Stimulation of Yield in the Cultivated Mushroom by Vegetable Oils

Effects of Sterols and Ethyl Linoleate

Lee C. Schisler* and Thomas G. Patton, Jr.

Supplementation of mushroom compost at casing with sterols and a sterol precursor failed to increase mushroom yield. Ethyl linoleate additions stimulated mushroom yield. Ethyl linoleate and safflower oil addition with equivalent linoleic acid concentration resulted in similarly increased yields. This fatty acid can be responsible for the stimulation of yield in *Agaricus bisporus* caused by vegetable oils.

Previous work by Schisler (1966, 1967) has shown that the supplementation of mushroom compost at spawning and at casing with various refined and crude seed oils resulted in substantial increases in mushroom yield. Evidence was presented for a relationship between lipid metabolism and the initiation of fruiting in the cultivated mushroom, *Agaricus bisporus* (Lange) Sing. Preliminary results suggested a possible involvement of sterols in the fruiting stimulation (Schisler, 1967).

Wardle and Schisler (1969) reported that the growth of mushroom mycelium *in vitro* was stimulated by the addition of lipids to the basal medium. The addition of nine commercial vegetable oils and beef and pork tallow caused increased growth of the same approximate magnitude. Esters of oleic and linoleic acid and oil additions with equivalent oleic and linoleic acid concentrations resulted in the similar increased growth of mycelium. They suggested that these fatty acids were largely responsible for the stimulation of growth.

Of interest is the possible correlation of increased mycelium production with increased sporophore yield due to oil supplementation in compost and, furthermore, whether compost supplementation using esters of oleic or linoleic acid can increase the yield of sporophores.

The research reported in this paper is an attempt to clarify this possible relationship.

MATERIALS AND METHODS

The same general method of mushroom culture as described by Schisler (1967) was followed, except that all supplementation was done after spawn growth and the experimental design was modified as follows.

The trays were filled in a series of eight replicates per treatment, and usually 10 treatments were combined in one experiment, making a total of 80 trays. All trays in a single test contained an equal amount of compost. Usually 18 to 22 Kg of wet compost (4.5 to 6 Kg oven dry weight) were used per 4 ft^2 tray.

The experimental data from the tests were subjected to a single or a combined analysis of variance to determine the least significant difference among the means.

RESULTS

A preliminary test (Table I) using sitosterols N.F. (a mixture of sitosterols from soybean comprised primarily of β-sitosterol with small amounts of other sterols) and squalene (a precursor to sterol synthesis) as supplements showed no increase in yields over the nonsupplemented control. The addition of cottonseed oil, however, did cause a significant increase in yield. Previous work by Schisler (1967, 1968) had shown that levels of addition of sterols greater than 1 g per tray were of no benefit, and in some cases had an adverse affect on yield. An additional experiment was performed testing the effects of 1 g of the sterols alone and in combination with Proflo flour (principally a nonhydrolyzed globular protein made from the embryo of cottonseed, Traders Oil Mill Co.). Neither squalene, sitosterols, nor stigmasterol caused a significant increase in yield over the nonsupplemented check (Table II). The addition of cottonseed oil or the addition of Proflo flour resulted in a 1 pound per ft^2 increase in yield. The combined addition of both materials caused an approximate 2 pounds per ft^2 increase in yield. However, when either of the sterols was combined with the Proflo flour, the increase in yield was not significantly greater from that obtained with the addition of Proflo flour alone. The combined addition of squalene and Proflo flour resulted in a significant decrease in yield from that obtained with the addition of Proflo flour alone.

A series of three experiments were performed on separate composts, the combined analysis of which is presented in Table III. Ethyl linoleate (98.7% purity, Procter and Gamble) was added in an amount approximately equivalent to that contained in 145 ml of safflower oil (Bailey, 1964). The addition of 110 ml of ethyl linoleate and the addition of 145 ml of safflower oil resulted in similar significant increases in yield. The addition of greater amounts of oil (both safflower and cottonseed) resulted in larger increases in yield. The addition of Proflo flour caused a similar increase in yield to that occurring when the greater amounts of oil were added. The combined addition of Proflo flour and ethyl linoleate resulted in a similar increase in yield, as did the combined addition of Proflo flour and 145 ml of safflower oil. The combined addition of Proflo flour and 290 ml of cottonseed oil caused a 1.4 pound per ft^2 increase. The addition of ground rye grain provided the greatest increase in yield (in excess of 1.75 pounds per ft^2).

DISCUSSION

The stimulation of yield in the cultivated mushroom by vegetable oils was again demonstrated in this series of experiments, and since the size of the individual mushrooms was not enhanced, the increase in yield was due to larger number of fruit bodies formed. The relationship between lipid metabolism and fruiting initiation was apparent.

It would appear that the linoleic acid in the oil can account for the increased yield resulting from the addition of vegetable oils. The addition of the ethyl linoleate caused a similar

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

* To whom correspondence should be addressed.

Table I. Effect of Sitosterols (N.F.) and Squalene on Mushroom Yield

Supplement	Amount added per tray	Yield (lbs/ft²)[a]
None		3.67
Sitosterols, N.F.	1 g	3.52
Squalene	1 ml	3.62
Cottonseed oil	400 ml	4.15

[a] Least significant difference, 0.05 = 0.29 lb/ft².

Table II. Effects of Some Sterols and Sterol Protein Combinations on Mushroom Yield

Supplement[a]	Yield (lb/ft²)[b]
None	2.68
Squalene (1 ml)	2.86
Sitosterols, N.F. (1 g)	2.78
Stigmasterol (1 g)	2.96
Cottonseed oil (320 ml)	3.66
Proflo flour (254 g)	3.58
Proflo flour (254 g) plus squalene (1 ml)	3.26
Proflo flour (254 g) plus sitosterols, N.F. (1 g)	3.30
Proflo flour (254 g) plus stigmasterol (1 g)	3.33
Proflo flour (254 g) plus cottonseed oil (320 ml)	4.55

[a] Amounts of supplements given represent amount added per tray.
[b] Least significant difference, 0.05 = 0.29 lb/ft².

Table III. Effect of Vegetable Oils, Proteins, Ethyl Linoleate, and Various Combinations of Them on Mushroom Yield

Supplement[a]	Yield (lb/ft²)[b]
None	2.76
Ethyl linoleate (110 ml)	3.02
Safflower oil (145 ml)	3.05
Safflower oil (290 ml)	3.27
Cottonseed oil (290 ml)	3.31
Proflo flour (254 g)	3.34
Proflo flour (254 g) plus ethyl linoleate (110 ml)	3.75
Proflo flour (254 g) plus safflower oil (145 ml)	3.83
Proflo flour (254 g) plus cottonseed oil (290 ml)	4.18
Ground rye grain (1135 g)	4.55

[a] Amounts of supplements given represent amount added per tray.
[b] Least significant difference, 0.05 = 0.21 lb/ft².

increase in yield as that caused by the addition of safflower oil containing an equivalent concentration of linoleic acid. Furthermore the combined addition of Proflo flour and ethyl linoleate resulted in a similar increase in yield, as did the combined addition of Proflo flour and an amount of safflower oil containing an equivalent concentration of linoleic acid.

A number of investigators (Al-Hassan and Fergus, 1968; Elliot *et al.*, 1964; Harnish *et al.*, 1964; Haskins *et al.*, 1964; Hendrix, 1965; Jefferson and Sisco, 1961; Klemmer and Lenney, 1965; Nelson *et al.*, 1967) have demonstrated the importance of sterols in the growth and reproduction of various fungi. This study suggests that sterols are not directly involved in the stimulation of fruiting in *Agaricus bisporus*, but that the fatty acids and linoleic acid, specifically, is involved. Moody *et al.*, (1968) found that rhizomorph production of *Armillaria mellea* was stimulated by oils and fatty acids. They reported that oleic and linoleic acids were effective in the stimulation. Whether morphological changes in other higher basidiomycetes might also be influenced by linoleic acid is an interesting speculation.

Since vegetable oils stimulate both mycelial growth and initiation of fruiting bodies, one might conclude that increased yield is a reflection of increased mycelial growth. However, the lipids are added after spawn growth just before the casing soil is applied. Perhaps the lipid addition provides for increased mycelial growth into the casing layer. Observations during the course of these experiments do not lend credence to this hypothesis, however. The mycelial density in the soil did not appear to be visibly increased and relatively little heat was evolved following the lipid addition.

Wardle and Schisler (1969) added mineral oil, a hydrocarbon exhibiting similar physical properties as vegetable oils, to their basal medium and found that it had no effect on the growth of mushroom mycelium *in vitro*. They concluded that the increased mycelial growth with lipids was not due to an alteration of the physical environment. Unpublished data by Schisler (1968), in which mineral oil was added to the compost after spawn growth, showed that mineral oil addition resulted in no increase in mushroom yield. Apparently the increased yield from the addition of vegetable oils is not due primarily to an altered physical environment.

Wardle and Schisler (1969) reported that five strains of *Agaricus bisporus* responded to lipid addition with increased mycelial growth, but the sixth strain did not. Research is in progress to determine the fruiting response of these same strains in an attempt to clarify any possible correlation of increased mycelial production with increased sporophore yield resulting from lipid addition to compost. Whether compost supplementation with ethyl oleate can increase yield of sporophores as it does mycelial growth is also under investigation.

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