EVALUATION OF PST INTERACTIONS WITH CANADA THISTLE IN NON-DISTURBED SITES

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Introduction

Canada thistle is the most invasive and challenging weed in grazing systems in the Upper Midwest (Doll, personal observation) and its membership on the noxious weed lists of many states demonstrates its tenacity against conventional control tactics. The resilience of this weed can be credited to its ability to effectively reproduce through both seed production/dispersal and its extensive root system. This is especially true in non-disturbed sites where few management strategies exist for its control. In pastures and non-crop sites, control is often not economically justifiable and only makes marginal gains against Canada thistle in the long run (Tichich and Doll 2001). However, non-disturbed sites appear to be a better fit for biological control than annual cropping systems as complete control is not required in the short run. Rather, one can afford to wait three or more years for a control tactic to reduce the infestation to acceptable levels.

Pseudomonas syringae pv. *tagetis* (PST), a phytopathogenic bacterium, infects many *Asteraceae* plants. PST was first described in Denmark affecting marigold producers (Hellmers 1955). In 1978, the pathogen was first reported in the USA in a sunflower field in Wisconsin (Styer and Durbin 1982). Common ragweed, giant ragweed, horseweed, common groundsel, bull thistle, annual sunflower, woollyleaf bursage and musk thistle have since been identified as hosts (Abbas et al. 1999; Gulya et al. 1982; Rhodehamel and Durbin 1985; Sheikh, et al. 2001; Styer and Durbin 1982).

PST produces a toxin (tagetitoxin) in host's leaves that results in chlorosis. As little as 0.5 ng of toxin can cause chlorosis in zinnia seedlings (Rhodehamel and Durbin 1989). The toxin is symplastically translocated to the apical growing areas of the plant. Upon its arrival at the meristem, the toxin binds to a chloroplastic enzyme (RNA polymerase III) which) that prevents chloroplast formation, resulting in apical chlorosis (Matthews and Durbin 1990, Steinberg et al. 1990). Despite the lack of chlorophyll and chloroplasts, the growth rate and morphology of newly formed tissue are unaffected and symptomatic leaves are not believed to recover (Gulya et al. 1982; Tichich and Doll, personal observations). This suggests that PST may be more effective than mowing as valuable root reserves are expended to form and maintain tissue that cannot provide a return on investment. Therefore, PST is an attractive candidate for biological control.

Researchers at the University of Minnesota demonstrated that the higher the population of PST on the Canada thistle leaf surface, the higher the infection probability (Gronwald et al. 2002). They established that if a PST population can be supported at a high population via an inoculating procedure for an extended period of time, the probability is high that significant levels of disease will be observed. Field research supported this claim by keeping the PST population artificially high for an extended period of time with five consecutive weekly applications of 10^8 PST colony forming units (cfu) ml^{-1}. Under this regime, emerging (from transplanted root stocks) Canada thistle plants were completely controlled and 5 to 30 cm tall plants were severely infected and stunted and seed production was reduced (Johnson and Wyse 1991).

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However, multiple applications of PST are costly and therefore not a practical solution for Canada thistle control. In 2000, researchers at the University of Wisconsin attempted to spread the disease by new means. They observed that naturally infected thistles commonly exist in non-disturbed sites (CRP lands, pasture, roadsides, etc.). A sample of infected thistles was harvested and the sap was extracted and applied to healthy thistles (at a non-disturbed site) with Silwet L-77 (organosilicone surfactant) to facilitate bacterial entry (Field and Bishop 1988; Zidack et al. 1992). The disease was successfully spread in this manner.

This success lead to many new questions about how to achieve optimal infection which the research presented in this paper addresses. These included: what spray volume and concentration should be used? What time of year should one apply? What are the effects of multiple applications? Does the disease infect for multiple years?

Materials and Methods

General Field Procedures

Site Description

Studies were conducted in either pastures or CRP sites in South Central or Southwestern WI in 2001 and 2002. Plant communities at these sites consisted of primarily bluegrass or bromegrass, and to a lesser degree burdock, plumeless thistle and wild carrot. These fields had extensive Canada thistle infestations (at least 1 to 2 plants ft^{-2}) that were essentially free of PST symptomology. The concentration and volume studies were conducted at the Lancaster Agriclutral Rearch Station (ARS) in 2001 and 2002 and at a site on a dairy farm in southeastern Dane County in 2002. The time of year studies were conducted at the Arlington ARS and Spring Green, WI in 2001. The multiple application studies were conducted at the Arlington ARS in 2001and the SE Dane County site in 2002. No grazing was done during the year of application except at the Lancaster ARS in 2001.

PST Applications

All PST applications (except those which examined variable volumes) were applied with a backpack sprayer at 40 gallons per acre (GPA) using CO_2 at 38 psi. All applications were made within 10 min of pressurization. The 5 ft boom employed four extended range 8006 flat fan nozzles on 15-inch spacing. Applications were generally made to Canada thistle plants in the vegetative to early bud stage, except treatments that examined seasonal timing or multiple applications.

Experimental Design

Treatments were organized into a randomized complete block design with four replications. Plot sizes were 10 by 10 ft with a 5 ft buffer between plots and 10 ft alleys between replications.

Spray Solution Preparation

Infected Canada thistle stems were cut from the upper portion of the plants from roadsides and other non-disturbed sites known to contain diseased thistles. The collected stems were stored on ice until the solution was prepared. Prior to solution preparation, the stems were cut into approximately 5-cm segments to facilitate the blending process. The desired amount of plant material (typically 65 g fresh wt) was weighed and added to tap water (typically one quart) in a standard kitchen blender. The thistles were blended using the “chop” key for 30 seconds with the blender running at full power. After blending, a foam/chopped biomass layer typically formed atop the solution and this was removed and squeezed to extract fluid.

The solution was then poured through a filtration system. This system was composed of a double layer of standard cheese cheesecloth with a piece of fiberglass window screen as a pre-filter. After filtration, the solution was brought back up to the original volume. Silwet (L-77) was then added at 0.3% v/v and the solution was placed on ice during transport to the field for application.

Data Collection and Analysis

Treatment impact data were collected at 28, 56 and approximately 390 d after application (DAA). Data collected included percent visual ratings for disease incidence (DI), disease severity (DS), and plant counts of infected and uninfected plants (at 56 and 390 DAA).

Disease incidence (DI) was determined by visually estimating the number of diseased (showing any PST symptomology) plants relative to the number of healthy plants. If no plants showed symptoms, then the plot received a zero for disease incidence. If all plants showed symptoms, then the plot would have scored 100.

Disease severity (DS) was determined by visually estimating the amount of chlorosis on the average plant showing the symptomology (percent of plant that is chlorotic). A plot received a disease severity rating of zero by default only if the disease incidence rating was also zero. A plot would have received a 100 if just one plant in the plot was infected, and it happened to be 100% chlorotic. If the amount of chlorosis on the average infected plant was 50%, then the DS rating would be 50.

In addition to the visual data, diseased and healthy plants were counted at 8 WAA. A 2 by 2 ft square was randomly placed per 25 ft^2 plot area and the number of infected and total thistles was recorded.

Data collected from the experiments were compiled and analyzed. ANOVA was the primary method used to assess treatment impact. Mean comparisons were made using Fisher's Protected T-Test (LSD). An alpha value of 0.05 was used for all comparisons. A two factor ANOVA was used for the concentration by volume experiments. Levene's test was used to test for unequal variance. When necessary, an angular transformation was performed to stabilize the variance.

Results

Concentration and Volume Study

Three volumes (20, 40 and 80 GPA) and two sap concentrations (65 and 130 g fresh wt of infected shoots per quart of spray solution) were considered. These were regarded as within the range of practicability for field applications. Neither concentration nor volume significantly impacted visual estimations of disease incidence or disease severity (Table 1). For the stand counts, the p values for the volume factor were below 0.20 for all three experiments, but still not significant at the 5% level. This suggests that PST applications could be practical at the field scale, as a single application caused infection using a manageable amount of infected thistle shoot material and commonly used spray volumes. Neither factor affected DS.

Timing Study

PST solutions were applied on June 15th, July 15th and August 15th. July 15th proved to be the best time to apply at based on the evaluations at 28 and 56 DAA (Table 1). Significant

differences were observed between locations in the levels of disease observed but the relative impact was consistent. This suggests that Canada thistle susceptibility to infection by PST varies by location/environment.

Microbial ecologists working with a different *Pseudomonas syringae* pathovar (*syringae*) found that rain events (specifically rain drop momentum) trigger population explosions (Hirano et al. 1996). It seems reasonable to apply this information to the PST/Canada thistle system. We therefore assessed whether PST applications preceding a period of wet, rainy weather were more successful than applications preceding dry weather.

We correlated results of these experiments to rainfall events during the 2001 and 2002 growing seasons and found that the most successful applications (July 15) were followed by a rain event within 14 days (Figures 1A and 1B). Less successful applications (June 15) occurred during extended dry periods. Time of application did not show a significant impact on disease severity.

Table 1. Influence of PST application date on disease incidence (DI) and disease severity (DS) on Canada thistle at Arlington, WI (Arl) and Spring Green, WI (SG) in 2001.

	----------DI----------			----------DS----------	
Treatment	Arl	SG	Arl	Arl	SG
	----------%Visual----------		%Counted[z]	----------%Visual---------	
28 DAA[x]					
June 15	0 b	4 b	na	0 c	4 bc
July 15	18 a	66 a	na	20 a	29 a
August 15	5 b	46 a	na	5 b	22 ab
Untreated	0 b	0 b	na	0 c	0 c
LSD (0.05)	12	*	na	*	19
p	0.028	0.002	na	0.001	0.022
56 DAA[y]					
June 15	5 b	36 b	7 b	18 a	26 a
July 15	35 a	73 a	29 a	15 a	27 a
Untreated	0 c	0 c	0 c	0 b	0b
LSD (0.05)	*	25	*	*	9
p	0.000	0.001	0.003	0.006	0.000

* indicates the data were transformed prior to analysis.

[x] Counts were not taken at 28 d after application.

[y] Observations were not made 56 d after application for the August 15th treatment at either site due to hard freeze in early October 2001.

[z] Counts were not taken at site 2001b at 56 d after application.

+Values with the same letter within a column (a-c) indicate they are not statistically different.

Multiple Applications

Two or more applications provided greater levels of disease than a single application (Table 2). The optimum number of applications varied with the season. One or two applications averaged 45% disease incidence and three or four applications averaged 67% in 2001. In the 2001 study, three applications appear to be optimal. In 2002, one application was not significantly different than the control. While two to four applications were significantly better than one, they did not differ significantly from each other and averaged 25%. Therefore, two

applications were optimal in this study. Disease severity was not greatly impacted by multiple applications in either year.

July 2002 at the Utica location was extremely dry (85% below the 30 year average) (Figure 1C). This may explain why higher levels of DI were not observed in 2002 as compared to 2001 (25 versus 67%) and therefore why additional applications were not beneficial.

Table 2. Influence of number and frequency of PST applications on disease incidence (DI) and disease severity (DS) of Canada thistle plants 56 d after the initial application at Arlington (Arl), WI in 2001 and Utica (Ut), WI in 2002.

	----------DI----------				----------DS----------	
Application Frequency	Arl	Ut	Arl	Ut	Arl	Ut
	--------% Visual-------		-------% Counted------		--------% Visual------	
1	45 bc	10 bc	35 a	9 bc	20 ab	7 ab
2 CW[x]	44 bc	17 ab	37 a	20 ab	24 ab	8 a
3 CW	68 a	23 ab	39 a	24 ab	31 a	10 a
4 CW	65 a	35 a	51 a	22 ab	29 a	12 a
2 EOW[y]	33 c	36 a	20 a	33 a	18 b	11 a
3 EOW	59 ab	41 a	44 a	26 ab	21 ab	9 a
0	0 d	1 c	0 b	3 c	0 c	2 b
LSD (0.05)	23	*	*	17	11	6
p	0.000	0.005	0.018	0.028	0.000	0.067

* indicates that the data were transformed prior to analysis.
[x] Consecutive weekly applications.
[y] Every other week applications.
\+ Values with the same letter (a-d) within a column indicate they are not statistically different.

Year Two Observations

The year after infection observations are not yet complete as additional data will need to be collected on the 2002 trials during the summer of 2003. However, preliminary analysis of the 2001 trails suggests that infections carry over into the following year. The 2002 data will need to be analyzed to substantiate this claim.

Discussion

If PST is to become an effective biological control agent in non-disturbed sites, levels of disease incidence must be increased. As evidenced by the data, DI levels typically ranged from 25 to 40%, which will not provide acceptable levels of suppression. To have a significant impact on Canada thistle populations in the long run, these levels will need to be increased to 70 to 85%. Our research has primarily examined the manipulation of inputs into the PST/Canada thistle system and subsequently treated that system as a "black box." This approach does not seem capable of reaching high levels of disease.

While we cannot currently recommend how to control Canada thistle with this technology, we can offer insight as to where future research will need to be directed. It seems that natural processes are the key drivers that influence PST populations and override our input manipulations into the system. As mentioned earlier, different sites showed different propensities for PST

infection, even though they appeared similar in terms of plant communities, land use, and site management. This suggests that there are differences at the leaf level. Therefore, it seems prudent to study the system at a finer scale, to no longer treat it as a "black box." This approach would undoubtedly involve the identification of population promoting and population constraining factors on the leaf surface.

These factors are either abiotic or biotic elements within the on the leaf surface. As previously mentioned, the mechanical action of rain drops strongly promoted the pathogenicity of PSS to snap beans (Hirano et al. 1996). Microbial ecologists have also demonstrated that the inundation of potato leaves with non-ice-nucleating strains of PS could competitively suppress ice-nucleating strains, resulting in reduced frost damage (Lindow 1995). This demonstrates the potential for microbial competition and exclusion on plant leaf surfaces.

Nutrient dynamics likely also play a role in community composition on the leaf surface where resource partitioning and composition can regulate bacterial population size. In many cases, nitrogen is likely a limiting factor for epiphytic microbe populations (Lindow 1991). Early in the growing season, bacterial population size is likely driven by the exudation of nutrients from the epidermis of the plant (Blakeman 1985). As the season progresses, organic debris (such as pollen, aphid honeydew and dust) are deposited. These depositions provide additional substrates for saprophytes, which play the role of scavengers and can be antagonistic to PS species such as PST (Blakeman 1985). It is clear that leaf surface dynamics can have a major effect on microbial populations and thereby their pathogenicity (or amount of toxin produced per leaf).

Ultimately to increase the pathogenicity of PST to Canada thistle, more toxin must be produced. This can be accomplished in one of two ways: by identifying or developing a new strain that produces more toxin per cell, or by working to increase the population of existing strains on the leaf surface. To achieve this, manipulations must be made at the leaf scale.

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References

Abbas, H.K., B.J. Johnson, C.D. Boyette, W.T. Molin and D.R. Johnson. 1999. Effect of *Pseudomonas syringae* pv. *tagetis* on weeds. Proceedings, Southern Weed Science Society 52:236.

Blakeman, J.P. 1985. Ecological succession of leaf surface microorganisms in relation to biological control, pp. 6-31 *In* C. Windels and S.E. Lindow (ed.) Biological control on the phylloplane. American Phytopathological Society Press, Minneapolis.

Field, R.J. and N.G. Bishop. 1988. Promotion of stomatal infiltration of glyphosate by an organosilicone surfactant reduces the critical rainfall period. Pesticide Science. 24:55-62.

Gronwald, J.W., K.L. Plaisance, D.A. Ide and D.L. Wyse. 2002. Assessment of PST as a biocontrol agent for Canada thistle. Weed Science 50:397-404.

Gulya, T.J., R. Urs, and E.E. Banttari. 1982. Apical chlorosis of sunflower caused by *Pseudomonas syringae* pv. *tagetis*. Plant Disease 66:598-600.

Hellmers, E. 1955. Bacterial leaf spot of African marigold (*tagetis erecta*) caused by *Pseudomonas tagetis* sp. n. Acta Agriculturae Scandinavica 5:185-200.

Hirano, S.S., L.S. Baker and C.D. Upper. 1996. Raindrop momentum triggers growth of leaf-associated populations of *Pseudomonas syringae* on field grown snap bean plants. Applied and Environmental Microbiology 62:2560-2566.

Johnson, D.R. and D.L. Wyse. 1991. Use of *Pseudomonas syringae* pv. *tagetis* for control of Canada thistle. Proceedings of the North Central Weed Science Society 46:14-15.

Lindow, S.E. 1991. Determinants of epiphytic fitness in bacteria, p. 295-314. *In* J.H. Andrews and S.S. Hirano (ed.) Microbial Ecology of Leaves. Springer-Verlag, New York, NY.

Lindow, S.E. 1995. Control of epiphytic ice nucleation-active bacteria for management of plant frost injury, pp. 239-256. *In* R.E. Lee, Jr., G.J. Warren, and L.V. Gusta (ed.) Biological ice nucleation and its applications. American Phytophathological Society, St. Paul, Minn.

Mathews, D.E. and R.D. Durbin. 1990. Tagetitoxin inhibits RNA synthesis directed by RNA polymerases from chloroplasts and *Echerichia coli.* The Journal of Biological Chemistry 265:493-498.

Rhodehamel, N.H. and R.D. Durbin. 1985. Host range of strains of *Pseudomonas syringae* pv. *tagetis*. Plant Disease 69:589-591.

Rhodehamel, N.H. and R.D. Durbin. 1989. Toxin production by strains of *Pseudomonas syringae* pv. *tagetis.* Physiological and Molecular Plant Pathology 35:301-311.

Sheikh, T., T.A. Wheeler, P.A. Dotray and J.C. Zak. 2001. Biological control of woollyleaf bursage (*Ambrosia grayi*) with *Pseudomonas syringae* pv. *tagetis.* Weed Technology 15:375-381.

Steinberg, T.H., D.E. Mathews, R.D. Durbin and R.R. Burgess. 1990. Tagetitoxin: a new inhibitor of eukaryotic transcription by RNA polymerase III. Journal of Biological Chemistry 265:499-505.

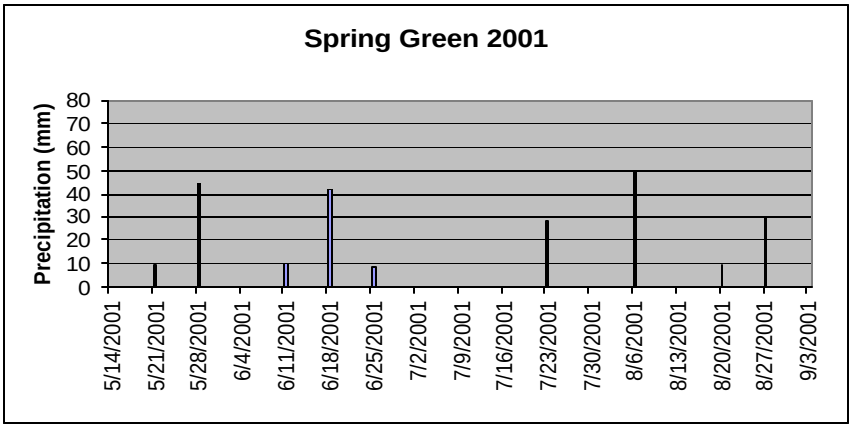
Styer, D.J. and R.D. Durbin. 1982. Isolation of *Pseudomonas syringae* pv. *tagetis* from sunflower in Wisconsin. Plant Disease 66:601

Tichich, R.P., and J.D. Doll. 2001. Herbicide options for Canada thistle control in pasture. Proceedings, North Central Weed Science Society (v) 56 (abst) 60.

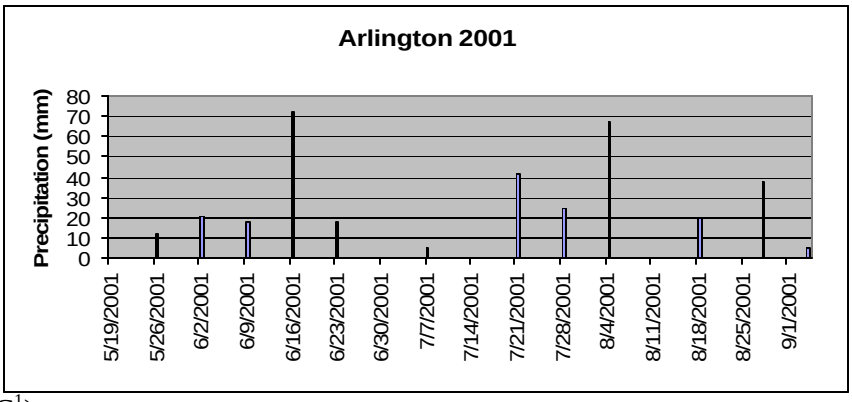
Zidack, N.K., P.A. Backman, J. J. Shaw. 1992. Promotion of bacterial infection of leaves by an organosilicone surfactant: implications for biological control. Biological Control 2:111-117.

Figure 1A-C. Rainfall summary for the studies comparing timing and the effects of multiple applications. Values above the date reflect the cumulative rainfall for the seven day period between dates.

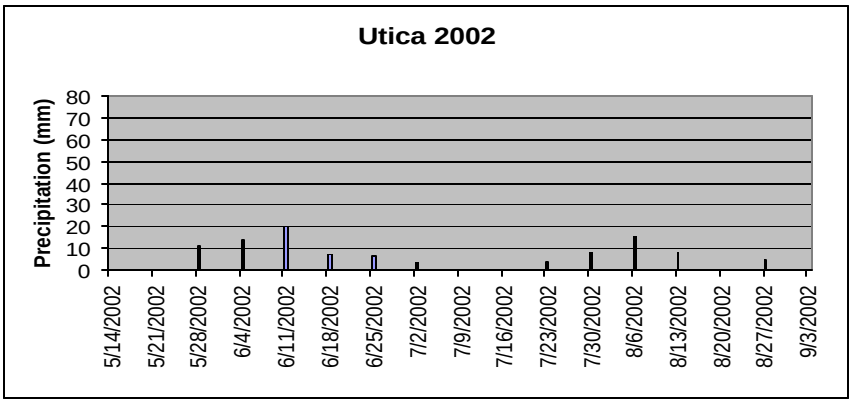
A[1])



B[2])



C[1])



[1] From the Cooperative Observer Network Data for Wisconsin website. Available: http://emily.soils.wisc.edu/cgi-bin/aws/opu.pl

[2] From the UW Automated Weather Observation Network (AWON) website. Available: http://www.soils.wisc.edu/wimnext/awon/awon.html