PUCCINIA CARDUI-PYCNOCEPHALI, A POTENTIAL AGENT FOR THE BIOLOGICAL CONTROL OF SLENDER THISTLES IN AUSTRALIA

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Summary. Progress towards the biological control of the slender thistles, *Carduus pycnocephalus* and *C. tenuiflorus* with the rust *Puccinia cardui-pycnocephali* is reported. A search for virulent strains of rust on both slender thistles was made from extensive collections in Southern Europe. Of these, some strains were found that are especially pathogenic. The effect of selected strains in decreasing the reproductive capacity of these winter annuals is documented. Recently, two selected strains have been imported into quarantine in Australia for further host specificity testing.

INTRODUCTION

The closely related slender thistles, *Carduus pycnocephalus* and *C. tenuiflorus*, are major weeds of sheep-grazed pastures. Both are winter annuals of Mediterranean origin that reproduce only by seeds. These two species are now widely distributed in New South Wales, Victoria (8.25 million hectares) and Tasmania, and also occur in South Australia, south-western Western Australia and south-eastern Queensland (10). High densities of slender thistles greatly reduce pasture availability and yield (by up to 66% in Victoria; 9), thereby causing notable economic losses.

Biological control represents a valuable strategy for long-term management of slender thistles because both these species are largely a problem of disturbed pastures and wastelands. Initially, research has focussed on the use of insects, which have not effectively controlled slender thistle populations in North America (4). In this regard, studies on the population dynamics of slender thistles in their native Mediterranean environment showed a remarkable population stability over sites and over years (12). Extensive surveys detected more than twenty insect species associated with slender thistles. However, by themselves, none of these appeared to be a limiting factor for these weed populations (12), a result which possibly reflects the short life span of the hosts and their partial asynchrony with the main period of insect activity (3).

Consequently, research efforts have shifted towards the evaluation of potentially useful pathogens, as they often show a short generation time, high reproductive capacity and dispersal, and a phenology matched to that of their hosts. Of these, the autoecious rust, *Puccinia cardui-pycnocephali*, is commonly found on slender thistles throughout the Mediterranean basin. Although at least one strain of this pathogen already occurs in Australia (9), it is not very effective in reducing growth or seeding of Australian genotypes of the two slender thistles. However, a significant decrease in growth and capitula production was found when glasshouse grown rosettes of Australian *C. pycnocephalus* were repeatedly inoculated with an isolate collected in southern France (8). This suggested that effective isolates could be found in Europe for the biological control of slender thistles in Australia.

METHODS

Rust surveys. Extensive field surveys for isolates of the rust were carried out in France, Italy, Spain and the former Yugoslavia in 1989-91. Two surveys were made in May 1989 in north-western Spain and Italy. Similar surveys were made in central-southern and north-eastern Spain and in Yugoslavia in 1990-91. Surveys in southern France were made on a more regular basis due to the proximity of sites to the CSIRO laboratory at Montpellier. Occurrence and abundance of the rust were scored on 50 to 100 plants per site using a visual scale similar to that used by Sheppard *et al.* (12).

Inoculation procedures. Glasshouse grown rosettes were inoculated at the 4-6 leaf stage with urediniospores using the method described by Hasan (7). Visual assessment of disease symptoms was made using a five point scale, as follows: 0- Immune (no symptoms); 1- Resistant (visible chlorosis and necrosis on leaves, no uredinia); 2- Moderately resistant (chlorotic and necrotic spots associated with a few minute pustules); 3- Moderately susceptible (numerous small and eruptive uredinia often associated with chlorotic halos) and, 4- Susceptible (leaves covered with eruptive uredinia, no plant reaction).

Effectiveness of the rust. An inoculation experiment in the field was designed to evaluate the effect of the rust on plants of both species of slender thistles. Two fully randomized plots were established with potted plants from 7 sites in Australia. Sterilised soil and timed drip-watering were identical for the 2 plots. Plants in one plot served as control and those in the other plot, situated 30 m away, were inoculated (at the 6-8 leaf stage) once only with the isolate FR3 to simulate natural levels of infection. Inoculation was achieved by spraying a suspension of urediniospores and water (1 mg/20 mL) onto the plants. Plants were monitored every fortnight for disease intensity, rosette growth and plant height. Ripe capitula were collected twice daily during the flowering period and seed number and viability assessed. Biomass of whole dried plants was measured after harvest. These data were analysed using Anovas.

RESULTS AND DISCUSSION

Rust surveys and selection of isolates. High levels and rates of infection by the rust were apparent at 11.6% of the 104 sites surveyed, especially in central and southern Spain. Overall, rust infection was noticed at 57.7% of sites visited, covering a wide range of environmental situations. Isolates of the rust were collected from these sites and evaluated in the glasshouse for pathogenicity on both species of slender thistles, including various Australian plant genotypes. The pathogenicity of isolates of *Puccinia cardui-pycnocephali* is highly variable and Australian accessions of both slender thistles present a continuum of infection type responses to the isolates within this rust fungus (Table 1).

Some isolates are extremely virulent on Australian genotypes of slender thistles. However, while isolates of rust collected from either one of the two host species will attack the other host, the isolates are most pathogenic and produce the severest symptoms on the host species from which they were originally collected. This was observed also for two isolates collected from *C. tenuiflorus* in Australia and could explain why the rust occurs particularly on this species in south-eastern Australia (9). These results indicated that it will be necessary to introduce and release at least two isolates initially, with the aim of affecting plants of both species of slender thistles. By attempting to overcome the genetic heterogeneity of these weed species for

resistance to the pathogen, the strategy used will be likely to minimize the problems raised by the existence of resistant biotypes (2).

Table 1. Summary of pathogenicity of *Puccinia cardui-pycnocephali* isolates from southern Europe on Australian slender thistles

Isolates collected from:	Infection type[a] on:		Number of isolates
	Carduus pycnocephalus	*Carduus tenuiflorus*	
Carduus pycnocephalus	4	0	1
	4	2	3
	4	3	2
	3	0	4
	1	5	3
	3	2	3
	1	0	
Carduus tenuiflorus	2	4	3
	2	3	3
	1	3	7
	0	1	4

[a] Infection type from 0= immune to 4= susceptible, heavy production of uredinia.

For *C. tenuiflorus*, comparative assessments made between two isolates of the rust already present in Australia and three of the highest ranking isolates from southern Europe suggested that none of these isolates was significantly more virulent on Australian plants of *C. tenuiflorus* than the other, as measured by the number of uredinia produced per unit of leaf area under uniform conditions (Sichez, unpublished data). However, isolate FR3 collected from Salin de Badon (southern France) was more agressive, producing the heaviest infection (number and amount of spores) on several Australian accessions of *C. tenuiflorus*. Thus, it was selected for further screening and subsequent host-specificity testing. Choice of this isolate was strengthened by the fact that it sporulated profusely on all plants collected from 10 sites in New South Wales and Western Australia. For *C. pycnocephalus*, isolate IT2 from Terlizzi (southern Italy) was by far the most virulent isolate on Australian plants. All plants collected from 11 geographically well separated sites in Australia showed high levels of infection after inoculation with this isolate. Hence it was chosen for the control of this species of slender thistle.

Impact of the rust on slender thistles. Uredinia first appeared on plants 15 days after inoculation. At least 6 generations of spores were produced over the course of the experiment. The highest levels of infection were recorded mainly on rosettes of *C. tenuiflorus* for which as many as 1560 uredinia per plant were counted. Plant height, biomass and the production of viable seeds of rusted plants were all significantly reduced (Table 2).

However, the two thistle species responded differentially to isolate FR3 of the rust (P=0.001), while within a thistle species, there was evidence of some geographic differences in the degree to which production of viable seed was reduced (Fig. 1; P=0.001). These results confirm the importance of introducing and releasing at least 2 isolates initially.

Table 2. Effect of *Puccinia cardui-pycnocephali* infection on plant height, dry weight and production of viable seeds of the slender thistles, *Carduus pycnocephalus* and *C. tenuiflorus*. Data from an outdoors inoculation experiment. Means (s.e.) are presented. Pairwise means are statistically different at the 1% level using one way Anova.

Species	Treatment	N	Height (cm)	Dry weight (g)	Seed production
C. pycnocephalus	control	20	87.30 (4.47)	19.36 (1.06)	173.97 (22.95)
	inoculated	20	38.80 (2.72)	11.27 (0.78)	32.31 (10.19)
C. tenuiflorus	control	21	52.90 (3.71)	16.85 (0.79)	84.44 (11.82)
	inoculated	21	25.43 (4.01)	7.26 (0.63)	20.65 (8.30)

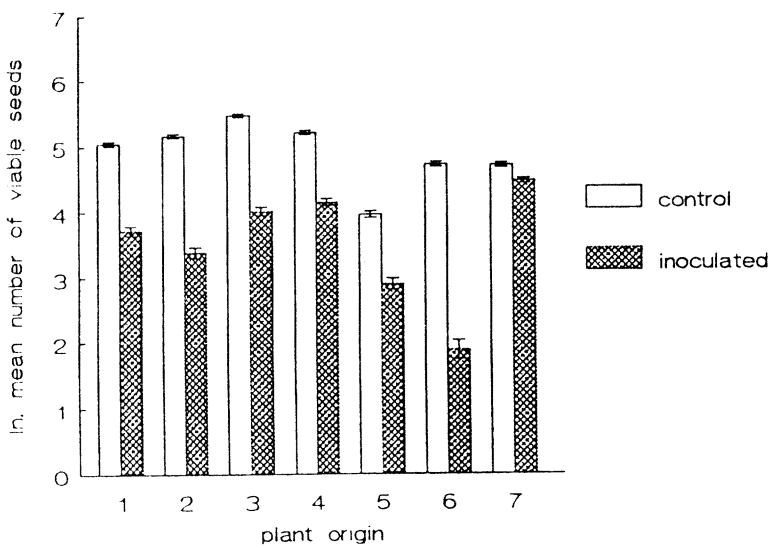

Figure 1. Production of viable seeds of slender thistles in relation to rust infection and plant origin. Origin 1 to 4: *C. pycnocephalus* plants collected from Denmark, Manjimup, Boyup Brook and Bridgetown (WA); 5 replicate plants per treatment and origin were used. Origin 5 to 7: *C. tenuiflorus* plants collected from Yaouk (NSW), Albany and Manjimup (WA); 7 replicate plants per treatment and origin were used.

Another field inoculation experiment confirmed that the selected isolates FR3 and IT2 curtailed the reproductive capacity of Australian genotypes of both slender thistles, even in the absence of plant competition (Chaboudez, unpublished data). As slender thistles are annuals, the significant reduction in seed production caused by rust infection is likely to reduce the size of infestations. However, the maintenance of a competitive perennial pasture during the limited period when slender thistles germinate (autumn rainfalls) should have a synergistic effect in controlling weed density, as has been shown for several other weed-pathogen systems (6, 11).

On the basis of the successful host specificity testing of the 2 candidate isolates, authorisation was given to import these into quarantine in Australia. From the actual distribution of the rust in Australia, it is anticipated that the introduced isolates will probably establish and spread under the same environmental conditions, if release is approved. These isolates have the potential to decrease vigour and reproductive capacity of slender thistles. However, the extent to which it will affect thistle density and thereby provide substantial control will ultimately depend on how well these biological control measures are integrated into pasture management (1, 5, 13).

ACKNOWLEDGMENTS

This project is funded by the Wool Research and Development Corporation. We would like to thank Caroline Sidi for skilled technical help, Jean-Paul Aeschlimann for collecting several isolates from Yugoslavia and north-western Spain and Dane Panetta for collecting seed of both species in Western Australia. We also thank David Briese, Guy Forrester and Andy Sheppard for helpful comments at various stages of this project.

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