Enhancing incidence of *Puccinia punctiformis*, through mowing, to improve management of Canada thistle (*Cirsium arvense*)

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Abstract

Biological control of Canada thistle using *Puccinia punctiformis* has been largely unsuccessful in part due to a low incidence of systemically infected shoots and heterogeneous distribution of teliospores in the soil. The present study investigated the feasibility of strategic mowing to improve incidence of systemically infected shoots, and enhance intra- and/or inter-season disease development in two unused pastures. Mid-season mowing of plots in July lead to a greater proportion of systemically infected shoots in experimental plots observed at seasons’ end compared to unmowed plots. Late-season mowing in September resulted in the highest levels of systemically infected shoots early the next summer. Over time, September mowing treatments significantly increased the proportion of systemically infected shoots compared to no mowing. The number of healthy shoots declined over time in mowed plots, whereas the number of healthy shoots in unmowed plots either increased or remained constant. These effects were observed in experimental plots in both pastures. It is proposed that mowing followed by regrowth of systemically infected shoots may help overcome the monocyclic nature of the pathogen and enhance severity of this disease.

Keywords: Rust; *Puccinia punctiformis*; Canada thistle; *Cirsium arvense*; Creeping thistle

1. Introduction

Canada thistle, *Cirsium arvense* (L.) Scop., is a noxious perennial weed occurring on many millions of acres of pastures, rangeland, and other agricultural lands in the United States and Canada (Morishita, 1999). It is particularly difficult to control. Chemical control of this vigorous weed has proven ineffective due to an extensive root system that survives even when aboveground shoots have been destroyed (Thomas et al., 1994). As a result, multiple herbicide applications are often required making control an expensive endeavor. In rangelands and natural areas, herbicide applications are prohibitively expensive and not an economical control option. Biological control using natural enemies could offer a noninvasive, host-specific, and low-cost management option for Canada thistle.

Although several antagonistic organisms have been evaluated for use as biological control agents on Canada thistle including fungi, bacteria, and insects (Green and Bailey, 2000; Gronwald et al., 2002; Ang et al., 1995), the obligate rust fungus *Puccinia punctiformis* (F. Strauss) Rohl., is perhaps the first plant pathogen proposed as a biological control agent for Canada thistle or any other weed. In 1893 a New Jersey farmer noticed Canada thistle patches diseased with the rust virtually disappeared after a couple of years, and he proposed that the rust should be widely disseminated to control the weed (Wilson, 1969). The fungus is an endemic, autoecious, brachy-form rust found on Canada thistle that can limit flowering and vegetative growth (French and Lightfield, 1990). In the spring, volatiles emitted from developing Canada thistle root buds induce germination of temperature-stratified diploid teliospores in the...
soil (Turner et al., 1986) leading to the production of infective haploid basidiospores. Systemic infections, arising from infection by basidiospores, give rise to spindly shoots that usually die before the plant flowers (Van den Ende et al., 1987). Leaves on systemically infected shoots bear pycnia containing orange-colored haploid pycniospores (Fig. 1) and receptive hyphae, which emit a characteristic sweet smelling fragrance (Thomas et al., 1994). Following fertilization of the receptive hyphae by compatible pycniospores, the hyphae give rise to brown primary uredinia, bearing dikaryotic urediniospores, which cause secondary infections of neighboring thistle shoots later in the season. Secondary infections result in the development of localized lesions, bearing urediniospores. Locally infected plants do not experience the growth abnormalities associated with systemic infections (Baka and Lösel, 1992). Primary uredinia convert to the production of teliospores later in the season as temperatures lower (Waters, 1928). Teliospores and mycelium in the rootstock serve as overwintering mechanisms for the rust (Bailiss and Wilson, 1967).

However, successful biological control of Canada thistle using *P. punctiformis* is hindered due largely to a heterogeneous distribution of teliospores in the soil leading to low incidence of systemically infected shoots. Furthermore, research has shown a steep dispersal gradient where teliospores are predominately deposited on soil directly under infected thistle shoots (Frantzen, 1994a). As a result, there are low probabilities of teliospore root bud contact and low incidence of systemically infected thistle shoots (Frantzen, 1994a). Heterogeneity of inoculum distribution effectively isolates the pathogen from a large part of the target weed population and the pathogen fails to utilize the full potential of its inoculum (Shrum, 1982).

Mowing Canada thistle patches seems only to prevent the patches from expanding (Willard and Lewis, 1939), and effective control may only be achieved when patches are mowed several times a year over several years (Amor and Harris, 1977). Haggard et al. (1986) proposed that mowing may be useful only to prevent seeding or to reduce weed dispersal. The effects of mowing combined with herbicide applications on Canada thistle control have also been investigated and the results have been variable (Amor and Harris, 1977; Beck and Sebastian, 2000). Alternatively, rotational cattle grazing in pasture and rangeland have provided significant weed suppression. De Bruijn and Bork (2006) reported that high intensity-low frequency rotational grazing systems reduced Canada thistle shoot density and biomass compared to season-long grazing. They further suggested that “uniform defoliation of all vegetation shifts the competitive balance among species in favor of those with rapid regrowth...[such as] forage grasses highly tolerant to grazing, with rapid regrowth from axillary buds”. Furthermore De Bruijn and Bork (2006) hypothesize that repeated defoliation might systematically deplete root carbohydrates.

Potted-plant experiments conducted by Kluth et al. (2003) demonstrated a synergistic effect of combined cutting and pathogen application, which reduces the reproductive fitness of Canada thistle. Kluth et al. (2003) further suggested that results from their potted-plant study are comparable to field conditions. However, due to the restriction of the thistle’s normally extensive root systems (Hayden, 1934) and prolific development of adventitious shoots, potted Canada thistle may respond differently to mowing than field-grown thistle. It is important to investigate the combined effects of mowing and pathogen infection on patch dynamics over time in natural settings.

The investigation of techniques to improve teliospore dispersal and thus increase the incidence of systemic infections in a Canada thistle patch was the motivation behind the study presented here. Mowing Canada thistle patches infected with *P. punctiformis* may enhance within-season disease development by removal of apical dominance leading to production of new systemically infected shoots. These new shoots, along with physical redistribution of infected plant parts by the mower, may increase teliospore dispersal for enhanced and more uniform infections in subsequent years. The objectives of the present research were to investigate the ability of strategically timed mowing to...
increase incidence of shoots systemically infected with *P. punctiformis* in targeted Canada thistle patches and to determine the timing and number of mowing events that results in the greatest increase in disease incidence both within and between seasons. Successful mowing strategies in combination with *P. punctiformis* may provide important insights into overcoming obstacles to effective Canada thistle biological control encountered in previous research.

2. Materials and methods

2.1. Thistle debris experiment

A preliminary experiment was conducted to determine whether in-season and/or inter-season teliospore/basidiospore infection of adventitious root buds would lead to new systemically infected shoots within a season. Potted healthy thistle plants were cut in mid-July to approximately 3 cm above soil level to simulate a mowing event. Three types of chopped thistle leaves and stems were weighed and spread over the pots to simulate redistribution of material from mowing. This experiment consisted of three treatments with four replications. Treatments included leaves and stems of systemically infected shoots (containing teliospores), locally infected shoots (containing probably only urediniospores), and healthy leaves. The respective treatments were applied on July 14th 2004 at a rate of about 10 g dried plant matter per 1 gallon pot. Pots were placed outside under direct sunlight and natural rainfall, but also received additional watering if necessary. Regrowth of shoots in all pots was monitored for signs of systemic infection between July and September. Pots remained outside until January to allow for cold stratification of teliospores. In early January, all pots containing frozen thistle shoots were moved into a greenhouse with a drip irrigation system. The dead above-ground shoots were trimmed and added to the soil surface in the pot. As new thistle shoots emerged, the regrowth of these shoots was monitored for systemic infections until April.

2.2. Teliospore development in primary and secondary lesions

Prior to conducting field experiments, leaves containing primary and secondary uredinia were collected weekly throughout the summer of 2003 for analyses in the laboratory. A single leaf from each of 10 systemically infected (exhibiting signs of pycnia at the beginning of the season) and 10 locally infected (without signs of pycnia at the beginning of the season) Canada thistle shoots were collected each week for 6 weeks. About 3–5 lesions from each leaf were sampled for microscopic examination. Primary uredinia were regarded as those arising from systemically infected shoots whereas secondary uredinia arose from local foliar infection by urediniospores. Spores from each lesion were wet mounted and observed under a microscope. Two-celled teliospores and one-celled urediniospores could be easily distinguished microscopically, and the presence or absence of teliospores was recorded for each lesion on each leaf sampled.

2.3. Field plot establishment

Two sites (designated A and B) separated by ca. 5 km on the north end of The Pennsylvania State University campus (University Park, PA) in unused pastures were selected for this experiment. Both sites contained heavy natural infestations of Canada thistle and moderate levels (typically varying from 0% to 5%) of thistle shoots systemically infected with *P. punctiformis*. The block of pasture at location A was surrounded by paved roads and other sections of pastureland were present beyond the roadway. Location B was situated at the edge of a large pasture with forested borders on the northwest and northeast sides. Research plots were selected from larger Canada thistle patches, marked using flags, and locations were recorded using a Trimble backpack GPS device (Sunnyvale, CA). To avoid any interference from systemically infected Canada thistle shoots outside the plot area, areas immediately surrounding experimental plots were cleared of all shoots using a string weed-cutter during the spring and summer months of 2004. In location A, 24 circular plots with a 3-m diameter (7.3 m²) were established within Canada thistle patches. In location B, 32 plots were established in the same way. All plots had similar densities of healthy and systemically infected Canada thistle.

2.4. Experimental design

This experiment was arranged in a completely randomized design with three mowing treatments in location A and four mowing treatments in location B and eight plots/patches (replications) per mowing treatment. Mowing treatments were randomly assigned to each of eight patches per mowing treatment per location. The four mowing strategy treatments included: unmowed; mowed once on July 13th (July); mowed once on September 30th (Sept); and mowed twice, once on July 13th and once on September 30th (July + Sept). The Sept treatment was conducted at location B only due to space restrictions at location A. A rotary blade mower was used to mow the plots. At each of the mowing dates, a large majority of the systemically infected stems contained mature teliospores. A systemically infected aboveground shoot was regarded as a single diseased shoot. Due to the clonal nature of Canada thistle, several to numerous aboveground shoots may constitute a single genetic entity. Frequently, a patch may originate from a single plant (Kay, 1985). For purposes of this research it was important to identify the number of spore-producing shoots rather than genetic individuals. The total numbers of Canada thistle shoots within each plot were estimated to calculate the proportion of systemic infection before and after mowing. Healthy Canada thistle shoots were counted in a 1-m square made from PVC piping arbitrarily placed into a plot. This count was repeated three times in each plot and the average number of thistle shoots...
per m$^2$ was calculated. This average was then multiplied by the total area of a plot (7.3 m$^2$). This procedure was conducted before mowing, 4 weeks after the July mowing and finally in June of 2005, the following summer season. Proportions were not calculated after the September mowing in 2004 due to the lack of regrowth as winter approached.

Exact centers of all plots were marked with a metal bolt that was driven into the ground so that only the head of the bolt was visible. This marking system was used to ensure that the plot center could be relocated after flags were removed and the plots mowed. After mowing, a metal detector was used to locate the metal bolts and the plot edges were re-marked with flags.

2.5. Data collection

Colored tags were attached to systemically infected Canada thistle shoots throughout the 2004 summer months. Systemically infected thistle shoots present during May were marked with red tags. Shoots present from June 1st until July 13th, when plots were mowed, were marked with yellow tags. Systemically infected shoots present after July 13th (counted in late August) were marked with blue tags. This color-coding system was used to follow the progression of disease spread over the course of the season in both mowed and unmowed plots and to avoid counting the same shoots twice. Disease incidence was also recorded in the spring/summer of 2005 to observe any inter-season effects of mowing treatments. Densities of healthy shoots were determined before and after mowing and in June 2005 using the 1 m$^2$-count method described previously. This procedure allowed for a comparison of the proportion of systemically infected shoots as well as numbers of systemically infected shoots before and after mowing and between the 2004 and 2005 seasons.

2.6. Statistical analyses

Data were analyzed using Statistical Analysis System (SAS) software version 9.1 TS level 1M3 for Windows XP_Pro platform (SAS Institute, 2002–2003, Cary, NC). Linear regressions of the number of systemically infected shoots present before and after mowing were conducted for each mowing treatment in each site using the REG procedure of SAS. These data were obtained by Ferdinandsen (1923) from two sites over the course of four years: 1915–1918 in one site and 1916–1919 in the other site. The data were incidence (%) of systemically infected shoots in undisturbed conditions over the course of the four years.

3. Results

3.1. Thistle debris experiment

There were no systemically infected shoots observed in the regrowth after treatments were applied in any of the pots from July through September of 2004. After a period of cold-temperature exposure into January, followed by 3 months in a greenhouse, systemically infected shoots were observed, by April 2005, in all pots receiving systemically infected plant material. Systemically infected shoots did not develop in pots receiving either the healthy or locally infected thistle debris.

3.2. Teliospore development in primary and secondary lesions

Urediniospores were observed in primary uredinia on all leaves sampled. Teliospores were observed in primary
uredinia in lesions of leaves sampled from late June to September. Urediniospores were found in all secondary lesions. Teliospores however were never observed within secondary lesions on locally infected leaves. Quantitative studies of teliospore production are required to further clarify this aspect of the rust life cycle.

3.3. Effects of mowing strategies on the number of systemically infected shoots within a season

The regressions of the number of systemically infected shoots before- versus after-mowing are presented in Fig. 2. There were significant differences in slopes among the different mowing treatments: both July and July + Sept slopes were significantly \( P \leq 0.05 \) steeper than the unmowed slope in location A. In location B, the July + Sept slope was significantly steeper than both the slopes of the unmowed and Sept treatment. In both locations the July + Sept treatment resulted in the greatest increase in number of systemically infected shoots after mowing.

3.4. Effects of mowing strategies on inter-season disease development

Proportion of systemically infected shoots for each mowing treatment over the course of the experiment best fit exponential growth curves (Fig. 3). In location A all three treatments (including unmowed) had similar rates of increase, but the July and July + Sept mowings resulted in a greater proportion of systemically infected shoots at the end of the experiment (June 2005). In location B, all mowing treatments showed greater rates of increase in proportion systemically infected shoots than the unmowed treatment.

At 150 days after start of the test, the mean proportion of systemically infected shoots was greater for the July (mean = 0.02) and July + Sept (mean = 0.02) strategies in location A than for the unmowed (mean = 0.007) strategy. These means were not, however, significantly different. By 360 days after start of the test, the mean proportion of systemically infected shoots was still greater for the July (mean = 0.06) and July + Sept (mean = 0.05) strategies in
Table 1
Average areas under disease progress curves (AUDPC) for the proportion of systemically infected Canada thistle shoots in each location and each mowing treatment.

<table>
<thead>
<tr>
<th>Mowing treatment (month)</th>
<th>Location A</th>
<th>Location B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean AUDPC</td>
<td>Standard error</td>
</tr>
<tr>
<td>July</td>
<td>11.10</td>
<td>3.057</td>
</tr>
<tr>
<td>July + Sept</td>
<td>9.35</td>
<td>13.44</td>
</tr>
<tr>
<td>Sept</td>
<td></td>
<td>12.32</td>
</tr>
<tr>
<td>Unmowed</td>
<td>5.54</td>
<td>6.83</td>
</tr>
<tr>
<td>LSD 0.10</td>
<td>7.44</td>
<td>6.02</td>
</tr>
</tbody>
</table>

* Proportions represent the number of systemically infected shoots over total number of Canada thistle shoots.

The mean area under the disease progress curve (AUDPC) for the proportion of systemically infected shoots was greater for every mowing strategy in both locations compared to the unmowed treatment (Table 1). In location A, AUDPCs for mowing treatments were 1.68–2.00× greater than the AUDPC for the unmowed treatment. The same applied to location B (range = 1.60–1.97× greater). However, because of variability, only the AUDPC of the July + Sept strategy was greater (P ≤ 0.10) than the unmowed strategy in location B (Table 1).

Exponential regressions on Ferdinandsen’s, 1923 data are presented in Fig. 4. Data from both sites fit well to the nonlinear exponential regressions, and the responses in both sites were similar to the responses shown in unmowed plots in both of locations of this study (Fig. 3). However, Ferdinandsen’s data were recorded over 4 years while the data in the present study were recorded over 1 year.

4. Discussion

The thistle debris experiment, designed to mimic mowing events in a controlled environment, suggested that teliospores produced and dispersed in a season only initiate systemic infection of Canada thistle in subsequent seasons following a period of cold stratification. In support of these observations, previous research has demonstrated that teliospores require a dark cold period before germination and formation of infective basidiospores (Turner et al., 1986; Frantzen, 1994b). In addition, it appears that secondary uredinia, produced on locally infected Canada thistle shoots, rarely convert to the production of teliospores in the mid-Atlantic region of the United States, since systemically infected shoots were never observed in pots receiving locally infected thistle debris. This result was surprising given previous reports of teliospore production within secondary lesions (Wilson and Henderson, 1966; Gäumann, 1959). We acknowledge that our examination of lesions from June through early September cannot exclude the possibility that teliospores may form within secondary lesions during October and November. Perhaps systemically infected shoots produce larger quantities of teliospores earlier in the season compared to secondary lesions. Temporal and quantitative comparison of teliospore production from primary and secondary uredinia has not been previously reported, to our knowledge, in the literature.

Mowing increased disease incidence in mowed plots as compared to plots that were not mowed. Thus, mowing in combination with the presence of plants systemically infected with *P. punctiformis* enhanced the incidence of systemically infected shoots beyond what was achieved without mowing. For all mowing treatments in both locations, mowing elevated the proportion of systemically infected shoots, in comparison to the unmowed treatment, in an additive manner consistent with the replacement of mowed systemically infected shoots with two or more new systemically infected shoots after mowing, i.e., generation of new systemically infected shoots after removal of apical dominance (Fig. 3). In location A, this appears to be the only effect since the rates of increase among all of the treatments are virtually the same. However, in location B, the Sept mowing shows a considerably greater rate of increase than the other treatments (Fig. 3). The exponential increase for this treatment beginning after 150 days (October 16) and continuing through the end of the study may well be due to the influence of teliospores leading to many newly infected shoots during the subsequent spring.
It is likely that mowing during the summer months when teliospores are present will increase disease incidence within the season by stimulating production of new systemically infected shoots. Cutting primary shoots eliminates apical dominance and is followed by the enhanced production of secondary adventitious root buds from both healthy and systemically infected rootstock. It is important that mowing occur soon after the preponderance of systemically infected thistle shoots bear mature teliospores so that the early summer inoculum is redistributed. After mowing, newly emerged systemically infected shoots develop and eventually produce a second crop of teliospores that become available for infection after winter stratification. Plots that are not mowed can be expected to contain teliospores produced from systemically infected shoots present in the spring plus those from the few new systemically infected shoots that develop over the summer months.

Timing of mowing is a key aspect in the enhancement of an epidemic because teliospores are the only spore stage that can lead to the development of systemically infected plants. Urediniospores themselves cannot cause systemic infections on Canada thistle (Van den Ende et al., 1987). They result instead only in local lesions that produce urediosori, that may convert to teliospore production at low levels during the summer months. Consequently, mowing before the development of mature teliospores (i.e., spreading infected plant material containing only urediniospores) will not likely result in the major increase in disease incidence that we observed when teliospores were present. This conclusion is supported by both the potted-plant experiment and field experiments. Within a season (Fig. 2) mowing in September was not as effective in increasing the number of teliospore producing shoots as mowing in July, possibly because of the limited time for shoot regrowth before winter frosts. When considering between-season disease dynamics, however, mowing in September actually increased proportion systemically infected shoots in location B compared to mowing in July (Fig. 3). These differences may be attributed to teliospore development and the length of time required for maturation. Perhaps there were fewer mature teliospores present on shoots mowed in July than on those mowed twice in July and September.

Although the highest proportion of systemically infected shoots was only 0.075 or 7.5% with the Sept mowing in location B, this amount might be adequate to control Canada thistle over an extended period. Predictions for the amount of time, in years, to reach 100% incidence of systemically infected shoots are presented in Table 2. These predictions were the result of linearizing (taking the natural logarithm of both sides of the equations) the corresponding exponential regression equations and solving for a proportion of 1. Although these predictions are beyond the range of the data in this study, they illustrate that, given exponential increase in disease, a value of 7.5% incidence may not be too low to arrive at 100% disease incidence within a relatively short period of time. When applied to the regressions on Ferdinandsen’s data, linearization of the equations and

<table>
<thead>
<tr>
<th>Mowing treatment (month/study)</th>
<th>Predicted number of years to reach 100% incidence</th>
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<tr>
<td></td>
<td>Location A</td>
</tr>
<tr>
<td>July (this study)</td>
<td>2.61</td>
</tr>
<tr>
<td>July + Sept (this study)</td>
<td>2.77</td>
</tr>
<tr>
<td>Sept (this study)</td>
<td>—</td>
</tr>
<tr>
<td>Unmowed (this study)</td>
<td>2.89</td>
</tr>
<tr>
<td></td>
<td>Site 1</td>
</tr>
<tr>
<td>Unmowed (Ferdinandsen, 1923)</td>
<td>4.97</td>
</tr>
</tbody>
</table>

solving for a proportion of 1 would result in 4.97 years for site 1 and 5.20 years for site 2 to reach 100% disease incidence even in unmowed plots. These predictions are not dramatically different from those in the current study (Table 2).

Ferdinandsen (1923) suggested that *P. punctiformis* does not have the potential for controlling Canada thistle in agricultural row-crop systems due to the continual disturbance by tillage burying the teliospores that give rise to basidiospores and systemic infections. Consideration of Ferdinandsen’s conclusions in concert with results of the present study suggest that management of Canada thistle with the rust is possible in undisturbed systems such as pasturelands, rangelands, and natural ecosystems but is not practical in agricultural row crops.

A factor that should be further considered is the physical movement by the mower of systemically infected plant material bearing teliospores. Frantzen (1994a) demonstrated that *P. punctiformis* is characterized by a steep dispersal gradient where teliospores are deposited on the soil very close to the infected plant from which they were produced. This phenomenon indicates reduced disease spread is probably a major limiting factor in producing widespread epidemics of *P. punctiformis* on Canada thistle. Physical redistribution of inoculum by mowing may assist pathogen movement within a thistle patch by creating a more uniform (homogenous) teliospore distribution. As a result, with homogenous rather than patchy distribution, there may be a greater probability of teliospore-root bud contact the following spring leading to a greater incidence of basidiospore infections, and ultimately enhanced levels of systemically infected shoots in the thistle patch. Van den Ende et al. (1987) reported viability of approximately 30% of teliospores stored in a refrigerator for 19 weeks. However, longer-term duration of viability of *P. punctiformis* teliospores has not been reported in the literature. Studies comparing teliospore distributions among mowing treatments are necessary to test this hypothesis.

It seems likely that a larger initial proportion of systemically infected shoots would lead to greater increase in disease incidence following a mowing event. Logically, the absence of systemic infections in a patch makes it unlikely
that mowing would cause any change in infected plant populations. This would require some introduction of teliospore-infested debris or, ideally, systemically infected plants actively producing teliospores from elsewhere to assure that systemic infections could be manipulated by mowing.

The Sept-only treatment in location B and the other two mowing treatments in both locations resulted in increases in proportion of systemically infected shoots (Fig. 3) and concomitant decreases in number of healthy shoots. Although some of the effect on number of healthy shoots might be due to a simple decrease as a result of increased number of diseased shoots, this was not the case with the unmowed treatments where the number of healthy shoots showed a linear increase (location A) or almost negligible decrease (location B). The decrease in number of healthy shoots in the same plots may be directly attributable to the increases in systemically infected shoots, but this will have to be tested further in other sites for a prolonged period of time. The synergistic effect of strategically timed mowing and the presence of \textit{P. punctiformis} may lead to acceptable levels of control when combined with other management practices particularly in areas where the use of herbicides are restricted or are not cost effective. It would be interesting to test the combination of high intensity-low frequency rotational grazing (De Bruijn and Bork, 2006) and \textit{P. punctiformis} to increase the incidence of systemically infected shoots and provide additional Canada thistle control.

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