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Stimulation of Yield in the Cultivated Mushroom by Vegetable Oils[1]

LEE C. SCHISLER

Department of Plant Pathology, The Pennsylvania State University, University Park, Pennsylvania 16802

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Supplementation of mushroom compost at spawning and at casing with various refined and crude seed oils resulted in 1 to 1.5 lb/ft² increases in mushroom yield. Supplementation at casing with ground seeds or protein-oil combinations caused 2 to 2.5 lb/ft² increases in mushroom yield. Further evidence is presented for a relationship between lipid metabolism and the initiation of fruiting in the cultivated mushroom, *Agaricus bisporus* (Lange) Sing. Preliminary results suggest the possible involvement of sterols in the fruiting stimulation.

Schisler and Sinden (6) reported a beneficial effect of supplementing mushroom compost with certain refined and crude vegetable oils at casing. The treatment was reported to increase mushroom yield, particularly in the first break or flush of mushrooms. This suggested a relationship between lipid metabolism and the initiation of fruiting in the cultivated mushroom. They used a compost consisting of either synthetic compost or a mixture of synthetic and straw-bedded horse manure.

The research reported in this paper is a continuation of this approach to compost supplementation. Various lipids and lipid-protein combinations were tested for their effect on yield of the cultivated mushroom, *Agaricus bisporus* (Lange) Sing., in an attempt to determine which component(s) in the oils was responsible for the fruiting stimulation. Whether the compost to which the oils were added had an effect upon the mushroom yield response was also of interest. In the present investigation, conducted at the Mushroom Research Center of The Pennsylvania State University, University Park, the compost consisted of straw-bedded horse manure.

MATERIALS AND METHODS

General method of mushroom culture used at the Mushroom Research Center. A standard procedure for all experiments in mushroom culture was followed with modification made only to suit the individual investigation.

Compost and composting. All composts consisted of wheat straw-bedded horse manure freshly collected from the college horse barns. It was prepared according to the method of short composting described by Sinden and Hauser (7). 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 was added without any leaching to the compost on the first two turnings. Little or no water was applied on the third turning. The compost was brought up to the desired moisture content (75 to 78%) when the compost was turned and filled. All supplements were added on the first two turns. Acto 88 (commercial supplement obtained from Mushroom Supply Co., Toughkenamon, Pa.) and brewers' grains were added at the rate of 40 and 50 lb per dry ton of horse manure, respectively, and gypsum was added at the rate of 75 lb per dry ton. The average nitrogen content of the compost at filling was 1.75%.

Phase I was followed by an indoor decomposition process (phase II) of 5 days' duration. The compost was filled into wooden trays 14 cm deep, which were placed into rooms under controlled atmospheric conditions of oxygen, air movement, and temperature. The "low-temperature" method of Sinden (*unpublished data*) was used. This procedure stresses the maintenance of compost temperatures between 45 and 55 C and as great a compost-air temperature differential as possible while maintaining the compost temperature in the desired range. An air temperature of 60 C was maintained for 4 hr on the 2nd day of phase II. This pasteurization process served to free the compost of injurious and antagonistic organisms that might be pathogenic to or merely interfere with the subsequent growth of the mushroom mycelium. The average nitrogen content after the completion of phase II was 2.35%.

Process of growing. The planting of the mushroom mycelium in trays, or spawning, consisted of mixing spawn (sterilized rye grains thoroughly impregnated with mushroom mycelium) with the compost as described by Hauser and Sinden (2). A 27.5-g quantity of spawn was used per ft² of bed area, or 110 g per tray. To be sure that the spawn was mixed evenly from top to bottom of the tray, the compost was filled in at least four layers, and equal portions of the

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weighed sample of spawn for each tray were spread over each layer. After spawning, all trays were placed in a controlled-environment room where the relative humidity was maintained at 95 to 98% and the temperature within the compost at 22 to 24 C. After 21 days of spawn growth, a 3.0- to 3.5-cm layer of pasteurized top soil was placed on the surface of the compost to induce sporophore production (this process is called casing). The average nitrogen content of the compost at this time was 2.65%. During the next 2 weeks, the ambient air temperature was gradually lowered to 15 C, and was maintained at this temperature for the remainder of the production period. Mushrooms matured 16 to 18 days after the casing layer was applied. Mushrooms appear in a cyclic pattern known as flushes or breaks. Seven such breaks were harvested in the 56-day picking period. All mushrooms were picked before the veil was broken. The bottom end of the stem was cut off to remove the clinging soil. The weight and number of mushrooms harvested were recorded daily for each tray. Subtotals of weight, number, and size were recorded at 8-day intervals. These corresponded roughly to the breaks of mushrooms. The "variety" of mushroom used has been the snow-white type commonly grown commercially in the United States. Multispore and single-spore isolates from the Penn State University culture bank carrying the numbers 310 and 318, respectively, were used in this investigation.

Supplementation at spawning modification. Since one of the experiments constituted an investigation of the effect of supplementation of the compost at the end of phase II, the general procedure outlined above was followed closely with the following modification. Just before the spawn was mixed into each layer of compost in a tray, an equivalent portion of any supplement used was added. The portion for each layer was mixed through the compost layer before the spawn was added to insure thorough mixing.

Supplementation after spawn growth, at casing, modification. After 21 days of spawn growth, the contents of all trays were dumped onto one pile. After shredding and thorough mixing, the contents were refilled into trays and supplemented at the same time with the desired amount of nutrient per tray. All trays were filled with an equal weight of compost. To be sure that the supplement was mixed evenly from top to bottom, the mycelium-impregnated compost was filled into the trays in at least four layers, and equal portions of the measured supplement for each tray were mixed thoroughly into each layer. The trays were placed in the production room at an ambient air temperature of 15 C. After 4 days, the trays were cased with a 3.5- to 4-cm layer of top soil. The heat generated in the trays, resulting from the respiration of the mushroom mycelium, was monitored by copper constantan thermocouple connected to a multipoint recorder. Temperatures were recorded at 1-hr intervals from the time of nutrient addition to the appearance of first-break mushrooms.

Experimental design. All tests were made in trays with an area of 0.37 m^2 and a depth of 14 cm. These were filled in a series of six replicates per treatment, and usually 16 treatments were combined in one experiment, making a total of 96 trays. All trays in a single test contained an equal amount of compost. Usually, 25 to 28 kg of wet compost or 6.5 to 7.5 kg of dry weight were used per tray.

The experimental data from each test were subjected to an analysis of variance to determine the least significant difference among the means.

RESULTS

Supplementation at spawning. The trays were spawned at the normal spawning rate described in the Materials and Methods section, and at four times the normal rate. Results in Table 1 show that both the ground soybeans and the refined cottonseed oil failed to increase yield when added to compost spawned at the normal spawning rate. However, both materials caused yield increases of approximately 0.5 lb/ft^2 when added to compost spawned at the higher spawning rate. There was no significant difference in size of mushrooms as a result of treatment variance. The yield increase from the ground soybeans occurred during the first three breaks of mushrooms, whereas the increase from the added cottonseed oil occurred primarily in the first break. It should also be noted that increasing the spawning rate alone resulted in a yield increase of 0.5 lb/ft^2.

Supplementation after spawn growth, at casing. A preliminary experiment was conducted in which the trays were supplemented after spawn growth with several protein concentrates, in addition to cottonseed meal to which they were being compared (Table 2). Cottonseed meal was added in the amounts of 250 and 500 g per tray of approximately 7 kg (dry weight) of compost. The other materials were added on the basis of an equivalent amount of nitrogen to that in the cottonseed meal. Crude and refined cottonseed

TABLE 1. *Effect of ground soybeans and cottonseed oil supplemented at spawning*

Treatment[a]	Yield (lb/ft^2)[b]
Normal spawn rate	2.01
Normal spawn rate plus ground soybeans (400 g)	2.09
Normal spawn rate plus cottonseed oil (250 ml)	2.11
Four times normal spawn rate	2.47
Four times normal spawn rate plus ground soybeans (400 g)	2.93
Four times normal spawn rate plus cottonseed oil (250 ml)	3.11

[a] Normal spawn rate was 110 g per tray. Amounts of supplements given represent amount per tray.

[b] Least significant difference, 0.05 = 0.40 lb/ft^2.

TABLE 2. *Preliminary experiment showing the effects of a few protein concentrates and vegetable oils on mushroom yield when supplemented at casing*

Supplement	Amt added per tray	Yield (lb/ft²)[a]
None		3.72
Cottonseed meal	250 g	3.91
Cottonseed meal	500 g	4.48
GB-18[b]	186 g	4.05
GB-18	372 g	3.43
GB-18	558 g	2.33
Cottonseed meats[b]	400 g	4.52
Cottonseed meats	800 g	2.75
Ground soybeans	286 g	4.48
Ground soybeans	572 g	4.36
Crude cottonseed oil (Proflo)[b]	200 ml	4.48
Crude cottonseed oil (Proflo)	400 ml	4.42
Refined cottonseed oil	200 ml	4.58
Refined cottonseed oil	400 ml	4.88
Refined soybean oil	200 ml	4.50
Refined soybean oil	400 ml	4.58

[a] Least significant difference, 0.01 = 0.41 lb/ft².
[b] Supplied by Traders Protein Division of the Traders Oil Mill Co. Fort Worth, Tex.

oil as well as refined soybean oil were added at the rate of 200 and 400 ml per tray. Increases in yield from the lower levels of addition of the various concentrates and from both levels of addition of the oils were substantial, as much as 1 lb/ft² in the case of the high concentration of refined cottonseed oil. This yield, expressed as grams of mushrooms produced per kilogram (dry weight) of compost plus supplement is 1,130. Both levels of addition of the oils used in this test caused similar significant increases in mushroom yield. The increases occurred primarily in the first break of mushrooms. The size of the mushrooms was not affected by the oil addition. Lower yields resulted from the higher levels of addition of GB-18 (cottonseed protein hydrolysate), cottonseed meats, and ground soybeans. This was probably due to excessive heating of the trays supplemented at this level of addition. The capacity for ventilation and cooling in the production room was inadequate for satisfactory removal of the heat produced.

A series of six experiments followed which were devoted to a study of the effects on mushroom yield of various protein concentrates, ground seeds, vegetable oils, and protein-oil combinations. Adequate ventilation and cooling capacities were provided for the experimental growing rooms so that temperatures could be maintained in the desired range as described under Materials and Methods.

Various lipids having a wide variation in concentrations of certain fatty acids were added to the compost at casing to determine whether certain fatty acids would affect the yield. The effect of a variety of vegetable oils on mushroom yield is shown in Tables 3 and 4. The lipids were also added at half the concentrations shown in the tables. The addition of the vegetable oils in each instance produced a substantial increase in yield. In the one experiment (Table 3), refined cottonseed, peanut, corn, and safflower oils each caused an increase of approximately 1 lb/ft² over that of the nonsupplemented trays. There was no significant difference between the increases caused by the addition of the various oils. In another experiment (Table 4), the check yield of 3.13 lb/ft² was lower than normal, and the yield increases caused by the addition of cottonseed and olive oils were approximately 1.5 lb/ft². The raw linseed oil and animal lard caused 1 lb/ft² increases in yield.

The effect of refining the cottonseed oil on the yield response of the mushroom is shown in Table 5. A crude cottonseed oil with the trade name of Proflo Oil, a miscella refined cottonseed oil (a majority of the sterols, free fatty acids, and phospholipids removed but not bleached, winterized, or hardened), and a refined cottonseed oil (Wesson) were added to the compost after spawn growth. There was no significant difference in the

TABLE 3. *Effect of various refined vegetable oils on mushroom yield when supplemented at casing*

Supplement[a]	Yield (lb/ft²)[b]
None	3.76
Cottonseed oil	4.74
Peanut oil	4.92
Corn oil	4.62
Safflower oil	4.76

[a] All supplements were added at 400 ml per tray.
[b] Least significant difference, 0.01 = 0.37 lb/ft².

TABLE 4. *Effect of various lipids on mushroom yield when supplemented at casing*

Supplement	Amt added per tray	Yield (lb/ft²)[a]
None		3.13
Cottonseed oil[b]	400 ml	4.64
Refined olive oil	400 ml	4.76
Raw linseed oil	400 ml	4.23
Animal lard	400 g	4.11

[a] Least significant difference, 0.01 = 0.53 lb/ft².
[b] Miscella refined cottonseed oil supplied by Traders Protein Division of the Traders Oil Mill Co., Fort Worth, Tex.

yield increases caused by the addition of each of these oils. The addition of the oils resulted in a 1 lb/ft² increase in mushroom yield.

The data presented in Table 6 are taken from several experiments and show the effect of cottonseed meal, cottonseed oil, and cottonseed meal-oil mixtures on mushroom yield when supplemented at casing. The cottonseed meal and the cottonseed oil when added separately caused an increase in yield in all experiments. The increase was greater from the cottonseed oil than from the cottonseed meal. The addition of the combination of cottonseed meal and cottonseed oil resulted in a yield increase which was purely additive in some experiments, but in others the increase was more than the sum of the increases obtained when the materials were added separately. In all instances, the yield increases resulting from the addition of the cottonseed meal-oil combination were very high. The yield from each of the first two breaks was generally better than 1.5 lb/ft² of good-quality mushrooms (Fig. 1).

TABLE 5. *Effect of refining cottonseed oil on mushroom yield when supplemented at casing*

Supplement[a]	Yield (lb/ft²)[b]
None	3.67
Crude cottonseed oil (Proflo)[c]	4.64
Miscella refined cottonseed oil[c]	4.82
Refined cottonseed oil	4.74

[a] All supplements were added at 400 ml per tray.
[b] Least significant difference, 0.01 = 0.24 lb/ft².
[c] Supplied by Traders Protein Division of the Traders Oil Mill Co., Fort Worth, Tex.

TABLE 6. *Effect of cottonseed meal, cottonseed oil, cottonseed meal-oil mixtures on mushroom yield when supplemented at casing*

Supplement	Yield (lb/ft²)[b]				
	Crop 46	Crop 48	Crop 52	Crop 53	Avg
None	3.76	3.13	3.96	3.66	3.63
Cottonseed meal (500 g)[b]	4.20	3.36	4.34	4.34	4.06
Cottonseed oil (400 ml)	4.74	4.14	4.64	4.72	4.56
Cottonseed meal (500 g)[b] plus cottonseed oil (400 ml)	4.96	4.93	5.61	5.22	5.18

[a] Least significant difference, 0.01 = 0.24 lb/ft²
[b] In crop 53, 454 g of cottonseed meal was used.

To determine whether any of the grains commonly used in the manufacture of mushroom spawn might be more beneficial in increasing mushroom yield, rye, kafir corn, and wheat were ground through a 1-mm mesh screen and added to the compost on the basis of an equivalent amount of nitrogen to that in the cottonseed meal to which they were being compared. The results of two such experiments are presented in Table 7. It can be seen that a small increase in yield occurred with the addition of cottonseed meal, but very large increases resulted with the addition of the various grains. Increases in yield of 2 to 2.5 lb/ft² over already high-yielding checks resulted. No significant differences in yield increases resulted from the use of the various grains. The increases in yield occurred throughout the crop. Mushroom yield from these trays remained high at the time the experiments were terminated (Fig. 2). Seventh break yields of 0.5 lb/ft² were common.

The effect of various proteinaceous materials, cottonseed oil, and protein-cottonseed oil combi-

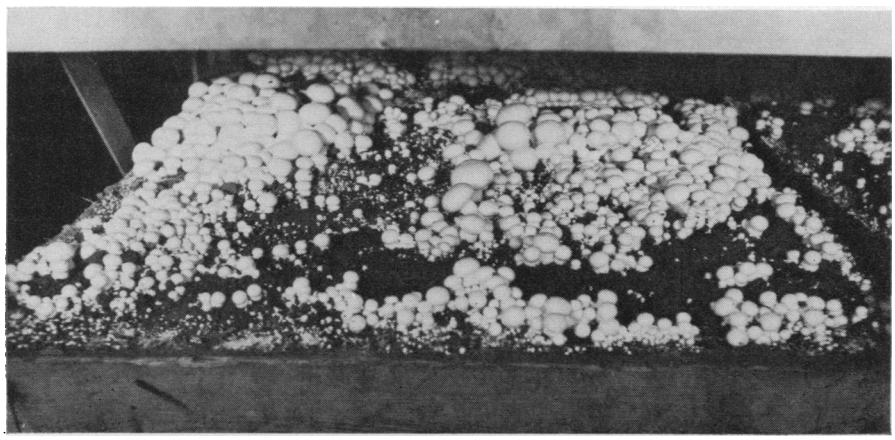

FIG. 1. *Second break of mushrooms enlarging on a tray supplemented at casing with a mixture of cottonseed meal and cottonseed oil.*

nations on mushroom yield when supplemented at casing is shown in Table 8. The proteinaceous materials were added on the basis of an amount of nitrogen equivalent to that in the cottonseed meal. A 400-ml quantity of cottonseed oil was added per tray. The yield increases as a result of the addition of cottonseed meal, Proflo flour (principally a nonhydrolyzed globular protein made from the embryo of cottonseed), and GB-18 were approximately 0.7 lb/ft². Ground rye grains, as was shown previously, caused an increase in yield of 2 lb/ft². The cottonseed oil addition resulted in an increase in yield of over 1 lb/ft². The addition of both the cottonseed protein and the cottonseed oil to trays caused large increases in yield. The combination of Proflo flour and cottonseed oil seemed to provide the greatest increase in yield. The combination of ground rye grain and cottonseed oil failed to cause a significant increase over the increase in yield which resulted from the addition of the ground rye grain alone.

TABLE 7. *Effect of some ground grains on mushroom yield when supplemented at casing*

Supplement	Crop 52[a]		Crop 53[b]	
	Amt added per tray (g)	Yield (lb/ft²)	Amt added per tray (g)	Yield (lb/ft²)
None		3.96		3.66
Cottonseed meal	500	4.34	454	4.34
Rye grain	1,605	6.30	1,460	5.68
Kafir corn	1,980	6.58	1,800	5.67
Wheat	1,560	6.24	1,420	5.62

[a] Least significant difference, 0.01 = 0.49 lb/ft².
[b] Least significant difference, 0.01 = 0.46 lb/ft².

The results of a preliminary test to study the effects of sterols on mushroom yield when supplemented at casing are shown in Table 9. Sitosterols (N.F.; a mixture of sitosterols from soybean comprised primarily of β-sitosterol with small amounts of other sterols) were added in the amounts of 1 and 10 g per tray. A significant increase in yield resulted from the addition of 1 g of sitosterols (N.F.) per tray. The increase occurred primarily in the first and second breaks of mushrooms.

DISCUSSION

Results of the test wherein supplements were added at spawning confirm previous work (4, 5) which showed that increasing the spawning rate

TABLE 8. *Effect of various proteinaceous materials, cottonseed oil, and protein-cottonseed oil combinations on mushroom yield when supplemented at casing*

Supplement	Yield (lb/ft²)[a]
None	3.66
Cottonseed meal (454 g)	4.34
Proflo flour[b] (330 g)	4.38
GB-18[b] (340 g)	4.36
Rye grain (1,460 g)	5.68
Cottonseed oil (400 ml)	4.72
Cottonseed meal (454 g) plus cottonssed oil (400 ml)	5.22
Proflo flour (330 g) plus cottonseed oil (400 ml)	5.80
GB-18 (340 g) plus cottonseed oil (400 ml)	5.58
Rye grain (1,460 g) plus cottonseed oil (400 ml)	5.99

[a] Least significant difference, 0.01 = 0.44 lb/ft².
[b] Supplied by Traders Protein Division of the Traders Oil Mill Co., Fort Worth, Tex.

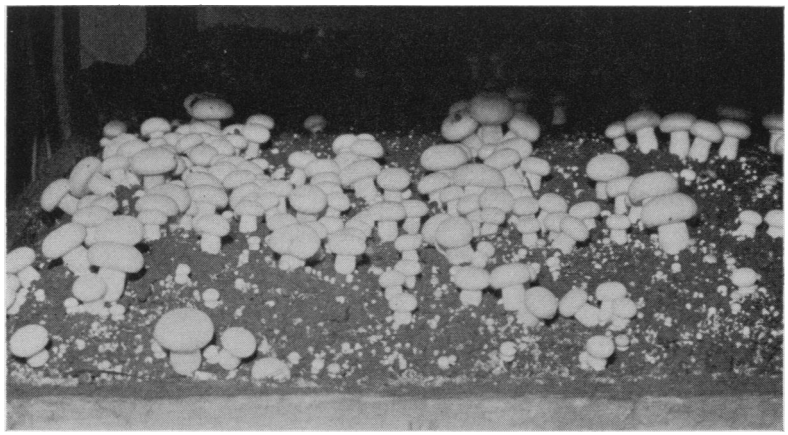

FIG. 2. *Seventh break of mushrooms on a tray supplemented at casing with ground kafir corn.*

increased the yield independently of the addition of supplement. Also, more efficient utilization of the supplement is made possible by increasing the spawning rate. The increased spawning rate probably makes the nutrient much more available to the mycelium of the fungus as a result of increased numbers of points of mycelial inoculation. Table 7 clearly indicates, however, that spawn grains can also act as supplemental nutrient. Inadequate ventilation in the production room when the mycelium reached the surface of the casing soil resulted in fruiting difficulties, especially on those trays spawned at the higher spawning rate. However, the combination of increasing the spawning rate and the addition of the cottonseed oil resulted in the greatest increase in yield. This increase occurred primarily in the first break, which reflects the degree of sporophore initiation. Any enhancement in first-break yield must be due to the initiation of a greater number of sporophores, since results showed that the size of the individual mushrooms was not increased. This relationship between lipid addition and fruiting stimulation was evident in this experiment as well as those in which the supplements were added at casing.

In all the experiments in which vegetable oils were added to the compost after spawn growth, large increases in total yield (1 lb/ft² or more) resulted. This is contrary to results reported previously (6), which showed an increase in total yield in some experiments but merely a stimulation of first-break yield in others as a result of lipid addition. The fact that the compost used in the former experiments consisted of either synthetic, or a mixture of synthetic and straw-bedded horse manure could account for the differences in response encountered in the two investigations. The hay present in the synthetic portion in the former investigation may have supplied sufficient lipid concentrations for adequate fruiting stimulation in some of the tests, whereas supplemental lipid addition was necessary in the present investigation in which the compost consisted entirely of straw-bedded horse manure.

A variety of lipids caused similar substantial increases in mushroom yield when added to the compost after spawn growth. A perusal of the fatty acid content of the various lipids failed to reveal a consistent correlation of specific fatty acids with yield stimulation. Observations suggested that a common component or components other than specific fatty acids were responsible for yield stimulation. It should be mentioned that a sufficient amount of critical fatty acids may have been present in all the lipids in the quantities added in these tests. However, even when half the amounts shown in Tables 3 and 4 were added, no correlation between specific fatty acids and yield stimulation was found. As can be seen from Table 5, refining the oil had little effect on the mushroom yield response to the oil addition. Apparently, a sufficient quantity of the "stimulator" remains in highly refined oil, or is unaltered in the refining process, to stimulate yield in the quantities added in this investigation.

Haskins et al. (1) concluded that the sexual reproduction of a *Pythium* sp. required the presence of a substance such as β-sitosterol or cholesterol, or one with similar structure. Hendrix (3) demonstrated that the growth of most species of *Pythiaceae* was stimulated by soybean oil and by cholesterol. He also found a requirement of exogenous sterol for sexual and asexual reproduction among several species of *Pythiaceae*. Preliminary results in this study (Table 9) suggest a fruiting stimulation in *A. bisporus* by soybean sitosterols. It will be of interest to learn whether a stimulation of growth occurs as well.

The check yield of 3.96 lb/ft² in crop 52 (Table 7), expressed as grams of mushrooms produced per kilogram (dry weight) of compost, was 1,070. This is more than double the better yields obtained in commercial mushroom production. Considering this, the increases in yield of from 2 to 2.5 lb/ft² resulting from the addition of ground grains commonly used in spawn-making are very high. The yield increase resulting from cottonseed meal addition, although added at an equivalent amount of nitrogen, was small in comparison. In Table 8, it can be observed that a combination of oil and protein concentrates resulted in yield increases equivalent to those obtained with ground grain. Apparently, a sufficient quantity of the lipid fruiting "stimulator" is present in the ground grains to enable the mushroom to utilize fully the nitrogen supplied by the ground grain. Supplemental lipid addition to the ground grains failed to cause a significant yield increase over that resulting from the ground grain alone.

Application of experimental results of supplementing mushroom compost at casing to com-

TABLE 9. *Effect of sitosterols (N.F.) on mushroom yield when supplemented at casing*

Supplement	Amt added per tray (g)	Yield (lb/ft²)[a]
None		3.66
Sitosterols, N.F.[b]	1	4.19
Sitosterols, N.F.	10	4.00

[a] Least significant difference, 0.05 = 0.36 lb/ft². Least significant difference, 0.01 = 0.49 lb/ft².

[b] Obtained from The Upjohn Co., Kalamazoo, Mich.

mercial production is not easy (8). One of the main difficulties is that the supplemented compost has a tendency to produce heat. The increased thermogenesis is probably the result of the increased metabolic rate of the mushroom mycelium in the presence of the supplement. Most commercial mushroom growing operations are not equipped with sufficient air-moving facilities to cope with removal of the heat produced. In the present studies, similar heating problems were encountered with the addition of proteinaceous materials. However, little increased thermogenesis resulted after lipid addition. This is of interest from a practical viewpoint, and it is also further evidence in support of the role of lipids in the fruiting mechanism rather than as nutrient factors.

Additional experiments are in progress to define more clearly the lipid stimulator(s) and to determine its mode of action.

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