Evaluating off-target movement of *Xanthomonas campestris pv. poannua* following application as a biocontrol agent for *Poa annua* on golf turf

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**Summary**

*Poa annua* var. *reptans* is a prostrate perennial grass and one of the most difficult to control weeds of golf course turf. *Xanthomonas campestris pv. poannua* (*Xcp*) has shown promise as a highly selective biological control agent for this species. Because *Xcp* must be applied at high concentrations and requires a wound for entry, limited off-target impacts have been assumed. However, since *Xcp* may infect some closely related species, an understanding of its movement and persistence is essential. A rifampicin-resistant strain of *Xcp* was applied to a simulated golf green at a rate of 150 mL of $1 \times 10^9$ cfu/mL to 26 cm² areas ($5.8 \times 10^9$ cfu/cm²) in the centre of 4 m² plots of *P. annua* var. *reptans* mowed thrice per week at 0.6 cm. Turf and thatch samples were extracted every other day from the point of inoculation, and at 35, 70, 105 and 140 cm in four directions for 49 days. *Xanthomonas campestris pv. poannua* was quantified by plating on selective medium. The experiment was conducted twice, in randomised complete block designs, with four replications. By nine days after inoculation (DAI), fewer than $1 \times 10^4$ cfu/cm² *Xcp* were recovered from the inoculated areas. By 49 DAI no *Xcp* was recovered from the area of inoculation. Off-target movement was minimal but detectable up to 140 cm from the point of inoculation. Maximum off-target *Xcp* recovery of between 75 and 400 cfu/cm² was observed 35 cm from the point of inoculation between 3 and 11 DAI. By 49 DAI no *Xcp* was recovered. No differences between quadrants were detected; therefore, mowing direction does not appear to influence off-target movement. These data suggest that the application of *Xcp* to golf turf does not present a significant risk of off-target movement and that *Xcp* populations at the site of inoculation will dissipate rapidly.

**Keywords:** dissipation, off-target movement, *Poa annua*, turf, *Xanthomonas campestris pv. poannua*.

**Introduction**

*Poa annua* L. (annual bluegrass or annual meadow grass) is one of the most widespread and difficult to control weeds of sports turf, especially golf greens (Bogart & Beard 1973). *Poa annua* is well adapted to frequent and close mowing, high nitrogen fertility, compaction, disturbance and frequent irrigation common to these sites.

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There are two distinct biotypes of *P. annua*: *P. annua* var. *annua*, a winter annual with an ascending growth habit and *P. annua* var. *reptans*, a perennial with a more prostrate growth habit (Warwick 1979). *Poa annua* var. *reptans* is the predominant form on golf greens in northern USA. Due to the species’ perennial life cycle, pre-emergence herbicides labeled for *P. annua* control in turf have not provided adequate control. Current control procedures rely upon selective plant-growth regulators and cultural practices that reduce the competitiveness of *P. annua* and encourage more desirable turfgrasses (Cooper et al. 1987, Gaussion & Branham 1989). However, these practices have not provided adequate control and must be carefully managed to avoid unacceptable injury to the desirable turfgrass.
**Xanthomonas campestris pv. poannua (Xcp)** is a facultative parasite that causes bacterial wilt in *P. annua* (Roberts et al. 1985). It is highly selective for *P. annua* and has shown promise as a selective biocontrol agent of *P. annua* in the USA and Japan (Savage 1991, Zhou & Neal 1995, Imaizumi et al. 1997). However, *P. annua* is often considered to be a desirable component of turfgrass swards. Consequently, an understanding of the fate of applied *X. campestris* pv. *poannua* and potential for off-target movement are imperative before widespread use. Therefore, the objectives of this study were to monitor *Xcp* populations in turfgrass following application as a biocontrol agent for annual bluegrass and to assay adjacent *P. annua*-infested turfgrass for off-target movement.

**Materials and methods**

The experiment was conducted on an established stand of *Agrostis stolonifera* (creeping bentgrass) ‘Seaside’ heavily infested with *P. annua var. reptans*. The experiment was conducted from July through September 1992, and was repeated from August through October 1992 on a separate turf sward. The experiment location was Ithaca, New York, USA. Each trial of the experiment was replicated four times in a randomised complete block design. Turfgrass was mowed three times a week at 6.5 mm and irrigated as needed.

A rifampicin-resistant strain of *Xcp* (from strain MSU-450) was selected on Kings B medium amended with 100 ppm rifampicin. All studies were conducted with this rifampicin-resistant strain. Inoculum was applied at a rate of 150 mL of approximately \(1 \times 10^9\) cfu/mL to 103 cm\(^2\) in the centre of each plot (about \(5.8 \times 10^9\) cfu/cm\(^2\)). Grass was mowed immediately following application (previously mowed borders separated each plot). Based on results from a preliminary experiment (Webber & Neal 1992), turf and thatch samples were extracted every other day for 2 weeks and weekly thereafter to 49 days after treatment. Samples were extracted using a 1-cm diameter cork borer from the inoculated centre area, and at 35, 70, 105 and 140 cm from the centre in four directions (Fig. 1). Turf and thatch samples were blended with sterile water then aliquots were quantified by plating on selective medium containing 100 ppm rifampicin.

**Results and discussion**

*Xanthomonas campestris* pv. *poannua* populations in the inoculated area declined rapidly following application. By 9 days after inoculation (DAI), fewer than \(1 \times 10^4\) cfu/cm\(^2\) of the bacterium were recovered from the inoculated areas (Fig. 2). Previous greenhouse and field trials have suggested that inoculum populations below \(1 \times 10^3\) cfu/cm\(^2\) produce no significant control of *P. annua* (Webber et al. 1992). By 49 DAI no *Xcp* was recovered. Similarly, Nishino et al. (1997) have reported rapid dissipation of a Japanese isolate of this bacterium in turfgrass. In their studies, populations were below their detection limit of \(1 \times 10^3\) cfu/g dry soil after 3 days in moist soil, and after 3 weeks in dry soil.

No differences in bacterium recovery were observed between quadrants; therefore data were pooled for analysis and presentation. Off-target movement was minimal but detectable up to 140 cm from the point of inoculation in both trials of the experiment (Fig. 3). Maximum off-target *Xcp* recovery in the July through September experiment was 75 cfu/cm\(^2\), 35 cm from the area of inoculation at 3 DAI. In the August through October trial, maximum recovery was 400 cfu/cm\(^2\) at the 35 cm sample points 11 DAI. When averaged over all data, the only recoveries statistically greater than zero were at 35 cm from the area of inoculation between 7 and 21 DAI (Fig. 3). By 49 DAI no *Xcp* was recovered in either trial of the experiment. This was consistent with results from preliminary tests on the same site in 1991. In contrast, Imaizumi & Fujimori (1999) have reported movement up to 16 m from the area of inoculation and the potential for secondary infections through movement on mowing equipment. Greater movement and persistence in the Japanese tests could be due to many factors including differences in virulence of the bacterial isolate used, the predominant biotype of annual bluegrass present or local environmental conditions.

These data demonstrate a rapid decline in populations of *Xcp* following application as a biological control agent for *Poa annua var. reptans*. This rapid decline in
bacterial populations may, in part, explain why repeated applications at high doses have been required to achieve suppression of the perennial type of *P. annua* (Zhou & Neal 1995). Furthermore, although research conducted in Japan suggests secondary infections and movement on mowing equipment is possible, we observed no evidence of this in our trials. These results suggest that the application of *Xcp* to golf turf does not present a significant risk of off-target movement in the northeastern United States, and that bacterial populations at the site of inoculation will dissipate rapidly.

References


