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Biological Control of *Bipolaris sorokiniana* on Tall Fescue by *Stenotrophomonas maltophilia* Strain C3

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ABSTRACT

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Stenotrophomonas maltophilia strain C3 was evaluated for control of leaf spot on tall fescue (*Festuca arundinacea*) caused by *Bipolaris sorokiniana*. In growth chamber experiments, C3 inhibited conidial germination on leaf surfaces and reduced lesion frequency and percent diseased leaf area compared with nontreated controls. The amount of leaf spot suppression was related to the C3 dose applied. The highest dose tested, 10^9 CFU/ml, prevented nearly all *B. sorokiniana* conidia from germinating on treated leaf surfaces and provided nearly complete suppression of lesion development. When colloidal chitin was added to C3 cell suspensions of 10^7 or 10^8 CFU/ml, biocontrol efficacy was significantly increased over C3 applied alone, whereas addition of chitin to a C3 cell suspension of 10^9 CFU/ml had no effect. In field experiments, application of C3 to tall fescue turf resulted in significant reductions in infection frequency and disease severity compared with nontreated controls. Strain C3 applied at 10^9 CFU/ml was more effective than C3 applied at 10^7 CFU/ml, and amendment of the lower dose with colloidal chitin enhanced its efficacy. Populations sizes of C3 established on foliage in a growth chamber and in the field were directly related to dose applied. Chitin amendments did not affect C3 population size.

Additional keywords: chitinase, *Cochliobolus sativum*, *Drechslera* sp., phyllosphere, turfgrass diseases.

Fungi formerly classified in the genus *Helminthosporium* are common pathogens of cool-season turfgrasses. *Bipolaris sorokiniana* (Sacc.) Shoemaker (teleomorph *Cochliobolus sativum* (Ito & Kuribayashi) Drechs. ex Dastur) is an example of this group and most commonly infects turfgrass foliage but also can attack crowns, rhizomes, and roots. *B. sorokiniana* can infect a wide range of turfgrass species, leading to symptoms ranging from necrotic leaf lesions surrounded by chlorosis to plant death and turf blight (20). Bipolaris leaf spot and other diseases caused by members of the genus *Helminthosporium* primarily are controlled by fungicides. Repeated fungicide applications, however, are associated with high costs, possible environmental and nontarget effects, and potential development of resistance in target pathogen populations (8). Cultivar resistance to leaf spot diseases in turfgrass is available, but breeding for host resistance is useful only when establishing new turf. As a result, microorganism-based biological control has been considered as a potential alternative management strategy. *Trichoderma harzianum* and several other fungal antagonists are inhibitory to *B. sorokiniana* on rye and wheat leaves (3,6). Strains of *Pseudomonas* spp. are reported to be antagonistic to *B. sorokiniana* (10) and *Drechslera poae* (18), another turfgrass pathogen in the genus *Helminthosporium*, but the bacterial strains used were not evaluated in the field.

The bacterial species *Stenotrophomonas maltophilia* (=*P. maltophilia* or *Xanthomonas maltophilia*) (14) has been isolated from the rhizospheres and phyllospheres of grasses (2,11,12). There are reports of rhizosphere strains being used as effective antagonists of fungal root pathogens (5,12). No attempts have been made, however, to use *S. maltophilia* for foliar disease control until strain C3, isolated from Kentucky bluegrass (*Poa pratensis*) foliage, was tested for inhibition of brown patch disease caused by *Rhizoctonia solani* (7). Strain C3 effectively colonized grass phyllospheres and reduced disease severity in the greenhouse but had limited efficacy in the field. It did suppress the growth of other fungi, including *B. sorokiniana*, on leaf surfaces in preliminary experiments. C3 also exhibited chitinolysis in vitro (24)—an attribute thought to be typical of the species (14). Enzymatic hydrolysis of chitin, the major cell wall component in most fungi, has been implicated as a mechanism of biological control (4,12). Application of chitin to foliage or soil has been reported to stimulate applied and resident chitinolytic microorganisms and enhance biocontrol efficacy (13,19), but its use as a nutrient substrate for *S. maltophilia* strains on plants has not been investigated.

The objectives of our study were to evaluate strain C3 for control of Bipolaris leaf spot on tall fescue (*Festuca arundinacea* Schreb.) and to determine the effects of chitin foliar applications on C3 phyllosphere population sizes and biocontrol efficacy. Preliminary reports have been published (22,23).

MATERIALS AND METHODS

Microorganisms and general microbiological methods. A spontaneous mutant isolate of *S. maltophilia* strain C3 resistant to rifampicin was used in all experiments. No differences in colony morphology, antimicrobial properties, chitinolysis, and growth rate were found between the mutant and wild-type in previous studies (7,24). C3 was cultured on tryptic soy agar (TSA; Difco Laboratories, Detroit) at 25°C. For application to plants, cells were collected from 2- or 3-day-old cultures and suspended in 0.01 M phosphate buffer, pH 7. Cell densities were adjusted initially to 10^9 CFU/ml by measuring turbidity with a spectrophotometer (Spectronic 20, Spectronic Instruments, Rochester, NY), and dilutions were made in phosphate buffer to desired levels. Strain C3 was enumerated by dilution-plating on TSA amended with rifampicin and cyclo-

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heximide at 100 µg/ml each. Dilution plates were incubated at 25°C for 3 days before bacterial colonies were counted. Population sizes were expressed as $\log_{10}$ CFU.

B. sorokiniana, isolated from Kentucky bluegrass in Nebraska, was cultured on potato dextrose agar (Difco) at 23°C. To maintain a high level of virulence, the pathogen was inoculated into tall fescue plants at regular intervals and reisolated from the resultant lesions. Cultures used for inoculum preparation were derived from a single conidium. Cultures were incubated under alternating cycles of 12 h of light and 12 h of darkness for at least 10 days. Conidia were harvested into phosphate buffer with a sterile soft-hair brush, and suspensions were filtered through four layers of sterile cheesecloth to remove hyphal fragments. Spore concentrations were determined with a hemacytometer and adjusted to 5×10^5 spores per ml for plant inoculations. Conidial germination rates were measured on depression slides after incubation at 23°C for 8 h.

Bacterial and fungal suspensions were applied to plants as sprays. To obtain more uniform dispersal of bacteria and conidia over leaf surfaces, a surfactant (Soydex 937, Setre Chemical Company, Memphis, TN), was added to all suspensions at 0.25% (vol/vol). The surfactant did not affect the viability of C3 or *B. sorokiniana*.

Growth chamber experiments. Tall fescue cv. Kentucky 31 was used in all growth chamber experiments. Approximately 200 seeds were sown per 15-cm-diameter pot, each containing pasteurized planting medium (Sharpsburg silty clay loam, vermiculite, and sand in equal volumes). After emergence, plants were incubated in a greenhouse under natural light at an average temperature of 25°C for 5 to 6 weeks. During the growth period, plants were watered daily with a nutrient solution (Peat-Lite Special [20-10-20, N-P-K], Scotts-Sierra Horticultural Products Co., Marysville, OH), containing 200 g of N per liter, and cut to a height of 5-cm every 10 days. Two days prior to application of microorganisms, plants were clipped to a height of 10 cm.

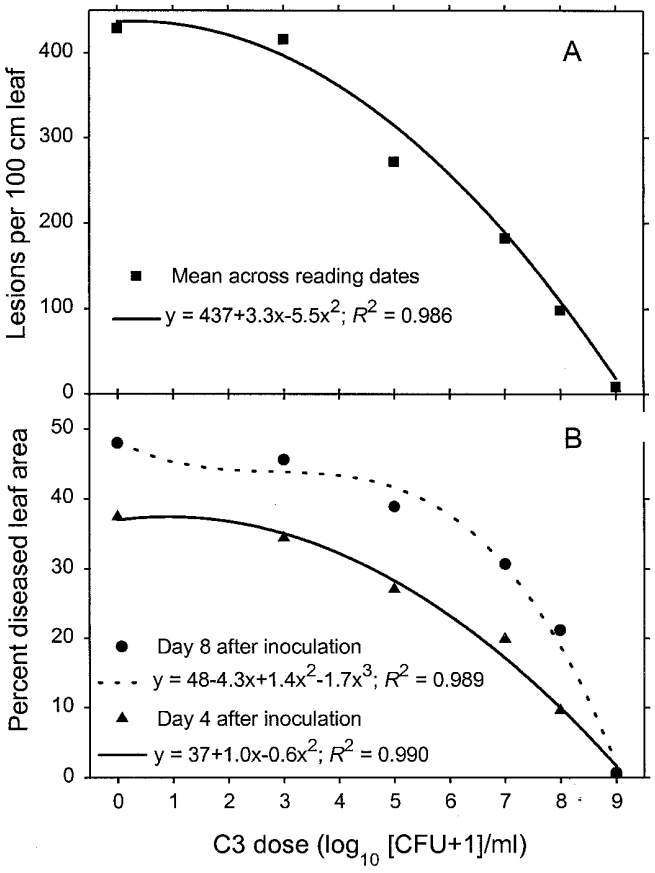

Fig. 1. Effects of different dosages of *Stenotrophomonas maltophilia* strain C3 on **A,** infection frequency and **B,** severity of leaf spot disease of tall fescue caused by *Bipolaris sorokiniana* under growth chamber conditions. All data points are means of two repetitions, each with six replicate units. Infection frequency measurements taken 4 and 8 days after inoculation were used to calculate the treatment mean, because the dose-reading date interaction was not significant. The dose-reading date interaction for disease severity was significant ($P < 0.001$).

Bacterial and chitin suspensions were applied to foliage by spraying until runoff with a hand-pump sprayer. Treatments were repeated six times, with a single pot of grass serving as a replicate. Pots of treated plants were arranged in a randomized designed in a growth chamber, with a 12-h photoperiod and constant temperature of 25°C and relative humidity (RH) of >93%. High levels of moisture were provided by an ultrasonic humidifier operated intermittently. After 24 h, 5 ml of *B. sorokiniana* conidial suspension was sprayed on each pot of grass, and pots were returned to the growth chamber. During the postinoculation period, temperature and humidity in the growth chamber were cycled between 25°C and <75% RH during the light period and 15°C and >93% RH during the dark period. In every experiment, some pots containing plants treated with biocontrol bacterial or chitin suspensions were incubated with no pathogen inoculum applied as negative controls. Because none of the controls exhibited disease symptoms, they were not considered in the statistical analysis and are not mentioned in the results.

Infection frequency and disease severity were measured 4 and 8 days after inoculation with *B. sorokiniana*. On each reading date, 20 leaf blades were collected at random from each pot. The number of lesions on each leaf blade was counted, and the linear length of each leaf blade was measured. Infection frequency (number of lesions per 100 cm of leaf) was calculated as the total number of lesions divided by the total leaf length in a sample. Disease severity (percent leaf area exhibiting lesions and chlorosis) was estimated for each leaf blade, and measurements from each pot were averaged prior to statistical analysis.

The population size of C3 on treated plants was monitored in one repetition of each experiment. Approximately 1.5 g of leaf blades was collected from each pot with sterilized scissors and forceps and put into a sterile plastic bag to which 10 ml of phosphate buffer was added. Leaves were crushed by rolling a wooden dowel over the bags. Serial dilutions of the resultant liquid were applied to agar plates. The weight of each plant sample was determined after drying in a 60°C convection oven for 3 days, and C3 population size was expressed on the basis of plant tissue dry weight. C3 population measurements were made at 2- to 3-day intervals and were continued for 1 week after the last disease severity assessment.

Two experiments were conducted in the growth chamber, and there were two repetitions of each experiment. In the first experiment, the effects of C3 dose were tested by applying C3 cell suspensions of 10^3, 10^5, 10^7, 10^8, and 10^9 CFU/ml to foliage. The control was a treatment with phosphate buffer alone. In the second experiment, the effects of chitin on C3 biocontrol activity were tested. C3 cell suspensions of 10^7, 10^8, and 10^9 CFU/ml were applied alone or with colloidal chitin. The three dosages were chosen because they provided disease control efficacy in the dose experiment, but control levels varied among dosages. Colloidal chitin was prepared from crude crab-shell chitin (Sigma Chemical Company, St. Louis, MO) according to the procedures of Roberts and Selintrennikoff (16), suspended in distilled water, autoclaved, and stored at 4°C until use. Colloidal chitin was added to bacterial suspensions at 0.2% (wt/vol) prior to application to grass plants. Colloidal chitin added to phosphate buffer was an additional control in this experiment.

In addition to infection frequency and disease severity, germination of *B. sorokiniana* conidia on leaf surfaces also was measured. Three leaf blades were collected at random from each pot 24 h after inoculation with conidia. One 3-cm-long section was excised

from each blade and placed on a microscope slide. The segment was stained with cotton blue for 1 min, covered with a coverslip, and destained by applying distilled water to one edge of the coverslip. The stained leaf segment was examined at 110× magnification under a compound microscope (Optiphot; Nikon Instrument Group, Melville, NY). The total numbers of germinated and nongerminated conidia on each leaf segment were counted. Results for three leaf segments from each pot were averaged prior to statistical analysis.

Data from the two repetitions of each experiment were combined in a pooled analysis of variance (ANOVA) after testing for homogeneity of variances. Data on percent conidial germination and disease severity also were analyzed after arcsine transformation, but because the results with and without transformation were similar in all cases, only analyses of nontransformed data are reported. The dose experiment was analyzed as a split-plot experiment, with dose (expressed as $\log_{10}$(CFU + 1)/ml) as the main treatment variable and repeated measurements (i.e., reading dates) as subplots. Trend comparison was applied to dose response data, and the function that best described each response was determined. The experiment involving chitin added to different dosages of C3 was analyzed as a factorial experiment, with reading date as a subplot. An LSD test was used to compare chitin and no chitin treatment means at each C3 dose. In the analysis of C3 population data in both experiments, LSD was used to compare C3 population means at each reading date. Statistical procedures were performed, in part, using the Proc Mixed and Proc Reg options of Statistical Analysis Software (release 6.10, SAS Institute, Cary, NC).

Field experiments. Two field experiments were performed in 1997 at the University of Nebraska-Lincoln Turfgrass Research Facility near Mead, NE. The first experiment was conducted on tall fescue cv. Kentucky 31 and the second on cv. Wrangler. Both plantings were more than 5 years old. Plants were fertilized monthly with 50 kg of N per ha in the form of urea, irrigated twice per week, and mowed weekly to a height of 8 cm, and clippings were removed. In each experiment, there were four 1.5 × 1.5-m replicate plots per treatment arranged in a randomized complete block design.

In the first experiment, a C3 cell suspension of 7×10^8 CFU/ml was applied on 19 May at a rate of 250 ml per plot with a backpack sprayer (Solo Inc., Newport News, VA). A second application was made 2 weeks later. Plots to which no C3 cell suspension was applied served as controls. A suspension of *B. sorokiniana* conidia was sprayed on all plots (250 ml per plot) on 9 June. Plots were sprinkler-irrigated shortly after inoculation. Due to the occurrence of drying winds and low humidity, the experimental area was covered with a polypropylene seed germination cover (Seed Guard, A.M. Leonard Inc., Piqua, IA) for 2 days to maintain high canopy moisture.

In the second experiment, strain C3 was applied at two cell densities: 10^7 and 10^9 CFU/ml. There also was a treatment in which 0.4% (wt/vol) colloidal chitin was added to the 10^7 CFU/ml C3 cell suspension. All treatments were applied on 2 September and again on 9 September, with 500 ml applied per plot during each application. Conidial inoculum was applied on 5 September at 500 ml per plot. The experiment area was irrigated just prior to inoculation, and canopy moisture was sufficient for infection without the polypropylene cover.

During each experiment, population sizes of strain C3 on foliage were determined at various intervals. Plants were clipped near the crown from three sites in each plot, pooled in sterile plastic bags, and transported to the laboratory on ice for processing within the same day. Population sizes of C3 in samples were measured by the methods described for growth chamber experiments.

To determine treatment efficacy, leaves were sampled 1 and 2 weeks after inoculation with the pathogen. Three subsamples, each containing more than 10 leaves, were collected from each plot and pooled. Infection frequency and disease severity in each sample were determined as previously described. To confirm that lesions were caused by *B. sorokiniana*, diseased leaves were placed on water agar, and the cultures were examined for pathogen sporulation.

ANOVA for a complete split-block design was performed on data from each experiment, with bacterial treatments as the main plots and reading dates as the subplots. LSD was used for mean comparisons. Analyses of arcsine-transformed disease severity data also were performed, but only the analysis of nontransformed data is presented, because results were similar.

RESULTS

Effects of C3 cell densities on leaf spot development. Bipolaris leaf spot development on tall fescue plants under growth chamber conditions was suppressed by applications of *S. maltophilia* strain C3; infection frequency and disease severity were inversely related to cell concentration applied (Fig. 1). The dose effect for infection frequency was significant ($P < 0.001$), but there was no significant dose-reading date interaction. The effect on infection frequency of increasing C3 dosages, averaged across reading dates, was best described by a quadratic equation (Fig. 1A). Plants treated only with phosphate buffer had more than 400 lesions per 100 cm of leaf, whereas application of C3 at 10^9 CFU/ml resulted in fewer than 10 lesions per 100 cm. The dose-reading date interaction for disease severity also was significant ($P < 0.001$); disease severity increased between the two reading dates at all dosages, except 10^9 CFU/ml (Fig. 1B). Disease severity response readings for days 4 and 8 were described by quadratic and cubic functions, respectively. By day 8, nearly 50% of the leaf surface in the control treatment exhibited symptoms, whereas ≈1% of the leaf surface exhibited symptoms in plants treated with C3 at 10^9 CFU/ml.

Population sizes of C3 detected on foliage differed depending on the C3 cell concentration applied (Fig. 2); dose effect, reading date, and dose-reading date interaction all were significant ($P < 0.001$) based on ANOVA. At all C3 dosages, population sizes of C3 on foliage increased over the first 6 to 8 days. On plants treated with C3 at 10^3 or 10^5 CFU/ml, the increase in numbers exceeded 3 log units. Population sizes on plants treated with higher C3 cell concentrations increased by ≈1.5 log units. After 8 days, population sizes declined gradually in most treatments.

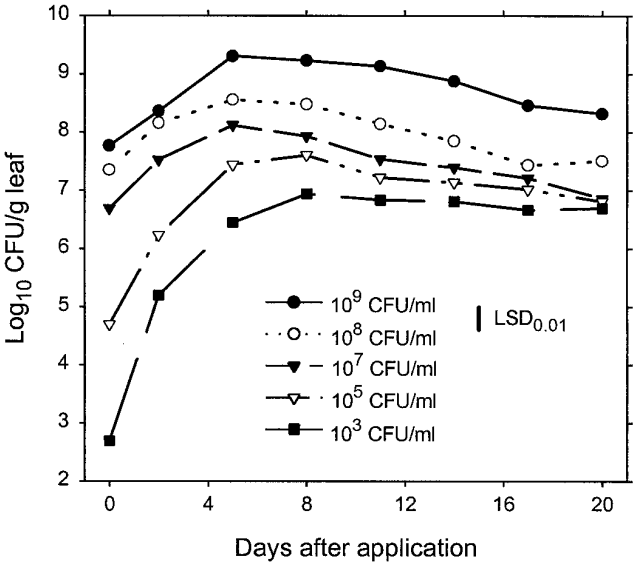

Fig. 2. Population sizes of *Stenotrophomonas maltophilia* strain C3 detected on tall fescue plants in a growth chamber after application of C3 cell suspensions at different cell densities. LSD was used to compare values on any given date.

Effects of chitin on C3 biocontrol efficacy. Chitin treatment and C3 dose had significant ($P < 0.001$) interactive effects on germination of *B. sorokiniana* conidia on leaves in the growth chamber (Table 1). Dosages of C3 suspensions of 10^7 to 10^9 CFU/ml, with and without chitin, reduced conidial germination frequency compared with controls. The amount of inhibition was related to C3 dose applied. Treatment with C3 at 10^9 CFU/ml prevented germination of nearly all *B. sorokiniana* conidia. Addition of colloidal chitin to C3 cell suspensions of 10^7 or 10^8 CFU/ml increased pathogen suppression, whereas amendment of a cell suspension of 10^9 CFU/ml with chitin had no additional effect. The application of chitin alone caused a slight, but significant, reduction in germination compared with the phosphate buffer control.

Combining colloidal chitin with C3 cells increased suppression of disease development more than C3 alone, depending on the C3 dose (Table 1). The dose-chitin interaction for infection frequency was significant at $P < 0.001$, but there was no significant interaction of main treatments with reading date. Addition of chitin to C3 cell suspensions of 10^7 or 10^8 CFU/ml reduced infection frequency compared with C3 alone at the same cell density, but chitin amendment to a C3 cell suspension of 10^9 CFU/ml had no additional effect. Chitin alone caused a slight decrease in infection frequency compared with the control. In relation to disease severity, dose-chitin and dose-chitin-reading date interactions were significant ($P < 0.001$ and $P = 0.03$, respectively). Chitin increased the effect of C3 on both reading dates only when it was added to a C3 cell suspension of 10^7 CFU/ml, and chitin alone reduced disease severity measured on day 4 compared with the phosphate buffer control.

Colloidal chitin had no influence ($P = 0.106$, 0.249, and 0.200 for chitin effect, dose-chitin interaction, and dose-chitin-reading date interaction, respectively) on the establishment of C3 populations on leaves (data not shown). C3 population sizes varied with C3 dose applied, following a trend similar to that of the dose experiment, but the addition of chitin did not alter C3 population sizes at any C3 dose or reading date.

Suppression of Bipolaris leaf spot in the field. In both field experiments, significant reductions in infection were observed in plots treated with strain C3 compared with nontreated control plots (Table 2). The number of lesions did not change significantly between the two sampling dates in each experiment. Inoculation with *B. sorokiniana* conidia led to high infection frequencies, with more than 170 lesions per 100 cm of leaf in control plots in both experiments. However, lesions did not expand appreciably in size, and there was very little chlorosis surrounding the lesions. Therefore, the percent diseased leaf area in all treatments was low (<10%) at the end of both experiments. Nevertheless, disease severity was significantly reduced by all C3 treatments compared with the control.

In the second experiment, in which C3 at two cell densities and a chitin amendment were tested, biocontrol efficacy was enhanced by applying C3 at the higher cell density and with colloidal chitin amendment (Table 2). Lower infection frequency and disease severity were measured in plots treated with a C3 cell suspension of 10^9 CFU/ml compared with those treated with a C3 cell suspension of 10^7 CFU/ml. With the addition of colloidal chitin to a C3 cell suspension of 10^7 CFU/ml, infection frequency and disease severity were intermediate between the two treatments with the bacterium alone.

C3 population sizes between 5.7 and 6.8 log CFU/g of plant tissue were detected throughout the first experiment (data not shown). In comparison, population sizes of C3 detected in the second experiment generally were higher (Fig. 3). Population sizes declined after each application by nearly 1 log unit and then remained level. C3 population sizes on plants treated with C3 at 10^9 CFU/ml were ≈1 log unit higher than on plants treated with C3 at 10^7 CFU/ml. The addition of colloidal chitin to C3 cell suspensions of 10^7 CFU/ml did not affect C3 population sizes significantly compared with the bacterium alone, except on one sampling date on which a higher number was detected in plots treated with chitin.

DISCUSSION

This is the first report of biological control of Bipolaris leaf spot on turfgrass under field conditions. Reductions in infection frequency and disease severity by *S. maltophilia* strain C3 were preceded by

TABLE 2. Suppression of Bipolaris leaf spot disease of tall fescue by cell suspensions of *Stenotrophomonas maltophilia* strain C3 and chitin amendments in two field experiments

Exp.	Treatment	No. of lesions/ 100 cm leaf[a]	% Diseased leaf area[b]
1	Nontreated control	190	9
	C3 at 7×10^8 CFU/ml	67	4
	$LSD_{0.05}$	37	2
2	Nontreated control	171	6
	C3 at 10^7 CFU/ml	115	4
	C3 at 10^7 CFU/ml + chitin	79	3
	C3 at 10^9 CFU/ml	51	2
	$LSD_{0.05}$	24	1

[a] Measurements were made 10 and 17 days after inoculation in experiment 1 and 7 and 14 days after inoculation in experiment 2. Because the treatment-reading date interaction was not significant in both experiments, values represent the means of measurements from four replicate plots on both sampling dates.

[b] Measurements were made 17 and 14 days after inoculation with the pathogen in experiments 1 and 2, respectively.

TABLE 1. Effects of chitin added to cell suspensions of *Stenotrophomonas maltophilia* strain C3 on germination of *Bipolaris sorokiniana* conidia and on frequency and severity of Bipolaris leaf spot of tall fescue in a growth chamber[a]

C3 dose (CFU/ml)	% Conidial germination[b]		Infection frequency (no. of lesions/100 cm leaf)[c]		Severity on day 4 (% diseased leaf area)[d]		Severity on day 8 (% diseased leaf area)[d]	
	No chitin	Chitin	No Chitin	Chitin	No chitin	Chitin	No chitin	Chitin
0	90	86	492	457	35	32	40	39
10^7	54	41	270	112	21	7	26	8
10^8	26	14	104	36	5	3	6	4
10^9	2	2	9	9	1	<1	1	1
	$LSD_{0.01} = 3.3$[e]		$LSD_{0.01} = 25.0$		$LSD_{0.05} = 2.6$		$LSD_{0.05} = 2.6$	

[a] Colloidal chitin was added at 0.2% (wt/vol) to C3 cell suspensions in phosphate buffer before application. Treatments were applied 1 day prior to inoculation with conidia.

[b] Conidial germination was measured in situ 1 day after inoculation. Values are means of two repetitions, each with six replicate units.

[c] Infection frequency was measured 4 and 8 days after inoculation. Dose-chitin-reading date interaction was not significant. Values are means of two repetitions, each with six replicate units and two measurement days per unit.

[d] Disease severity was measured 4 and 8 days after inoculation. Because the dose-chitin-reading date interaction was significant ($P = 0.03$), the data for each reading date are presented. Values are means of two repetitions, each with six replicate units measured on 1 day.

[e] LSD values are for comparison of chitin and no chitin treatments at a given C3 dose and for C3 treatments with a no bacterium control in the same column.

suppression of conidial germination. Although C3 can inhibit the growth of hyphae of *B. sorokiniana* and other fungi on leaf surfaces (7,24), we surmise that C3 is probably more active against spore germination or germ tube development and that biocontrol efficacy depends on the biocontrol bacterium inhibiting the early prepenetration stages of pathogen infection. As evidenced by the low degree to which C3 affected brown patch disease on turfgrass (7), antagonism by C3 is probably less effective against pathogens, such as *R. solani*, that have an extended epiphytic phase prior to penetration (20).

The suppressiveness of strain C3 to leaf spot was related to the bacterial dose applied. This finding supports other studies on biological control in the phyllosphere, in which high doses of biocontrol agents were necessary to achieve acceptable disease control (1,21). In the growth chamber, strain C3 multiplied on leaves after application, and therefore, it is conceivable that low numbers of C3 applied to leaves could provide protection if given sufficient time to increase before the arrival of pathogen inoculum. When C3 was applied at a lower dose under field conditions, however, it did not reach the same population size as when it was applied at a higher dose, probably due to abiotic conditions and microbial competition in the field restricting multiplication only to a rate that balanced cell death. Therefore, application of C3 at high cell densities is important for biocontrol efficacy in the field.

Biocontrol activity of C3 at submaximum dosages was enhanced when the bacterium was amended with chitin. The utility of chitin as a substrate for chitinolytic biocontrol agents has been demonstrated in several other host-pathogen systems (9,13,19). Researchers (9,13,19) have suggested that chitin can serve as an exclusive substrate for chitinolytic microorganisms, thereby increasing their population sizes and shifting the microflora composition. It also is thought that chitin can serve as a physical barrier to spore germination and penetration processes (13). In addition, chitin, or the oligomers contained in colloidal chitin, can elicit lignification and chitinase activity in plants (15,17). In our study, the beneficial effects of chitin did not appear to be related entirely to stimulation of C3 population growth. A direct effect of chitin on the pathogen or an indirect effect through host resistance may be involved, because conidial germination and infection frequency in the growth chamber were affected to a small degree by chitin alone. Although the exact role of chitinolysis in biological control by C3 has not been established, stimulation of chitinase production by C3 on leaf surfaces by chitin is another possible mechanism. In laboratory experiments, chitinase production by C3 in vitro and on leaves was stimulated by chitin (24). Regardless of the modes of action, our results show that chitin amendments can be useful in achieving effective control when it is not possible to apply bacteria in high numbers.

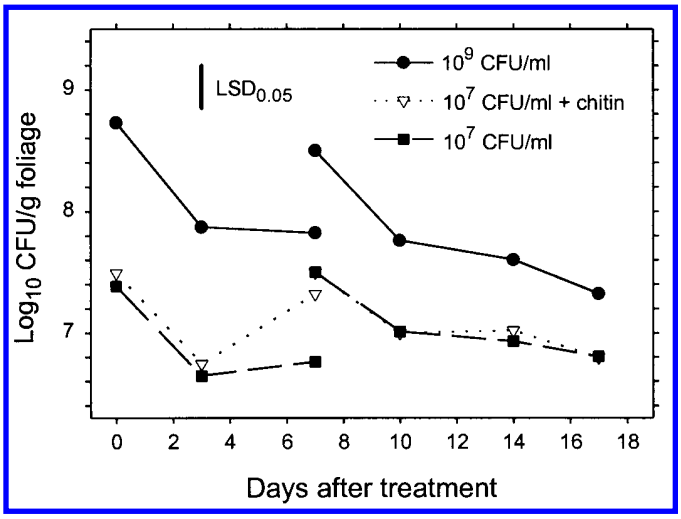

Fig. 3. Population sizes of *Stenotrophomonas maltophilia* strain C3 detected on tall fescue cv. Wrangler plants in field experiment 2 after application on 2 September 1997. The higher of the two data points for each treatment on day 7 represents the population size after a repeat application of the treatment. LSD is used to compare values on any given date.

Toxicological and environmental safety questions need to be addressed before the commercial potential of strain C3 or the *S. maltophilia* sp. can be considered. As a model system, the suppression of Bipolaris leaf spot disease by *S. maltophilia* strain C3 supports the integration of bacterial biocontrol agents into turfgrass disease-management systems. Further study is needed to determine the mechanisms responsible for antagonism by C3 and to elucidate the influence of microclimate factors. Additional knowledge will aid in the development of improved and more predictable control measures. Combining biocontrol agents with nutrient substrates, such as chitin, that may affect the applied antagonist, resident microbes, pathogen, and host may be one approach for improving control.

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