SHORT COMMUNICATION

Bioherbicidal potential of Xanthomonas campestris for controlling Conyza canadensis

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The effects of environmental parameters on bioherbicidal activity of the bacterium Xanthomonas campestris, against glyphosate-resistant and – susceptible Conyza canadensis (horseweed), were studied under greenhouse conditions. Rosette leaf-stage plants were more susceptible than older plants, and increasing inoculum from 10^5 to 10^9 cells/mL caused significantly greater plant mortality and biomass reduction of plants in both the rosette and bolting growth stages. A dew period at 25°C was required to cause an 80% and 60% mortality of plants in the rosette and bolting growth stages, respectively. Results indicate that X. campestris can infect and kill horseweed, demonstrating its bioherbicidal potential.

Keywords: bioherbicide; biological control; Xanthomonas campestris; Conyza canadensis; horseweed

Horseweed (Conyza canadensis L. Cronq.), aka mare’s tail or Canada fleabane, is an annual Asteraceae weed that is native to, and widespread in, North America (Weaver, 2001). This weed is problematic in conservation tillage in cotton production (Brown & Whitwell, 1988), grain sorghum (Vencill & Banks, 1994), corn (Buhler, 1992; Buhler & Owen, 1997) and soybean (Buhler & Owen, 1997). It is also a serious problem during fallow periods in the southern Great Plains (Wiese, Salisbury, & Bean, 1995) and can be a challenge in marketing of container-grown ornamentals (Gallitano & Skroch, 1993). C. canadensis is also a host of the tarnished plant bug [Lygus lineolaris (Palisot de Beauvois)] and of a mycoplasma disease (aster yellows) transmitted by the aster leaf hopper [Macrosteles fascifrons (Stal)] (Weaver, 2001).

Various biotypes of C. canadensis have become resistant to certain herbicides. Populations of horseweed resistant to paraquat (Lehoezki, Laskay, Gaal, & Szigeti, 1992; Smisek, Doucet, Jones, & Weaver, 1998), triazines (Lehoczki, Laskay, Polos, & Mikulas, 1984) and others (Heap, 2014) have been reported. Glyphosate can provide a high level of control of C. canadensis (Bruce & Kells, 1990; Scott, Shaw, & Barrentine, 1998; VanGessel, Ayeni, & Majek, 2001), but glyphosate-resistant C. canadensis in North America was first reported in Delaware in 2000 (VanGessel, 2001) and now has been documented in 10 countries, including 24 states in the USA (Heap, 2014). Generally, post-emergence herbicides that adequately control glyphosate-resistant C. canadensis are lacking. Alternatively, new crop technologies such as...
dicamba and 2,4-D-resistant cultivars should help alleviate this glyphosate-resistant problem in situations where control with pre-emergence herbicides fails. Preplant tank mixtures of glyphosate of saflufenacil, saflufenacil/dimethenamid-p, metribuzin, cloransulam or flumetsulam also can provide acceptable control of *C. canadensis* (Byker et al., 2013).

Due to this resistance and its aggressive growth characteristics, this weed has become a severe problem. Because conventional chemical herbicide weed control measures may not control this weed, alternative strategies such as biological control may be warranted.

The discovery, virulence, host range and epidemiology of a bacterial pathogen, *Xanthomonas campestris* (isolate LVA987) has been evaluated as a bioherbicide against *Xanthium strumarium* L. (common cocklebur) (Boyette & Hoagland, 2013a, 2013b). Host-range studies revealed that this bacterium also infected and killed *C. canadensis* seedlings under optimal growth stage and environmental conditions (Boyette & Hoagland, 2013a). The present study was undertaken to determine the effects of dew duration, air and dew temperature, inoculum concentration and plant growth stages on biocontrol efficacy of this pathogen for both glyphosate-resistant and susceptible *C. canadensis* seedlings. Knowledge of these epidemiological parameters is essential for evaluating candidate bioherbicial microbials as effective weed control agents, especially in instances of herbicide-resistant weeds.

*C. canadensis* seeds were kindly provided by D. Chacalis, USDA-ARS, Stoneville, MS. Seeds were germinated on dampened filter paper in a darkened moisture chamber [100% relative humidity (RH)] for 48 h, then planted in a 2:1 mixture of jiffy mix: sandy soil contained in 10 cm² pots and grown in a greenhouse. Greenhouse conditions were 28–32°C, 40–60% RH, ~14-h day length and 1650 µE m⁻² s⁻¹ photosynthetically active radiation (PAR) as measured at midday. The plants were watered by sub-irrigation with de-ionised water and fertilised [N:P:K (13:13:13)] biweekly. Various growth stages of both glyphosate-susceptible and -resistant weed biotypes were tested: 5–8, 11–15 rosette leaves or bolting to 5–15 cm and 16–30 cm tall. A fully expanded leaf was considered to be a true leaf.

A stock culture of *X. campestris* [isolate LVA987, originally isolated from diseased cocklebur leaves from Chicot County, AR, USA; (Boyette & Hoagland, 2013a)] was streaked on Bacto™ nutrient agar (Becton Dickinson and Co., Sparks, MD) plates and a single colony (isolate LVA987) from this plate was used as inoculum for 100 mL of nutrient broth (Bacto™) in a 250-mL baffled Erlenmeyer flask. The liquid culture was grown overnight at 30°C and 300 rpm in a rotary shaker incubator. This whole culture was mixed 1:1 (v/v) with a sterile 20% glycerol solution and 2.0 mL aliquots were stored in cryo-vials at −80°C for use as stock cultures.

Previous studies revealed that *X. campestris* cell growth was most rapid at temperatures of 25–30°C when evaluated in several media (Boyette & Hoagland, 2013b). Because there were no significant differences in inoculum production among these media, nutrient broth (NB) was selected for inoculum production in all subsequent testing. Log phase cells were obtained by inoculating NB with cells obtained from streaked nutrient agar plates followed by growth of these cultures for 12–16 h in a rotary shaker incubator at 30°C and 300 rpm. Inocula were prepared by centrifuging whole cultures at 8000×g for 10 min at 23°C, decanting the supernatant and resuspending the cell pellet in sterile potassium phosphate buffer
(12 mM, pH 6.8). Sterile distilled water was used for dilution to the desired inoculum concentrations.

To test the effect of air and dew temperature on pathogen efficacy, *C. canadensis* were sprayed until leaves were fully wetted (ca. 200 L/ha) with a formulation containing a surfactant (Silwet-L77™; OSi Specialties, Inc., Danbury, CT), *Xanthomonas* cells and distilled water. Final concentration in the formulation was 1.0 × 10⁹ cells/mL in 0.20% surfactant (v/v). Control plants were sprayed with 0.20% surfactant in distilled water. In this test, plants were placed in individual darkened dew chambers (100% RH) at temperatures of 15, 20, 25, 30 or 35°C for 20 h. The plants were then transferred to individual growth chambers (Conviron, Model E-7, Pembina, ND) with day/night air temperatures of either 20/10°C, 25/15°C, 30/15°C, 35/25°C or 40/30°C. Photoperiods were: 14 h at 65% RH and 820–840 µE m⁻² s⁻¹ (PAR). Plants were watered daily. Mortality and dry weight reductions were recorded 14 days after treatment. Dry weight measurements were determined in untreated and treated plants by excising at soil level followed by tissue drying in an oven (85°C, 48 h).

The effect of dew duration on *X. campestris* efficacy was examined by spraying *C. canadensis* seedlings at the various growth stages described. Inoculated plants were then placed in darkened dew chambers at 25°C and 100% RH for periods of 0, 4, 8, 12, 16, 20 or 24 h. Following the dew treatments, plants were placed on sub-irrigated trays on greenhouse benches and monitored for disease development. Mortality and dry weight reductions were recorded 14 days after treatment. *X. campestris* inoculum concentration effects versus *C. canadensis* plant growth stage were evaluated by spray applications of several bacterial cell suspension concentrations (1.0 × 10⁵ to 1.0 × 10⁹ cells/mL) to plants in the stages of growth described above. After application, the plants were held in a dew chamber for 20 h at 25°C. Control plants were sprayed with 0.20% surfactant in distilled water. After dew treatments, plants were moved to the greenhouse and mortality and dry weight reductions were recorded as described.

All experiments were conducted using triplicate sets of 12 plants for each treatment in each experiment. Treatments were arranged in a randomised complete block design, and all experiments were repeated. Means were pooled, subjected to analysis of variance and compared using Fisher’s LSD (P = 0.05) or SEM analysis via SAS (Version 9.1, SAS Institute, Inc., Cary, NC) statistical software. Since the data from glyphosate-resistant and – susceptible plants were not significantly different, only data from the glyphosate-resistant plants are presented.

Optimal mortality (80%) and dry weight reduction (80%) of *C. canadensis*, caused by *X. campestris* (10⁹ cells/mL) occurred at day/night/dew temperatures of 30/25/20°C and 35/30/25°C of plants in the rosette and bolting growth stages (Figure 1A and 1B). Both weed mortality and biomass reduction were significantly reduced at day/dew/night temperatures lower or higher than these optimal values (Figure 1A and 1B). Similar results were found in studies of the efficacy of this pathogen on cocklebur control (Boyette & Hoagland, 2013b).

The bacterium caused mortality of *C. canadensis* of rosette and bolting plants over a range of dew period durations from 8 to 24 h at 25°C (Figure 2A). A dew period of at least 20 h was required to cause about 80% mortality of plants in the rosette stage or 60% in the bolting stage (Figure 2A). In contrast, a dew duration period of only 16 h was required to achieve 80% control of cocklebur treated with
this bioherbicide (Boyette & Hoagland, 2013b). No mortality or dry weight reduction of plants occurred at dew periods ≤4 h (Figure 2A and 2B). Although 100% mortality was not achieved by any dew period duration (Figure 2A), plants were significantly stunted as reflected in the dry weight reduction data even by an 8 h dew period (Figure 2B).

Figure 1. Effects of day, night and dew temperatures on the efficacy of X. campestris on C. canadensis seedlings at two growth stages. A = mortality; B = dry weight reduction. Light grey histogram bars = rosette stage (five to eight leaves); dark grey histogram bars = early bolting stage, 5–15 cm tall.
Mortality and dry weight reductions were increased in plants at all (four) growth stages by increasing the inoculum concentration (10^5–10^9 cells/mL) (Figure 3A and 3B). Larger plants exhibited more resistance to infection than younger plants tested as shown in the mortality and dry weight reduction data (Figure 3A and 3B). This trend was also exhibited by cocklebur treated with this X. campestris isolate.

Mortality and dry weight reductions were increased in plants at all (four) growth stages by increasing the inoculum concentration (10^5–10^9 cells/mL) (Figure 3A and 3B). Larger plants exhibited more resistance to infection than younger plants tested as shown in the mortality and dry weight reduction data (Figure 3A and 3B). This trend was also exhibited by cocklebur treated with this X. campestris isolate.
Figure 3. Effects of *X. campestris* inoculum concentration on the bioherbicidal control of *C. canadensis* seedlings at several growth stages. A = mortality; B = dry weight reduction. Closed circles (●), solid lines = rosette stage five to eight leaves; open circles (○), short-dashed lines = rosette stage (11–15 leaves); closed triangles (▲), dotted lines = early bolting stage (5–15 cm tall); open triangles (Δ), long dash-dot lines = late bolting stage (16–30 cm tall). For the effects of cell concentration of *X. campestris* on mortality of *C. canadensis*, the relationship for the five to eight leaf rosette stage is best described by the third-degree polynomial equation: 

\[ Y = 35.67 - 56.12X + 23.69X^2 - 2.19X^3, \quad R^2 = 0.99; \]

11–15 rosette stage by the third-degree polynomial equation: 

\[ Y = 29.33 - 45.73X + 18.90X^2 - 1.70X^3, \quad R^2 = 0.99; \]

early bolting stage by the second-degree polynomial equation: 

\[ Y = -4.00 - 1.00X + 2.14X^2, \quad R^2 = 0.96 \]

and the late bolting stage is best described by the second-degree polynomial equation: 

\[ Y = -3.00 + 1.21X + 0.36X^2 = R^2 = 0.88. \]

For effects of cell concentration of *X. campestris* on dry weight reduction of *C. canadensis*, the relationship for the five to eight leaf rosette stage is best described by the third-degree polynomial equation: 

\[ Y = 28.33 - 49.30X + 23.07X^2 - 2.20X^3; \]

11–15 rosette stage by the third-degree polynomial equation: 

\[ Y = 27.67 - 47.54X + 21.81X^2 - 2.07X^3, \quad R^2 = 0.99; \]

early bolting stage by the second-degree polynomial equation: 

\[ Y = -11.20 + 5.56X + 1.36X^2, \quad R^2 = 0.94 \]

and the late bolting stage by the second-degree polynomial equation: 

\[ Y = -5.00 + 2.14X + 0.71X^2, \quad R^2 = 0.87. \]
Generally, mortality and dry weight reductions of C. canadensis were directly proportional to inoculum concentration applied. The preponderance of microbes evaluated as bioherbicides has been fungi, with few bacterial phytopathogens evaluated for this purpose (Gurusiddaiah, Gealy, Kennedy, & Ogg, 1994; Gealy, Gurusiddaiah, & Ogg, 1996; Johnson, Wyse, & Jones, 1996; Charudattan, 2001; Caldwell, Hynes, Boyetchko, & Korber, 2012). Several Xanthomonas pathovars, however, have been evaluated as bioherbicides, as exemplified by several reports. X. badrii was reported as a pathogen of cocklebur in India (Patel, Kulkarni, & Dhande, 1950), and several pathovars of X. campestris have been identified on a wide range of plants including crop plants and weeds (Anonymous, 1970). X. campestris pv. poae effectively controlled annual bluegrass (Poa annua L.) (Imaizumi, Nishino, Miyabe, Fujimori, & Yamada, 1997) with control directly proportional to the bacterial concentration applied (Imaizumi, Tateno, & Fujimori, 1998). A stable bacterial cell formulation of this organism was developed (Jackson, Frymier, Wilkinson, Zorner, & Evans, 1998) into a commercial bioherbicide (Camperico®) (Nishino & Tateno, 2000). However, successful control of annual bluegrass required wounding (via mowing) of weeds prior to application.

One constraint of X. campestris isolate LVA987 (as well as most microorganisms evaluated as bioherbicides) is the requirement for a rather lengthy period of free moisture (dew) following inoculation. At least 20 h of dew was required to achieve acceptable levels of weed control (~80% mortality), often unlikely in cropping situations where this weed is problematic. Water-in-oil formulations can be useful to overcome this requirement of some mycoherbicidal fungi (Boyette et al., 1996; Boyette, Bowling, Vaughn, Hoagland, & Stetina, 2010; Boyette, Gealy, Hoagland, Vaughn, & Bowling, 2011) and possibly could be used with this bacterial pathogen. Additionally, proper timing of X. campestris applications to C. canadensis in its most vulnerable growth stage(s) would optimise success.

These experiments demonstrated that glyphosate-resistant and glyphosate-susceptible C. canadensis plants were equally controlled by this X. campestris isolate under proper conditions. Although this pathogen may not possess sufficient virulence to be a potent bioherbicide against C. canadensis under field conditions, formulation improvements or synergistic interactions with certain chemicals such as herbicides could enhance its efficacy and this approach is currently being pursued. More research to optimise this bioherbicide candidate is currently being undertaken.

References


