Nematode Dissemination in Commercial Mushroom Houses

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ABSTRACT

One hundred and six mushroom production runs were periodically sampled during their production cycle to determine the type and number of nematodes present. *Aphelenchoides composticola*, the only parasite recovered, occurred in 36% of the samples.

This study established that nematodes did not survive pasteurization and farm workers or wind-blown debris were not major sources of contamination. Dipterous insects, principally Sphaerocerid flies, were the primary means by which newly spawned production runs became contaminated with nematodes. Modification of the insect control program designed to exclude or prevent migration of flies resulted in practical control of *A. composticola*.

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The importance of nematodes in reducing the production of commercial mushrooms, *Agaricus bisporus* (Lange) Imbach, and methods for their control have been reported by several workers (1, 2, 4, 5). This study reports the results of an investigation on the frequency of occurrence and the population density of nematodes at a mushroom production unit located at Lacey, Washington, and the means whereby the mushroom crops become contaminated with nematodes.

MATERIALS AND METHODS.—The normal cultural practices at this location included: phase 2 composting for 9 to 11 days during which a pasteurization temperature of approximately 63 C was maintained in the compost for 72 to 96 hours. Casing soil (sandy loam mineral soil) was pasteurized at 86 to 96 C for 1 hr and 309 ml of 40% formaldehyde was added to each cubic meter of soil through the steam system. At the completion of the cropping sequence all houses were steamed to obtain a compost temperature of about 65 C for 2 hr and an air temperature of 72 C for 5 to 6 hr. Following removal of compost, all houses were washed with a 0.2% solution of DNOC (4, 6, dinitro-*O*-cresol). Prior to filling with new compost, the houses were again treated with steam to obtain an air temperature of approximately 72 C for 6 hr.

Additional sanitary precautions to prevent introduction of the nematodes into the houses included: clean smocks for supervisory personnel before entering newly pasteurized houses, and clean clothing for workers when spawning houses and working in nematode-contaminated houses at the end of the work period. Entry to all houses were provided with a foot-dip disinfectant pad containing Nemagon (1,2,dichloropropane) or chlorine-containing compounds.

Standard insecticide programs at the start of this study were: treatment of the houses with dichlorvos (Vapona) twice weekly (thermo-fogger), dusting beds with 4% malathion twice weekly, and painting of the uprights in the house with Diazinon 50 WP.

Frequency and population density survey.—The nematode survey was conducted by collecting compost samples at seven intervals during each cropping sequence: filling, immediately prior to casing and 14, 28, 42, and 56 days after casing. Each sample consisted of seven subsamples collected from pre-determined areas within each house. Successive samples were collected from the same seven areas. Samples obtained after casing were collected by first removing the casing layer and then obtaining one handful of compost from the surface 3 to 4 inches. (The subsamples were mixed and 15 g of moist compost assayed for nematodes.) Samples to be assayed were incubated for 24 hr at 21 C on screens covered with a single ply of wet-strength facial tissue suspended in a 14-cm diameter spun aluminum funnel. The funnels were filled with water to a level to saturate the sample but not submerge it in water. The casing soil was assayed by the same technique using 50-g samples and incubating for 48 hr. After the incubation period 10 ml of water was removed from the bottom of the funnel and 1-ml aliquots examined for living nematodes.

Origin of nematode contaminants.—To determine whether nematodes were surviving the pasteurization process of phase 2 composting, nine separate crops were sampled by collecting compost 24 to 48 hr after exposure to pasteurization temperatures. Six 500-g samples were collected from each crop, placed in 0.025 mm (1.25 mil) polyethylene bags, and incubated at room temperature for 8 weeks. Two 500-g samples were collected from three areas in each house. The compost was collected from the side to the center of the bed and compost in contact with the bedboard was included in each sample. One-half of the paired samples were inoculated with 1,000 saprophagous nematodes and the remaining samples were not inoculated. After the incubation period, all samples

were assayed employing the previously described technique.

Concurrent with the collection of the 500-g compost samples, additional material was collected. These samples contained scrapings from the bedboard, other wooden structures that came in contact with the compost and material in cracks and crevices of the bedboard. Three 10-g samples were collected from each of the nine houses sampled.

Dissemination of nematodes by supervisory and farm personnel was studied by rinsing hands, footwear, and smocks in water and examining the wash water for nematodes.

Wind-blown debris was assayed as a nematode carrier by exposing 2% water agar in petri dishes at various locations at the farm for 8 hr. The dishes were incubated at room temperature and examined after both 7 and 14 days.

Data from the debris study suggested that dipterous insects might disseminate nematodes. Therefore, dipterous insects were collected, mascerated in water, and this concoction examined directly for nematodes. Flies collected for this study were obtained by placing clean nematode-free paper in close proximity to ventilation openings - a location where high densities of dead flies accumulated.

RESULTS.—*Frequency and population density survey*.—The survey results of over 100 crops are summarized in Table 1. *Aphelenchoides composticola* Franklin (3) was recovered from 3% and 2% of the individual crops at spawning and casing, respectively. However, 42 and 56 days after casing, 32- and 36% of the compost samples were infested with *A. composticola*, respectively. *Aphelenchoides composticola* occurred in low populations until after casing (1 per sample) and then increased rapidly until the final sample which occurred 56 days after casing. The average population of *A. composticola* recovered from 15 g of infested compost 56 days after casing was 2,599. The only parasitic nematode recovered from compost at this farm was *A. composticola*.

Saprophagous nematodes recovered from the compost samples were not identified to genus. Approximately 70% of the different batches of compost used for the 102-crop survey contained an average of 411 saprophagous nematodes per 15 g at filling; i.e., prior to pasteurization. Phase 2 composting, which includes pasteurization, reduced the recovery rate at spawning to 25% and the average number of nematodes recovered per 15-g sample was 115 (Table 1). The frequency of recovering saprophagous nematodes increased from time of spawning, and reached its maximum of 97%, 56 days after casing. The population density of saprophagous nematodes varied considerably during the production cycle.

Origin of nematode contaminants.—Individual lots of casing soil, examined following pasteurization and before use in the houses, were found to be nematode-free.

The rapid colonization of compost by saprophagous nematodes indicated either that nematodes were introduced into the compost immediately after pasteurization, that they survived in the house from previous crops, or that the pasteurization was ineffective. Twenty-seven 500-g compost samples collected after the pasteurization in phase 2 composting from each of nine compost batches were free of nematodes following room temperature incubation for 8 weeks. However, the 27 samples seeded with 1,000 saprophagous nematodes yielded over 200,000 nematodes each when assayed following the same incubation period.

Samples collected from bedboards and other wooden supports for the beds, were nematode-free. Hands, footwear, and smocks of supervisory personnel also were determined to be free of nematodes.

The possibility of nematodes being introduced into the houses by wind-blown debris was studied by collecting this material on petri dishes exposed at various locations at this farm. One dish of the 200 exposed was contaminated with a mixed population of *A. composticola* and saprophagous forms. A close

TABLE 1. Frequency of occurrence of *Aphelenchoides composticola* and saprophagous nematodes in compost from mushroom crops

	Sampling time						
	Fill[a]	Spawn	Case	14	28	42	56
	102[b]	100	102	100	101	96	73
A. composticola							
% samples infested	6	3	2	7	20	32	36
avg no. nematodes[c]	1	5	1	14	287	1,338	2,599
saprophagous forms							
% samples infested	71	25	68	61	79	93	97
avg no. nematodes	411	116	639	408	477	1,122	923

[a] Stage in production cycle at which samples were collected, 14 refers to case + 14 days, etc.

[b] Number of samples assayed for nematodes from 106 mushroom crops.

[c] Average number of nematodes refers to $\frac{\text{number of nematodes recovered}}{\text{number of infested samples}}$

TABLE 2. Frequency of occurrence of *Aphelenchoides composticola* nematodes in mushroom compost as influenced by fly control programs designed to eliminate flies from mushroom houses or to kill flies after their entrance into a mushroom house

	Sampling times (numbers are days after casing)						
	Fill[a]	Spawn	Case	14	28	42	56
A. composticola							
flies killed in house[b]							
no. of samples	48	47	48	47	48	46	35
% samples infested	6	4	2	15	37	57	63
avg no. nematodes[c]	24	5	1	11	167	1,714	3,201
flies excluded from house[d]							
no. of samples	54	53	53	53	53	45	43
% samples infested	7	0	4	0	6	7	9
avg no. nematodes	23	0	1	0	130	46	310

[a] Stage in production cycle at which samples were collected; 14 refers to case + 14 days, etc.

[b] Number of samples collected before change in fly control program.

[c] Average number of nematodes refers to $\frac{\text{number of nematodes}}{\text{number of infested samples}}$.

[d] Number of samples collected after change in fly control program with major emphasis on excluding flies from pasteurized compost.

examination of this petri dish revealed that a dipterous insect was impinged on the agar. Following this observation, flies (principally Sphaerocerid flies) from several nematode-infested mushroom houses were mascerated in water and examined for nematodes. *Aphelenchoides composticola* and saprophagous nematodes were consistently recovered from flies in infested houses. However, flies collected from noninfested houses were relatively, but not completely, free of nematodes. *Aphelenchoides composticola* and saprophagous forms were also recovered from water-filled trays placed in mushroom houses infested with nematodes and flies. These data established that dipterous insects are capable of transporting nematodes in mushroom houses.

Additional information was needed to determine whether nematodes associated with flies could survive the dichlorvos (Vapona) and malathion insecticide program used at this production facility. Thirty-two dead flies collected from a nematode and fly-infested house were examined and 27 carried live *A. composticola* and/or saprophagous nematodes.

The nematode survey was continued following production management changes which were designed to exclude flies from entering new mushroom crops. When the flies were excluded from the mushroom crops, the percent infested as well as the nematode population within compost, was reduced to a very low level. The percent of infested houses was reduced from 63% to 9%, 56 days after casing. Also the density of *A. composticola* per sample was reduced from 3,201 to 310 at the 56-day sampling period (Table 2). This change in management practice eliminated *A. composticola* as an economic factor in mushroom production at this facility. The data from the nematode survey together with the negative results from the compost incubation tests, bedboard samples, wind-blown debris, and supervisory farm personnel indicated that another means of nematode entry into the pasteurized compost occurred at this farm.

DISCUSSION.—The uniform infestation of nematodes in the houses would indicate that farm personnel and/or ventilating wind currents were not the major means by which nematodes entered the houses. Following the establishment of an association between nematodes and flies, changes in the cultural practices were made. These included: (i) screening of newly filled and spawned houses to exclude flies; (ii) modifying the control program to fumigate fly-infested houses more frequently; (iii) spray compost piles and other areas adjacent to the production facility with malathion and/or dichlorvos; (iv) all outside vent ports painted with ronnel (Korlan) or fenthion (Baytex); and (v) screen all ventilating ports and doors to prevent migration of flies into as well as out of mushroom houses.

The data from the nematode survey indicated that either the pasteurization period in phase 2 composting was not adequate to kill nematodes or that the nematodes were being introduced into the houses immediately after pasteurization. Compost samples collected within 48 hr after pasteurization were nematode-free. Also scrapings from bedboards and other wooden supports in the houses that came in contact with the compost were nematode-free. These data, in conjunction with negative findings with respect to nematode transport by supervisory farm personnel and wind-blown debris, indicated that another agent (or agents) was responsible for nematode introduction into the pasteurized compost.

Following the establishment that nematodes were being transported by Sphaerocerid flies, a change in the insect control program was established to prevent movement of flies out of infested houses, and entrance of flies into houses containing recently pasteurized compost. This change in insecticide program resulted in practical control of *A.*

composticola. It is the opinion of the authors that dipterous insects were the major agent for transport of nematodes at this production farm.

LITERATURE CITED

1. ARROLD, N. P., & C. D. BLAKE. 1966. Some effects of Ditylenchus myceliophagus and Aphelenchoides composticola on the growth on agar plates of cultivated mushroom Agaricus bisporus. Nematologica 12:501-510.
2. CAIRNS, E. J. 1952. Nematode disease and their control in mushroom crops. Phytopathology 42:4 (Abstr.).
3. FRANKLIN, M. T. 1957. Aphelenchoides composticola n. sp. and A. saprophilus n. sp. from mushroom compost and rotting plant tissue. Nematologica 2:306-313.
4. GOODEY, J. B. 1960. Observations on the effect of the parasitic nematodes Ditylenchus myceliophagus, Aphelenchoides composticola and Paraphelenchus myceliopthorus on the growth and cropping of mushrooms. Ann. Appl. Biol. 48:655-664.
5. LAMBERT, E. B., G. STEINER, & C. DRECHSLER. 1949. The "Cephalothecium disease" of cultivated mushrooms caused by a nematode (Ditylenchus spp.) evidenced by surface development of predacious fungi. Plant Dis. Reptr. 33:252-253.