Mushroom Brown Blotch: Effects of Chlorinated Water on Disease Intensity and Bacterial Populations in Casing Soil and on Pilei

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ABSTRACT

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Severity and incidence of brown blotch caused by *Pseudomonas tolaasii* (=*P. fluorescens*) in the cultivated mushroom (*Agaricus bisporus*) was reduced by routine waterings with chlorinated water. Both disease severity and incidence were closely correlated with the occurence of *P. tolaasii* on mushroom pilei, but not with numbers of *P. tolaasii* in the casing soil. Furthermore, bacterial populations in the casing soil were not correlated with those on the surface of mushroom pilei. Thus, control of brown blotch apparently is due to protection of pilei rather than reduction of bacterial populations in casing soil. The efficacy of chlorinated water was not significantly increased by acidification (pH 3.5) with hydrochloric acid.

Additional key words: calcium hypochlorite, *Pseudomonas fluorescens* biotype Va, selective medium, *A. brunnescens*.

Bacterial bloch of cultivated mushrooms, first described by Tolaas (14), is characterized by brown spots or blotches on the pilei and, in more severe cases, on the stipes. Tolaas (14) identified the causal organism as a pathogenic strain of *Pseudomonas fluorescens* Migula, but Paine (9) working with other isolates found differences in their action on nitrates and starch and proposed the name *P. tolaasii* Paine. Lelliott et al (8) showed that *P. tolaasii* was indistinguishable from some isolates of *P. fluorescens* and suggested that *P. tolaasii* could be regarded as a normal constituent of the microflora of mushroom beds.

Chemical control of bacterial blotch with water chlorinated with 100–200 μg/ml of active chlorine has been practiced according to the recommendation of Ayers and Lambert (1). They observed that disease severity gradually decreased with successive waterings and suggested that blotch was being controlled by eliminating the blotch organism in the soil rather than protecting the surface of the mushroom caps. Wuest and Patton (16–18) obtained control with chlorinated water, but suggested efficacy was influenced by specific environmental regimes. Some mushroom growers routinely acidify chlorinated water to pH 3.5 prior to application (P. J. Wuest, *personal observation*) because it is purported to increase efficacy.

The purposes of this study were: to determine the effects of chlorinated water on *P. tolaasii* populations in casing soil and on mushroom caps, to determine the relation of disease intensity to observed populations, and to determine whether chlorinated water acidified to pH 3.5 is more effective in reducing disease intensity than chlorinated water alone.

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MATERIALS AND METHODS

Compost preparation. The standard procedures followed for mushroom crop tests at The Pennsylvania State University's Mushroom Research Center, University Park, PA were used as described by Carroll and Schisler (2). All compost was prepared according to the short method of Sinden and Hauser (13) with wheat straw-bedded horse manure. This required two phases. The outdoor composting (phase I) had a duration of 7 days with turnings at 0, 2, 5, and 7 days. As much water as possible (without leaching) was added to the compost on the first two turnings. Little or no water was applied on the third turning. The compost was adjusted to the desired moisture content (73–76%) when the compost was turned and filled. All supplements were added on the first two turnings. Dried chicken manure and brewers' grains each were added at the rate of 22.7 kg/ton of dry horse manure. Gypsum was added at the rate of 34 kg/ton (dry). The average nitrogen content, determined by the Kjeldahl method, of the compost at filling was 1.75%.

Phase I was followed by an indoor decomposition/pasteurization process (phase II). Nitrogen content of the compost after the completion of phase II was 2.2%.

Spawning. Pasteurized compost was mixed with a specially designed compost turner and emptied into one pile. As the tared trays were refilled, the compost was spawned at the rate of 110 g of spawn (sterilized rye grains thoroughly colonized with mushroom mycelium) per 0.37 m^2 (4 ft^2) tray. A smooth white strain of mushroom (culture 310 from The Pennsylvania State University Mushroom Culture Collection) maintained as outlined by Jodon and Royse (6) was used in this investigation. After spawning, all

trays were placed in a controlled-environment room in which the relative humidity was maintained at 95–98% and the temperature within the compost at 22–24 C.

Casing. Soil used for casing was moistened to a moisture content of 17–18% (wet weight basis) as it was filled into boxes for pasteurization. Air temperature in the pasteurization room was set and maintained at 73.9 C (165 F) with live steam. When the lowest soil temperature reached 68.3 C (after approximately 10 hr) the steam was turned off and the soil was allowed to cool. The Hagerstown silty clay-loam soil had the following properties: pH 7.5, cation exchange capacity 19.6, organic matter 2.2%, and milliequivalents of potassium, magnesium, and calcium per 100 g soil were 0.49, 1.1, and 18.0, respectively. A 3.0- to 3.5-cm layer of the pasteurized soil was placed on the surface of the compost to induce basidiocarp formation.

Bacterial seeding of casing soil. After casing, 100 ml of an aqueous suspension (3.6×10^8 cells per milliliter) of the bacterial blotch pathogen were sprayed onto the surface of the casing layer for each tray. The bacterial blotch pathogen, isolate 5462 of *P. tolaasii,* originally was isolated from infected mushrooms at the Plant Disease Clinic of The Pennsylvania State University. Pathogenicity of the isolate was confirmed according to Koch's postulates and a 24-hr culture of the bacteria in nutrient broth was used as inoculum. During the next 2 wk after casing, the ambient air temperature was gradually lowered to 15 C, and was maintained at this temperature for the remainder of the production period. Relative humidity was maintained at 85–95% to encourage blotch development. Trays were arranged in a randomized complete block design with six replications.

Application of HTH dry chlorinator. When mushroom primordia were well formed (9 days after casing) and were beginning to enlarge, waterings (11) with chlorinated water were substituted for regular routine waterings where appropriate. Regular and chlorinated waterings were applied with a 50-cm-long wand nozzled with a 750-hole rose face. The water pressure was adjusted before watering began so that a fine spray would fall in a gentle arc onto the surface of the soil approximately 50 cm from the rose face. Care was taken to maintain a uniform height of the rose face to minimize destruction of aggregation at the soil surface. Treatments included: (i) calcium hypochlorite ($CaOCl_2$ [HTH dry chlorinator, Olin Corp., 120 Long Ridge Road, Stanford, CT 06904]) at 175 μg/ml a.i., (ii) calcium hypochlorite at 175 μg/ml a.i. acidified to pH 3.5 with concentrated HCl, (iii) water acidified to pH 3.5 with HCl, and (iv) nonchlorinated tap water only (pH 6.5).

Selective medium for bacterial recovery. The selective medium used for recovery of bacteria from soil and mushroom pilei consisted of King's medium B (7) adjusted to pH 7.2 with 0.1N. NaOH prior to autoclaving. Antibiotics added to 940 ml of melted (45 C) basal medium after autoclaving for 15 min at 121 C included: penicillin G (75,000 units), novobiocin (45 mg), and cycloheximide (75 mg) (Sigma Chemical Co., St. Louis, MO 63178). The antibiotics were combined in 3 ml of 95% ethanol, then diluted with 50 ml of sterile distilled water (10). After 20 ml of the amended medium was added, the plates were allowed to dry over night and then refrigerated in polyethylene bags until use a few days later.

Recovery of bacteria from infested casing soil. Two 1-g soil samples were collected at five random areas from each tray of each treatment with a plastic teaspoon. One sample was used to determine the moisture content. The second soil sample was placed in a 16 × 150-mm culture tube to which 10 ml of sterile distilled water was added. After agitation with a Vortex mixer for 1 min, serial dilutions (1:10) were made and 0.1-ml aliquots were plated on the selective medium. Plates were incubated for 4 days at 25± 1 C and the bacterial colonies were counted. Raw data were transformed to raise all numbers to the same basis; ie, bacterial cells per gram of dry soil.

Disease assessment. Ten randomly selected mushrooms from each tray were rated for disease severity (percent tissue area affected) according to the Horsfall-Barratt grading scale (5). The readings were converted with Elanco conversion tables (Eli Lilly and Co., Indianapolis, IN 46206) into percentage pileus area infected. Disease incidence ([number of mushrooms infected]/[number of mushrooms examined] × 100) also was recorded. Assessments were made at the peak of days of the first and second break or flush.

Recovery of bacteria from mushroom pilei. Following disease assessment, mushroom pilei were aseptically excised (at the base of the pileus) from the stipe, weighed, and placed in a 1-L Erlenmeyer flask to which 300 ml sterile distilled water was added. After 1 min of steady, uniform, hand-swirling, 3-ml aliquots were removed, stored at 4 C, and plated on selective medium within 6 hr as described above. Raw data were expressed as numbers of bacteria per gram of fresh mushroom tissue.

RESULTS

Bacterial populations on pilei and in casing soil. Bacterial populations recovered from casing soil were not significantly ($P = 0.05$) different regardless of treatment (Tables 1 and 2). This was consistent within first and second breaks. There was, however, a substantial increase in the numbers of bacteria isolated from casing soil at the second break compared with the first break.

Pseudomonas tolaasii populations were significantly ($P = 0.05$) higher on mushroom pilei watered with tap water only than on pilei watered either with HTH dry chlorinator alone or HTH dry chlorinator at pH 3.5 (Tables 1 and 2). This result was consistent for both breaks, implying that HTH dry chlorinator controlled blotch by reducing or inhibiting bacterial reproduction on the surface of the pileus.

Bacterial blotch intensity. Blotch developed rapidly in the environmental regime used in this study. Blotch incidence was 90% or more in control treatments for both breaks (Tables 1 and 2). Both HTH dry chlorinator and HTH dry chlorinator acidified to pH 3.5 with HCl gave significant reduction in both severity and incidence for both breaks. HTH dry chlorinator acidified to pH 3.5 with HCl did not result in significantly better control than HTH dry chlorinator alone at either of the two breaks examined (Tables 1 and 2). Disease severity remained relatively the same at both breaks when HTH dry chlorinator or HTH dry chlorinator at pH 3.5 were

TABLE 1. Bacterial blotch intensities and *Pseudomonas tolaasii* populations on mushroom pilei and in casing soil at the time of first break

Treatments	Disease intensity		Bacterial populations ($\times 10^6$)	
	Incidence (%)	Severity (%)[u]	Casing Soil[v]	Mushroom pilei[w]
Control (tap water, pH 6.5)	96.7 a[x]	13.8 a	5.60 a	20.43 a
HTH dry chlorinator[y]	51.7 b	2.1 b	5.58 a	1.89 b
HTH acidified to pH 3.5[z]	46.7 b	2.0 b	3.87 a	0.37 b
Acidified H_2O, pH 3.5[z]	93.3 a	9.0 a	5.20 a	4.25 ab

[u] Data are Horsfall and Barratt's scale of 1-12 (Phytopathology 35:655) converted to percentage of disease severity.
[v] Expressed as number of bacteria per gram of dry soil.
[w] Expressed as number of bacteria per gram (fresh weight) of mushroom tissue.
[x] Numbers followed by the same letter are not significantly different at $P = 0.05$ according to Duncans's multiple range test.
[y] HTH is 65% calcium hypochlorite applied at 175 μg/ml (a.i.) as a routine watering.
[z] Acidified with concentrated HCl prior to bed application.

TABLE 2. Bacterial blotch intensities and *Pseudomonas tolaasii* populations on mushroom pilei and in casing soil at the second break

Treatments	Disease intensity		Bacterial populations ($\times 10^6$)	
	Incidence (%)	Severity (%)[u]	Casing Soil[v]	Mushroom pilei[w]
Control (tap water, pH 6.5)	90.0 a[x]	7.2 a	45 a	20.9 a
HTH dry chlorinator[y]	63.3 b	2.0 b	60 a	0.2 b
HTH acidified to pH 3.5[z]	43.3 b	1.8 b	96 a	0.1 b
Acidified H_2O, pH 3.5[z]	88.3 a	5.9 a	12 a	11.6 ab

[u] Data are Horsfall and Barratt's scale of 1-12 (Phytopathology 35:655) converted to percentage of disease severity.
[v] Expressed as number of bacteria per gram of dry soil.
[w] Expressed as number of bacteria per gram (fresh weight) of mushroom tissue.
[x] Numbers followed by the same letter are not significantly different at $P = 0.05$ according to Duncan's multiple range test.
[y] HTH is 65% calcium hypochlorite applied at 175 μg/ml (a.i.) as a routine watering.
[z] Acidified with concentrated HCl prior to bed application.

TABLE 3. Correlations among disease assessments and *Pseudomonas tolaasii* populations on mushroom pilei and in casing soil at two consecutive breaks or flushes

	Disease intensity		Bacterial populations	
	Incidence (%)	Severity (%)[a]	Casing Soil	Mushroom pilei
Disease intensity				
Incidence				
Break 1		0.85**[b]	0.19	0.58*[c]
Break 2		0.88**	0.16	0.52*
Severity (%)				
Break 1			0.08	0.68*
Break 2			0.27	0.58*
Bacterial populations				
Casing Soil				
Break 1				0.11
Break 2				−0.15

[a] Data are Horsfall and Barratt's scale of 1-12 (Phytopathology 35:655) converted to percentage of disease severity.
[b] Significant at the 0.01 probability level.
[c] Statistically significant, $P = 0.05$.

used for routine waterings. Disease severity was slightly less at the second break for all treatments than at the first break. This occurrence was more pronounced for tap water only and tap water at pH 3.5 than for either of the HTH dry chlorinator treatments (Table 1 and 2).

Correlation coefficients (r) between *P. tolaasii* populations on mushroom pilei and disease incidence at break 1 (r = 0.58) and break 2 (r = 0.52) were significant (Table 3). *Pseudomonas tolaasii* populations on mushroom pilei and disease severity ratings were significantly ($P = 0.05$) correlated at break 1 (r = 0.68) and break 2 (r = 0.58). The correlations between disease severity and incidence at break 1 and break 2 (r = 0.85 and r = 0.88, respectively) were highly significant. Casing soil populations of *P. tolaasii* and either disease incidence or disease severity were not correlated at either break. Correlation coefficients between *P. tolaasii* populations in the casing soil and on mushroom pilei were low and nonsignificant.

DISCUSSION

The infection court of the blotch bacterium is the outer surface of the mushroom at any stage of its growth (3, 4, 12). The bacteria are splashed onto the mushroom from the soil during watering, or they may reach the surface of the mushroom during the early stage of their development while the young pileus is still in contact with the casing soil. Under favorable conditions, incubation time can be as little as a few hours (12). If the surface of the mushroom dries within an hour or two, no infection occurs. Therefore, control requires either drying the surface of the mushroom quickly or protecting it with a material that prevents bacterial reproduction or a combination of both.

Our results indicate that chlorinated water reduces bacterial reproduction on the surface of the mushroom and not in the soil as suggested by Ayers and Lambert (1). Our results also indicate that the bacterial population on the mushroom pileus is not necessarily related to the population in the soil. We contend that blotch severity is determined by the ecological factors at the mushroom surface. These findings substantiate, in part, the contentions of Sinden (12) who stated that slight fluctuations in temperature at the surface of the mushroom are of utmost importance in providing the moist conditions necessary for pathogenesis.

Wuest (15) related various dew point/ambient temperature (DP/AT) differentials to disease incidence. He found that disease incidence decreased as the number of days with 15 hr or more of DP/AT differentials of 3 F or more increased. According to Sinden (12) brown blotch is in some cases not a problem where the relative humidity is usually around 99% and where air temperatures rarely fluctuate more than 0.5 C.

More research on the relationship of mushroom surface wetness to disease progress is urgently needed. On aboveground farms where the relative humidity is maintained at 80–82% (3), brown blotch is no problem. Under those conditions, however, certain much-used mushroom cultivars become too scaly or colored for premium marketing.

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