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Aspects of the Biology of the Spear Thistle Rust Fungus in Victoria, Australia

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Abstract

The autoecious rust fungus *Puccinia cnici* was commonly found attacking *Cirsium vulgare* throughout its area of distribution in Victoria. The rust also occurs in South Australia, New South Wales, and Queensland. Due to the biennial life cycle of the host plant, urediniospores were found throughout the year but were the dominant spore type encountered in spring and summer. Tellospores were found on flowering and senescent plants in late summer. Pycnia and aecia were found in winter and spring, mainly on seedlings growing near dead plants from the previous season. Laboratory studies showed that the optimum temperature for urediniospore germination is 20°C and that continuous light or darkness has no influence on germination. The optimum dew period for infection was 24 h. Under optimum conditions, the germination of freshly collected urediniospores was only 32%. Twice weekly inoculations with urediniospores for four wks had no effect on rosette dry weight but significantly reduced root dry weight of *C. vulgare* seedlings. Host specificity tests on 24 species of thistles and knapweeds available in Victoria showed that *P. cnici* can infect five species of thistles not recorded as natural hosts, including globe artichoke. Only species in the sub-tribe Carduineae were infected. *P. cnici* could be maintained on globe artichoke seedlings for only three generations.

Introduction

Spear thistle, *Cirsium vulgare* (Savi) Tenore (Asteraceae) is a Eurasian plant which is considered weedy in 29 countries around the world (Holm *et al.* 1979). In Australia it occurs in all States and is a declared weed in Victoria, South Australia, New South Wales and Tasmania. In Victoria, Lane *et al.* (1980) found spear thistle to be the most widespread noxious weed. He estimated the total infestation at 9.7 million ha of which 340,000 ha was dense, 1.5 million ha medium and 7.86 million ha scattered.

A preliminary survey of the predators and pathogens of thistles (Asteraceae: Cardueae) in Victoria showed that spear thistle is commonly attacked by the rust fungus *Puccinia cnici* Mart. (Uredinales: Pucciniaceae). Since spear thistle has been proposed as a candidate weed for biological control in Australia and *P. cnici* has been proposed as a possible agent for the biological control of spear thistle (Hasan 1980) investigations were carried out on aspects of the biology of this pathogen in Victoria.

Materials and Methods

The life cycle of *P. cnici* was determined by regular inspections of a *C. vulgare* infestation at the Keith Turnbull Research Institute. Samples of the host were collected and inspected for the presence of different spore types over a three-year period.

The distribution of the rust in Victoria was determined by inspection of spear thistle infestations throughout the State over a two-year period.

The effect of the rust on the biomass of *C. vulgare* was determined by inoculating plants twice per week for 4 wks with freshly collected urediniospores. One ml of urediniospore suspension (1 mg/ml water) was applied to each plant from a height of 30 cm using an

atomiser. Ten potted seedlings of the same age were inoculated a total of 8 times, and 10 plants were kept as controls. Fourteen days after the last inoculation they were removed from the soil, washed and dried to constant weight. A comparison of leaf and root dry weights between the inoculated and control plants was carried out.

The effect of temperature and light on urediniospore germination and germ tube growth was determined by sprinkling freshly collected urediniospores on glass microscope slides coated with 2% water agar. These were placed in petri dishes with a little water to maintain humidity and incubated at 0, 5, 10, 15, 20, 25, 30, 35 and 40°C in cabinets under 24 h light. Some of the dishes at each temperature were wrapped in aluminium foil to exclude the light. For the germ tube studies, growth was stopped after 6 h of incubation by addition of methylene blue to the agar. Germ tube length was measured on 50 germinated spores on two replicate slides. Germination percentage was determined from observations of 200 spores on two replicate slides after 18 h incubation.

The effect of dew period on infection by urediniospores was determined by exposing inoculated plants to varying periods of wetness. The leaves on four replicate potted plants were marked and inoculated with a urediniospore suspension (1 mg/ml) as described previously and incubated at approx. 20°C in a glasshouse under natural daylength. The surface of the inoculated plants was kept moist by sealing them in a plastic bag with some water for the duration of the treatment. Treatments were: dry spores (plants placed in bag for 6 h with no water), O, 1, 2, 3, 6, 12, 24 and 48 h of wetting. Fifteen days after inoculation the pustules on the marked leaves were counted and the leaf area measured. Infection was expressed as pustules/cm^2.

The host-specificity of *P. cnici* was determined by inoculating 24 species of thistles and knapweeds available in Victoria with freshly collected urediniospores. Five replicate plants of each species (Table 1) were inoculated and incubated in a glasshouse as described previously and *C. vulgare* was used as a control plant throughout the tests. Leaves were kept moist for the first 18 h. Plants were observed daily for disease development and latent periods for the production of symptoms and uredinia were recorded. Twenty days after inoculation plants were evaluated using the following disease ratings:

0 - immune (no host reaction);
1 - highly resistant (flecks, chlorotic or necrotic areas on leaves, no sporulation);
2 - resistant (flecks, chlorotic or necrotic areas on leaves, limited sporulation with small uredinia);
3 - susceptible (numerous small to medium uredinia); and
4 - highly susceptible (abundant sporulation comparable to that on host plant).

Results and Discussion

Observations on the life cycle of *P. cnici* revealed all spore stages in the field except basidiospores. All attempts to germinate teliospores in the laboratory or to inoculate plants with teliospores collected on previous season plants were unsuccessful, however Kellerman (1903) obtained aecia when he inoculated *C. vulgare* with teliospores.

Urediniospores were found throughout the year. This is due to the extended seed germination and biennial habit of the host which means that plants at different stages of growth are present throughout the year. Urediniospores were the most common spore type in late spring and summer whilst teliospores were more common on flowering plants in late summer. These mature on the dead flowering stalks which may remain standing for up to two years. Pycnia and aecia were noticed on seedlings and rosettes growing close to dead previous season plants in late winter to late spring. Although the effect of the rust on its host was not studied in the field, no sites were observed where the pathogen had an obvious deleterious effect on its host.

The rust was collected at 35 locations throughout the distribution of *C. vulgare* in Victoria, in all the climatic areas represented in the State. The rust was also collected by the authors in southeastern New South Wales and in southeastern South Australia and has been recorded in

Queensland (J. Alcorn, pers. comm.). It is therefore likely that the distribution of *P. cnici* in Australia extends to the distribution of *C. vulgare*.

Table 1. Reaction of species of Tribe Cardueae (Asteraceae) to *Puccinia cnici* Mart.

Species	Disease Rating	Latent periods (days) for Symptoms	Uredinia
Sub-Tribe Carduinae			
Carduus pycnocephalus L.	1	14	-
C. tenuiflorus Curtis	0	-	-
C. nutans L.	0	-	-
Cirsium arvense (L.) Scopoli	0	-	-
C. vulgare (Savi) Tenore	4	8	10
Cynara cardunculus L.	3	8	11
[1]*C. scolymus* L.	2	8	11
Onopordum acanthium L.	3	10	12
O. acaulon L.	2	11	14
O. illyricum L.	1	11	-
O. tauricum Willd.	1	11	-
Picnomon acarna (L.) Cass.	1	15	-
Silybum marianum (L.) Gaertn.	3	8	11
Sub-Tribe Centaureinae			
Acroptilon repens (L.) DC.	0	-	-
Carthamus dentatus (Forsk.) Vahl	0	-	-
C. lanatus L.	0	-	-
[1]*C. tinctorius* L.	0	-	-
Centaurea calcitrapa L.	0	-	-
[1]*C. cyanus* L.	0	-	-
C. melitensis L.	0	-	-
[1]*C. moschata* L.	0	-	-
C. nigra L.	0	-	-
C. solstitialis L.	0	-	-
Rhaponticum australis (Gaudisch) Soskov	0	-	-

[1] Cultivated or ornamental species.

In laboratory studies, no significant difference was found between the above ground biomass of inoculated seedlings and that of control plants, however, the root biomass of inoculated seedlings was significantly less than that of control plants (P 0.05) (Fig. 1). These results are in contrast to those found by Politis (1986) with a highly virulent isolate of *Puccinia carduorum* Jacky on *Carduus nutans* L. where dry weights of rosette and root tissue of inoculated plants were significantly less than those of the control plants even after two inoculations.

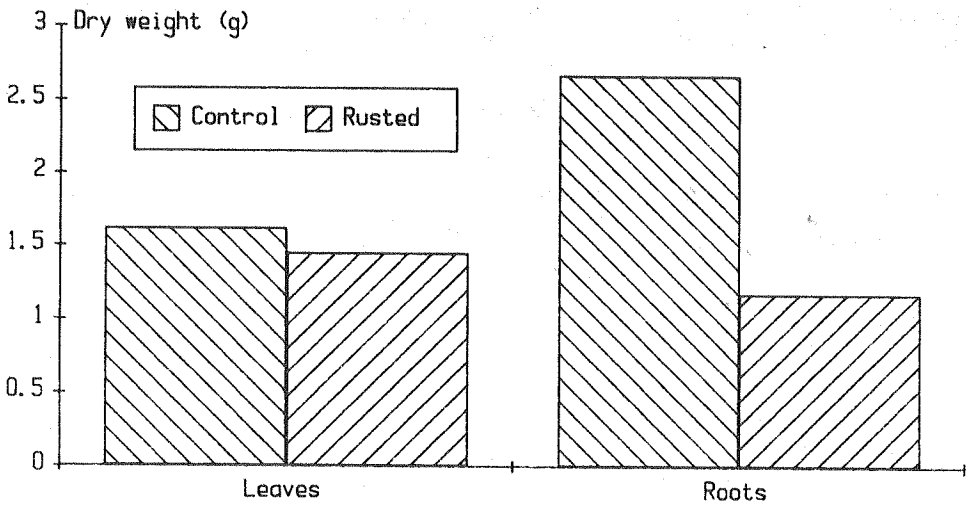

Figure 1. Effect of repeated inoculations of *Puccinia cnici* Mart. on plant biomass.

There were no significant differences in germination percentage and germ tube growth between urediniospores incubated in the light and in the dark (P 0.05). Germination occurred between 10 to 25°C with a maximum at 20°C at which only 32% of freshly collected spores germinated. Maximum germ tube growth also occurred at 20°C (Fig. 2).

The optimal dew period for infection with urediniospores was 24 h (Fig. 3). No significant differences could be found between the inoculation of dry spores on dry plants and 0, 1, 2, 3, 6 and 12 h dew periods however there was a highly significant difference between 12 and 24 h dew periods (P = 0.05). No significant difference was found between 24 and 48 h dew periods. The long dew period requirement may explain why no difference was recorded between germination of urediniospores in the light and in the dark. The long dew period requirement and low germination percentage of freshly collected urediniospores indicates the presence of germination inhibitors. In a preliminary experiment to test this hypothesis, germination of fresh urediniospores was increased to 46% at 20°C by washing them in distilled water for 15 min.

The combination of high temperature requirement, long dew period and low germination percentage of urediniospores may explain why in Victoria the isolate of *P. cnici* present does not seem to reach damaging levels on its host. In Victoria, spear thistle generally germinates in autumn after the seasonal rains and develops an extensive root system during the winter (Parsons 1973). The rosette continues to grow during the spring and produces a flowering stem in summer. Germination also occurs in winter and spring and any resulting rosettes which have not received adequate winter chilling flower the following summer. For *P. cnici* to be a successful biological control agent it would need to damage spear thistle during the winter/spring before the plant can build up reserves in the root system and produce a flowering stem. Obviously, the germination requirements of urediniospores do not allow the rust to develop to epidemic proportions in winter/spring under Victoria's climatic conditions.

Results of host-specificity tests (Table 1) show that *P. cnici* has a restricted host range. All the infected species are members of the sub-tribe Carduinae, as was found with *Puccinia carduorum* Jacky, a rust of *Carduus* spp. by Politis *et al.* (1984). As with other rusts of Cardueae, the host range of *P. cnici* was expanded by inoculation under laboratory conditions. Watson (1985) lists three other rusts of Cardueae (*P. carduorum, P. jaceae* Otth. and *P. centaureae* DC.) which have had their ranges expanded in laboratory tests. In the laboratory, *P. cnici* was able to infect *Cynara cardunculus* L., *C. scolymus*, *Onopordum acanthium*, *O. acaulon* and *Silybum marianum* (L.) Gaertner. None of these have been recorded as hosts of this rust in extensive field surveys (Bruzzese & Heap, unpubl. data),

even though their distribution in Victoria often coincided with that of *C. vulgare* and the rust. It can therefore be concluded that under field conditions *P. cnici* is restricted to *C. vulgare*. *C. scolymus* (globe artichoke) is the only cultivated plant which was infected by *P. cnici*.

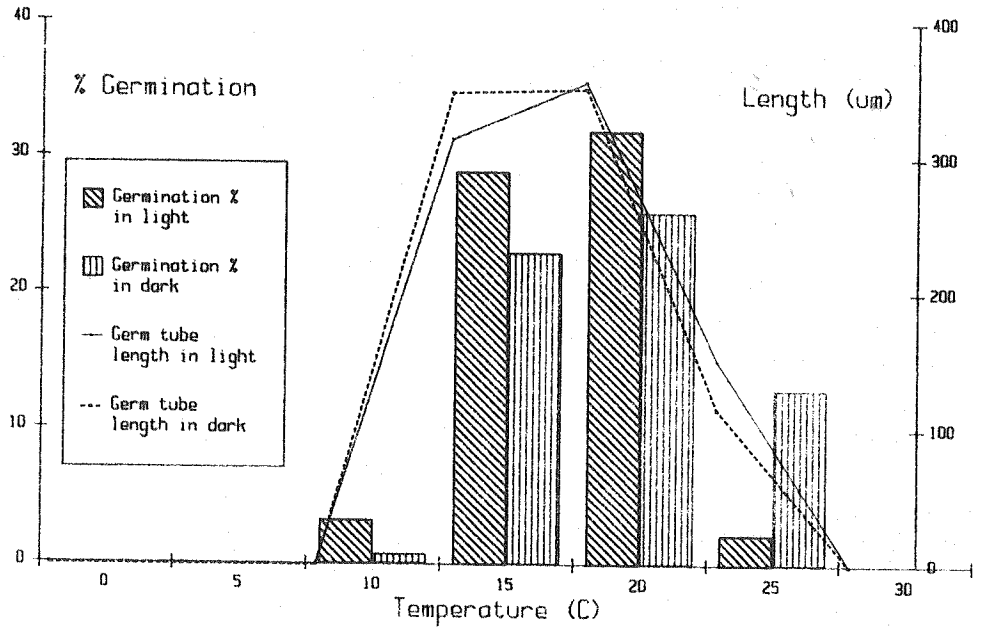

Figure 2. Effect of temperature and light on germination of *Puccinia cnici* Mart. urediniospores.

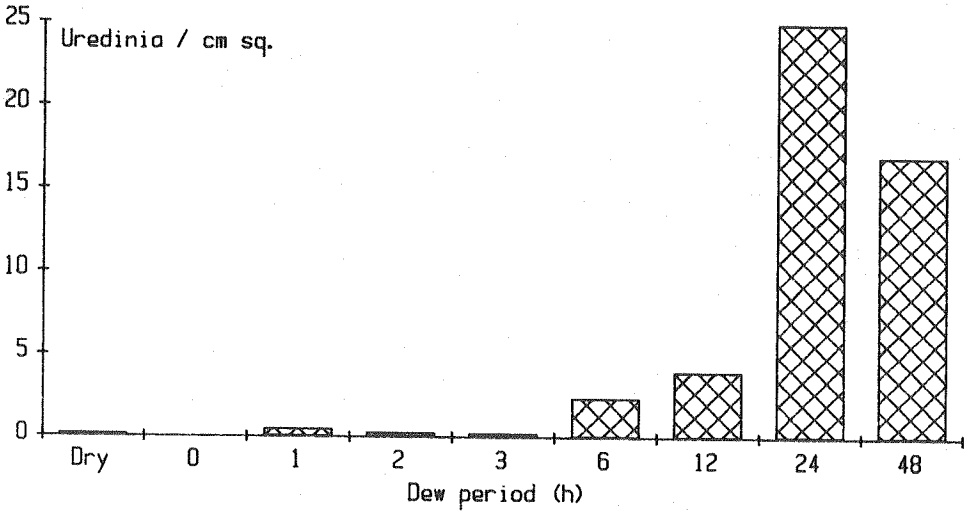

Figure 3. Effect of dew period on infection by *Puccinia cnici* Mart. urediniospores.

An experiment was carried out to study the potential risk of *P. cnici* to globe artichoke. Four seedlings of cv. Grande Beurre were inoculated with a suspension of urediniospores and any resulting urediniospores were collected and inoculated again onto new seedlings of cv. Grande Beurre. Production of urediniospores declined steadily and by the third generation on globe artichoke, no further collections could be made. In a similar experiment with *C. cardunculus* (artichoke thistle), no rust could be collected after the fourth generation. This is further proof that repeated passage by an obligate parasite through a resistant host, results in progressive reduction in inoculum, to the point where insufficient inoculum is produced for infection to occur (Gaumann 1950, Watson 1985). Similar to our findings, Bruckart *et al.* (1985) were unsuccessful in maintaining *P. carduorum* on globe artichoke. It is therefore highly unlikely that globe artichoke will be at risk if further strains of *P. cnici* are imported into Australia for the control of spear thistle.

The strain of *P. cnici* currently in Australia does not seem to have a deleterious effect on its host under the Mediterranean, winter rainfall conditions of south-eastern Australia. This rust is however host-specific in the field and has a very restricted host range under laboratory conditions. The importation of further strains of this rust, more synchronised to attacking its host at crucial periods in its life cycle, may be advantageous for the classical biological control of spear thistle in Australia.

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