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## Biological Control of Septoria Leaf Spot Disease of Hybrid Poplar in the Field

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### ABSTRACT

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Biological control of Septoria leaf spot of hybrid poplars was investigated using disease-suppressive *Streptomyces* strains. Field experiments were conducted in 1998 and 1999 on potted trees placed in a hybrid poplar plantation near Rosemount, MN, and on field-planted trees in 1998 at St. Paul. At both locations, one resistant and three susceptible hybrid poplar clones were sprayed with *Streptomyces* spore suspensions and exposed to natural field inoculum of *Septoria musiva*. In the 1998 potted-tree experiment, strains GS-93-3, 93, and Mycostop in Tergitol or Triton X-100 solutions applied every 7 days significantly reduced leaf disease by 29 to 83% compared with the controls. In the 1999 potted-tree experiment, *Streptomyces* strain mixtures in Tergitol solution applied every 5 days significantly reduced leaf disease by 50 to 87% compared with the controls. In the 1998 plantation experiment, strains GS-93-3, 93, or Mycostop in Tergitol solution applied weekly, bi-monthly, or monthly significantly reduced leaf disease in all treatments by 64 to 78% compared with the controls.

Additional keywords: gram-positive bacteria, *Mycosphaerella populorum*, *Populus* spp.

Interspecific hybrid poplar clones are planted in many parts of the world for the production of wood, fiber, and energy (12). The area planted with poplars is rapidly increasing in the north-central region of the United States. However, the expansion of poplar culture is limited because many poplar clones are susceptible to leaf spot and canker disease (7,8) caused by *Septoria musiva* Peck (teleomorph = *Mycosphaerella populorum* G. E. Thompson) (14). Ascospores and conidia of *S. musiva* are present in plantations throughout the growing season. On susceptible clones, *S. musiva* can cause early-season defoliation, and stems and branches often break at cankers in strong winds, which can result in plantation failure (7).

Various agronomic practices can be used for disease prevention (9), but high-yielding, susceptible clones require repeated fungicide applications (7), raising environmental concerns. Use of resistant clones is one of the best disease-control strategies (9); however, the number of highly resis-

tant clones is limited, making biological control an attractive disease-control alternative (8).

The potential of biological control of Septoria leaf spot has been investigated in previous studies. Yang et al. (16) used a spore suspension and culture filtrate of *Phaeothea dimorphospora* to inhibit *S. musiva* under laboratory and greenhouse conditions. Shimizu (11) found that suppressive strains of *Streptomyces* inhibited growth of several *Septoria musiva* isolates in culture and leaf-disk assays. Two of the *Streptomyces* strains used in Shimizu's study also were used in this work.

*Streptomyces* spp. are gram-positive, filamentous, soilborne bacteria that also occur in the phylloplane (6), especially on dust-covered leaves (4). Hodges et al. (2) used *Streptomyces* spp. to control leaf pathogens of *Poa pratensis* in the greenhouse.

The objective of this study was to evaluate the potential of *Streptomyces* strains to control Septoria leaf spot disease of hybrid poplar exposed to natural inoculum of *Septoria musiva* in the field.

### MATERIALS AND METHODS

**Selection of *Streptomyces* strains.** The co-plating method of Liu (3) and the bacteriocin assay described by Vidaver et al. (15) were used to select *Streptomyces* spp. strains for their ability to compete with and produce antibiotics against *Septoria musiva* isolates in agar plate assays. Selected *Streptomyces* strains with the best biocontrol potentials also were evaluated using a single leaf plantlet assay (1). A fully expanded healthy leaf on a 10- to 12-

cm-long stem was excised from a greenhouse-grown stock plant, and the entire abaxial surface was inoculated with a spore suspension of *Septoria musiva* ( $10^6$  conidia/ml) and allowed to air dry. A piece of glass was laid over one half of the leaf and the exposed half was sprayed with a spore suspension ( $10^6$  to  $10^8$  spores/ml) of a single strain of *Streptomyces*. Inoculated single leaf plantlets were planted in plastic trays (53.5 by 27.7 by 7.3 cm) containing 3,000 ml of sterile sand, moistened with 2.0 g of MIRACID Soil Acidifier Plant Food (N:P:K = 30:10:10) in 1,000 ml of sterile deionized water. A plastic-coated wire mesh was placed approximately 2 cm above the sand to support the plantlets until they rooted. The plantlets were covered with a transparent plastic lid to maintain high humidity and grown at room temperature under an 18-h light and 6-h dark period. Thirty-one days after inoculation, the percentage of leaf necrosis was measured using a leaf disease severity scale of 0 to 8, where 0 = 0, 1 = 1, 2 = 2 to 3, 3 = 4 to 8, 4 = 9 to 17, 5 = 18 to 25, 6 = 26 to 50, 7 = 51 to 75, and 8 = 76 to 100% necrosis. Differences in disease severity between the two sides of the same leaf were determined.

Three *Streptomyces* strains (93, GS-93-3, and Mycostop) that showed the highest inhibition in the co-plating, bacteriocin, and the single-leaf plantlet assays were selected for the field experiments. *Streptomyces* strains 93 and GS-93-3 (not related strains) used in these field experiments were isolated from a Minnesota potato scab suppressive soil (3,10), and the Mycostop strain of *Streptomyces* was obtained from peat soil in Finland (13). Two additional *Streptomyces* strains were used in the 1999 potted field trials. Strains LR1 and LR2 were re-isolated from hybrid poplar leaves 197 days after spraying with GS-93-3 or Mycostop, respectively, in the single-leaf plantlet assay (1).

**Plant material.** Greenhouse-grown potted trees of four interspecific hybrid poplar clones were used in all field experiments at the University of Minnesota Experiment Station near Rosemount. Clone NM2 (*Populus nigra* L. × *P. maximowiczii* A. Henry) was resistant and the other three clones—NE242 (*P. deltoides* Bartr. ex Marsh. × *P. nigra* var. *plantierensis*), DTAC26 (*P. deltoides* × *P. trichocarpa* Torr. & A. Gray) and NE299 (*P. nigra* var. *betulifolia* × *P. trichocarpa*)—were susceptible to *S. musiva*.

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Two rooted cuttings of the same poplar clone were planted in a plastic pot (21.7 cm in diameter and 20.5 cm high) containing steamed soil (4 h), vermiculite, and peat moss in a ratio of 5:1:1. Trees were grown in the greenhouse under a cycle of 18 h of light and 6 h of dark at temperatures of 25 and 18°C (day and night, respectively) and received Peters water-soluble fertilizer (N:P:K = 20:20:20) every 3 weeks. Monthly applications of Enstar II (S-Kinoprene [CAS # 65733-20-2]) and Pentac (Decachloro bis[2,4-cyclo-pentadiene-1-yl]) were used to control insects and mites. Six weeks before trees were placed in a hybrid poplar plantation, they were pruned to 3- to 4-cm stumps. When the new shoots on the stumps were 2 to 3 cm tall, all but two shoots of similar size on each stump were removed. In the field, wooden frames were used to support four pots and two 2-liter plastic bottles of water inverted into a saucer under each pot that provided a weekly supply of water for the trees.

**Preparation of inoculum.** *Streptomyces* strains were grown on oatmeal agar (3) and incubated in the dark at 30°C for 7 days. Tergitol (0.05%) and Triton X-100 (0.05%) were used to keep *Streptomyces* spores in suspension. Spores were harvested from the oatmeal agar and suspended in the adjuvant solution ( $10^6$  to  $10^7$  spores/ml) 1 day before application.

**Evaluation of single *Streptomyces* strains on potted exposure trees.** In 1998, *Streptomyces* strains GS-93-3, 93, and Mycostop in 0.05% Tergitol or 0.05% Triton X-100 solutions were applied every 7 days to potted trees placed within a hybrid poplar plantation containing a large number of interspecific poplar clones susceptible to *Septoria musiva*. Trees were sprayed until runoff using an electric mist blower in the field. After treatment, the trees were placed in the wooden frames using a randomized complete block design. Trees were exposed to natural *S. musiva* inoculum for 3 to 4 weeks and then returned to the greenhouse. Sets of 96 trees were exposed in five inoculum exposure periods (IEPs) starting 5 June (IEP1), 1 July (IEP2), 22 July (IEP3), 19 August (IEP4), and 16 September (IEP5). One tree with two shoots was sprayed until runoff with a single *Streptomyces* strain spore suspended in 0.05% Tergitol solution, and the other tree in the pot was sprayed with the same strain but in 0.05% Triton X-100. Each combination (*Streptomyces* strain  $\times$  adjuvant  $\times$  clone) was replicated three times. Three controls were used: one tree was left untreated, one shoot of the other tree in the same pot was sprayed with 0.05% Tergitol only, and the other shoot with 0.05% Triton X-100. The mist blower was disinfected with 1% NaOCl and rinsed with deionized water between applications of different *Streptomyces* strains. At the end of each IEP, the trees were returned to the greenhouse and

evaluated for *Septoria* leaf spot severity and defoliation 5 weeks after being placed in the hybrid poplar plantation. Eight randomly selected treatment leaves and six leaves from Tergitol or Triton X-100 controls were scored from each treatment using the 0-to-8 leaf disease severity rating scale. Defoliation was determined by visually estimating the percentage of missing leaves on each stem.

**Evaluation of *Streptomyces* strain mixtures on potted exposure trees.** In 1999, three combinations of *Streptomyces* strains were evaluated for their ability to control *Septoria musiva* on poplar clones in the field. A method described by McQueen et al. (5) was used to test compatibility reactions between paired *Streptomyces* strains. *Streptomyces* selections consisted of pairs exhibiting varying levels of inhibition, ranging from none to high, to determine if different levels of antagonism between *Streptomyces* strains can increase the efficacy of biocontrol. *Streptomyces* strain pairs either were grown together (+) on the petri plates or were plated separately (/) and then mixed when inoculum was harvested for spraying. *Streptomyces* strains were suspended in 0.05% Tergitol solution. As in the 1998 experiment, one tree with two shoots was sprayed with a *Streptomyces* mixture (+) and the two shoots of the other tree in the same pot was sprayed with the same *Streptomyces* strain pair but mixed after growing them separately (/). Three replications of each combination were evaluated. For controls in each replication, one tree in a pot was left untreated and the other tree was sprayed with the Tergitol solution only. Five sets of 96 trees each were sprayed and exposed to field inoculum between 3 June and 25 August 1999. During the first three IEP exposures, starting on 3 June (IEP1), 16 June (IEP2), and 30 June (IEP3), the trees received the *Streptomyces* spray every 5 days and were exposed to natural *Septoria musiva* inoculum for 2 weeks. From 14 July (IEP4) and 4 August (IEP5), trees were sprayed every 7 days and were exposed to natural inoculum for 3 weeks. As in 1998, eight randomly selected leaves from each treatment were scored for disease severity using the 0-to-8 scale and the extent of defoliation was determined on each stem 5 weeks after the trees were placed in the hybrid poplar plantation.

**Evaluation of *Streptomyces* spray schedules in a plantation.** Fifty trees each of the four hybrid poplar clones were planted at a 2.4-by-2.4-m spacing using a randomized complete block design in spring 1997 at the University of Minnesota Experiment Station, St. Paul. The trees were exposed to natural inoculum of *S. musiva* from a nearby hybrid poplar plantation. In early spring 1998, all trees were pruned to a height of 90 to 100 cm. Starting the first week of June 1998 until mid-September, new shoots were sprayed

weekly, bimonthly, or monthly with spore suspensions of one of three *Streptomyces* strains (GS-93-3, 93, or Mycostop) suspended in 0.05% Tergitol, using a SOLO Junior 410 gasoline-powered mistblower. Each tree received a 100- to 150-ml suspension of *Streptomyces* spores, and the sprayer was disinfected with 1% NaOCl and rinsed with deionized water between applications of different *Streptomyces* strains. Untreated control trees were covered with transparent plastic sheets during spray applications. After the *Streptomyces* spray application was completed, the control trees were uncovered and five branches on each tree were sprayed with a 0.05% Tergitol solution. Each combination (*Streptomyces* strain  $\times$  each spray period  $\times$  clone) was replicated five times. The foliage of each tree was scored for *Septoria* leaf disease severity using the 0-to-8 scale the first week of August and the first week of September, and the extent of defoliation was recorded.

**Experimental design and data analysis.** A split-plot experimental design was used in all potted tree experiments. The growing season was split into IEPs in both years to test the effectiveness of *Streptomyces* strains, mixtures, and spray schedules. The 1998 and 1999 potted-tree experiments were evaluated separately and each IEP was treated as a subplot in the statistical analysis. A randomized complete block design was used in every IEP and an analysis of variance (ANOVA) was done according to the split-plot design. Initially, all interaction terms were tested (clone, treatment, and IEP) at the  $P = 0.05$  level. An ANOVA indicated significant ( $P < 0.0001$ ) differences among the IEPs in both years for leaf disease severity and also for defoliation; therefore, each IEP was analyzed separately for leaf disease severity and defoliation. Next, an ANOVA was done to determine if there was a clone-treatment interaction and also if the variation was due to the treatment or the clone. If there was no significant clone-treatment interaction and the treatment effect was significant ( $P < 0.05$ ), the PROC GLM procedure of Duncan's multiple range test (DMRT) of SAS (SAS Institute Inc., Cary, NC) was used to compare treatment means ( $P < 0.05$ ). If there was a significant clone-treatment interaction, data on resistant and susceptible clones were compared separately. In most cases, by analyzing resistant and susceptible clone data separately, the significant ( $P < 0.05$ ) clone-treatment interaction was eliminated, but if the treatment effect was significant ( $P < 0.05$ ), the DMRT was used to compare treatment means. However, if the clone-treatment interaction was still significant, the data for each clone were analyzed separately. If the ANOVA showed that the treatment effect on the clone was significant ( $P < 0.05$ ), the DMRT was applied to treatment means.

In the 1999 plantation experiment, a randomized complete block design was used. As in the potted-tree experiments, an ANOVA was used to determine whether the variation was due to the treatment or to the clone and, also, whether there was a significant clone-treatment interaction.

## RESULTS

**Single strains on potted trees.** The results of using single *Streptomyces* strains to control Septoria leaf disease on potted trees in 1998 are presented in Table 1. Only IEPs 1 to 3 are included because severe leaf rust on trees in IEPs 4 and 5 prevented us from determining Septoria leaf spot disease severity. An ANOVA indicated that the clone-treatment interaction was significant ( $P < 0.0001$ ) only in IEP2; thus, data for individual clones are presented. Septoria leaf disease was most severe in IEP1 and, in five of six *Streptomyces* treatments, leaf disease severity was significantly ( $P < 0.0001$ ) reduced compared with the adjuvant controls.

Leaf disease reduction was calculated by converting each mean disease rating scale value to a percentage of leaf area diseased. We considered that the diseased area on the adjuvant control was 100% and calculated the percentage of disease reduction in each *Streptomyces* treatment compared with the adjuvant control. The Mycostop strain provided the best control in IEP1, reducing the disease by 52% compared with the adjuvant controls. In IEP2, significant leaf disease control was achieved on all three susceptible clones, NE242 ( $P = 0.0002$ ), DTAC26 ( $P < 0.0001$ ), and NE299 ( $P = 0.006$ ), compared with the adjuvant controls. The best control was on clone DTAC26, and leaf disease was reduced 83% with the Mycostop/Triton X-100 treatment compared with the Triton X-100 control. In IEP3, leaf disease was significantly reduced ( $P < 0.0001$ ) with all six *Streptomyces* treatments. The most effective control was with the GS-93-3/Tergitol treatment, which reduced disease by 44% compared with the Tergitol control.

Defoliation was significantly reduced by the *Streptomyces* treatments in IEP1 ( $P = 0.0017$ ) and IEP2 ( $P = 0.0302$ ), as shown in Table 1. The ANOVA of the defoliation data did not indicate a significant clone-treatment interaction in any IEP. Defoliation was most severe between 5 June and 1 July (IEP1), and defoliation was significantly reduced by 15% with the GS-93-3/Tergitol and the 93/Tergitol treatments compared with the Tergitol control. In IEP2, the 93/Tergitol treatment significantly reduced defoliation by 33% compared with the Tergitol control.

**Strain mixtures on potted trees.** The 1999 results of biocontrol of Septoria leaf disease with strain mixtures in IEPs 1 to 3 are presented in Table 2. As in 1998, the potted trees exposed in IEPs 4 and 5 had severe leaf rust, and these data were omitted from the analysis. Septoria leaf disease developed on clone NM2 only in IEP1; thus, NM2 data from IEPs 2 and 3 were not included in the statistical analysis.

**Table 1.** Septoria leaf spot disease severity and defoliation caused by *Septoria musiva* on potted poplar trees sprayed with single *Streptomyces* strains in 1998<sup>a</sup>

Treatments <sup>z</sup>	Leaf disease severity					Defoliation (%)			
	IEP1 <sup>y</sup>		IEP2			IEP3	IEP1	IEP2	IEP3
	All	NM2	NE242	DTAC26	NE299	All	All	All	All
Untreated control	5.9 a	1.4 a	3.8 a	3.9 a	3.8 a	4.4 a	66 abc	16 a	14 a
Tergitol control	5.9 a	0.5 b	2.9 b	4.5 a	3.4 ab	3.9 bc	68 ab	15 ab	14 a
Triton X-100 control	5.8 ab	0.7 b	2.4 bc	4.4 a	3.0 abcd	4.0 ab	70 a	13 abc	13 a
GS-93-3/Tergitol	5.0 cd	0.7 b	1.8 c	2.9 bc	2.8 bcd	3.2 d	58 d	11 bc	8 a
93/Tergitol	4.9 cd	0.5 b	1.5 c	2.8 bc	2.8 bcd	3.4 d	58 cd	10 c	16 a
Mycostop/Tergitol	4.7 cd	0.5 b	1.9 c	2.5 bc	2.3 cd	3.4 d	60 bcd	12 abc	8 a
GS-93-3/Triton X-100	5.2 bc	0.5 b	1.9 c	2.8 bc	3.2 ab	3.4 d	62 bcd	9 c	10 a
93/Triton X-100	4.7 cd	0.6 b	1.5 c	3.0 bc	3.1 abcd	3.4 d	58 d	12 abc	14 a
Mycostop/Triton X-100	4.5 d	0.5 b	1.5 c	2.1 c	2.3 d	3.5 cd	58 d	11 bc	8 a

<sup>a</sup> Mean leaf disease severity using a 0-to-8 scale and mean percentage defoliation of each treatment. Significant ( $P < 0.0001$ ) treatment-clone interaction in IEP2 required the analysis of leaf disease severity data by clone. All = all clones. Within each column, the means of three replications with unlike letters were significantly different ( $P < 0.05$ ) according to Duncan's multiple range test.

<sup>y</sup> Potted trees were exposed to natural *S. musiva* inoculum in three inoculum exposure periods (IEPs): 5 June to 1 July (IEP1), 1 July to 22 July (IEP2), and 22 July to 16 August (IEP3). At the end of each IEP, trees were returned to the greenhouse for evaluation and a new set of trees was used in the following IEP.

<sup>z</sup> Spores of all *Streptomyces* strains were suspended in either 0.05% Tergitol or 0.05% Triton X-100 and the trees were sprayed every 7 days, starting on the first day of each IEP that the trees were in the plantation.

**Table 2.** Septoria leaf spot disease severity and defoliation caused by *Septoria musiva* on potted poplar trees sprayed with mixtures of *Streptomyces* strains in 1999<sup>a</sup>

Treatments <sup>z</sup>	Leaf disease severity					Defoliation (%)					
	IEP1 <sup>y</sup>		IEP2		IEP3	IEP1		IEP2		IEP3	
	All	NM2	Susc.	NM2	Susc.	NM2	Susc.	NM2	Susc.	NM2	Susc.
Untreated control	4.4 a	0	5.0 a	0	5.6 a	32 ab	32 a	0	55 a	0	72 a
0.05% Tergitol	4.2 a	0	4.0 b	0	5.5 a	46 a	23 a	0	35 b	0	74 a
GS-93-3 + LR1	3.2 b	0	1.8 c	0	3.8 b	11 bc	26 a	0	17 c	0	57 ab
GS-93-3/LR1	3.2 b	0	1.8 c	0	3.5 b	8 bc	25 a	0	19 c	0	45 b
93 + LR2	2.9 b	0	1.8 c	0	3.8 b	11 bc	30 a	0	24 bc	0	55 ab
93/LR2	2.8 b	0	2.0 c	0	3.4 b	3 c	27 a	0	23 bc	0	59 ab
Mycostop + LR2	2.8 b	0	1.8 c	0	3.3 b	30 abc	24 a	0	18 c	0	53 b
Mycostop/LR2	2.7 b	0	1.5 c	0	3.5 b	4 bc	22 a	0	11 c	0	47 b

<sup>a</sup> Mean leaf disease severity using a 0-to-8 scale and mean percentage defoliation. All = all clones and Susc. = susceptible clones. Within each column, the means of three replications with unlike letters were significantly different ( $P < 0.05$ ) according to Duncan's multiple range test.

<sup>y</sup> Trees were exposed to natural *S. musiva* inoculum in three inoculum exposure periods (IEPs): 3 June to 16 June (IEP1), 16 to 30 June (IEP2), and 30 June to 14 July (IEP3). At the end of each IEP, trees were returned to the greenhouse for evaluation and a new set of trees were used in the following IEP.

<sup>z</sup> Spores of all *Streptomyces* strains were suspended in 0.05% Tergitol and trees were sprayed every 5 days, starting on the first day of each IEP that the trees were in the plantation. Inoculum of the *Streptomyces* pairs were prepared from cultures grown together (+) or on separate (/) petri plates. LR1 was derived from GS-93-3 and LR2 was derived from Mycostop.

Septoria leaf spot was most severe on the susceptible clones in IEP3. In IEPs 1 to 3, all *Streptomyces* treatments significantly ( $P < 0.0001$ ) reduced Septoria leaf disease compared with the Tergitol controls and there were no significant clone-treatment interactions. A disease reduction of 64 and 87% in the IEP1 and IEP2, respectively, was achieved with a mixture of Mycostop/LR2 (derived from Mycostop) when strains were plated separately compared with the Tergitol controls. In IEP3, the same strain mixture grown together provided the greatest disease reduction (70%) compared with the Tergitol control. However, no significant differences were found whether the strain pairs were plated together or separately, and no significant difference in disease reduction was found among the different paired strains of *Streptomyces*.

The effectiveness of *Streptomyces* mixtures in reducing defoliation also is presented in Table 2. An ANOVA of the percentage defoliation in IEP1 showed significant ( $P = 0.01$ ) clone-treatment interaction when the data of all four clones were analyzed together. When data of the resistant and susceptible clones were analyzed separately, there was no significant clone-treatment interaction ( $P = 0.0631$ ). In IEP1, the ANOVA of the defoliation data of clone NM2 indicated significant ( $P = 0.0182$ ) treatment differences. Defoliation of clone NM2 in IEP1 was significantly less in five of six treatments compared with the Tergitol control, and disease was reduced 93% with the 93/LR2 strain mixture. Defoliation of susceptible clones was significantly reduced in IEP2 ( $P < 0.0001$ ) and IEP3 ( $P = 0.0090$ ) compared with the Tergitol controls. The Mycostop/LR2 strain mixture provided the best reduction (69%) in defoliation in IEP2; and, in IEP3, the GS-93-3/LR1 (derived from GS-93-3) strain mixture was the most effective, reducing defoliation 39% compared with the Tergitol control.

***Streptomyces* spray schedules in a plantation.** Only the August data were

used in the statistical analysis because of severe leaf rust on the trees by the first week of September. The ANOVA did not indicate a significant clone-treatment interaction, and treatment effect was significant ( $P < 0.0001$ ). Disease was reduced 63 to 75% with all *Streptomyces* treatments compared with the untreated control (Table 3). No difference in disease severity was noted between the 0.05% Tergitol-treated and nontreated part of the same tree, and these data were not included in the statistical analysis. Generally, the weekly and bimonthly applications provided slightly better control than the monthly application, but these differences were not significant ( $P = 0.05$ ).

There was no significant difference in defoliation among the different spray schedules (Table 3). The ANOVA indicated significant treatment effect ( $P = 0.0011$ ) in reducing defoliation of the susceptible clones but no significant clone-treatment interaction. All *Streptomyces* treatments significantly reduced defoliation from 55 to 91% compared with the untreated control.

## DISCUSSION

This is the first report of effective biological control of Septoria leaf spot disease of hybrid poplar using *Streptomyces* strains in the field. Septoria leaf disease was less severe at the St. Paul than at the Rosemount location, and the level of control varied between years and locations. The potted-tree experiments enabled us to test spray schedules during different times of the growing season and inoculum exposure periods.

The selection of single strains of *Streptomyces* for this study was based on their competitive and antibiotic activity against *Septoria musiva* isolates in vitro and on the single-leaf plantlet test. Although use of these isolates singly reduced disease in the field, it has been suggested that mixtures of strains may enhance and provide more permanent disease control (15). Our

experiments with strain mixtures were limited; however, slight increases in disease control were achieved. Weekly or bimonthly applications resulted in somewhat better control than monthly applications; however, the differences were not significant.

Septoria canker does not become a significant problem in hybrid poplar plantations until the third or fourth year after planting (7). Few cankers developed on susceptible trees in our experiments because of the short exposure times of the potted trees to natural Septoria inoculum, and the planted trees were exposed to inoculum for only one field season. Therefore, the effectiveness of *Streptomyces* to prevent or reduce the incidence of Septoria canker is unknown.

Genetic resistance is considered to be the most effective way to reduce the damage caused by Septoria leaf spot (9). However, the number of resistant clones that are available at this time are limited; therefore, biocontrol using *Streptomyces* may be an effective way to manage this disease in nurseries and plantations. Further research is needed on selection of *Streptomyces* strains and combinations of strains, and on the longevity of their activity in plantations.

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**Table 3.** Effectiveness of different *Streptomyces* strains and spray schedules in controlling Septoria leaf spot in a hybrid poplar plantation

Treatments <sup>z</sup>	Leaf disease severity <sup>x</sup>		Defoliation (%) <sup>y</sup>	
	All clones		NM2	Susceptible clones
Untreated control	3.0 a		0	11 a
GS-93-3, monthly	1.5 b		0	1 b
GS-93-3, bimonthly	1.9 b		0	2 b
GS-93-3, weekly	1.8 b		0	3 b
93, monthly	1.3 b		0	2 b
93, bimonthly	1.4 b		0	2 b
93, weekly	1.8 b		0	1 b
Mycostop, monthly	1.5 b		0	5 b
Mycostop, bimonthly	1.5 b		0	3 b
Mycostop, weekly	1.4 b		0	1 b

<sup>x</sup> Leaf disease severity was assessed using a 0-to-8 scale. Means of five replicates in the column with unlike letters were significantly different ( $P < 0.05$ ) according to Duncan's multiple range test.

<sup>y</sup> Means of five replicates in the column with unlike letters were significantly different ( $P < 0.05$ ) according to Duncan's multiple range test.

<sup>z</sup> Single *Streptomyces* strains were sprayed (starting the first week of June and ending in mid-September in 1998) on poplar clones planted in a field plot in St. Paul, MN.

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