

MORTALITY OF CANADA THISTLE DUE TO *Puccinia punctiformis*A.K. Watson¹ and W.J. Keogh^{1,2}

ABSTRACT

Puccinia punctiformis (Str.) Rohl. is a systemic, autoecious brachy form rust which is coextensive with its host, Canada thistle, *Cirsium arvense* (L.) Scop. in North America. Initially diseased and healthy shoots were monitored throughout the growing season in two natural habitats. By the end of July, 98 and 100 per cent of the diseased shoots had died at the two sites. Secondary infections (2^o urediosori) were observed on all healthy plants, but did not contribute to mortality. Multiple inoculations with urediospores is proposed as a technique to increase systemic infection levels in field populations.

INTRODUCTION

Canada thistle (*Cirsium arvense* [L.] Scop.) is a cosmopolitan weed and is naturalized throughout Canada as far north as 59° N lat. (Moore 1975). A complex of natural enemies are associated with this weed in Canada (Maw 1976, Savile 1970, Watson and Shorthouse 1979). The objectives of this study were: 1) to determine the abundance of natural enemies on Canada thistle in field populations; and 2) to investigate the impact of the rust, *Puccinia punctiformis* (Str.) Röhl., on Canada thistle.

MATERIALS AND METHODS

Field survey

Three field populations of Canada thistle were surveyed for natural enemies during the 1978 growing season. Plants were selected at random and the per cent occurrence of natural enemies was recorded. In addition, 10 healthy and 10 thistle shoots infected with *P. punctiformis* were selected at random at one site and height and number of flower buds were recorded.

Effect of *P. punctiformis* on Canada thistle

In 1979 two Canada thistle populations were selected for intensive study of the rust on its host. Site A was a relatively dry, gravelly location, situated in a roadside right-of-way with a mean density of 11.9 Canada thistle shoots per m². Site B, a relatively moist location, was located in ungrazed pasture with a thistle density of 12.2 shoots per m². Quadrats were established at random within the two sites and diseased and healthy shoots were tagged and monitored throughout the growing season. At site A, a total of 149 shoots (97 healthy and 52 diseased) were monitored. At site B, 116 shoots (63 healthy and 53 diseased) were monitored.

Photosynthetic and respiration rates of diseased and healthy leaf tissues were measured in a Gilson respirometer (Umbreit and Burris 1964). Leaves with pycnia, leaves with uredia and healthy leaves were collected from the field. Leaf discs (2 cm diam.) were cut from these leaves and six leaf discs were placed into each reaction vessel. Flasks were initially wrapped in tin foil for respiration

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measurements and then the foil was removed and photosynthesis measured. Experiments were conducted at 27°C with readings recorded every 15 min. until a constant volume of gas was evolved. Each of the three treatments were replicated three times and the experiment was repeated on three different dates. Data was analysed by the analysis of variance.

Biology of *P. punctiformis*

Field collected urediospores were placed on 1 per cent water agar in petri dishes. Three replicates were placed into incubation chambers set at 5, 10, 15, 20, 25 and 30°C. Per cent spore germination was recorded after four hours.

Eight seedlings (2-leaf stage) each were placed in three different controlled environment chambers with 14 hr light (1800 ft-candles) and 10 hr dark periods and light/dark temperatures of 25/20°C, 20/15°C and 15/10°C. The cotyledons of each seedling were inoculated with freshly collected, hydrated urediospores by the spatula method. Plastic bags were placed over the inoculated plants and subsequently removed after 10 hr in the dark.

In another experiment 10 emerged shoots from non-infected rootstocks, grown in a growth chamber at 20°C light (1800-ft-candles) for 14 hr and 15°C dark for 10 hr, were inoculated with freshly collected, hydrated urediospores as described above. All leaves (two to five) of each shoot were inoculated.

RESULTS AND DISCUSSION

Field survey

The seed-head fly, *Orellia ruficauda* (Fab.), was the most common natural enemy observed in Canada thistle populations (Table 1). The beetle, *Cassida rubiginosa* (Mull.), and the rust, *P. punctiformis*, were the next most frequent parasites observed on Canada thistle. Rust infected shoots had significantly fewer flower buds than healthy shoots (Table 2).

Table 1. Per cent occurrence of natural enemies on Canada thistle — 1978.

Natural enemy	Site			
	Ungrazed pasture (n=325) ¹	Roadside right-of-way (n=439)	Grazed ² pasture (n=200)	Combined (n=964)
<i>Cassida rubiginosa</i> (Mull.)	15.1	28.0	33.5	24.8
<i>Cleonus piger</i> Scop.	15.4	8.0	15.5	12.1
<i>Orellia ruficauda</i> (Fab.) ³	63.4	44.4	32.3	36.5
<i>Puccinia punctiformis</i> (Str.) Röhl.	17.2	4.3	57.5	19.7
<i>Ditylenchus dipsaci</i> (Kuhn) Filipjev	4.6	10.7	26.0	11.9
Mycoplasma ⁴	5.2	0.7	0.0	2.1
Unknown pathogen ⁵	16.3	3.2	0.0	7.0

¹ Number of plants examined at each site.

² Data collected only once (26 August, 1978) while data for the other two sites accumulated over June, July, August, 1978.

³ Flower heads collected during last two weeks of August (n=54, 34, 323, 411).

⁴ Typical aster yellows symptoms, possibly caused by a mycoplasma-like organism (Schaber and Balsbaugh 1974).

⁵ Circular lesions similar to *Cassida* damage, but caused by an unidentified pathogen.

Table 2. The effect of *Puccinia punctiformis* infection on height and flower bud production of Canada thistle.

Pathogen	Flower buds (no.)	Height (cm)
Present	1.8a ¹	75.3a
Absent	46.6b	99.8a

¹ Means in the same column sharing the same letter are not significantly different at the 1 per cent level according to the *t* test.

The effect of the disease can be very dramatic in reducing vigour and causing the ultimate death of its host. Early reports (Halsted 1893 in Wilson 1969, Cockayne 1915) noted the localized destructiveness of this pathogen and suggested means should be found to increase infection beyond that occurring naturally. Systemically infected shoots, when compared to healthy shoots, are chlorotic, have smaller leaf area and usually fail to produce seed (Bailiss and Wilson 1967).

Effect of *P. punctiformis* on Canada thistle

In the two natural habitats (Site A and Site B), all shoots systemically infected with *P. punctiformis* (except for one shoot at site B) had died by the end of July (Table 3 and Table 4). Over the same period 36.4 and 19.0 per cent of the 'healthy' shoots had died in site A and site B, respectively. Although all 'healthy' shoots developed minor 2^o infections (2^o urediosori) of *P. punctiformis*, the major causes of the mortality to 'healthy' shoots was due to other biotic stresses (*Cassida* and *Cleonus* attack) and climatic stresses. Mortality rates for diseased and healthy shoots at each site were transformed into survivorship graphs based on an arbitrary initial cohort of 1000 individuals in each sample (Figure 1 and Figure 2). These results demonstrate the stress imposed by natural infection of *P. punctiformis* on Canada thistle.

Laboratory investigations of the photosynthetic and respiration rates of healthy and diseased leaves demonstrated the physiological stress of disease (Table 5). Respiration rates were significantly higher for diseased tissue when compared to healthy tissues. The higher value of uredia infected leaves as compared with the pycnia infected leaves could possibly be explained by respiration of the urediospores. No significant differences were observed between the rates of photosynthesis in this experiment.

Biology of *P. punctiformis*

The rust overwinters as mycelium in infected roots and the adventitious buds emerge as systemically infected shoots which usually produce pycnidia or occasionally urediosori (Buller 1950, Menzies 1953). In our studies only two of the 105 emerging diseased shoots had the uredia infection while all others (103) had the pycnidia infection. All pycnidia in one shoot are of the same mating type and evidence suggests that insects are responsible for mixing ('diploidization') of the two mating types (Buller 1950, Menzies 1953). After the pycnidial stage, urediospores and teliospores are produced on diseased shoots. Infected plants produced an abundance of urediospores throughout the early part of summer, but production decreased as the season progressed, which was probably due to higher temperatures. The presence of teliospores in collected spore samples increased linearly from 0 per cent on 7 July to approximately 80 per cent by mid October.

Table 3. Mortality of Canada thistle caused by the rust, *Puccinia punctiformis*, in a roadside habitat (Site A).

	Cumulative per cent mortality						
	May 25	June 07	June 14	June 22	July 04	July 17	July 27
Healthy plants ¹	0.0	5.1	8.2	15.5	28.9	33.0	36.4
Diseased plants ²	3.9	21.2	36.5	80.1	94.2	98.1	100.0

¹ No symptoms of disease in early spring, mortality caused by other organisms and environmental factors.

² Systemically infected in early spring, mortality primarily a function of the disease.

Table 4. Mortality of Canada thistle caused by the rust, *Puccinia punctiformis*, in an ungrazed pasture (Site B).

	Cumulative per cent mortality				
	June 12	June 18	July 03	July 17	July 31
Healthy plants ¹	1.6	1.6	3.2	12.7	19.0
Diseased plants ²	1.9	18.9	64.2	83.0	98.1

¹ No symptoms of disease in early spring, mortality caused by other organisms and environmental factors.

² Systemically infected in early spring, mortality primarily a function of disease.

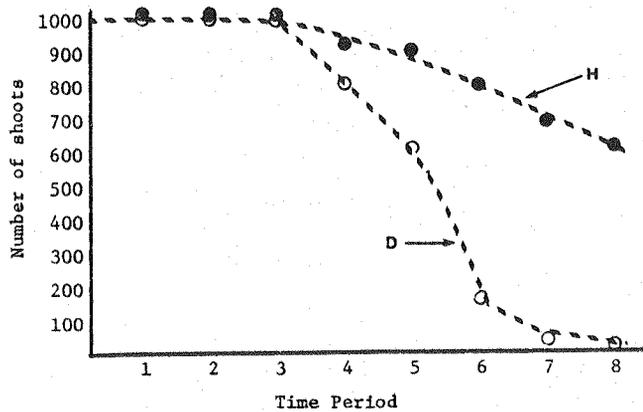


Figure 1. Survivorship of natural populations of diseased and healthy Canada thistle – Site A.

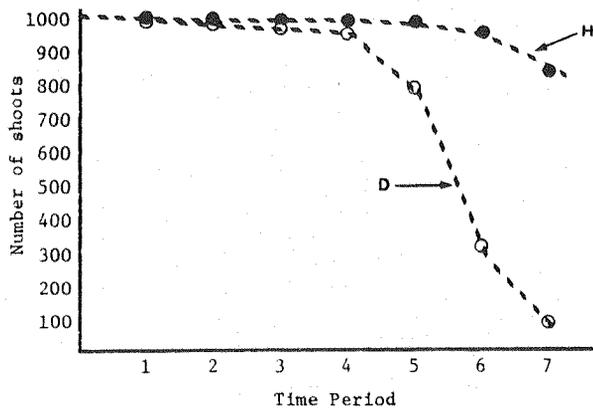


Figure 2. Survivorship of natural populations of diseased and healthy Canada thistle – Site B.

Table 5. Photosynthesis and respiration rates of healthy and diseased Canada thistle leaf tissue.

Leaf tissue	Photosynthesis ($\mu\text{l O}_2/\text{cm}/0.25 \text{ hr}$)	Respiration ($\mu\text{l CO}_2/\text{cm}/0.25 \text{ hr}$)
Control (healthy)	29.75a ¹	10.58c
Uredia infected	31.37a	31.60a
Pycnia infected	27.45a	25.53b

¹ Means sharing the same letter in the same column are not significantly different at the 1 per cent level according to LSD test.

Secondary infections arise from air-borne urediospores which land on leaves, germinate and develop sori which produce additional urediospores and subsequently, teliospores (Bailliss and Wilson 1967). At the end of July, 2^o urediosori were observed on 93.8 per cent and 86.5 per cent of the 'healthy' shoots at site A and site B, respectively. However, these sori were small and covered less than 1 per cent of the leaf area of any shoot. Primary urediosori are not eruptive until approximately mid-June and subsequent infection and development of 2^o urediosori is probably limited by the absence of favourable conditions for urediospore germination and penetration of host leaf tissues.

In laboratory studies it was found that 15°C was the optimum temperature for urediospore germination (Fig. 3). However, the rate of disease development was slightly faster in warmer temperatures than compared with cooler temperatures (Table 6). Urediosori developed on 74.3 per cent of the inoculated leaves and all infected leaves died within two weeks. No further development of the rust was observed and none of the infected plants died.

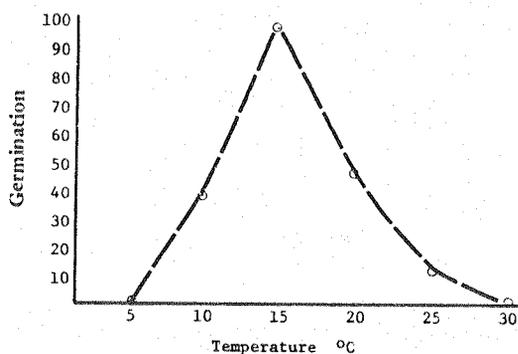


Figure 3. Effect of temperature on germination of *P. punctiformis* urediospores.

Table 6. Effect of temperature on length of time for uredia to appear after inoculation of seedlings of Canada thistle.

Temperature (°C)		Days
Day (14 hr)	Night (10 hr)	
25	20	12
20	15	13
15	10	14

The life cycle of the rust presumably continues with the fungal mycelium, produced from uredial infections on leaves, losing their dikaryotic character, growing down the stem into the roots and giving rise to systemically infected shoots the following spring (Buller 1950, Menzies 1953). This mycelial growth is very slow as Menzies (1953) observed that the fungus travels only a few centimeters down the stem in two months after young leaves were successfully inoculated with urediospores. Bailiss and Wilson (1967) observed the mycelium only intercellularly and primarily in parenchymatous tissues. It is therefore possible that young shoots emerging in the fall may become infected with urediospores and the resulting mycelium is able to reach root tissue prior to winter dormancy. Alternatively, the fungus may overwinter as teliospores and establish localized infections the following spring from resulting basidiospores. However, teliospores are extremely difficult to germinate in the laboratory (Buller 1950, Menzies 1953).

The results presented in this paper demonstrated the effectiveness of *P. punctiformis* in severely stressing its host, Canada thistle. Studies are presently under way to investigate the possibility of increasing the number of systemically infected plants through multiple inoculations of urediospores and other techniques. Despite the general occurrence of Canada thistle throughout Canada (Moore 1975), it is not considered a serious weed in eastern Canada (Deschenes and Anderson, pers. comms.). We propose that the complex of natural enemies (see Table 1) present in eastern Canada are providing effective natural control in at least some habitats.

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