Specificity and host specialization of *Puccinia chondrillina*

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**SUMMARY**

Various strains of *Puccinia chondrillina*, the *Chondrilla* rust, were collected during surveys in the Mediterranean, undertaken to discover biological control agents suitable for use in Australia against skeleton weed, *Chondrilla juncea*. Only the strain collected at Vieste (S.E. Italy) was highly virulent against the common Australian form of *C. juncea*. The specificity of this strain of the rust was tested by inoculation of many unrelated cultivated plants, related cultivated plants and closely related wild plants; all were immune to the rust. These tests satisfied the Australian plant quarantine authorities and this strain of the rust has now been introduced into Australia as a biological control agent for *C. juncea*.

**INTRODUCTION**

The specialization of parasitism in the Uredinales has been the subject of extensive research for the last 50 years. Most rusts studied have been those of cereal or other crop plants and little work has been done on the rust diseases of wild plants. Recently, increased interest in the use of plant pathogens for the control of weeds (Wilson, 1969) has awakened interest in the specificity and host specialization of fungi attacking wild plants.

In 1967 a project was undertaken to examine the possibility of using, as a biological control agent, *Puccinia chondrillina* Bubak & Syd., a very damaging rust of *Chondrilla juncea* L. (skeleton weed), a Composite plant of considerable importance as a weed in South-Eastern Australia (McVean, 1966).

This communication discusses the specificity and host specialization of the *Chondrilla* rust and the methods used to evaluate these two important aspects of the biology of a biological control organism.

*Puccinia chondrillina* Bubak & Syd

The *Chondrilla* rust is macrocyclic and monoecious. It is active throughout the year and in the Mediterranean it multiplies solely by the uredo-stage. The rust damages all the aerial parts of the *Chondrilla* plant. In autumn and spring the leaves of rosettes regenerating from the previous year's rootstock are heavily damaged by brown eruptive uredosori. These appear on the flowering shoot in the late spring and then occur on the plant throughout the summer and even on the flower buds. Teleutosori appear at the end of the season, but play no part in the life-cycle of the rust in the Mediterranean (Hasan, 1970). Heavily attacked plants are either killed or severely
S. Hasan

damaged and seed production and root storage reserves are reduced (Hasan & Wapshere, personal communication).

The rust has been recorded only from the genus Chondrilla. In southern Russia it occurs not only on C. juncea but on the Chondrilla species endemic to that region, C. brevirostris Fisch. and Mey., C. ambiguа Fisch. ex Kar. and Kir., C. coroniferа Iljin. and C. piptocoma Fisch. and Mey. (A. Johnston, personal communication). Of these species C. brevirostris is closely related to C. juncea but the others belong to other subgenera of the genus Chondrilla. The rust also occurs in North America on C. juncea, the only member of that genus found there (Arthur, 1934). It has been observed during the present study in Iran, Turkey, Greece, Yugoslavia, Italy, France, Spain and Portugal on C. juncea. It has never been recorded in Australia (McAlpine, 1906; J. Walker, personal communication).

The rust clearly has a very wide climatic and geographic range, extending from the cold continental region of southern Siberia and hotter continental regions of Kazakhstan, Uzbekistan, etc., in southern Russia, westwards to the hot Mediterranean climates of Portugal and North Africa and northwards to northern France. It can be considered to occur everywhere in Eurasia where Chondrilla species are found.

The study of host specialization and specificity

C. juncea is a triploid and an obligate apomict (Rosenberg, 1912) and there is strong evidence that the geographical separation of the stands of the plant in Eurasia has led to the development of a large number of morphologically and genetically different clones, most of which differ considerably from the Australian types (McVean, 1966). Several morphologically different types sometimes coexist in the same place. It has also been found (Hull & Groves, personal communication) that three different morphological forms are distinguishable in the Australian populations of the plant. These are a narrow-leaved form which is by far the most common, a broad-leaved form and a form with leaves intermediate between the two other types.

During surveys for and studies of the biological control organisms of C. juncea in Portugal, Spain, France, Italy, Greece and Turkey, plants infected by the rust species were collected at many sites and seeds of the various forms of C. juncea were forwarded to R. Groves in Canberra, Australia, to compare with the Australian forms of the plant.

Two series of infection experiments were carried out. The first was to expose the three forms of Australian C. juncea to spores of P. chondrillina from a large number of European sites, to discover the extent of host specialization and to discover the strain of rust most adapted to the Australian forms of the plant. The second was to expose a large number of cultivated plants to infection to determine whether, if introduced into Australia, the rust species would infect any important crop plants, or whether it was completely specific to the genus Chondrilla. These experiments were carried out in a greenhouse.

MATERIALS AND METHODS

Individual plants grown in 8 x 8 cm plastic pots were placed on inverted lids of square plastic containers which were covered with sawdust. The body of the container
was inverted and placed over the plant and pot, and fitted tightly into the lid to form an infection chamber. The boss of the lid, in which holes had been pierced, was immersed in water to maintain high humidity in the infection chamber. Uredospores of *P. chondrillina* were either dusted on to the plants within the infection chambers, and the plants then sprayed with droplets of water, or spores were sprayed with a hand atomizer on to the plant in a dilute aqueous suspension.

Inoculations were made in the late afternoon and the plants were left overnight in the infection chambers. The absence of light and the low temperature overnight in the greenhouse favoured spore germination. Next day at noon the inoculated plants were removed from the infection chambers and left in the greenhouse at night temperatures of 15–20 °C and day temperatures of 15–35 °C. Low night temperatures, with retention of water droplets, favoured spore germination and germ-tube development but subsequent development of the rust was best at 20–30 °C (Hasan & Wapshere, personal communication). Production of sori occurred in 7–10 days, according to conditions in the greenhouse.

The infection chambers were used to inoculate the Australian forms of *C. juncea* with *P. chondrillina* from various sources. Spore germination and subsequent disease development was followed using Shipton & Brown’s (1962) whole leaf clearing and staining technique. In this way the *P. chondrillina* strains of most efficacy against the Australian *C. juncea* types were selected. Once the strain of *P. chondrillina* that was most pathogenic to the Australian forms of *C. juncea* had been discovered its pathogenicity was tested against a large number of cultivated plants and several Cichoraceous weeds related to *Chondrilla*. The tests were carried out in 1969 and 1970 in batches of five to ten plant species. Each species was represented by five to ten plants and with each batch of plant species there were ten control plants of the common Australian form of *C. juncea*.

RESULTS

Specialization of the rust to *Chondrilla juncea* forms

Initially, when work was first commenced on *P. chondrillina* in 1967, it was found impossible to obtain any infection of the Australian forms of the plant with *P. chondrillina* spores collected in the region of Montpellier and Ales in southern France. Many attempts were made with a strain from Aniane, near Montpellier, where there was a heavy infestation of *C. juncea*, but all were unsuccessful. Later attempts with material from northern Italy and Spain were also equally unsuccessful.

It was found that the *C. juncea* forms from these regions differed considerably from the Australian.

The pathogenicity of the various strains of *P. chondrillina* against the Australian *C. juncea* was assessed in two ways. The first was to determine the proportion of plants on which uredosori developed; the second was to grade the susceptibility of the Australian narrow-leaved form of *Chondrilla* to *Puccinia* spores from the various sources, using a series of readily distinguishable reaction types, similar to those used for studies of susceptibility of wheat varieties to rust species (Gassner & Straib, 1932; Purdy & Allan, 1963). For this study the following reaction types were readily distinguished:
Penetration of germ-tube occurs but there is no mycelial development in the host tissue. There is no sign of infection, no host reaction and no soral formation.

(1) Only restricted development of the fungal mycelium occurs within the host tissue. Small dark-brown, round or irregular sunken lesions appear on the epidermis.

(2) Less-restricted growth of mycelium within the host tissue, larger dark-brown depressions on the epidermis with occasional development of small sori.

(3) Small eruptive sori occur on the epidermis, each surrounded by a yellow halo. Sori rarely fuse with each other and give rise to only a small number of spores.

(4) Many large, eruptive, soral pustules occur over much of the plant epidermis with a heavy production of uredospores in a thick, leathery mass. As the disease progresses the sori fuse together to give a complete, uniform covering over the plant surface.

Table 1. The pathogenicity of the Chondrilla rust from various localities to the Australian narrow-leaved form of Chondrilla juncea

<table>
<thead>
<tr>
<th>Collection sites</th>
<th>Plants on which uredosori were produced (%)</th>
<th>Plant reaction (type no.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Costa Brava</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Murcia</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Grenada</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>France</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aniane (Montpellier)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Le Soler (Perpignan)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Cap Ferret (Bordeaux)</td>
<td>100</td>
<td>2</td>
</tr>
<tr>
<td>Bauz Vernou (Orléans)</td>
<td>60</td>
<td>2</td>
</tr>
<tr>
<td>Italy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parma (Po Valley)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Montalbano (Taranto)</td>
<td>80</td>
<td>3</td>
</tr>
<tr>
<td>Vieste</td>
<td>100</td>
<td>4</td>
</tr>
<tr>
<td>Termoli</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Greece</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agrinion</td>
<td>80</td>
<td>2</td>
</tr>
<tr>
<td>Anavysso (Athens)</td>
<td>50</td>
<td>3</td>
</tr>
<tr>
<td>Thebes</td>
<td>100</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 1 shows that the Australian narrow-leaved form of C. juncea was completely attacked and extremely susceptible to a strain of P. chondrillina from Vieste (near Foggia) in south-eastern Italy. This rust strain also attacked the other two forms of Australian Chondrilla – the broad-leaved and intermediate types – though less virulently. The sori produced were very large and developed extremely rapidly with no sign of host resistance, and seedling plants were rapidly overcome. The Vieste strain of the rust was used for all subsequent testing of the cultivated plant species and on all occasions it produced the same virulent disease symptoms on the control Australian C. juncea plants. Other strains of P. chondrillina which were effective, i.e. giving a high percentage attack and causing severe plant reaction (3), were found at Montalbano, Taranto, in south-eastern Italy in the same region as the Vieste site and at Thebes.
Puccinia chondrillina 261

in Greece. R. Groves (personal communication) found that C. juncea types from south-eastern Italy and Greece resembled the Australian types and that in particular the Vieste C. juncea type is morphologically extremely similar to the Australian narrow-leaved type.

Table 1 also indicates how patchily distributed are the effective strains of P. chondrillina, which reflects the patchy distribution of the forms of C. juncea. It was notable, for instance, that the rust strains from Termoli, which is only 80 km north of Vieste, were completely non-pathogenic to Australian C. juncea. It was also found that the rust strains from Vieste would not infect the French (Aniane) form of C. juncea.

Specificity of Puccinia chondrillina to the genus Chondrilla

Using the method described above, many plants were tested by exposure to the spores of the Vieste strain of P. chondrillina. These included common Cichoraceous weeds closely related to C. juncea, all of which are known to be attacked by various species of Puccinia other than P. chondrillina, namely Cichorium intybus L., Urospermum dalechampii Desf., Crepis taraxaciformea Thuill., C. foetida L., Hypochaeris radicata L., Hieracium pilosella L., Sonchus asper All., S. oleraceus L. and Taraxacum officinale Wiggers. Two other Composite weeds common in Australia, Onopordon acaulon L. and Carthamus lanatus L., were also tested. A group of cultivated plants prescribed by the quarantine authorities in Australia was also tested. Of these, those most closely related to C. juncea were the two members of the Cichoraceae, Lactuca sativa L. (cvs. Trocadero and Great Lakes) and Cichorium endivia L. (cv. Endive de Bruxelles) and, slightly less closely related, the Compositae belonging to other sub-families, Helianthus annuus L. (cv. Peredovic), H. tuberosa L., Chrysanthemum sp., Dahlia sp., Bellis sp., Zinnia sp., Tagetes sp., Carthamus tinctorius L. (cv. Horowitz) and Cynara scolymus L.

The remaining cultivated plants, not related to C. juncea, which were also tested were as follows: Beta vulgaris L. (cv. Rouge Globe), Ipomaea batatas Lam., Brassica oleracea L. (cv. Cavalier), Brassica rapa L. (cv. Blanc des Halles), Cucurbita maxima Duch. (cv. Vert d’Espagne), Cucumis melo L. (cv. Cantaloup Charentais), Citrullus vulgaris L., Triticum sp. (cv. Endive de Bruxelles) and, slightly less closely related, the Compositae belonging to other sub-families, Helianthus annuus L. (cv. Peredovic), H. tuberosa L., Chrysanthemum sp., Dahlia sp., Bellis sp., Zinnia sp., Tagetes sp., Carthamus tinctorius L. (cv. Horowitz) and Cynara scolymus L.

Of these plants only Lactuca sp., Cichorium endivia, Helianthus annuus, H. tuberosa, Carthamus tinctorius, Chrysanthemum, Tagetes, Triticum sp., Zea mays, Sorghum
S. Hasan

vulgare, Lolium perenne, Allium cepa, Gossypium sp. and Daucus carota have ever been recorded as attacked by a Puccinia species.

There was no sign of infection on any of the plants tested, no development of sori and no brown mark on the plants, indicating a resistant action. Conversely on all occasions the common Australian form of C. juncea always showed a rapid development of large uredosori.

DISCUSSION

The results described above demonstrate clearly the specialization of P. chondrillina to geographically separated forms of C. juncea and indicate, with the study made by Hull and Groves (personal communication), that there is a clear relationship between host form and susceptibility to certain forms of the rust. The result of the very large testing programme against a wide variety of cultivated plants and against plants closely related to C. juncea must certainly be considered to confirm the specificity of P. chondrillina to the genus Chondrilla. These two features are considered to show that there is no need to fear an extension of the host range of the rust when introduced into Australia and that it will remain specific to C. juncea. This fact, and the estimated potential of the rust under Australian climatic conditions (Wapshere, 1970, 1972), indicate strongly that the rust should be used in Australia as a biological control agent against C. juncea. Permission for its introduction has now been granted by the Australian plant quarantine authorities and it was established in the field there during 1971.

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REFERENCES


