at the beginning of the germination process followed by incubation in the dark. In April 1995, the rust was unexpectedly found on *H. pilosella* at Lincoln, New Zealand. A preliminary experiment was then conducted to determine the pathogenicity of the newly-discovered rust towards plants grown from the same seed collections as those used in the European study. Variation in disease response of plants within and between different seed collections was also observed with the New Zealand rust isolate, although an overall higher proportion of plants than that recorded in the European study developed uredinia after inoculation. The results suggest that several forms of the weed exist in New Zealand and that each form may vary in its susceptibility to different rust isolates.

Introduction

Hieracium pilosella L. (mouse-ear hawkweed) is an aggressive weed of native grasslands that threatens one of New Zealand's distinctive ecosystems and causes major losses to pastoral production (Hunter 1991). It is the most widespread, weedy *Hieracium* species (Scott 1984) and has potential to expand its current range. It reproduces apomictically by seeds and vegetatively by stolons, forming dense prostrate mats (Makepeace 1985). Control by intensive methods such as the use of herbicide, or oversowing and topdressing with fertilizer (Scott 1993) has proved uneconomical or inappropriate for most areas where *H. pilosella* is a problem. Classical biological control using insects (Syrett and Sárospataki 1993; Syrett *et al.* this Volume) and fungi (Scott 1984; Jenkins 1995) may offer a sustainable solution for this troublesome weed.

Rust fungi are considered excellent biological control agents because they sporulate rapidly and prolifically, cause disease epidemics, disperse efficiently, and are highly host-specific (Evans and Ellison 1990; Watson 1991). The autoecious, macrocyclic rust, *Puccinia hieracii* (Röhl.) H. Mart. var. *piloselloidarum* (Probst) Jørst. has been studied in Europe with the intention of introducing it as a biological control agent for *H. pilosella* in New Zealand (Jenkins 1995). In April 1995, the rust was unexpectedly found at Lincoln, New Zealand, and it appeared to fit descriptions of *Puccinia hieracii* var. *piloselloidarum* (E.H.C. McKenzie personal communication). Two other varieties of this rust, var. *hieracii* and var. *hypochoeridis* (Oudem.) Jørst., have been widely established in New Zealand for some years (McKenzie 1981, 1987). The three varieties are distinguished by the position of the germ pores on the urediniospores, and have been recorded from a different range of hosts (Wilson and Henderson 1966; Cummins 1978; Pennycook 1989). Fortunately, *P. hieracii* var. *piloselloidarum* does not represent a threat to non-target plants in New Zealand because of its putative very limited host-range (Jenkins 1995). In initial tests, the rust found at Lincoln did not infect any other *Hieracium* species belonging to the same subgenus (*Pilosella*) as *H. pilosella* (unpublished data).

This paper presents results from recent preliminary studies conducted in Europe and New Zealand which investigated the disease response of *H. pilosella* plants, grown from seed collected at 14 different sites in New Zealand, to two isolates of *P. hieracii* var. *piloselloidarum*. It also describes the effect of light regime and temperature on germination of urediniospores on water agar.

Methods

Maintenance of rust in Europe

Rust-infected *H. pilosella* plants collected from an approximately 2 m^2 area at Pitlochry, Scotland (October 1994) were transplanted in steam-sterilized loam (John Innes No. 2) contained in 7-cm diameter pots. The plants were maintained at the Institute of Environmental and Biological Sciences, Lancaster University, United Kingdom, in an insulated growth room fitted with artificial lights (discharge lamps, metal halide fluorescent kolorarc, 400WMBIF/BU GESE40, Thorn EMI Lighting Ltd, UK) and set at day and night temperatures of 18±1°C and 15±1°C, respectively, with a 16-h photoperiod (410 $\mu Em^{-2}s^{-1}$). Plants were regularly inoculated by collecting urediniospores from infected leaves, using a camel hair paint brush, and dusting the brush onto the adaxial surface of newly-produced leaves. Inoculated plants were misted with water and placed in plastic trays (34x48x24 cm) containing water to a depth of 3 cm. Trays were then placed inside opaque plastic bags (to provide a high humidity environment) and placed in the growth room for 18-24 h. Plants were subsequently removed from the bags and trays.

Maintenance of rust in New Zealand

Rust-infected *H. pilosella* plants collected from an approximately 10 m^2 area at Lincoln, New Zealand (May 1995) were transplanted in steam-sterilized peat pumice contained in 9-cm diameter pots and maintained in a glasshouse (15-28°C) with no artificial lights at the Mt. Albert Research Centre, Auckland. Regular inoculations of plants were performed as described above. Inoculated plants were placed, in the late afternoon, in a glasshouse mist-chamber controlled with a wetness sensor, at 18-21°C for 18-24 h, and then transferred to the glasshouse.

Germination of urediniospores

Newly-produced urediniospores (four days after previous harvest) from each of four randomly selected infected leaves, each from a different plant collected at Pitlochry (see above) were harvested by brushing the leaf surface with a camel hair paint brush. Urediniospores were then brushed onto the surface of 1.5% water agar discs (12-mm diameter) kept at room temperature. Inoculation of discs was performed under a dissecting microscope to ensure that the surface of all discs was covered with a similar density of urediniospores. Groups of four discs, inoculated with urediniospores of each of the four leaves, were placed in plastic petri dishes. Half of the petri dishes were wrapped with aluminium foil and incubated at 6, 13, 18, 21, 25, 30 and 37±1°C respectively for 16 h. The other petri dishes were first exposed to a 2-h light treatment in a growth room (conditions as above), then wrapped with aluminium foil and incubated with the other dishes at the various temperatures for 14 h. Petri dishes were then transferred to a refrigerator (4°C) until germination was assessed. Several randomly selected fields of view were examined using a compound microscope until a total of 200 urediniospores per disc (replicate) had been evaluated. Urediniospores were considered to have germinated when the length of at least one of the germ-tubes was greater than the width of the spore. A completely randomized factorial design with four replicates was used. The experiment was performed twice. Data were analyzed using a binomial General Linear Model with logit link, and allowing overdispersion (McCullagh and Nelder 1989). Results for the trials were not pooled for regression analysis because a significant (P = 0.0247) interaction between trials and light regimes was observed. Polynomial regression equations were generated for each light regime and trial. For each light regime, curvilinear functions were fitted to the data by averaging regression coefficient estimates across trials.

Plant production and inoculation with the Pitlochry rust

Seed of *H. pilosella* collected from 14 different sites in New Zealand (Fig. 2) were sown in steam-sterilized loam contained in 15-cm diameter pots (one seed collection per pot) and grown in a glasshouse (15-30°C), fitted with artificial lights (Son-T Agro 400 W, Philips, Belgium) set at a 16-h photoperiod (485 $\mu Em^{-2}s^{-1}$). Two weeks after sowing, seedlings of similar size from each of the seed collections were transplanted equidistant (2 cm) from each other in steam-sterilized loam, contained in plastic trays (36x30x5 cm) (two seed collections per tray). The experiment was performed twice. An average of 40 and 37 seedlings representing each seed collection were used in the first and second trials of the experiment, respectively. Three weeks after transplanting, plants were inoculated by evenly sprinkling a dry mixture (1:50 w/w) of urediniospores and talc onto leaves using a salt shaker. Approximately 120 mg of fresh urediniospores, harvested from field-collected plants, were used in each trial. Trays containing inoculated plants were placed in the late afternoon in a glasshouse mist-chamber controlled with a wetness sensor, at 15-20°C for 18 h, and then transferred to the glasshouse. Plants were assessed for presence or absence of mature uredinia three weeks after inoculation.

Plant production and inoculation with the Lincoln rust

Seeds of *H. pilosella*, collected from 14 different sites in New Zealand (Fig. 2), were placed onto the surface of 1.5% water agar contained in 9-cm petri dishes, in a dark incubator at 25°C for 5-6 days. For each seed collection, 27 germinated seeds were then sown equidistant (2 cm) from each other in steam-sterilized peat pumice contained in plastic trays (42x30x6 cm). (three seed-collections per tray), and grown in a glasshouse (15-25°C) with no artificial lights. The experiment was performed twice. An average of 19 seedlings survived transplantation for each of the seed collections in each trial. Plants grown from seeds collected at Twizel, Mt. Cook, and Hanmer Springs were included in only one trial. Plants grew very slowly because of the short photoperiod during the winter months when the trials were performed. Seventeen weeks after sowing, plants were inoculated by brushing leaves of stock plants covered with uredinia (see above) on their foliage (approximately 20 stock plants used in each trial). Trays containing inoculated plants were placed, in the late afternoon, in a glasshouse mist-chamber controlled with a wetness sensor, at 18-21°C for 18 h, and then transferred to the glasshouse. Plants were assessed for the presence or absence of mature uredinia two weeks after inoculation.

Results and discussion

Germination of urediniospores

Fresh urediniospores of *P. hieracii* var. *piloselloidarum* germinated over a wide range of temperatures, but highest germination rates were recorded when spores were exposed to a short period of light at the beginning of the germination process, followed by a dark treatment (Fig. 1). Optimum temperature for germination was approximately 21°C for both photoperiod regimes. This optimum was similar to that reported by Jenkins (1995) for germination of other isolates of *P. hieracii* var. *piloselloidarum* exposed to darkness, but we found lower overall germination values in our study. Germination of urediniospores of *Puccinia* spp. on artificial surfaces is known to vary considerably depending on the experimental method used (Rowell 1984), the moisture content (Chang *et al.* 1973) and age (Burrage 1970) of spores, and environmental conditions (Kochman and Brown 1976; Knights and Lucas 1980).

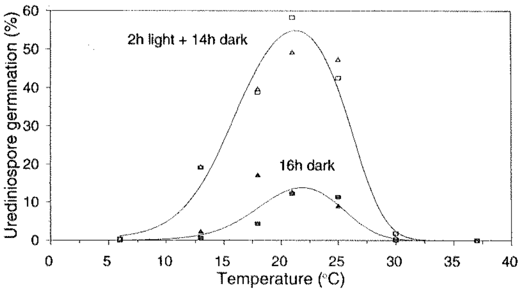

Fig. 1. Effect of temperature on germination of urediniospores of *Puccinia hieracii* var. *piloselloidarum* (Pitlochry, Scotland) on water agar discs subjected to two different light regimes. Data points represent means of four replicates (rectangles - trial 1; triangles - trial 2). Polynomial curves were fitted using the averaged estimate of regression coefficients estimates across trials. Regression equations: (16 h dark regime) $\log_e(y/(100-y)) = -8.2680-0.0355x+0.0436x^2-0.001304x^3$; (2 h light/14 h dark regime) $\log_e(y/(100-y)) = -5.205-0.0835x+0.0436x^2-0.001304x^3$, where x = temperature and y = percentage of germination.

In this study, germination of urediniospores of *P. hieracii* var. *piloselloidarum* on an agar surface was stimulated by exposure to light. Stimulation of urediniospore germination by a 1-min red light (660 nm) irradiation has been reported for *Puccinia graminis* Pers. f. sp. *tritici* (Erikss. and Henn.) Guyot and attributed to the possible existence of a photoreceptor system which, when activated, initiated the germination process (Schneider and Murray 1979). Continuous light has been observed to inhibit germination of urediniospores in some *Puccinia* spp., but the inhibitory effect was found to vary with light intensity and hydration of spores before exposure (Chang *et al.* 1973; Kochman and Brown 1976; Knights and Lucas 1980).

It has been widely demonstrated that results obtained in studies on the germination of urediniospores performed on artificial surfaces often differ from those on host leaves (Burrage 1970; Knights and Lucas 1980; Rowell 1984). Examination of the infection process of *P. hieracii* var. *piloselloidarum* on host plants is required not only to confirm the validity of the optimum range of temperatures for urediniospore germination *in vivo* but also to determine the effect of temperature, light and the presence of available water on the pre-penetration and penetration phases. This knowledge will assist development of a climate model to predict the geographical and seasonal limitations for rust epidemics in the field.

Disease responses of plants to the rust

In the preliminary studies presented here, *H. pilosella* plants grown from seed collected at 14 different sites in New Zealand differed considerably in their responses to rust isolates from Pitlochry, Scotland and from Lincoln, New Zealand (Fig. 2). Plant disease response to these rust isolates varied both within and between seed collections. However, results for each of the two isolates should be compared with caution because the two experiments involved plants of different age (5- and 17-weeks old) and were conducted in two different countries using slightly different conditions. Nevertheless, differences between the Lincoln isolate and other European isolates of *P. hieracii* var. *piloselloidarum* have recently been observed in an experiment which simultaneously compared their pathogenicity towards *H. pilosella* (Jenkins unpublished report 1995).

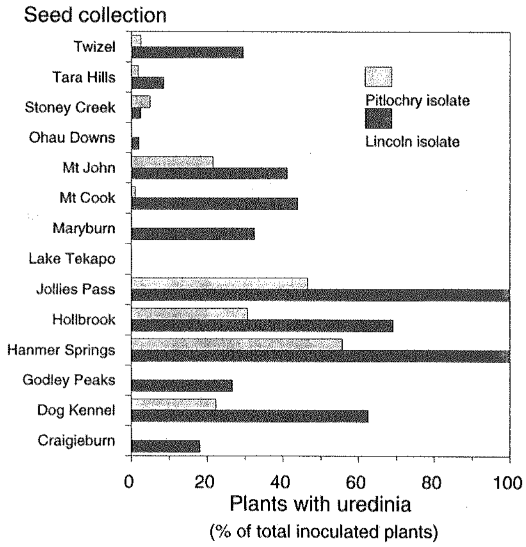

Fig. 2. Disease response of *Hieracium pilosella* plants grown from seed collected from various sites in New Zealand to isolates of the rust *Puccinia hieracii* var. *piloselloidarum* collected at Pitlochry, Scotland and Lincoln, New Zealand. Separate experiments were conducted with each of the isolates under slightly different conditions. For each experiment, results are from pooled trials because similar trends were observed.

In the experiment conducted in the UK, plants grown from seeds collected at Craigieburn, Godley Peaks, Lake Tekapo, Maryburn, and Ohau Downs did not develop any uredinia (hence, exhibiting a resistance response) after inoculation with the isolate from Pitlochry (Fig. 2). In contrast, uredinia developed profusely (susceptible response) on plants grown from the other seed collections inoculated with this isolate, although the proportion of plants that developed uredinia varied for each of the seed collections (Fig. 2). The highest proportions of plants with uredinia were observed for the seed collections from Hanmer Springs (46%) and Jollies Pass (56%).

In the experiment conducted in New Zealand with the Lincoln isolate, an overall higher proportion of plants, than that recorded in the European experiment, developed uredinia (Fig. 2). All plants grown from the Hanmer Springs and Jollies Pass seed collections became infected by this rust isolate and developed uredinia. In contrast, no uredinia developed on plants grown from seeds collected at Lake Tekapo. A varying proportion of plants grown from the other seed collections developed uredinia after inoculation with the Lincoln isolate, although proportions were higher than those recorded in the experiment with the isolate from Pitlochry.

It is unlikely that unfavourable environmental conditions prevented the rust infecting and producing uredinia on some of the plants because care was taken to ensure that all plants were grown and inoculated under uniform conditions in each of the experiments. No obvious morphological differences were found between plants that did or did not develop uredinia. Differences in the disease response of morphologically similar populations of weeds to various rust isolates have been reported in the host-pathogen systems, *Chondrilla juncea* L. - *Puccinia chondrillina* Bubák and Syd. (Emge *et al.* 1981) and *Carduus nutans* L. - *Puccinia carduorum* Jacky (Politis *et al.* 1984).

In both experiments, small chlorotic flecks were observed on some of the plants that did not develop uredinia. These flecks arose most probably from hypersensitive host reactions to fungal invasion. Incompatible interactions between host plants and races of rust often lead to a hypersensitive response by the host, which results in the collapse of invaded cells and consequently inhibits growth of the pathogen (Bushnell 1982). Such hypersensitive responses strongly suggest that resistance of *H. pilosella* plants to the rust is at least partly under genetic control. Therefore a gene-for-gene system may operate in this rust-plant interaction, based on the premise that virulence genes of the pathogen population are recognized by resistance genes in the host population (Flor 1956). Several genotypes of *H. pilosella* that differ in their susceptibility to the rust probably exist in Europe and New Zealand. If so, it is likely that a number of rust genotypes will be present, at least in Europe, as a result of coevolution of the plant and pathogen.

We propose that the seed collections used in these studies were samples from populations of *H. pilosella* that comprise a number of genotypes which differ in their susceptibility to the rust isolates investigated. Figure 3 is a diagramatic representation of a possible explanation for our results. Detailed studies, involving systematic sampling of populations of *H. pilosella*, are required to determine the genetic variation in New Zealand populations of the weed and to evaluate thoroughly whether both specific (qualitative) and general (quantitative) resistance are present. Preliminary allozyme studies indicated the presence of different genotypes of *H. pilosella* in New Zealand (Jenkins 1995; H.M. Chapman personal communication). *Hieracium* species belonging to the subgenus *Pilosella* are facultative apomicts that produce most of their seed clonally, but also have other breeding mechanisms by which genetic diversity can be increased (Bicknell 1995).

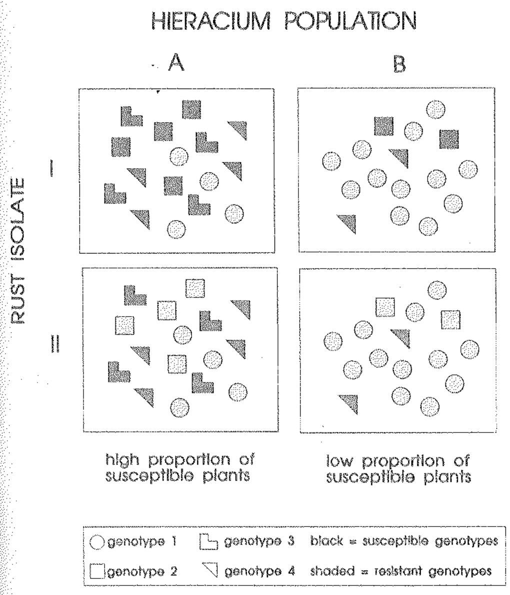

Fig. 3. Diagram illustrating a hypothetical interaction between two rust isolates and two populations of *Hieracium pilosella* that are structured differently: population *A* consists of a mixture of four different genotypes and population *B* of three genotypes. Rust isolate *I* is capable of infecting three genotypes (# *2*, *3*, and *4*) while isolate *II* is pathogenic towards only two genotypes (# *3* and *4*). Genotype #*1* is resistant to both rust isolates. Population *A* comprises a large number of genotypes susceptible to the rust isolates while population *B* mainly consists of the resistant genotype #*1*. The genetic structure of the populations may explain differences in the proportion of plants that become infected by the rust and developed uredinia.

Although it is too early to assess the impact that the recently discovered rust in New Zealand will have on populations of *H. pilosella*, our results suggest that other isolates of the rust will have to be introduced to affect all variants of the weed found in New Zealand. Monitoring the spread of the rust and development of epidemics in the field will assist in identifying resistant and susceptible populations of *H. pilosella* and, in addition, areas where suitable environmental conditions for disease development prevail.

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