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Role of 10-oxo-*trans*-8-decenoic Acid in the Cultivated Mushroom, *Agaricus bisporus*

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1 INTRODUCTION

Mushrooms are a product of high commercial value and one of the major agricultural crops in Pennsylvania. The cultivation of common button mushrooms (*Agaricus bisporus*) involves several different stages including composting, spawning, casing and picking (Gaze 1985). The limiting factor in the cultivation of a mushroom crop is the time required for the colonization of the compost and subsequently the casing layer by the mycelium and the initiation of fruiting bodies. Despite numerous studies, the factors that promote mycelial growth and initiate fruiting are still not clearly defined. Reduction of the growth period following spawning and casing would improve the economics of mushroom cultivation, and the identification of growth and fruiting stimulator(s) or hormone(s) would contribute toward this goal.

Recent research has led to the discovery of a mushroom metabolite with potent hormonal properties (Mau 1992). This compound was identified as 10-oxo-*trans*-8-decenoic acid (ODA), which is a product formed coincidentally with 1-octen-3-ol, the major mushroom aroma component, by two-enzyme catalyzed reactions when tissues of fruiting bodies or filaments of mycelium are disrupted (Grosch and Wurzenberger 1984, Schindler 1989). Also, 1-octen-3-ol has been found in other fungi, such as *Aspergillus* and *Penicillium* spp. (Kaminski 1974), thus by inference, it is apparent that ODA is also concurrently produced. ODA has a structure similar to "queen substance" (9-oxo-*cis*-2-decenoic acid, the sex pheromone of honey bees) (Butler *et al.* 1959) and traumatin (12-oxo-*trans*-10-decenoic acid, a wound hormone in higher plants) (Zimmerman and Coudron 1979). As a homologue, ODA might be expected to have similar physiological effects on mushrooms or fungi.

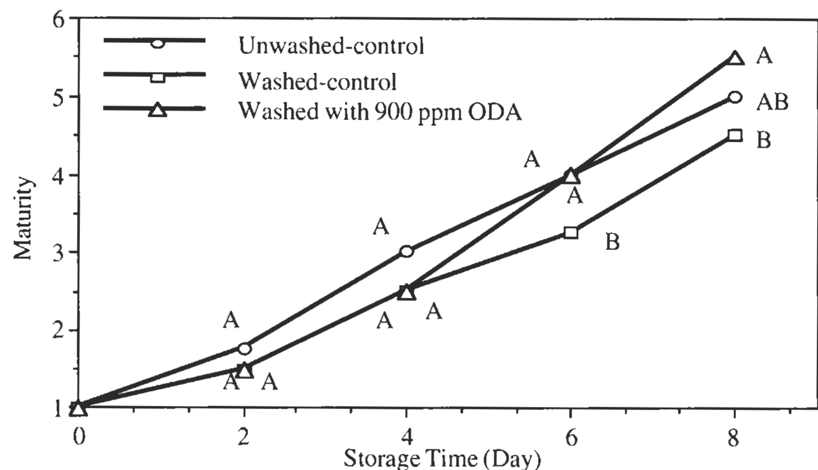

Figure 1. Maturity of *Agaricus bisporus* (hybrid off-white) washed with or without 10-oxo-trans-8-decenoic acid during postharvest storage at 12°C. Values within a day with the same letter are not significantly difference (p = 0.05).

This paper will focus on research conducted at Penn State University related to elucidating the physiological effects of ODA on mushrooms that were demonstrated by its influences on the growth of mushroom mycelia, the postharvest development of mushrooms after washing, and the elongation of mushroom stipes. The effects of ODA, in the form of mushroom powder, on mushroom crops were also included.

2 INFLUENCE ON GROWTH OF MUSHROOM MYCELIA

The methods for the estimation of mycelial growth include measurements of the linear growth on agar plates and the extracellular laccase activity and dry matter in liquid cultures. Although the role of laccase in growth and development of *Agaricus bisporus* is not well defined, it has been observed that laccase production correlated with mycelial biomass of this fungus (Wood 1979, Matcham *et al.* 1985, Claydon *et al.* 1988).

It was observed that all mycelia grew and spread out at constant rates from the center of the potato dextrose yeast (PDY) agar plates with or without ODA supplemented. The results showed that mycelia grown on agar plates containing 10^{-4} and 2 x 10^{-4} M ODA (18.4 and 36.8 ppm) grew significantly faster than mycelia grown at lower concentrations and control. The relative linear growth rate of mycelium was slightly increased by ODA at the concentration of 10^{-5} M (1.84 ppm) to 1.14, and greatly

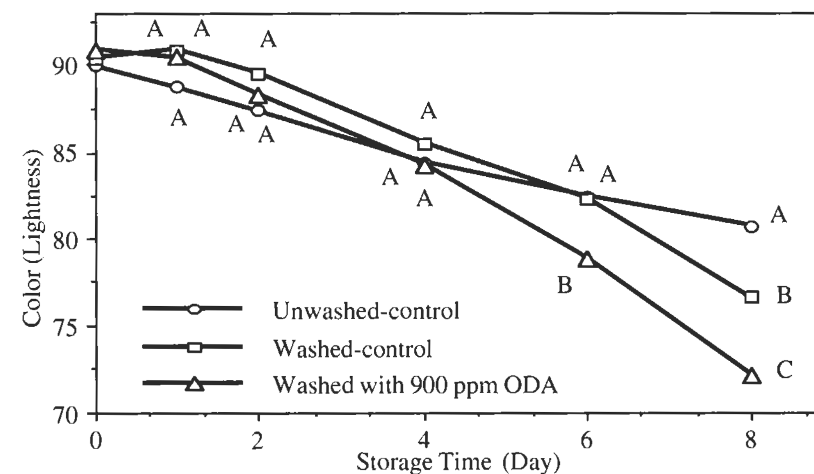

Figure 2. The color of *Agaricus bisporus* (hybrid off-white) washed with or without 10-oxo-trans-8-decenoic acid during postharvest storage at 12°C. Values within a day with the same letter are not significantly difference (p = 0.05).

increased at concentrations of 2 x 10^{-4} and 10^{-4} M (36.8 and 18.4 ppm) to 1.88 and 1.50, respectively, for the control being 1.00.

In PDY broth, the laccase activity was apparently stimulated by ODA at all tested concentrations from 10^{-7} to 2 x 10^{-4} M during the first two weeks, and then maintained fairly constant through the fifth week, while the laccase activity of the control only gradually increased at a lower constant rate. After 5 weeks of growth in liquid culture, the dry matters of the mycelia at ODA concentrations of 10^{-6} and 10^{-5} M (44.1 and 48.5 mg, respectively) were about three-fold higher than mycelia grown on the control medium (15.6 mg). Both the laccase activity and dry matter indicated that the mycelial growth was stimulated by ODA at 10^{-6} and 10^{-5} M (0.184 and 1.84 ppm). ODA at a concentration as low as 10^{-7} M still stimulated laccase activity, while its dry matter (16.9 mg) did not differ from that of control at the end of incubation (5 weeks).

Using the measurement of linear growth, dry matter and laccase activity, ODA was found to have a significantly stimulatory effect on mushroom mycelial growth on PDY agar and in broth. The effective concentrations were much lower in liquid cultures than on agar plates. The slow diffusion of ODA to the mycelia grown on top of agar plates might be a limiting factor accounting for the differences in effective concentrations.

As reported by Grove (1981) and Schindler (1989), 1-octen-3-ol was found in mushroom mycelia. Thus, by inference, it is apparent that ODA is also produced when the filaments of mycelia are damaged or disrupted. ODA produced in damaged filaments may migrate to adjacent fila-

ments, and there, stimulate the mycelial growth. Like traumatin in higher plants, ODA may stimulate mycelial growth in response to wounding.

3 INFLUENCE ON POSTHARVEST GROWTH OF WASHED MUSHROOMS

Washing of mushrooms recently gained commercial popularity in order to improve quality, control deterioration, and increase consumer acceptance of fresh mushrooms. Normally, washing was employed as a means of removing dirt particles and for the application of chemicals aimed at the prevention of browning, microbial decay, or senescence (McConnell 1991). However, Guthrie (1984) reported that bacterial growth and deterioration were significantly greater for washed mushrooms during storage, although washed mushrooms initially had lower bacterial numbers and an improved appearance as compared to unwashed mushrooms. In this research, the effect of ODA on postharvest changes of mushrooms was studied by washing mushrooms with ODA of 900 ppm.

During storage at 12°C, unwashed mushrooms and mushrooms washed with or without ODA continued their postharvest development, including both maturation and senescence, in a manner similar to that for mushrooms allowed to mature on the bed. Both washed mushrooms matured at the same rate slightly higher than unwashed control from days 0 to 4 (Fig. 1). On day 6, mushrooms washed with 900 ppm ODA matured similarly to the unwashed control and significantly higher than the washed control. On day 8, significant differences in maturation in term of cap opening were observed.

Browning, as indicated by lowered L-values (lightness), occurred continuously during storage in all treatments (Fig. 2). All washed mushrooms showed higher L-values after washing and browned faster thereafter. On day 6, the lightness of mushrooms washed with 900 ppm ODA was significantly lower. On day 8, mushrooms washed with 900 ppm ODA browned the most, while the unwashed mushrooms had the lightest color.

Mau (1992) washed mushrooms with ODA at the concentrations from 18.4 ppm to 0.00184 ppm, and no differences in maturation and browning were observed. However, at the concentration of 900 ppm, ODA showed its influences on postharvest growth of washed mushrooms as evidenced by the faster maturation and browning. The effective concentration thereof seemed to be much higher than those effective in mycelial growth. The short washing time (5 min) and the slow diffusion of ODA from the surface to inside tissues of fruiting bodies might be the reasons accounting for the high effective concentration.

4 INFLUENCE ON POSTHARVEST GROWTH OF MUSHROOM STIPES

Due to its rapid elongation during postharvest storage, the mushroom stipe has been a subject of study since 1956 (Urayama 1956). Hagimoto and Konishi (1959, 1960) studied the postharvest development of mushrooms and found evidence for the existence of a growth hormone or hormones promoting stipe elongation and curvature. Separated stipes, dissected from the mushrooms, were used to study elongation as influenced by crude preparations of ODA (mushroom supernatants) and purified ODA added to the test agar. Since the factor promoting stipe elongation was previously found to be mainly produced in the gills (Hagimoto and Konishi 1959, 1960, Gruen 1963, 1967, 1982), upper stipes were placed in contact with test agar to simulate the normal growth conditions. In this study, the elongation was specified into two undivided stages: 1) the initial elongation that is triggered virtually by the cutting; 2) the additional elongation that is stimulated by mushroom supernatants or ODA in the test agar. The elongation of stipes in excess of the control is referred to as the additional elongation.

During storage in petri dishes with the test agar containing various mushroom supernatants (A, B, C and controls) at 12°C, the upper portion of separated stipes lengthened more than the lower portion, although the lower portion made up 65-70% of the original length before storage. This indicates that the upper stipe is the major elongation portion during storage. This finding was in general agreement with those reported by Bonner *et al.* (1956) and Eilers (1974). Addition of crude preparations of ODA had significant impact on the elongation of upper stipes, but no difference in the elongation of lower stipes was observed. The main difference among three supernatants (A, B and C) was the concentrations of linoleic acid prior to blending, i.e., the ODA concentrations after blending (3.4×10^{-6}, 6.0×10^{-5} and 1.2×10^{-4} M, respectively). These crude preparations of ODA significantly enhanced upper stipe elongation. However, compounds other than ODA might have been responsible for the stimulation of elongation above that of the control.

ODA is formed as a result of mechanical damage of mushroom tissues, such as blending and cutting (Grosch and Wurzenberger 1984, Schindler 1989). During separated stipe preparation, mushroom stipes had been cut at both ends. ODA, or some other factor, released during cutting might have triggered the elongation of separated stipes (both upper and lower portions) in the control. In this study, it seems that the lower stipe responds only to the cutting since no differences were shown in the lower stipe elongation as a function of added ODA. This may be due to the limited diffusion of ODA from upper to lower stipes.

Since the upper stipe was the major portion that responded to crude preparations of ODA, the following experiment used only upper stipes and applied pure ODA solutions (from 10^{-4} to 10^{-8} M and control) to the test agar. The results showed no differences in the elongation of the upper stipes among treatments, indicating that no additional upper stipe elongation occurred other than that which occurred in response to the cutting during sample preparation.

Because no differences were observed using upper stipes alone, separated stipes with both upper and lower portions were used to re-examine the effect of ODA. The results showed that ODA had a significant effect on additional upper elongation at all levels (from 10^{-4} to 10^{-8} M). This indicates that ODA stimulated the elongation of upper stipes in excess of the control, but the response occurred only when the lower portions were present. The additional elongation of upper stipes was stimulated by ODA at a concentration as low as 10^{-8} M (0.00184 ppm or 1.84 ppb).

The movement of dry matter from the stipe to the cap reportedly occurs even after harvest (Hammond and Nichols 1975, Murr and Morris 1975), and is apparently associated with mushroom development. The lower stipe has been found to be high in mannitol content (28% of dry weight) as compared to mannitol content in the gills (10%) and the upper stipe (19%) (Ajlouni 1991). Mannitol is considered to be the main respiratory substrate used by harvested mushrooms (Hammond and Nichols 1975) and it can be translocated between the various tissues (Schmidt 1977). Also, translocation of mannitol and water from the lower to upper stipes can cause (support) growth and cell enlargement of upper stipes (Ajlouni 1991). Therefore, with the lower stipe, the upper stipe could respond to the stimulatory effect of ODA. Without the lower stipe, the upper stipe elongation may be limited by mannitol availability. This is consistent with the finding that trimming the stipe of nameko (*Pholiota nameko*) (Minamide 1985) and *Agaricus bisporus* mushrooms (Ajlouni 1991) to 5 mm from the cap prevented the elongation of stipes and opening of caps, and improved shelf life of fresh mushrooms. In short, with the presence of the lower stipe with the large pool of mannitol and water contained herein, the upper stipe could respond to the stimulatory effect of ODA produced *in situ* or translocated from the gills as could be the case in whole intact mushrooms.

Comparing the above results, it can be concluded that ODA is the factor contributing most to the additional elongation of upper stipes, and possibly the factor that triggers the initial stipe elongation after cutting, since it is produced when the tissues are damaged. The formation of ODA during cutting could be recognized because of perceptibility of the mushroom aroma, 1-octen-3-ol. During normal development of fruiting bodies, ODA produced in the gills may translocate to the upper stipe, and there, stimulate stipe elongation. Therefore, ODA can be considered a hormone of mushroom fruiting bodies. In order to confirm that the elongation observed in the control is mainly triggered by ODA, rather than some other factors, further investigations are needed.

5 INFLUENCE ON MUSHROOM CROPPING

The effect of ODA on mushroom cropping was first studied by supplementing mushroom powders containing various concentrations of ODA to the casing layer at casing or 24 hours prior to pinning. In a mushroom cropping experiment at the MRC, the supplementation of mushroom powder A, prepared directly from freeze-dried whole mushrooms without blending and presumably containing no ODA, resulted in significantly lower mushroom yield and numbers as compared to the control (Table 1). Mushroom powders B and C supplemented to the casing layer at casing were more effective than those supplemented 24 hours prior to pinning. Mushroom powders B and C supplemented to casing layer at casing resulted in significantly higher mushroom yields and numbers at the first flush (break), while the average mushroom size was not affected by treatments (Table 1). However, no significant differences in mushroom yield or numbers were observed in the later flushes of the crop cycle. This may indicate that ODA was involved in the initiation of fruiting bodies, but it may be short-lived in its activity.

In another mushroom crop conducted at MRC, ODA supplemented to compost at spawning in the form of mushroom powder resulted in significantly higher mushroom numbers and lower mushroom size at the first flush (Table 2). The mushroom yields at the first and second flushes were both higher than those of the control, but unfortunately, not significantly. No differences were observed in the mushroom yield, size and numbers at the third and forth flushes. Overall, the mushroom yield from the four flushes was significantly increased about 10.4%, and mushroom numbers increased about 24.6% (Table 2). Since ODA was found to be stimulatory to mycelial growth on agar and in broth, the results in Table 2 further showed that ODA also stimulated the mycelial growth in compost.

Table 1. Mean values of *Agaricus bisporus* (hybrid off-white) production parameters as influenced by mushroom powder supplementation to casing layer at casing or 24 hours prior to pinning.

Treatment[a]	ODA applied (mg/kg casing layer)	Yield (kg/ m^2)		Size (g/ mushroom)		Quantity (mushroom/ m^2)	
		1st Flush	Total[c]	1st Flush	Total	1st Flush	Total
1	—	4.30 C[b]	11.85 AB	25.1 A	14.0 ABC	175 B	854 B
2	—	4.65 BC	10.37 C	23.5 A	14.5 AB	212 B	721 C
3	1.2	5.25 AB	12.11 AB	22.7 A	13.7 BC	236 AB	887 AB
4	5.3	5.50 A	12.40 A	18.9 A	13.0 C	295 A	960 A
5	1.2	4.38 C	12.14 AB	26.0 A	15.5 A	174 B	788 BC
6	5.3	4.33 C	11.41 B	25.3 A	14.5 AB	174 B	787 BC

[a] Treatment 1: Control. Treatments 2, 3 or 4: Mushroom powders A, B or C (12.5 g/ tray each) added to casing layer at casing, respectively. Treatments 5 and 6: Spraying of powders B or C (12.5 g/ tray) to casing layer 24 hr prior to pinning, respectively.
[b] Values within a column with the same letter are not significantly different (p = 0.05).
[c] Values represent data obtained from the four flushes of the crop cycle.

6 CONCLUSION

ODA was found to stimulate mycelial growth, postharvest development and stipe elongation in *Agaricus bisporus*. Also, it might be involved in the initiation of fruiting bodies. It can be considered a mushroom hormone. However, the observations of the influence of ODA on mushrooms mentioned herein indicated that it has unique growth promoting properties that could have profound influence of commercial value. To cultivate mushrooms more economically and beneficially, further study in practical mushroom production is needed.

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Table 2. Mean values of *Agaricus bisporus* (hybrid off-white) production parameters as influenced by mushroom powder supplementation to compost at spawning.

Treatment	Flush	Yield (kg/m^2)	Size (g/mushroom)	Quantity (mushroom/m^2)
Control	1st	2.74 A[b]	18.8 A	142 B
	2nd	4.81 A	11.4 A	425 A
	3rd	1.26 A	8.1 A	159 A
	4th	0.16 A	14.4 A	11 A
	1st - 4th	8.97 B	12.2 A	737 B
Mushroom[a] Powder	1st	3.19 A	14.8 B	219 A
	2nd	5.26 A	10.4 A	506 A
	3rd	1.27 A	7.0 A	180 A
	4th	0.18 A	14.0 A	13 A
	1st - 4th	9.90 A	10.9 A	918 A

[a] Mushroom powder was supplemented to compost at the rate of 20 g per 22.7 kg wet compost. The ODA concentration in the compost was 7.75 mg per kg wet compost.
[b] Values with the same letter within the same flush of a column are not significantly different at the level of 0.05.

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