Effect of an Extract from Saprozoic Nematode-Infested Compost on the Mycelial Growth of *Agaricus brunnescens*[1]

T. D. KAUFMAN, J. R. BLOOM, and F. L. LUKEZIC[2]

Abstract: Extracts from compost infested with *Caenorhabditis elegans* suppressed mycelial growth of *Agaricus brunnescens*. An extract from uninfested compost also inhibited mycelial growth but to a lesser degree. The critical role of compost bacteria and/or other compost micro-organisms is implicated by these results. *Key words:* free-living nematodes, mushrooms, leachate.

Journal of Nematology 15(4):567-571. 1983.

Many theories have been offered to explain the degenerative disease condition of the cultivated mushroom associated with saprozoic nematode infestations. One such theory suggests that a metabolic toxin may be produced by the nematodes and/or other compost micro-organisms associated with the nematodes. The role of compost bacteria and other micro-organisms may be critical to the establishment of this degenerative disease. Cairns and Thomas (5) were the first to propose the toxin theory but did no experimentation. According to Moreton (12), Sarazin observed that nematode-free water leached from mushroom beds infested with parasitic nematodes caused a suppression in yield when it was applied to uninfested beds. Using the quantity of extracellular laccase present in a compost extract as a measure of mycelium development, we examined the effect of an extract from nematode-infested compost on growth of mushroom mycelium in compost.

MATERIALS AND METHODS

Two experiments with the same basic experimental design having four different treatments per experiment were conducted. Each treatment consisted of 20 replicates. The off-white mushroom cultivar PSU-348, used in all experiments, was obtained from the culture collection maintained at Pennsylvania State University. Treatments for both experiments were a compost control, a spawned control, and a nematode-infested compost extract. A nematode-inoculated treatment was used in the first experiment and a control compost-extract treatment in the second. An extract from compost containing a population of *Caenorhabditis elegans* (Maupas) Dougherty and associated micro-organisms was applied to the infested compost (10). The Waller-Duncan K-ratio t-test (13) was used to analyze the weekly means.

The moisture content of the compost used for the two studies was determined after adding water. The moisture content for the first experiment was 78.4%. At this level, water was easily squeezed by hand from the compost. The moisture of the compost used for the second experiment was 75.1%. This compost did not exude water when squeezed.

Each treatment included 50 g of pasteurized wheat straw-bedded horse manure compost (15) placed in a 100-ml beaker. Spawned treatments received 1 g of grain spawn which was mixed with the preweighed compost before it was placed in the beaker.

The nematodes used for the inoculated treatments were extracted from compost cultures using a modified Baermann funnel technique (3). Approximately 150 viable nematodes in 3 ml of water were placed into each beaker compost.

In the first experiment, one treatment was exposed to an extract derived from compost infested with *C. elegans*. One treatment in the second study received a similar extract, and a second was exposed to an extract obtained from compost containing no nematodes. Each inoculated treatment received 15 ml of extract solution applied to the surface of the compost at the beginning of the experiment. The control treatments in both studies received 15 ml of the buffer solution used in the extraction technique.

Compost used for extraction was prepared 4 weeks prior to processing. Addi-

Received for publication 14 December 1982.

[1]Contribution No. 1374, Department of Plant Pathology, Pennsylvania Agricultural Experiment Station. Authorized for publication 12/1/82 as Journal Series Paper No. 6563.

[2]Respectively, former Graduate Research Assistant and Professors, Department of Plant Pathology, Pennsylvania State University, University Park, PA 16802. Current address of senior author: 607 W. Seventh Avenue, Lenoir City, TN 37771.

tional water was added to the compost prior to pot filling to increase the moisture content thus favoring nematode colonization and development. Approximately 2,200 g of compost was placed into two 25-cm × 13-cm plastic pots. The pots to be infested received approximately 3,000 *C. elegans* in a 50-ml aliquot of water applied over the surface of the compost. The control treatment received 50 ml of water without nematodes. A thin layer of a 1:1 (v/v) sphagnum peat moss and limestone mixture was applied to the surface of the compost to a depth of 1.3 cm. This material was kept moist throughout the preextraction period. The pots were maintained at a temperature of 20–22 C.

After 4 weeks, 1 kg of compost from each treatment was processed for laccase (11). Two hundred and fifty grams of compost and 250 ml of 0.01 M potassium-phosphate buffer, pH 7.0, were placed in a 1,000-ml Erlenmeyer flask and shaken for 20 min. The liquid was then poured through four layers of cheesecloth and centrifuged at 8,000 g for 20 min and at 40,000 *g* for another 20 min. The supernatant was filtered through 0.45-μm pore membrane filters.

The amount of laccase in each sample of compost extract was measured polarographically using an oxygen electrode (17, 18). A 1.0-ml sample of compost extract was combined with 1.7 ml of sodium 0.02 m acetate-acetic acid buffer, pH 5.0, in the electrode chamber. Water at 25 C circulated around the chamber. Oxidation reaction was initiated by injecting 0.3 ml of 0.1 M p-phenylenediamine into the electrode chamber. Assuming that the solubility of molecular oxygen per liter is 8.5 mg and defining a unit of laccase as the amount of enzyme needed to consume 1 μ mole of molecular oxygen per minute, the amount of laccase per milliliter of extract was calculated. The dry weight (mg) of mycelium per gram dry weight of compost was computed by assuming that there are 0.1 enzyme units/mg dry weight of mycelium and using the percent dry matter of the compost used for each experiment.

RESULTS

An estimate of the dry weight of mycelium per gram dry weight of compost was calculated for all replications at weekly intervals (Table 1). The control treatment produced more mycelium than did the other treatments over the duration of the experiment. The treatment exposed to the extract derived from nematode-infested compost produced more mycelium at the second, third, and fourth harvests than did the nematode-inoculated treatment. The inoculated treatment actually showed a slight decrease from week 1 to week 4.

The mean values of the three spawned treatments were not different at the first harvest, but were significantly higher than the control (Table 1). The values for the spawned control treatment at the second, third, and fourth harvests were significantly higher than those for the other treatments. The means of the spawned extract treatment and nematode-inoculated treatment were not different except at week 4. The nematode-inoculated treatment was

Table 1. Effects of a *Caenorhabditis elegans* infestation and an extract from infested compost on mycelial growth of *Agaricus brunnescens* cv. PSU-349 in compost.

Treatment	Mycelial growth (mg dry wt mycelium/g dry wt compost)			
	1	2	3	4
PSU-348*	0.69 a	1.17 a	4.32 a	10.94 a
PSU-348 NE†	0.55 a	0.74 b	1.07 b	2.18 b
PSU-348 N‡	0.59 a	0.34 bc	0.21 b	0.21 c
CK§	0.25 b	0.26 c	0.03 b	0.02 c

*Spawned control.
†Infested compost extract.
‡Nematode inoculated.
§Unspawned control.
Numbers in the same column followed by the same letter are not significantly different according to the Waller-Duncan K-ratio t-test, $P = 0.05$.

not different from the compost-control treatment after the first week harvest.

In the second study as in the first, the treatment spawned with PSU-348 produced noticeably more mycelium after 1 week than did the other treatments (Table 2). The treatment exposed to the extract produced from the uninoculated compost had a higher mean value of mycelium than did the infested compost extract treatment after 2 weeks. At the first two harvests, the estimated means for these two treatments were very similar. Overall, the spawned control and the infested compost extract treatments produced more mycelial growth in the second experiment than did the same treatments in the first experiment.

From week 2 on, the PSU-348 spawned treatment had a significantly higher value for the estimated mycelium as compared to the other treatments (Table 2). Mycelial growth in compost treated with compost extract was not significantly different from the infested compost extract at week 2, but was significantly greater at weeks 3 and 4.

DISCUSSION

The white and off-white types are the most commonly grown mushrooms in the United States. The off-white cultivar PSU-348 was chosen for this study because of its extensive use in the industry.

Compost infested with *C. elegans* was used to derive an extract which was evaluated for toxic characteristics. This parthenogenic nematode was also used in the first experiment to initiate the disease condition in one treatment. During the survey described by Kaufman et al. (11), *C. elegans* was the second most commonly encountered species.

An excessive amount of water was inadvertently added to the compost used for the first extract experiment and the adverse effect caused by excessive water was reflected in the production of mycelium (Table 1). The mycelial development was noticeably lower than in the second extract study (Table 2). PSU-348 produced a higher rate of mycelium under the less water-stressed conditions during the experimental period. High moisture conditions are known to favor the growth and development of nematodes. The infested treatment in the first experiment produced only a minimal amount of mycelium over the duration of the experiment. The excessively high water content of the compost probably aided in the ultimate damage caused by the saprozoic nematode disease complex.

Observers of this disease condition in a production situation have reported a variety of mycelial symptoms. The described symptoms range from a reddening of the mycelium (16) to an inhibition of mycelial growth (6) or a complete degeneration which creates dark watery patches in the compost (2,3,14). Most of the controlled studies designed to determine the effect of nematode infestations have been based on yield data. A study by Blake and Conroy (2) using a rye grain medium demonstrated that *Rhabditis* sp. noticeably reduced mycelial growth. No research had quantita-

Table 2. Effect of extracts from *Caenorhabditis elegans* infested and uninfested compost on the mycelial growth of *Agaricus brunnescens cv.* PSU-348 in compost.

Treatment	Mycelial growth (mg dry wt mycelium/g dry wt compost)			
	1	2	3	4
PSU-348*	1.20 a	7.26 a	19.79 a	27.22 a
PSU-348 CE†	1.26 a	1.74 b	8.94 b	17.01 b
PSU-348 NE‡	0.77 b	2.61 b	5.16 c	12.46 c
CK§	0.06 c	0.21 c	0.27 d	0.12 d

*Spawned control.
†Compost extract treated.
‡Infested compost extract.
§Unspawned control.
Numbers in same column followed by the same letter are not significantly different according to the Waller-Duncan K-ratio t-test, $P = 0.05$.

tively verified mycelial decline until the present studies summarized by Kaufman (10) demonstrated for the first time that saprozoic nematode infestations can significantly reduce the production of mycelium in compost.

A popular theory suggested by numerous investigators has been that a metabolic toxin produced by the saprozoic nematodes inhibits the growth or causes a degeneration of mycelium (2,3,4,8,9). Another theory suggests that the toxin is produced by bacteria and that the development of a large and harmful bacterial population is stimulated by metabolites produced by the nematodes (3,4,5,7,8). The results of the extract experiments support the theory that toxins are produced by a combined population of nematodes and bacteria. The extract from compost infested with *C. elegans* and bacteria in both experiments limited the production of mycelium (Tables 1 and 2). The question of whether or not the nematodes, bacteria, or both are responsible for the production of the inhibitory substance remains unexplained.

In the second experiment, an extract from uninfested compost significantly reduced mycelial production (Table 2). However, the adverse affect was significantly less than that seen in the treatment receiving the extract from infested compost. The inhibitory substance produced in the absence of nematodes may have been derived from compost bacteria. A possible explanation for this phenomenon is that if compost is allowed to stand, the bacteria produce substances inhibitory to mushroom mycelial growth. The presence of saprozoic nematodes in large numbers may simply increase the rate at which this process occurs. Waste metabolites produced by the nematodes may stimulate the bacterial population size by providing a food substrate. The ability of nematodes to carry bacteria superficially (1,4,12) could aid in food finding and thus population development.

The laccase assay for mycelial biomass estimation proved to be ideal for this research investigation. This technique provides an acceptable means of acquiring quantitative estimates of mycelium in a sample of compost.

This study should help understanding of the degenerative disease condition of the cultivated mushroom associated with saprozoic nematodes. The potential for damaging infestations continues to remain a reality. High energy costs have forced conservation on many mushroom farms, and this trend has led to a revival of the natural cook-out as an energy saver (19). But, "natural cook-out" and other techniques which rely on lower temperatures for pasteurization, increase the possibility of nematode survival and potential problems. For this reason, the incidence of nematode infestations is not likely to diminish in the near future.

LITERATURE CITED

1. Atkins, F. C. 1966. Mushroom growing today. New York: The Macmillan Company.

2. Blake, C. D., and R. J. Conroy. 1959. Some nematodes as factors in yield reduction and spawn degeneration in the cultivated mushroom Agaricus hortensis (Cke.) Imai. J. Aust. Inst. Agric. Sci. 25:213-216.

3. Bloom, R. J. 1977. Nematodes (eelworms): Sources of infestation and methods of testing. Mushroom News 25(12):9-11.

4. Cairns, E. J. 1952 Nematode disease and their control in mushroom crops. Phytopathology 42:4 (Abstr.).

5. Cairns, J., and C. A. Thomas. 1950. Nematodes causing mushroom crop losses. Mushroom Sci. 1:89-91.

6. Conroy, R. J., and C. D. Blake. 1959. Nematode pests of mushrooms. Ag. Gaz. of New South Wales 70:646-648.

7. Hesling, J. J. 1966. The effect of some microphagus saprobic nematodes on mushroom culture. Ann. Appl. Biol. 58:477-486.

8. Hesling, J. J. 1979. The role of eelworms (nematodes) in the culture of mushrooms in the UK. Mushroom J. 82:423-429.

9. Ingratta, F. J., and Th. H. A. Olthof. 1978. The influence of saprophagous nematodes on the production of Agaricus brunnescens (bisporus). Mushroom Sci. 10 (Part 2):397-405.

10. Kaufman, T. D. 1982. A study of degenerative disease conditions of the cultivated mushroom, Agaricus brunnescens Peck associated with saprophagous nematodes. M.Sc. thesis. Pennsylvania State University, University Park.

11. Kaufman, T. D., J. R. Bloom, and F. L. Lukezic. 1983. Saprophagous nematodes. Mushroom News 31(2):9-10.

12. Moreton, B. D. 1953. Eelworms as pests of mushrooms. MGA Bull. 41:149-152.

13. Ott, L. 1977. An introduction to statistical methods and data analysis. North Scituate, Massachusetts: Duxbury Press.

14. Sanderson, F. R., J. W. Marshall, G. E. Ovenden, and H. M. Stengs. 1981. Problems in New Zealand mushroom production associated with bacterial feeding nematodes (Mesorhabditis

sp.). Mushroom Sci. 11:609-619.

15. Sinden, J. W., and E. Hauser. 1950. The short method of composting. Mushroom Sci. 1:52-59.

16. Thomas, C. A., and G. H. Mitchell. 1951. Eelworms (nematodes) as pests of mushrooms. MGA Bull. 22:61-71.

17. Wood, D. A. 1979. A method for estimating biomass of Agaricus bisporus in a solid substrate, composted wheat straw. Biotechnology Letters 1(6):255-261.

18. Wood, D. A., and P. W. Goodenough. 1977. Fruiting of Agaricus bisporus. Changes in extracellular enzyme activities during growth and fruiting. Arch. Microbiol. 114:161-165.

19. Wuest, P. J. 1977. Managing air during phase II composting and the nematode nemesis. Mushroom News 25(1):7-10, 16.