The Effect of Water Extracts of Spent Mushroom Compost on Apple Scab in the Field

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This article was prepared with the support of a grant from the Agriculture in Concert with the Environment (ACE) Program, which is jointly funded by the U.S. Department of Agriculture, Cooperative State Research, Education and Extension Service, and the Environmental Protection Agency Program under Cooperative Agreement 94-COOP-1-0809.

Any opinions, findings, conclusions, or recommendations expressed herein are those of the authors and do not necessarily reflect the views of the U.S. Department of Agriculture or the Environmental Protection Agency.

We thank J. Floyd, Gourmet's Delight Mushrooms, Eden, WI, and C. Broomall, Terry Farms Mushrooms, Princeton, IL, for supplying the composts used in these experiments; E. Ela and R. Willard, Ela Orchard, Rochester, WI, for their collaboration; P. M. Crump for assistance in data analyses and J. L. Chen, M. J. Cronin, B. C. Grant, C. M. Simmer, R. N. Spear, and R. P. Voland for assistance in the laboratory and field; and R. F. Harris, P. S. McManus, and W. R. Stevenson for their comments on an early version of this manuscript.

Accepted for publication 24 May 1996.

ABSTRACT

Yohalem, D. S., Nordheim, E. V., and Andrews, J. H. 1996. The effect of water extracts of spent mushroom compost on apple scab in the field. Phytopathology 86:914-922.

To control apple scab disease caused by *Venturia inaequalis*, aqueous extracts from two sources of spent mushroom substrate (SMS), anaerobically fermented for 7 days and amended with spreader-sticker, were applied at weekly intervals to apple trees (cv. McIntosh) from green-tip to petal-fall and biweekly thereafter. Trials were conducted for three seasons at two locations in Wisconsin. Both extracts significantly reduced ($\alpha = 0.05$) the leaf area affected by scab relative to water and spreader-sticker controls as evaluated by the Horsfall-Barratt scale. Disease incidence was similarly decreased but to a lesser extent. Extracts were not as effective in inhibiting disease as captan sprayed at the same intervals. No difference was detected between extracts with and without spreader-sticker. Higher populations of bacteria, which persisted for at least 1 month after the final spray, were detected on leaves treated with the extracts. No differences were found in total numbers of fungi. Inhibitory activity of extracts, assessed as in vitro inhibition of *Venturia* conidia germination, was monitored over time for extracts prepared from SMS stored under different conditions. For one source of SMS, neither time nor storage conditions (outdoors uncovered or indoors in sheds) affected inhibitory activity of extracts. Decline in efficacy of the other source was apparent by 13 weeks relative to unstored compost, although not between storage regimens.

Additional keywords: compost tea, filtrate, manure, *Spilocaea*, sustainable agriculture.

Apple scab, caused by the fungus *Venturia inaequalis* (Cooke) G. Wint. (anamorph *Spilocaea pomi* Fr.:Fr.), is, if uncontrolled, the limiting factor for apple (*Malus* × *domestica* Borkh.) production in moist, temperate climates worldwide (2). Although resistant cultivars are being planted more extensively, disease management still hinges on multiple applications of fungicides. However, the use of many fungicides is being restricted because of the development of pathogen resistance and because of environmental and public health concerns (6). Consequently, there is an increasing need for alternative, sustainable strategies.

Over the last decade, there have been reports of the use of water extracts from composts for control of foliar diseases (7,10,15,16, 23,26,27,29). Extracts are prepared by mixing compost and water and incubating the resulting slurry without agitation for several days. The slurry is filtered through cheesecloth, and the filtrate, termed an extract or compost tea, is sprayed onto the aerial surfaces of plants. This approach to biological control, if effective in practice, is a potentially attractive alternative to fungicides that is consistent with sustainable agriculture.

Although much of the information on compost extracts is anecdotal, the consensus is that various manure-based compost extract formulations are more effective than extracts prepared from composts that lack manure (7,23,27,29). Using extracts from horse or cattle manure-based composts, Wetzien (27) observed good control of gray mold (*Botrytis cinerea*) on strawberries, various powdery mildews, and late blight of potato (*Phytophthora infestans*) relative to untreated controls and, in some cases, to fungicide preparations. At least partial control was reported for gray mold (*B. cinerea*) on tomato, pepper foliage, and grape berries (7) and grape leaves (12) and for shoot blight on red pine caused by *Sphaeropsis sapinea* (29). Tränkner and Kirchner-Bierschenk (24) reported a decrease in apple scab lesions from 2.5 per fruit on water-sprayed controls to 1.5 per fruit with a manure-straw-soil extract. However, there have been failures in field applications of extracts that appeared promising under more controlled conditions (9,10).

Spent mushroom substrate (SMS) is the by-product of commercial *Agaricus bisporus* (Lange) Imbach cultivation after the material has been removed from production. The substrate is derived from horse manure with bedding straw and various other ingredients, typically including chicken litter, soybean meal, and gypsum (19, 28). The mixture is composted in tunnels by standard methods and sown with commercial mushroom spawn. After casing (mulching) with peat and cultivation of the crop for 3 to 4 months, the compost is removed from beds. SMS is somewhat variable from lot to lot (8,22), and several important chemical properties change with time (21). We have screened extracts from more than 40 different

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Publication no. P-1996-0617-02R

composts, including the components of fresh mushroom substrate, noted above, and the composted substrate prior to the mushroom crop, for biocontrol efficacy (D. S. Yohalem, R. P. Voland, X. Zhang, and J. H. Andrews, *unpublished data*; [29]). Extracts prepared from SMS were the most effective in inhibiting germination of conidia of *V. inaequalis* in an in vitro assay and in reducing disease on apple seedlings in growth chambers (29).

The objective of this research was to assess the efficacy of SMS extracts for scab control under conditions of severe and moderate disease pressure. Our primary hypothesis was that SMS would control scab as well as a protectant fungicide under moderate disease pressure. Further, we postulated that spreader-sticker amendment of SMS would enhance control and that inhibitory activity of SMS, as evaluated by in vitro assay of extracts, would decrease more rapidly for unsheltered than for sheltered composts. We present evidence, from 3 years of field experiments, that (i) severity and incidence of scab were reduced relative to water controls but not by as much as treatment with captan; (ii) no difference could be attributed to spreader-sticker amendment; and (iii) over compost storage time, extracts from one substrate, but not the other, exhibited a decline in inhibitory activity that was marginally greater in uncovered storage than in covered storage. A preliminary report has been published (30).

MATERIALS AND METHODS

Composts and extract production. The composts used in these experiments were spent substrates from commercial mushroom (*A. bisporus*) production provided by Gourmet's Delight Mushrooms, Eden, WI (GDM) (1993 to 1995), and Terry Farms Mushrooms, Princeton, IL (TMF) (1994 and 1995). The substrates were prepared by the mushroom growers according to standard protocols (28), sown with commercial mushroom spawn, cased with peat, and retained in production for 3 to 4 months. GDM spent substrate was removed with no additional treatment and discarded by the operators; TMF spent substrate was pasteurized in situ at 80°C, removed from the beds, coarsely sieved, and discarded. Each year we collected the composts during April in ~2-m^3 lots and stored them without turning, as described below.

The quantities of compost needed for particular applications were mixed with water (1:2, wt/vol, compost/water) in plastic containers and incubated without agitation in the laboratory or a storage shed (15 to 25°C) for 7 to 8 days. After incubation, the container contents were stirred, and SMS extracts were obtained by filtration through a single layer of cheesecloth. This filtration procedure yielded maximum particle sizes between 100 and 125 μm in cross-section diameter.

Field experiments. All field research was performed as branch trials in which randomly assigned branches within apple trees (cv. McIntosh) received applications of compost extract or, depending on the experiment, another treatment or water (control). The branches used were scaffold limbs ~1.5 to 2.0 m above ground and spaced, insofar as possible, equidistantly around the trunk (branch position is commented on below). All leaves on the selected branches were treated. Experiments were performed at two sites during the growing seasons of 1993, 1994, and 1995. Severe disease pressure was expected at the first location, a 0.5-ha experimental orchard of approximately 15-year-old trees located at the University of Wisconsin West Farm Experiment Station in Madison, which was not managed to control scab. Moderate disease pressure was expected at the second site, a commercial orchard of mixed cultivars located in Rochester, WI, where scab is normally controlled with minimal applications of eradicant fungicides. The McIntosh trees at Rochester were 10 years old. Distilled water was used in the preparation of all slurries at Madison; well water was used at Rochester.

Treatments were applied with manually pressurized hand or backpack sprayers equipped with Multi-Spray (H.D. Hudson Mfg. Co., Chicago) nozzles adjusted to a conical spray pattern. Sprays were applied according to standard recommendations for protectant fungicide applications (14) on a weekly basis throughout the period of ascospore release (i.e., green-tip during early April until 1 week after petal-fall, generally during late May or early June). This was followed by biweekly applications until 3 weeks before harvest, generally in mid-September, for a total of 10 to 12 applications per year. To avoid excessive drift or wash-off caused by inclement weather, spray applications occasionally were delayed by 1 or 2 days. Captan was applied at rates recommended by the manufacturer (0.84 g/liter) at ~0.5 liters per treated branch. All other sprays were administered to runoff at approximately 1 liter per branch (~3,000 liters/ha).

In 1993, four treatments were applied to branches of 12 trees at the Madison site and 6 trees at the Rochester site: (i) GDM extract; (ii) cow manure-based compost extract (27); (iii) water (negative control); and (iv) captan 50 WP (Chevron Chemical Corp., San Rafael, CA) (positive control). The treatments were applied to four main branches within trees and arranged as randomized complete blocks with trees as the blocks. Interbranch interference was minimized by the presence of untreated branches that acted as buffers between the treated branches and by applying treatments at dawn or dusk when drift was minimal.

In 1994 and 1995, five treatments were applied at both sites, with modifications as follows: (i) Latron B1956 (Rohm and Haas Corp., Philadelphia), a nonionic modified phthalic glycerol alkyd resin spreader-sticker, alone; (ii) GDM amended with 0.6 ml of Latron B1956 per liter of extract (GDM+); (iii) TMF amended with 0.6 ml of Latron B1956 per liter of extract (TMF+); (iv) water; and (v) captan 50 WP (0.84 g/liter). Informal analysis of the 1993 data led us to invoke a second blocking factor, branch position (compass orientation), in the design of experiments for the Madison location in 1994 and 1995. Position number 1 in randomization schemes was the scaffold branch oriented in the western-most direction from the trunk. Other treatments were assigned clockwise on branches nearest 72.5 degrees from the previous branch. Thus, although the design remained a randomized complete block with five treatments per each of six trees at Rochester in 1994, it was changed to three Latin squares (20) with trees (five per square) and branch orientation (five per tree) as blocking factors for the distribution of the five treatments at Madison. In 1995 at Rochester, four treatments were used on each of 12 trees. Branches of 12 trees were treated with GDM+, TMF+, and water. Six of these trees received captan as a fourth treatment, and the other six received a Latron control as a fourth treatment. Treatments were randomized independently for each year of the study among the same group of trees (with six additional trees in 1995).

The effects of spreader-sticker compounds were evaluated in 1994 and 1995 in a related series of trials on different trees from the Latin-square experiment at the Madison site. In 1994, GDM and TMF extracts, with and without spreader-sticker (Latron B1956), were tested and compared on the branches of six trees at the Madison site in a randomized complete block design. In 1995 GDM, GDM+, fish oil, and GDM amended with fish oil at 2.5 ml/liter were compared on six trees. (Fish oil was included because it is an acceptable spreader amendment for orchardists who wish to market their crop as organically grown produce.)

Laboratory assays. We monitored in vitro inhibition of conidial germination by extracts at each spray date and evaluated differences between covered and uncovered SMS storage regimes relative to freshly collected (not aged) SMS over the course of the apple production season, as previously described (5). In brief, to facilitate microscopic examination (discussed below), aliquots (1.5 ml) taken from each compost slurry were spun in a microcentrifuge (16,000 × *g*) for 10 to 20 min to remove debris. Although centrifugation caused the loss of some microbes from the supernatant, their presence was not required in the assay system for inhibition to be realized (5). Suspensions of *V. inaequalis* conidia, produced by the wick bottle method (11) and containing approxi-

mately 500 spores in 10 µl of MilliQ (Millipore Corp., Bedford, MA) water, were placed in each of six sterile 96-well Falcon Microtiter III (Becton-Dickinson, Cockeysville, MD) plate wells loaded with 40 µl of the clarified compost extract. Microtiter plates were incubated at approximately 20°C in the dark for 48 h, after which 5 µl of 1% ethyl(sodium-*o*-mercaptobenzoato)mercury (Thimerasal) was added to each well as a killing solution. Spore germination was assessed at 400× magnification with an inverted microscope (model TMS, Nikon Instrument Group, Melville, NY). Inhibition of conidial germination was calculated as $1 - (G_T/G_C)$, where G_T represents the proportion of germinated conidia in the treatment and G_C is the proportion of germinated spores in a MilliQ water check.

TABLE 1. Analysis of variance for apple scab severity data (inverse proportion of leaf area affected) for 1994 at Madison, WI[a]

Source	df	Type III MS	*F*	*P*
Square[b]	2	140,682.9	11.57	0.002
Error (square)	12	12,157.2	2.16	0.037
Position[c]	4	3,807.8	0.68	0.612
Treatment[d]	4	132,403.4	23.55	0.0001
Square × position	8	9,176.4	1.63	0.150
Square × treatment	8	3,430.4	0.61	0.763
Error (branch)	36	5,621.1		
Leaf type[e]	1	709,475.2	110.09	0.0001
Treatment × leaf type	4	4,273.2	0.66	0.620
Position × leaf type	4	5,961.2	0.93	0.455
Tree × position × treatment × leaf type (square)	66	6,444.5	2.93	0.0001
Error (leaf type)	1,350	2,193.7		
Surface[f]	1	737,019.5	572.18	0.0001
Leaf type × surface	1	34,701.2	26.94	0.0001
Treatment × surface	4	8,621.2	6.69	0.0001
Treatment × leaf type × surface	4	1,581.1	1.23	0.297
Position × surface	4	4,215.5	3.27	0.0111
Position × leaf type × surface	4	773.4	0.6	0.662
Error (leaf surface)	1,482	1,288.1		

[a] Test statistics and *P* values were calculated with the appropriate error terms, i.e., the indicated mean square (MS) for the closest source of error below the evaluated term in the first column. Error (square) = tree (square); error (branch) = tree × position × treatment (square); error (leaf type) = position × treatment × leaf type × leaf (square × tree); error (leaf surface) = position × treatment × leaf type × surface × leaf (square × tree). A nominal *P* value of 0.0001 signifies ≤0.0001.

[b] Square = Latin square (row within orchard).

[c] Position = branch orientation (compass direction).

[d] Treatment = captan, GDM+ (Gourmet's Delight Mushrooms, Eden, WI, amended with Latron), TMF+ (Terry Farms Mushrooms, Princeton, IL, amended with Latron), Latron, or water.

[e] Leaf type = lateral (spur) or terminal.

[f] Surface = adaxial or abaxial.

Estimation of fungal and bacterial populations on leaves. The following assays were done to determine the overall impact of SMS applications on fungal and bacterial populations. In 1993 a single leaf was collected from each treated branch 7, 14, and 28 days after the last spray application at the Madison orchard. The leaves were shaken individually for 1 h in solutions of 50 ml of sterile distilled water with 0.5 ml of Tween 20 per liter. Four 10-fold dilutions of the suspension were prepared, and eight replicate 10-µl drops were plated on potato dextrose agar (25) amended with 250 mg of chloramphenicol per liter and on King's Medium B (13) amended with 50 mg each of cycloheximide and nystatin per liter for most probable number (MPN) estimation (17) of fungal and bacterial populations, respectively. An identical assay was performed in 1994, except that only two of the three Latin squares (10 trees) were sampled.

During July 1995, 5 randomly selected leaves were collected from each of the 15 trees and pooled from branches that had been treated with extracts or water. Each 5-leaf sample was bulked and assayed as described above, except that 100 ml of wash solution was used and 0.1% tryptic soy agar (Difco Laboratories, Detroit) replaced King's Medium B for enumeration of bacteria. A sample was collected immediately after application; additional 5-leaf samples were collected 3, 5, 7, and 12 days thereafter and assayed for microbial populations.

Compost age and weathering. In 1993, composts were stored uncovered and outdoors during the growing season. In 1994 and 1995, all the composts used in field experiments were stored in a shed (Madison) or under tarps (Rochester). To assess the impact of weathering, a portion of the compost also was stored as uncovered piles outdoors. Three 500-g samples (31) of SMS were collected from both covered (shed) and uncovered composts at monthly intervals from the Madison site in 1994 and approximately every 6 weeks in 1995. Slurries (1:4, wt/vol, compost/water) were prepared from each lot of compost, incubated for 7 to 8 days, and

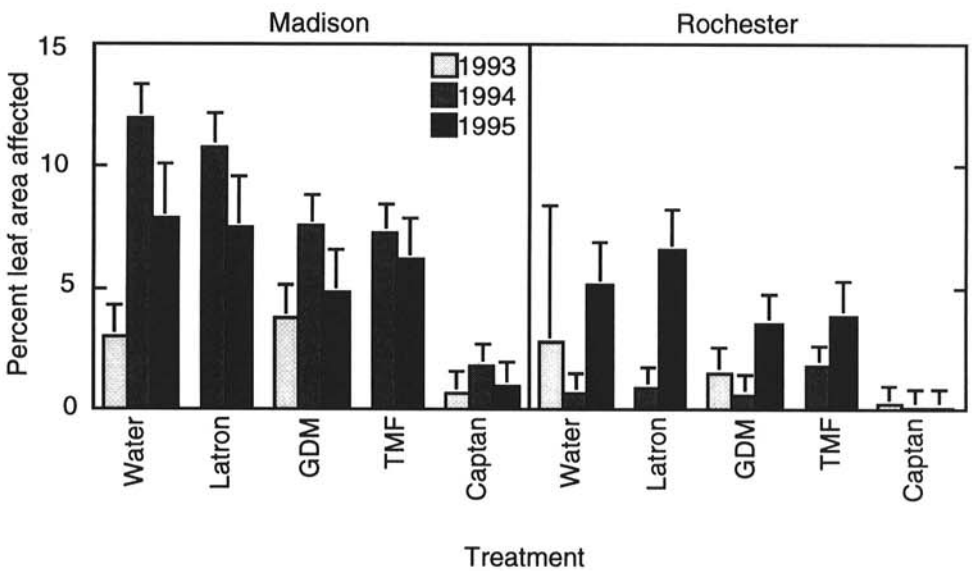

Fig. 1. Apple scab severity as measured by the Horsfall-Barratt scale and converted to mean percent leaf area affected for 3 years at two locations (Madison and Rochester, WI). Data for Rochester in 1994 are from 23 August. Vertical lines represent the upper half of the 95% confidence intervals and were calculated on the transformed (inverse) scale. Due to the nonlinearity of the transformation, the back-transformed confidence interval is not symmetrical. Only the larger, more conservative part is shown. Latron = spreader-sticker; GDM = spent mushroom substrate from Gourmet's Delight Mushrooms, Eden, WI; TMF = spent mushroom substrate from Terry Farms Mushrooms, Princeton, IL.

extracts were assayed for inhibition of conidial germination. To assess the impact of aging, fresh (not aged) collections were obtained from growers at approximately 6-week intervals in 1995 (7, 12, and 18 weeks for TMF and 7, 13, and 19 weeks for GDM) and assayed as described above.

Disease assessment and data analyses. *Method one.* The first method of obtaining apple scab data was to collect quantitative data for all experiments with leaf type (lateral spur and terminal leaves) nested within each branch. Adaxial and abaxial surfaces of leaves were nested within leaf type. Trees were evaluated for scab

TABLE 2. Protected *t* tests[a] for apple scab severity

Comparison[b]	Madison 1994		Rochester 1994[c]		Madison 1995		Rochester 1995[d]	
	t	*P*	*t*	*P*	*t*	*P*	*t*	*P*
Captan vs. GDM+ and TMF+	53.02	0.0001	12.16	0.002	183.1	0.0001	109.5	0.0001
GDM+ vs. water and Latron	4.20	0.048	2.92	0.103	10.79	0.002	14.67	0.002
TMF+ vs. water and Latron	6.72	0.014	3.62	0.072	9.59	0.004	5.13	0.038
GDM+ vs. TMF+	0.22	0.640	0.03	0.868	0.03	0.871	3.91	0.098
Water vs. Latron	0.41	0.526	1.51	0.234	0.43	0.515	0.65	0.433

[a] The probability of obtaining a more extreme overall result (*F* test) by chance in each case was ≤0.0001. The error terms were tree × position × treatment (square) for Madison, WI, and tree × treatment for Rochester, WI, orchards.

[b] GDM+ = Gourmet's Delight Mushrooms, Eden, WI, amended with Latron; TMF+ = Terry Farms Mushrooms, Princeton, IL, amended with Latron.

[c] Data collected on 22 August 1994.

[d] The contrasts, calculated on the basis of randomized complete blocks for Rochester in 1995, were for 12 trees for comparisons of GDM+ to TMF+, and each versus water (in the entries indicating water and Latron), GDM+ and TMF+ versus captan, and water versus Latron were based on 6 trees.

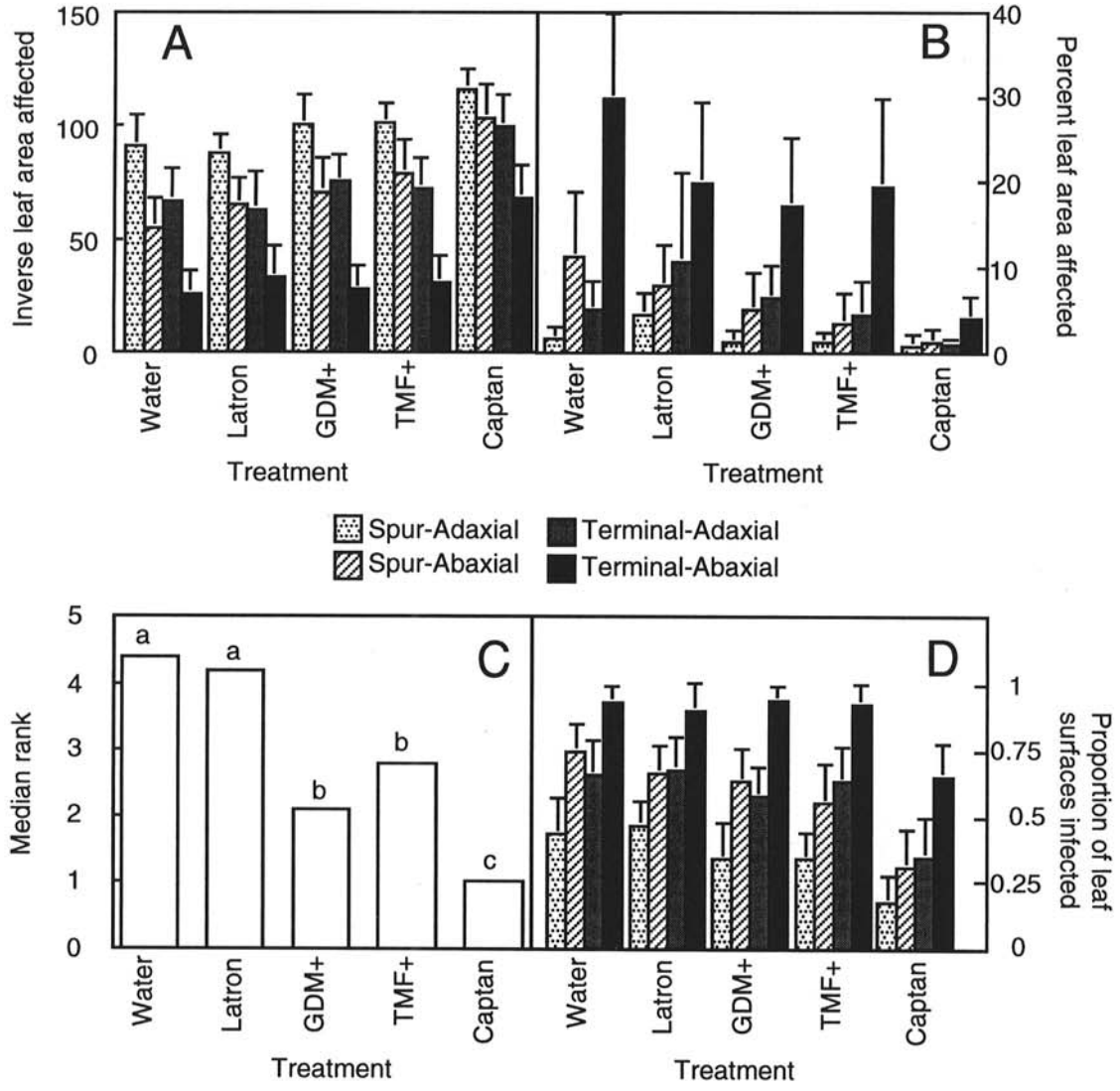

Fig. 2. Four methods for evaluation of apple scab as illustrated by data from 11 September 1994 at Madison, WI. **A,** Disease severity expressed as the inverse of the mean proportion of leaf area affected, with the upper half of the 95% confidence intervals for the three-way interaction among treatment, leaf type (spur or terminal), and leaf surface (adaxial or abaxial). **B,** Disease severity expressed as the percent leaf area affected, with means and the upper half of the 95% confidence intervals calculated on the untransformed means for the three-way interaction among treatment, leaf type, and leaf surface. **C,** Median ranks for the treatments (1 = best, 5 = worst control) with trees considered as the sole blocking factor. Columns topped by same letter are not significantly different at $P = 0.10$. **D,** Disease incidence expressed as the proportion of leaf surfaces infected, with the upper half of the 95% confidence intervals for the interaction among treatment, leaf type, and leaf surface. Latron = spreader sticker; GDM+ = spent mushroom substrate from Gourmet's Delight Mushrooms, Eden, WI, amended with Latron; TMF+ = spent mushroom substrate from Terry Farms Mushrooms, Princeton, IL, amended with Latron.

severity on a Horsfall-Barratt (H-B) scale (3) at least once during each growing season. In 1993, adaxial and abaxial surfaces of 100 leaves on each branch (50 spur and 50 terminal leaves) were evaluated once on the H-B scale from 28 to 30 July. In 1994, estimates were made, twice (23 August and 13 September) at Rochester and once (22 August) at Madison, for adaxial and abaxial surfaces of 10 spur and 10 terminal leaves on each branch. In 1995, adaxial and abaxial surfaces of 10 spur and 10 terminal leaves on each branch were evaluated once at each site (2 July at Madison and 3 July at Rochester). During each season, treatments also were compared at several dates by ranking treated branches within each tree for apple scab.

For all experiments, the branch was the experimental unit with subplots for leaf type (spur and terminal) nested within the branch and subsubplots for surface (adaxial and abaxial) nested within leaf type. Multiple observations of leaves within branch × leaf type × leaf surface combinations were interpreted as subsamples. As noted above, in 1994 and 1995 both tree and branch orientations were invoked as blocking factors for the Latin-square experiment at Madison, whereas in all other cases trees were the sole blocking factor.

Analyses of variance (ANOVA) and contrasts of interest (protected t tests) were calculated for all branch experiments and evaluated at $\alpha = 0.10$. H-B classes for disease severity (3) were converted to the midpoint of the range of the affected percentage of leaf area appropriate to the designated value (e.g., H-B 0 = 0% infected tissue, H-B 1 = 1.5%, and H-B 2 = 4.5%). The analyses we present are based on these data, transformed to their inverses after percents were converted to proportions and 0.0075 (one-half of the smallest value greater than 0) was added (to avoid division by 0).

Disease incidence, i.e., the proportion of symptomatic leaf surfaces in a treatment × leaf type × leaf surface combination, was analyzed by conversion of H-B data to binary (presence/absence) values. From these binary data for individual leaf surfaces, proportions of leaves within a class, i.e., within a leaf type × leaf surface combination, were calculated. ANOVA and contrasts of interest were calculated for the proportion of leaf surfaces in a treatment showing any symptoms of scab. Analysis of residuals indicated no need for transformation of these data.

Method two. The second method by which apple scab data were collected was ranking of treated branches within trees for overall scab severity. Rankings within trees were analyzed by the Friedman test (4). This is a nonparametric analog of two-way ANOVA with blocks; for this analysis, treatments were considered as completely randomized within trees. Where statistically significant differences were detected, pairwise differences were calculated (4).

Fruit yield (weight and quantity) and quality data were collected from all treated branches, but statistical analyses are not presented because fruit set was erratic among branches, trees, and years. Furthermore, commercial fruit grading involves assessment of location of scab lesions, not merely presence. Hence, a formal presentation of fruit data is unwarranted.

MPN in the estimation of microbial population sizes were expressed as logarithms and analyzed by split-plot ANOVA and by regression (18). From an initial analysis with repeated measures, the results suggested no violation of the assumptions underlying the split-plot analysis. Further, there were several missing data, which caused some minor difficulties in the formal repeated measures analyses; however, there was no impact on the conclusions. Thus, we focus on the split-plot analyses.

To fulfill ANOVA assumptions, particularly the assumption of homogenous variance, conidial germination-inhibition data were subjected to angular transformation (arcsine $\sqrt{x}$) and scaled by dividing by $\pi/2$. For each assay the design was nested with microtiter wells within incubations. Laboratory experiments were evaluated at $\alpha = 0.05$. Statistical analysis was performed using SAS version 6.09 (SAS Institute, Cary, NC) running under a UNIX operating system or by Minitab version 10 for Windows (MINITAB Corp., University Park, PA).

RESULTS

We collected data on the effect of GDM extract for all 3 years (1993 to 1995) at both locations; data on the effect of TMF extract were obtained for 2 years (1994 and 1995) at both locations. In general, the pattern of results was similar across years and sites. For simplicity, unless discrepancies exist among the analyses, we present details only from the larger experiment conducted in 1994 at Madison.

Disease severity. Regardless of treatment, trees at Rochester were consistently less affected by scab than those at Madison (Fig. 1). At the Madison orchard, disease severity (percent leaf area affected) on branches treated with water controls was highest in 1994;

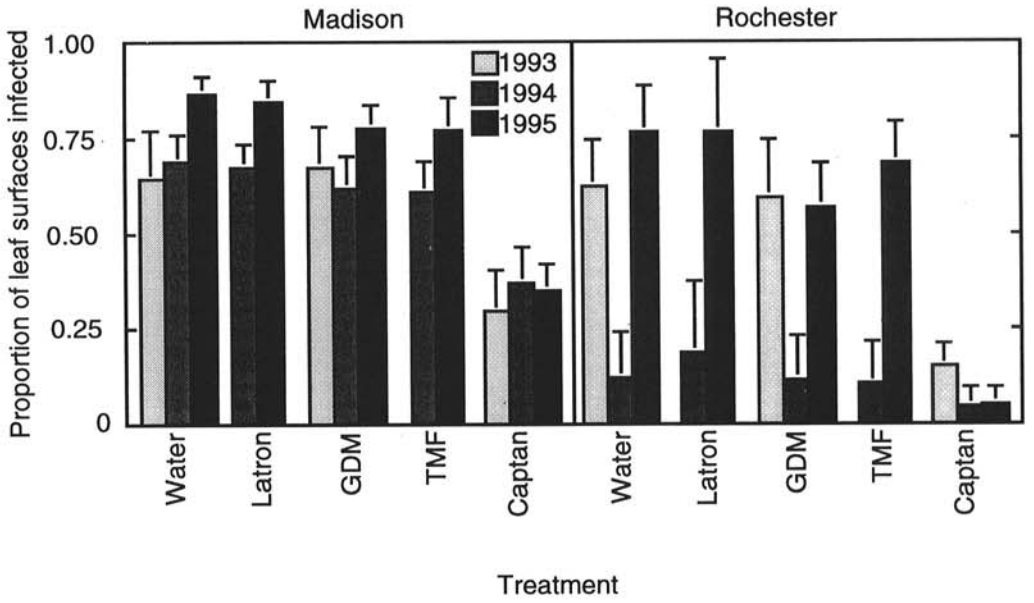

Fig. 3. Apple scab disease incidence (proportion of leaf surfaces infected) for 3 years at two locations (Madison and Rochester, WI). Data for Rochester in 1994 are from 23 August. Vertical lines represent the upper half of the 95% confidence intervals. Latron = spreader-sticker; GDM = spent mushroom substrate from Gourmet's Delight Mushrooms, Eden, WI; TMF = spent mushroom substrate from Terry Farms Mushrooms, Princeton, IL.

it was most severe at the Rochester orchard in 1995. No statistically significant difference was detected between GDM and water treatments (TMF not tested) in 1993 at either site. In 1994 (Table 1) and 1995, statistically significant differences in disease severity were detected among Latin squares (rows within the orchard) and trees within Latin squares but not among branch orientations at Madison. We attribute the differences among Latin squares to the position of the rows relative to inoculum and prevailing winds. Statistically significant differences among trees were detected for all experiments at Rochester.

In 1994 and 1995 statistically significant differences in disease severity attributable to treatment were detected at both sites. Treatment effects were pooled over branches and partitioned into contrasts (Table 2). Less disease was observed on leaves treated with captan than on those treated with compost extracts. Differences were statistically significant (but marginal for 1994 at Rochester) for both the GDM+ and TMF+ extracts compared to the negative controls (water and Latron). No differences were found between GDM+ and TMF+ (with the exception of 1995 at Rochester, where GDM+ was marginally [$P = 0.098$] superior to TMF+ in reducing disease severity) nor between water and Latron. Treatment effects and their approximate relative magnitudes were not different for evaluations on dates 5 weeks apart at Rochester in 1994 (data not shown).

Statistically significant effects of leaf type and surface, their interaction, and the interaction between leaf surface and treatment (Table 1) are explained, in part, by the uniformly effective control afforded by captan across leaf types and surfaces, as opposed to the more variable effects found with the other treatments (Fig. 2A). The pattern of severity among leaf types and surfaces was the same for GDM+, TMF+, and Latron-treated leaves. Unlike the other treatments, disease on water-treated controls was more severe on abaxial surfaces of spur leaves than on adaxial surfaces of terminal leaves. In 1994, no difference was observed among treatments (including captan) on adaxial surfaces of spur leaves. The pattern of disease severity differed across years, by leaf surfaces and types, probably varying with tree phenology during infection periods. In 1995 adaxial surfaces of terminal leaves were the most severely affected; abaxial surfaces of spur leaves were least affected by disease (data not shown). Several transformations (logarithmic, angular, and raw H-B ratings) were attempted to reduce problems associated with heterogeneity of variances among treatments. The success of the inverse transformation in homogenizing variance may be inferred from Figure 2A; that it was needed is apparent in Figure 2B.

Assessment and ranking of treated branches by overall symptomatology (Fig. 2C) with analysis by the Friedman test revealed patterns of difference similar to the more time-consuming semi-continuous classifications derived from the H-B scheme (Fig. 2A, B, and D). However, assessment by ranking lacked the sensitivity to detect small differences. When differences could be detected, treatments with GDM+ and TMF+ were better than spreader-sticker and water controls but not different from each other. Captan was always the most effective treatment. Once established, patterns of differences among treatments generally were consistent through the season.

With respect to fruit quality and yield, in 1993 only the captan treatment differed from the water treatment at either site. Fruit set and maturation were erratic at both sites, and disease severity on fruit was high: only about 70% of the fruit on branches treated with captan could have been sold at market, although many fruits from all treatments were suitable for cider. Because of a late frost at Rochester, few fruits were produced in 1994. At Madison, fruits were more abundant on extract-treated branches than those on water- and Latron-treated control branches, but they were of unmarketable quality. In 1995, possibly because of high temperatures and a drought at Madison, all fruit that set during May and June dropped by mid-July. Drought stress was lower at Rochester, and although disease severity was high, trees produced many fruit that were evaluated by rank, as well as foliage, at harvest. Several

TABLE 3. Analysis of variance for incidence of apple scab (proportion of leaf surfaces infected) at Madison, WI, in 1994

Source	df	Type III MS	F[a]	P
Square[b]	2	0.953	11.11	0.002
Error (square)	12	0.086	1.76	0.095
Treatment[c]	4	1.014	20.55	0.0001
Position[d]	4	0.033	0.66	0.624
Square × position	8	0.076	1.53	0.180
Square × treatment	8	0.030	0.61	0.764
Error (branch)	36	0.049		
Leaf type[e]	1	5.018	102.83	0.0001
Treatment × leaf type	4	0.037	0.75	0.562
Position × leaf type	4	0.036	0.74	0.567
Error (leaf type)	66	0.049		
Surface[f]	1	5.070	211.62	0.0001
Leaf type × surface	1	0.071	2.94	0.089
Treatment × surface	4	0.037	1.56	0.188
Treatment × leaf type × surface	4	0.025	1.03	0.392
Position × surface	4	0.044	1.83	0.126
Position × leaf type × surface	4	0.008	0.34	0.851
Error (leaf surface)	132	0.024		

[a] Test statistics and P values were calculated with the appropriate error terms, i. e., the indicated mean square (MS) for the closest source of error below the evaluated term in the first column. Error (square) = tree (square); error (branch) = tree × position × treatment (square); error (leaf type) = tree × position × treatment × leaf type (square); error (leaf surface) = tree × position × treatment × leaf type × surface (square). A nominal P value of 0.0001 signifies ≤0.0001.

[b] Square: Latin square (row within orchard).

[c] Treatment: captan, GDM+ (Gourmet's Delight Mushrooms, Eden, WI, amended with Latron), TMF+ (Terry Farms Mushrooms, Princeton, IL, amended with Latron), Latron, or water.

[d] Position: branch orientation (compass direction).

[e] Leaf type: lateral (spur) or terminal.

[f] Surface: adaxial or abaxial.

TABLE 4. Protected t tests[a] for disease incidence

Comparison[b]	Madison 1994		Rochester 1994[c]		Madison 1995		Rochester 1995[d]	
	t	P	t	P	t	P	t	P
Captan vs. GDM+ and TMF+	49.32	0.0001	13.63	0.001	188.55	0.0001	82.11	0.0001
GDM+ vs. water and Latron	3.17	0.084	2.79	0.110	6.70	0.014	7.45	0.010
TMF+ vs. water and Latron	4.26	0.046	3.80	0.065	7.87	0.008	1.35	0.254
GDM+ vs. TMF+	0.06	0.807	0.06	0.812	0.04	0.851	1.16	0.284
Water vs. Latron	0.14	0.714	1.76	0.199	0.38	0.543	0.01	0.906

[a] The probability of obtaining a more extreme overall result (F test) by chance in each case was ≤0.0001. The error terms were tree × position × treatment (square) for Madison, WI, and tree × treatment for Rochester, WI, orchards.

[b] GDM+ = Gourmet's Delight Mushrooms, Eden, WI, amended with Latron; TMF+ = Terry Farms Mushrooms, Princeton, IL, amended with Latron.

[c] Data collected on 22 August 1994.

[d] The contrasts, calculated on the basis of randomized complete blocks for Rochester in 1995, were for 12 trees for the comparisons of GDM+ to TMF+ and each versus water (in the entries indicating water and Latron),GDM+ and TMF+ versus captan, and water versus Latron were based on 6 trees.

fruits on extract-treated branches escaped serious infection. Those on branches treated with GDM+ and TMF+ were not different from each other but were marginally better than those on branches treated with water or Latron.

Disease incidence. Disease incidence, as with disease severity, was consistently lower at the Rochester orchard than at the Madison orchard (Fig. 3). This difference was most marked in 1994, when far fewer leaves were symptomatic at Rochester than at Madison. As with disease severity, significant differences were discerned for the effects of location in the orchard (Latin square and tree within Latin square) but not for position within a tree (Table 3). The only statistically significant interaction was for leaf type × leaf surface, which indicates that different frequencies of infection within leaf types were not uniform for leaf surfaces (or, conversely, that different frequencies of infection within leaf-surface classes were not uniform across leaf types [Fig. 2D]). This implies that infections on different surfaces of a leaf were independent.

Captan significantly reduced the proportion of diseased leaf surfaces in all 3 years at both sites (Fig. 3). Branches that received GDM treatment did not differ in disease incidence from water-treated branches in 1993 at either site. Treatment differences were significant and partitioned into contrasts in 1994 and 1995 (Table 4). Disease incidence manifested a pattern of differences very much like that for disease severity: captan was effective in reducing the frequency of infection, whereas GDM+ and TMF+ extracts were generally better at inhibiting infection than water or Latron. Disease incidence was reduced by GDM+ but not by TMF+ treatment at Rochester in 1995, whereas GDM+ was no better than negative controls at Rochester in 1994.

Spreader-sticker adjuvants. The addition of Latron had no effect on disease severity ($F_{3,15} = 0.03$, $P = 0.992$) nor did the addition of fish oil ($F_{3,15} = 1.27$, $P = 0.320$). Disease incidence was not different among treatments in these experiments.

Effects of compost extracts on phyllosphere microflora. For at least 12 days after extract application, phylloplane bacterial population densities were elevated by at least 1 order of magnitude ($P < 0.0001$) (Fig. 4A). No significant time × treatment interaction was observed for bacteria ($P = 0.548$). The differences in bacterial population density diminished and were not significant 28 days after the final spray application in 1994 (Fig. 4B). Fungal population densities were not higher relative to water controls for the 12 days after applications of treatments (Fig. 5A, $P = 0.266$). Regardless

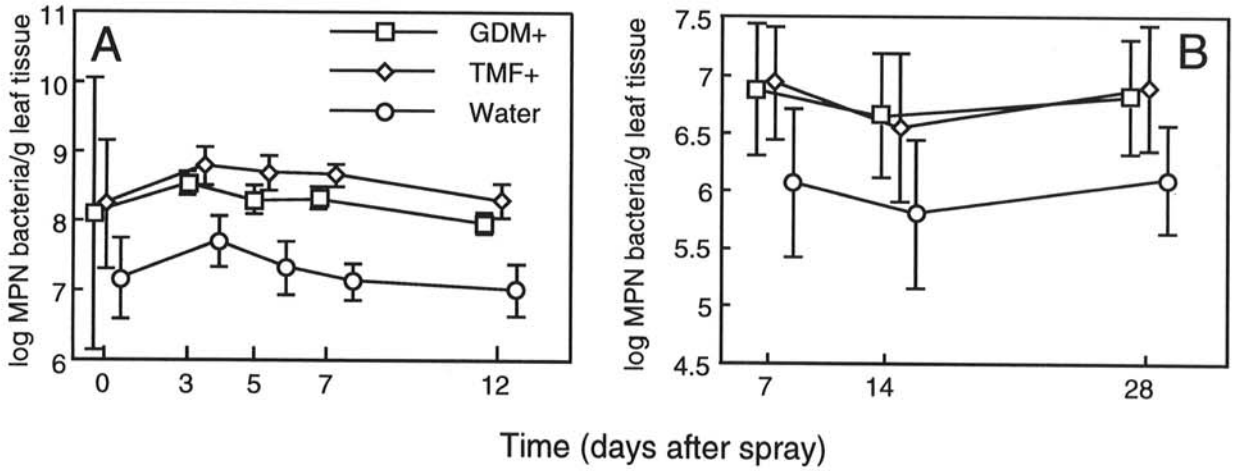

Fig. 4. Most probable numbers (MPN) of bacteria recovered from apple leaf washings **A,** immediately after spraying and 3, 5, 7, and 12 days later during July 1995 (symbols displaced for easier viewing) and **B,** at 7, 14, and 28 days (symbols displaced) after the last spray during August to September 1994. Vertical lines represent the 95% confidence intervals. Greenhouse-Geiser $\varepsilon = 0.83$ and 0.94 for **A and B**, respectively, indicating no autocorrelation of data (18). GDM+ = spent mushroom substrate from Gourmet's Delight Mushrooms, Eden, WI, amended with Latron; TMF+ = spent mushroom substrate from Terry Farms Mushrooms, Princeton, IL, amended with Latron.

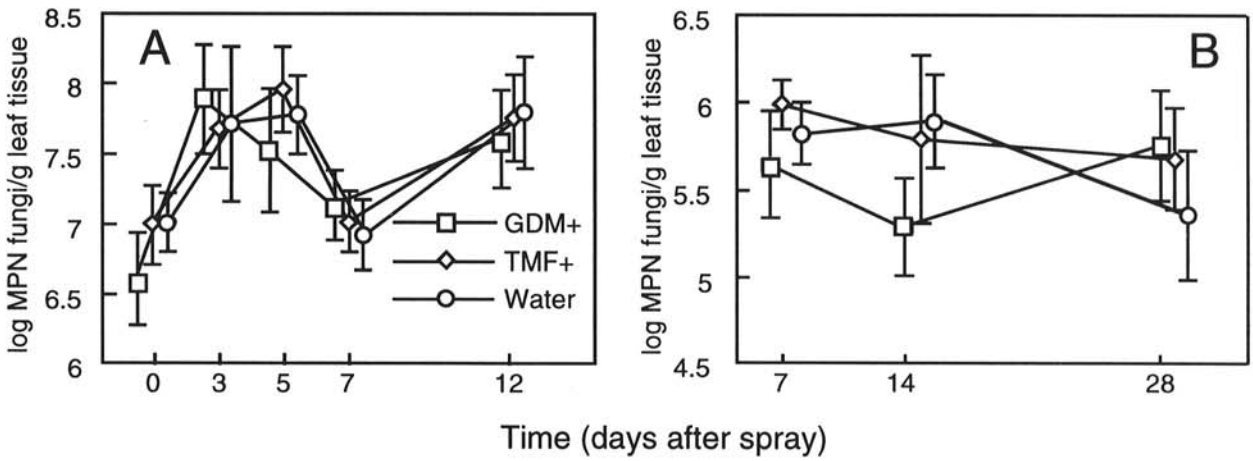

Fig. 5. Most probable numbers (MPN) of fungi recovered from apple leaf washings **A,** immediately after spraying and 3, 5, 7, and 12 days later during July 1995 (symbols displaced for easier viewing) and **B,** at 7, 14, and 28 days (symbols displaced) after the last spray during August to September 1994. Vertical lines represent the 95% confidence intervals. Greenhouse-Geiser $\varepsilon = 0.80$ and 0.92 for **A and B**, respectively, indicating no autocorrelation of data (18). GDM+ = spent mushroom substrate from Gourmet's Delight Mushrooms, Eden, WI, amended with Latron; TMF+ = spent mushroom substrate from Terry Farms Mushrooms, Princeton, IL, amended with Latron.

of treatment, fungal population size varied significantly with time (Fig. 5A, $P \leq 0.0001$; Fig 5B, $P \leq 0.0001$).

Compost weathering. A decline in in vitro inhibitory activity of extracts assayed during the spring and summer of 1993 prompted us to compare methods of compost storage. Because we lacked fresh material for some dates in 1994 a rigorous comparison was not possible, although the data (not shown) supported the 1995 results (Fig. 6). There was no difference in inhibitory activity among extracts from stored (covered and uncovered) TMF composts relative to fresh material throughout the 18-week sample period.

GDM composts, however, had declined in efficacy after 13 weeks of storage under both covered and uncovered conditions. We infer from results of weighted least squares regression (used because of increasing error variance over time) over the period from 7 to 19 weeks after collection that there was a significant decline in inhibitory activity ($P = 0.018$) but no significant difference between the storage regimes ($P = 0.562$). Simple linear regression resulted in $R^2 = 0.70$ and 0.62 for weighted and unweighted models, respectively. Weights for the regression were calculated on standardized inverse variances (20). The common slope for the rate of decline for extracts from GDM compost between 7 weeks and the end of the experiment was –0.028 (transformed inhibition) per week with a standard error of 0.006 ($t = 5.14$, $P < 0.0001$). There was less than 1% decline in inhibition of conidial germination by GDM extracts for each week of compost storage.

DISCUSSION

SMS from two sources significantly reduced apple scab in the field, though it was less effective even under moderate disease severity than the fungicide captan. This negative assessment is somewhat ameliorated by recognition that this biocontrol agent is being compared to a chemical treatment for which optimal rates and formulations are known. Moreover, disease severity at the Madison site was unrealistically high relative to levels typically observed in commercial orchards.

In 1994 and 1995, lesions were smaller and less frequent on extract-treated leaves than on negative controls. We inferred that the extracts inhibited infection and lesion expansion. Tränkner and Kirchner-Bierschenk (24) similarly reported a decrease in the number of scab lesions, but few escapes, on fruit.

Evaluation of individual leaves was no more powerful for detection of differences among treatments than the far quicker method of establishing relative ranks within trees (Fig. 2). However, analysis of ranking allows no estimate of the magnitude of differences among treatments. Hence, we feel the more laborious method is merited, once per season, for estimating quantitative distinctions among treatments.

Achimu and Schlösser (1) showed that protection imparted by extracts against downy mildew of grape was not translocated across leaf surfaces nor persistent after removal of compost-extract residues by washing. Jongebloed et al. (10), working with late blight of potato, and Tränkner and Kirchner-Bierschenk (24), working with apple scab, suggested removal of extract by rainfall as a possible cause of the failure of extracts under field conditions. Thus, we had hoped that adding spreader-sticker would increase the adherence and improve the distribution of SMS extracts. However, no difference could be detected among extracts with and without spreader-sticker. Either distribution/adhesion of extracts was not improved under our conditions, or if it was, the enhancement did not measurably affect efficacy.

Larger numbers of bacteria were recovered from leaves to which SMS extracts had been applied than from control leaves. Whether this is indicative of high survival rates of applied bacteria in the suspension (12,27) or nutritional supplementation of indigenous populations is not known. Elevated populations of pseudomonads, enterics, actinomycetes, and bacilli in compost extracts have been reported, and extract activity has been attributed to their presence (12,23,27). Evidently, populations of easily detached sporulating fungi are not affected by treatment with SMS extracts. The apparently benign or stimulatory effects of SMS extracts on nontarget microbiota could be important in developing SMS extracts as a component of an integrated biocontrol strategy.

We observed no decline in the in vitro ability of TMF extracts to inhibit conidial germination over time in storage, whereas extracts produced from GDM from both storage regimens became less effective than those produced from fresh (not aged) material sometime after 7 weeks of storage. Consistent with earlier observations (31), variability increased inversely with decline in in vitro efficacy. The observed greater stability of TMF extracts may have been due to the greater uniformity of the substrate relative to the unsieved GDM; to the aerated steam treatment, which may have given a competitive advantage to members of the antagonist community in colonizing the material; or to stabilization of the material prior to collection.

Lack of economically meaningful scab control, combined with more promising control of red pine shoot blight (29), indicates the need for further research into the suppressive mechanism(s) of SMS extracts so efficacy can be enhanced. Cronin et al. (5) reported that sterilized SMS extracts inhibited *V. inaequalis* conidial germination as effectively as untreated filtrates and that a thermostable, nonprotein, low molecular weight product of anaerobic fermenta-

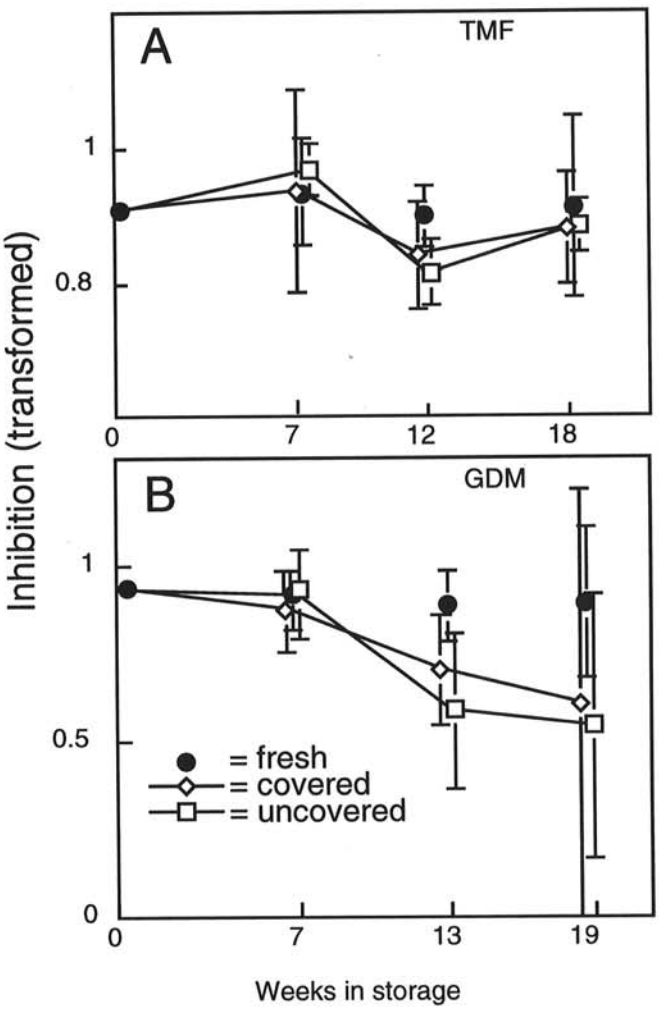

Fig. 6. In vitro inhibition of *Venturia inaequalis* conidia by extracts produced from fresh or stored (covered or uncovered) composts in 1995. Vertical lines represent the 95% confidence intervals. Values for the first date (4 April) are for 1:1 dilutions of pooled field extracts, hence no estimate of sample error could be made. **A**, TMF (spent mushroom substrate from Terry Farms Mushrooms, Princeton, IL) extracts; **B**, GDM (spent mushroom substrate from Gourmet's Delight Mushrooms, Eden, WI) extracts.

tion apparently was responsible. Further, if SMS extracts are to become realistic alternatives to fungicides for scab control, their performance will have to be increased. One approach to accomplishing this is further characterization of the chemical(s) and the microbial population(s) involved in its production.

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