

***Entyloma hieracii* and *Puccinia hieracii*, two promising pathogens for the biological control of *Hieracium* spp. (Asteraceae, hawkweeds) in North America**

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Abstract. Hawkweeds (*Hieracium* spp.) are perennial, rangeland weeds in New Zealand and are becoming a problem in several states of the United States of America and in provinces of Canada. During 1994 and 1995, surveys were conducted in the plant's native range, in Europe, for pathogens attacking hawkweeds. Several fungal pathogens were isolated, of which the rust *Puccinia hieracii* and the leaf smut *Entyloma hieracii* were found to infect *H. pilosella* and other species of *Hieracium*. Because of their damage to the plants and their probable host specificity, the two pathogens seem to be promising biological control agents. Both kill infected leaves and in severe cases, the whole plant. Further studies on their biology, effectiveness and host specificity are underway.

Introduction

The genus *Hieracium* (Asteraceae, Cichorioideae) (hawkweeds) occurs in temperate regions of the world except Australasia (Heywood 1978). It is native to the Old World and most species occur in Europe (Fiori 1974; Bremer 1994), where the plant causes no problem to agriculture. The plants are small, hairy herbaceous perennials that have yellow or orange flowers. *Hieracium* spp. have been introduced, intentionally or accidentally, into New Zealand and into North America, where they have become problem weeds in pastures and rangelands (Callihan *et al.* 1989; Roché 1992; Makepeace 1985). *Hieracium caespitosum* Dumortier (= *H. pratense* Tausch), *Hieracium aurantiacum* L. and *H. pilosella* L. are among the main problem species in the United States of America. These plants compete very aggressively with native plants and desirable grasses, which are displaced (Guyot 1951; Duquénais *et al.* 1956; Dawes and Maravolo 1973; Makepeace 1976; Widera 1978; Callihan *et al.* 1989; Roché 1992). Hawkweeds are very hardy and able to adapt to a variety of soil and climatic conditions. They are especially resistant to drought (Duquénais *et al.* 1956; Hopkins 1978; Makepeace 1985). Their numerous stolons allow the rapid vegetative spread of the plants. Hawkweeds

produce many small seeds which can disperse long distances. In North America, the ranges invaded are expanding and *Hieracium* spp. have the potential to spread more extensively (Callihan *et al.* 1989; Roché 1992). Chemicals are too expensive for the control of hawkweeds on low-value land and are not environmentally sound. The purpose of this investigation is to identify pathogens for the control of *Hieracium* spp.. Studies are being conducted to show the potential of these pathogens as biological control agents for hawkweeds.

Materials and methods

Preliminary field work was conducted during the summers of 1994 and 1995 to locate populations of *Hieracium* spp. and fungal pathogens attacking the plants. The areas to be surveyed were identified in the French Pyrenees, southern Massif Central of France and western Alps of France and Italy, where populations of *Hieracium* species are relatively common. Samples of all fungi suspected of being pathogenic on *Hieracium* species were collected and dried in a plant press. Infected plants were also transferred to small plastic pots and brought to the laboratory for detailed studies. Dry spores from rust pustules were collected using a cyclone spore collector.

These were stored in an ice box during the survey.

Isolations from diseased plant parts, as well as direct plating of sporulating structures were made on potato dextrose agar (PDA) or 20% water agar. Subcultures were maintained on V8-juice agar and carrot juice agar in a growth chamber at 20°C with a photoperiod of 12 h.

Plants of *H. pilosella* were grown from seeds collected in France in individual 10x10 cm plastic pots, in a mixture of soil/humus/sand in equal proportions (v/v/v). Plants of *H. caespitosum* from Idaho were also grown in the same way. The plants were maintained in a greenhouse at temperatures of 15-25°C night and 15-35°C day with natural light during May - September.

Urediniospores of the rust fungus, sucked from infected leaves and suspended in distilled water, were spread on microscope slides coated with water agar (1% Difco Bacto). The slides were then immediately enclosed in Petri dishes with moist filter paper on the bottom and incubated at 20°C. Teliospores of the smut fungus, obtained by dissecting infected leaves of *H. pilosella*, were suspended in distilled water with 0.05% Tween[®] 80 (poloxyethylene sorbitanmonooleate; Sigma Chemical, St. Quentin Fallavier, France). The spore suspension was homogenized by stirring with a glass rod. The spore suspension was then spread on microscope slides and incubated as described above.

To study spore germination, penetration of germ-tubes and further development of the pathogen, leaf segments were cleared and stained using the technique of Bruzese and Hasan (1983) at 48 h and six days after inoculation. Leaf portions were cut into 1-2 cm long segments and immersed in a mixture of Carnoy's solution (acetic acid, chloroform, ethanol - 1:2:3/vol.) and lactophenol-cotton blue (phenol 10 g, glycerin 10 ml, aniline blue 0.02 g) in a proportion of 2:1 (v/v). After 48 h the leaf segments, by then completely decolorized, were rinsed with distilled water and transferred to 50% aqueous solution of chloral hydrate for 30 min. and then mounted on microscope slides in a permanent mounting medium (Omar *et al.* 1978) for microscopic examination.

Dry spores of the rust organism were transferred to the upper surface of the leaves of six-week-old plants using a camel hair brush. Inoculated plants were placed on inverted saucers in an incubation chamber (1 m x 1 m x 0.5 m high, made of light-proof plastic, each fitted with a 1 m x 1 m x 3 mm thick air-tight glass top) and lightly misted with distilled water using an

atomizer. Tepid water to a depth of about 1 cm was added at the bottom of the chamber and the plants were again misted lightly with distilled water. The glass cover of the incubation chamber was then replaced and the lights were turned off. The plants were held for a minimum of 8 h in darkness at an overnight temperature of about 20°C, with artificial light (10:14 L:D photoperiod). The lights in the room were turned off after inoculation. Inoculations were made in the afternoon and the plants were left in the incubation chamber for approximately 16 h before being transferred to the greenhouse (night / day temperatures: 15-25/15-35°C) for disease development.

Teliospores of the smut fungus, obtained by dissecting infected leaves of *H. pilosella*, were suspended in distilled water containing 0.05% Tween[®] 80. The spore suspension was homogenized by stirring with a glass rod. For inoculation, the spore suspension was sprayed onto the plants and the same procedures, as described above, were used, except that the inoculated plants were kept in the incubation chamber for 56 h before being transferred into the greenhouse.

Smut teliospores obtained by dissecting infected leaves at the periphery of lesions were suspended in sterilized distilled water with 0.05% Tween[®] 80. The suspension, stirred to separate teliospores, was sprayed on water-agar. Forty-eight and 56 h later the agar plates were examined under a stereoscopic microscope and the germinated spores were picked up with a fine needle. These spores were then transferred to potato-dextrose-agar-Difco (PDA) containing 0.1 mg/ml streptomycin sulphate and 0.03 mg/ml penicillin for further growth. Other media tested for the growth of the fungus were potato carrot agar (PCA), morphology agar (MA) and malt-extract agar (MEA).

Results

Field surveys

During the field surveys in 1994 and 1995, *H. pilosella* was the species most commonly found. Among various pathogens found in France, two fungal plant pathogens, a rust and a smut were found to be highly damaging to hawkweeds in Europe. The rust was identified as *Puccinia hieracii* (Schumann) Mart. (Wilson and Henderson 1966) and the leaf smut as *Entylooma hieracii* H. & P. Sydow *ex* Ciferri (Vánky 1985). These pathogens were found on *H. pilosella*, which belongs to the subgenus *Pilosella*, the same subgenus to which *H. aurantiacum* and *H. caespitosum*

belong. So far they have been reported only on *Hieracium* spp. (Gaumann 1959; Vánky 1985).

Puccinia hieracii was found on *H. pilosella* and *H. lactucella*, but more commonly on *H. pilosella* in the French Pyrenees and French and Italian Alps. The uredinia appeared on the upper leaf surface and stems as small yellowish spots, turning to a cinnamon brown colour. These are usually scattered, but in severe infections, pustules became confluent, covering the leaves with a brown powdery mass. Urediniospores are globose to ellipsoid (21-30 μm x 16-25 μm), with echinulate walls and supra-equatorial pores (Wilson and Henderson 1966). They germinated within a few hours, on 1% water agar, and mostly gave rise to one germ-tube from each spore. The infected plants were often poorly developed and showed restricted growth of the rosettes as well as of the stolons. In severe infections, the rust killed the rosette leaves and often the entire plants.

The rust has been given various taxonomic treatments by different authors. Wilson and Henderson (1966) recognize *P. hieracii* as a species with three varieties, one of which, *P. hieracii* var. *piloselloidarum* is restricted to the stoloniferous species of *Hieracium* subgenus *Pilosella*. Gaumann (1959) refers to the rust infecting the plants in the subgenus *Pilosella* as *P. piloselloidarum* Probst. Within each rust variety there are also specific strains, as shown by Probst (1909).

Entyloma hieracii was found on *Hieracium* spp., but more commonly on *H. pilosella* in the Pyrenees. This fungus caused round or angular lesions on leaves, at first yellowish but later brown in colour with a paler border and often with a perforated centre, 2-10 mm in diameter. The lesions are flat, later becoming swollen. They contain globose to subglobose teliospores, single or adhered in irregular groups, with sub-hyaline to yellow, smooth, evenly thickened walls. The fungus caused mortality of the infected leaves and, in severe infections, of the whole plants. In advanced stages, the disease covers the entire leaves, killing them and often destroying the rosettes. No external spores or conidial stage could be found on the leaf surface, as described for *Entyloma ageratinae* sp. nov. (Barreto and Evans 1988).

Microscopic examination

In cleared and stained leaves infected by *E. hieracii*, thin-walled, fine, scarcely visible hyphae were found in the lesions. These hyphae were mixed

with dark thick-walled teliospores in the centre of the lesions. Some of these teliospores were found germinating while still in the host tissue, giving rise to needle-shaped sporidia.

Spore germination

The teliospores of *E. hieracii*, spread on water-agar, gave rise to germ-tubes after 24-48 h. These widen, almost at once, but remain constricted at the basal attachment to the teliospores. On the distal part of the germ-tubes appear four slender, straight or sometimes slightly curved sporidia. These sporidia easily detach and germinate on the surface of agar, often at both ends, to give a narrow, sparsely branched mycelium.

Cultures of *Entyloma hieracii*

On germination, the teliospores usually produced four primary sporidia and gave rise to a few hyphae on PDA with antibiotics, but did not grow further. Similarly, the fungus was unable to grow on PCA and MA and MEA media. Further investigations are underway to find suitable media for the growth and sporulation of *E. hieracii*. PDA, PCA and MA media have already been used with success to grow colonies of *E. ageratinae* (Barreto and Evans 1988). However, Trujillo (1988) states that the teliospores of *E. ageratinae* cannot be grown *in vitro*. The fact that a few hyphae are produced on PDA shows that further development of the fungus requires a more suitable medium. Urediniospores of *P. hieracii* successfully infected hieracium plants brought from the field and from which spores were collected. The rust pustules which appeared about 10 days after inoculation were multiplied in the greenhouse for a few generations, on the same plants. Efforts to make cross inoculations using different collections of *Hieracium* species were unsuccessful.

The leaf-smut fungus developed small pale green spots on leaves 12 days after inoculation. Later these became prominent, whitish, with necrotic lesions in the centre and without external sporulation.

Discussion

From the limited pathogenicity tests it appears that the rust has host-specific strains. It infected mostly the same hieracium plants on which it was collected. Probst (1909) had similar results in 29 inoculation experiments using *P. hieracii*, though two of the rust

strains infected more than one species/variety of *Hieracium*.

The rust is highly damaging to the plants and is common at high elevations in Europe. The powdery urediniospores are likely to be easily dispersed. These characteristics are suitable for its rapid adaptation in the cooler climatic areas of the western USA and for the establishment of the fungus.

Similarly, the leaf smut seems to be well adapted to the cooler conditions at high elevations. The teliospores may be disseminated in the dry leaf fragments and thus dispersed over long distances. Their germination, while still within the leaf tissue, presents an additional advantage for disease establishment and dispersal.

Both the fungi seem to be promising agents for the control of hawkweeds. *Entyloma hieracii* has not been studied for the purpose of biological control. However, a species of *Entyloma*, *E. compositarum* Farlow (*E. ageritinae* sp. nov.) has already been used successfully in Hawaii for biological control of Hamakua pamakani (*Ageratina riparia*) (Trujillo 1985, 1988; Barreto and Evans 1988). *Puccinia hieracii* is being studied in New Zealand and will probably be used for the biological control of *Hieracium* spp. there.

Further studies on the biology, effectiveness and host specificity of *P. hieracii* and *E. hieracii* are underway prior to their possible use for the biological control of hawkweeds in North America.

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