Effects of Tannic Acid on Rhizomorph Production by *Armillaria mellea*

P. C. Cheo

Chief, Research Division, Los Angeles State and County Arboretum, 301 N. Baldwin Ave., Arcadia, CA 91006.
The technical assistance of Mrs. Janet Pennycook, member of Las Voluntarias, California Arboretum Foundation, is gratefully acknowledged. Appreciation is also expressed to Francis F. Lombard, Forest Products Laboratory, Madison, WI, for providing cultures of three wood-rotting fungi for this study.
Accepted for publication 17 September 1981.

ABSTRACT

Cheo, P. C. 1982. Effects of tannic acid on rhizomorph production by *Armillaria mellea*. Phytopathology 72:676-679.

On a defined basal medium, the fungus *Armillaria mellea* produced scanty mycelial growth with no rhizomorphs. When tannic acid (TA) was added to the medium, rhizomorph development as well as mycelial growth were strongly stimulated. Stimulation of mycelial growth was observed at 0.001% (w/v) TA. Stimulation of rhizomorph development, however, did not occur at TA concentrations <0.05%. The best production of rhizomorphs occurred at 0.3 to 1.0% TA. TA did not serve as a sole carbon source for the growth of *A. mellea*. The incorporation of TA in a basal medium, on the other hand, inhibited the growth of many other fungi. *Fomitopsis annosa*, a wood-rotting fungus, and *Verticillim dahliae*, a wilt-producing fungus, were strongly inhibited by TA. Gallic acid, as well as some other phenolic compounds, also stimulated to a lesser extent the development of rhizomorphs in *A. mellea*. This reaction to TA and other phenolic compounds may confer a competitive advantage in its parasitism.

Rhizomorphs play an important role in dissemination, survival, and pathogenicity of *Armillaria mellea* (Vahl.) Quel. (5,8,10,16). Most isolates of this fungus produce rhizomorphs readily on undefined media such as potato-dextrose agar (PDA). Rhizomorphs, however, are not readily produced on defined mineral media unless additional growth-promoting substances are added. A figwood extract that stimulated rhizomorph growth on mineral medium and PDA was reported by Raabe (13), and its active ingredient was determined to be indole-3-acetic acid (18). Later Weinhold (17) reported a stimulatory effect on rhizomorph production with ethanol and other low-molecular-weight alcohols. Moody et al (11) and, later, Moody and Weinhold (12) disclosed the stimulatory effect of oils and fatty acids. Garraway (7) further claimed that *o*- and *p*-aminobenzoic acids, but not *m*-aminobenzoic acid, stimulated the production of rhizomorphs in *A. mellea*. These rhizomorph-stimulating agents are plant metabolites, and their presence could facilitate and enhance the infection process by *A. mellea*.

The association of *A. mellea* with root infection of many species of woody plants and its parasitic invasion in the zone between wood and bark prompted this study on its growth response to tannic acid (TA), which is generally considered to be an antifungal compound (6). This study, however, indicates a stimulatory effect of TA on the growth and rhizomorph development of *A. mellea*.

MATERIALS AND METHODS

The culture of *A. mellea* used for this study was an isolate obtained from infected roots of California live oak (*Quercus agrifolia* Nee). It produces rhizomorphs abundantly on PDA but not on the basal medium that was used throughout the study. The basal medium consisted of 5 g of D-glucose, 2 g of $(NH_4)_2HPO_4$, 1.75 g of KH_2PO_4, 0.75 g of $MgSO_4 \cdot 7H_2O$, 1 mg of thiamin hydrochloride, and 20 g of agar in 1 L of distilled water (19). The pH of the medium was adjusted to 6.25–6.5 with 1 M KOH after the addition of TA (Allied Chemicals, New York, NY 10006). The medium was dispensed in 20-ml aliquots into 120-ml (4-oz) prescription bottles and autoclaved at 121 C for 15 min. Each treatment included five replicates.

Inoculum for the growth study was derived from 3- to 5-wk-old colonies of *A. mellea* grown on PDA. Agar plugs 3 mm in diameter were cut from the margin of the mycelial colony and placed, one per bottle, on the flat center of the solid medium. Cultures were then incubated in the dark at 23 C for 2–4 wk. The fungal tissue was harvested by filling the bottles with water and heating in an autoclave for 3 min. This was followed by repeated rinsings of the mycelial mat with hot water and two final rinses with distilled water. The fungal tissue was then collected, placed on tared aluminum dishes for drying (48 hr at 85 C), and weighed.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

0031-949X/82/06067604/$03.00/0

RESULTS

When TA was incorporated into the basal medium, a stimulatory effect of thallus growth and rhizomorph production was observed (Fig. 1). An increase in growth was apparent as the TA concentration in the medium was increased from 0.075% (w/v) to 0.6% (Table 1). Thallus weight increased more than 10-fold when the TA concentration in the medium was 0.3 or 0.6%. In a separate experiment, an increase in mycelial growth was observed on a basal medium containing 0.001% (10 μg/ml) of TA. Rhizomorph production was initiated only when the TA concentration was 0.05% or above. The maximal stimulation of rhizomorph production occurred at or above 0.3% TA. Even though the medium containing 1% TA did not solidify, there was abundant rhizomorph production.

Plain TA agar with no other nutrients or salts added did not provide an adequate substrate for the growth of *A. mellea*. However, average increases of 0.5 and 1.0 cm in diameter of mycelial growth beyond inoculum plugs were observed on 0.1 and 0.2% TA agar, respectively, compared with growth on water agar controls. Thallus growth in this comparison was too sparse for accurate dry weight determination.

TA is ineffective as a sole carbon source for the growth of *A. mellea* in a basal medium. Rhizomorphs were not produced on TA-supplemented basal medium without glucose. Abundant rhizomorph production was apparent only on media containing both glucose and TA. The growth of *A. mellea* on the basal medium increased in response to an increase in glucose concentration within the range of 0.25 to 4.0% (Table 2). At 4% glucose rhizomorphs were initiated after 1 mo of incubation, but further development was aborted. For a comparison, basal medium containing 0.5% TA with different concentrations of glucose was tested. Rhizomorph initiation and development occurred at all concentrations of glucose in the presence of TA (Table 2).

Different isolates of *A. mellea* varied in cultural characteristics

on PDA (14). Several isolates were tested for response to TA and variation was observed; those that did not produce rhizomorphs on PDA did not respond to TA stimulation.

Weinhold (16) reported a stimulatory effect of ethanol on rhizomorph formation in *A. mellea*. A comparison was made using TA at 0.3 and 0.6% and ethanol at 0.025 and 0.05% (250 and 500 μl/ml). Profuse development of rhizomorphs was evident with both supplements (Fig. 2). The average dry weights of rhizomorph growth and mycelial growth after 2 wk of growth were (respectively): 8.9 and 12.3 mg at 0.3% TA; 11.3 and 21.5 mg at 0.6% TA; 10.5 and 26.0 mg at 0.025% ethanol; and 12.2 and 39.5 mg, at 0.05% ethanol. In the absence of either TA or ethanol there was no rhizomorph development, and the total thallus dry weight was only 4.3 mg.

Commercial TA has a formular weight of 1,700 ($C_{76}H_{52}O_{46}$). A 0.6% TA solution is equivalent to 3.5×10^{-3}M, whereas a 0.05% solution of ethanol is equivalent to 1×10^{-2}M. Therefore, on a molecular basis TA is quite efficient in its stimulatory action. Autoclaving, however, might break down its molecular integrity.

The growth of many plant pathogenic fungi is inhibited by the presence of TA in the culture media (3). Several common plant pathogenic and wood-rotting fungi were tested in the same manner to ascertain their response to different concentrations of TA. These results are shown in Table 3. Among the fungi tested, *Fomitopsis annosa* (Fries) Karsten, a wood-rotting fungus, and *Verticillium dahliae* Klebahn were the most strongly inhibited by TA. Growth of both fungi was reduced at the lowest TA concentration (0.075%) that was tested. *Fusarium roseum* Link was intermediate in sensitivity, (Fig. 1, Table 3) whereas the other two wood-rotting fungi, *Coriolus versicolor* (Fries) Quelet, *Poria placenta* (Fries) Cooke, and *Alternaria solani* (Ellis et Martin) Sorauer were more

TABLE 1. Effect of tannic acid on total thallus growth of *Armillaria mellea* in a basal medium

Tannic acid concentration (%)	Average dry weight (mg)[a]	
	15 days	30 days
0.0	2.3 ± 0.8	3.7 ± 1.6
0.075	9.6 ± 2.1	25.3 ± 4.4
0.15	15.7 ± 8.2	29.7 ± 5.9
0.3	24.8 ± 7.8	41.9 ± 6.2
0.6	26.8 ± 5.6	45.4 ± 7.0

[a] Mean values with standard deviation(s).

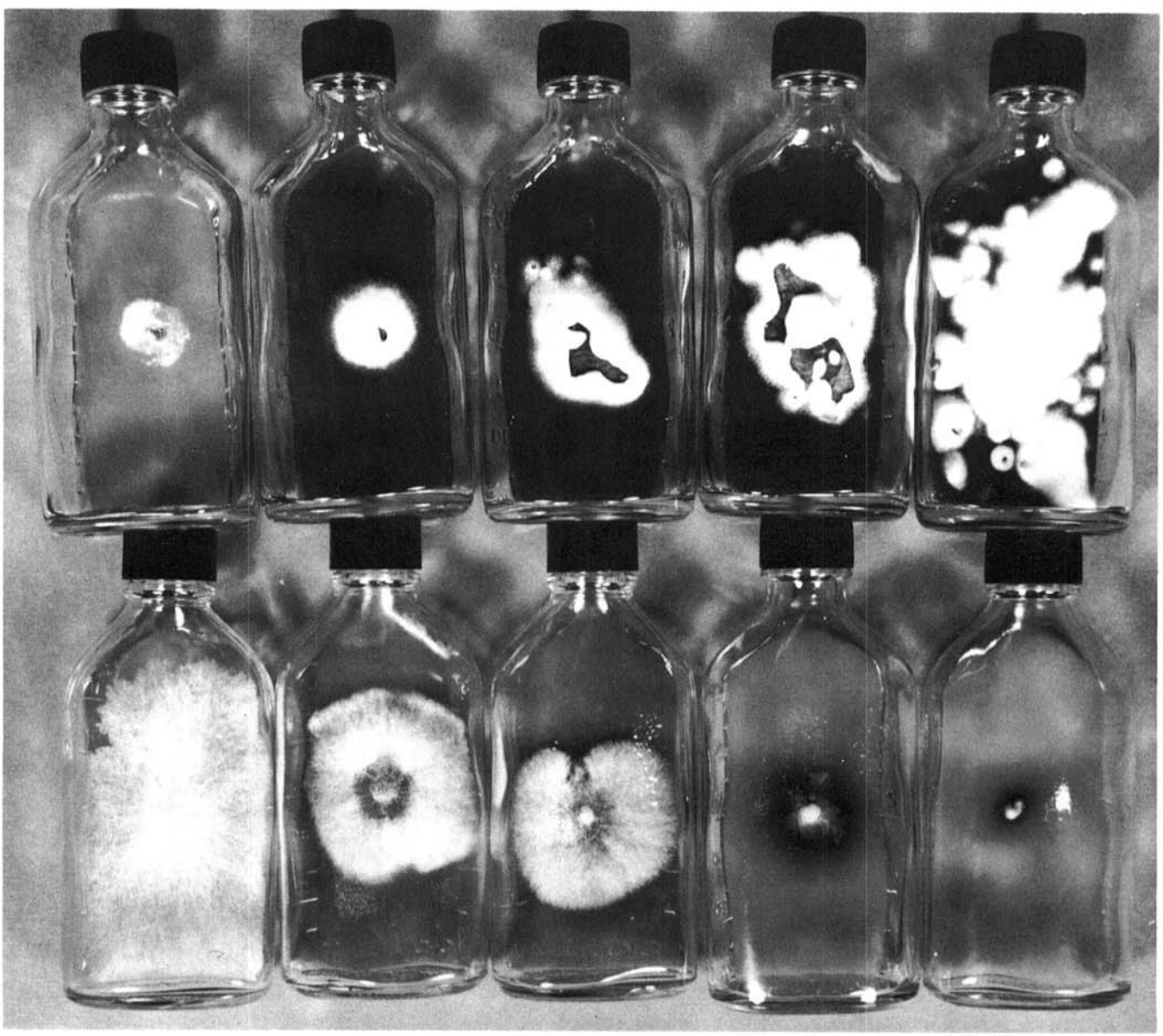

Fig. 1. The effect of tannic acid (TA) on growth of *Armillaria mellea* (upper row) and *Fusarium roseum* (lower row) on a basal medium. From left to right: TA content in the medium at 0, 0.075, 0.15, 0.3, and 0.6%. *A. mellea* was incubated at 23 C for 28 days, and *F. roseum* at 23 C for 7 days. The dark diffusion zone is characteristic of the "oxidase" reaction reported by Bavendamn (1).

tolerant of TA.

Mycelial growth and rhizomorph initiation of *A. mellea* were not only enhanced by the presence of TA, but also by its hydrolyzed form, gallic acid. Abundant development of rhizomorphs occurred at all concentrations of gallic acid (0.0725 to 0.6%). A slight inhibitory effect on mycelial growth occurred at the highest concentration (0.6%), but strong stimulation of rhizomorph development at that concentration resulted in an increase in total dry weight. Other phenolic compounds were also tested at concentrations ranging from 0.025 to 0.2%. Chlorogenic acid and guaiacol stimulated growth and rhizomorph development. At 0.2%, there were fourfold and sixfold increases, respectively, in total dry weight in response to chlorogenic acid and guaiacol. Salicylic acid, catechol, and hydroquinone were stimulatory at lower concentrations (0.025 and 0.05%) but were inhibitory at 0.1%. No growth occurred in the presence of catechol or hydroquinone at $\geq$0.2%. Phloroglucinol, coumarin, and caffeine were highly inhibitory to growth, but not to rhizomorph initiation at 0.025%.

DISCUSSION

Tannic acid (tannin, polyphenols) is widely distributed throughout the plant kingdom and occurs in practically all parts of flowering plants. High concentrations of TA can be found in the bark of trees and in the coats of seeds and ovaries. TA is generally considered to be an antifungal compound (6) and several reports have suggested that TA is responsible for disease resistance in plants (4,9,15). Cook and Taubenhaus (3) concluded that TA has a tendency to retard or inhibit the growth of fungi and that parasitic forms are more sensitive than saprophytes. One fungus, *Glomerella psiddi* was reported (3) to be resistant to TA, but it did not grow at a concentration >0.4%.

A. mellea is a common facultative parasite of woody as well as herbaceous plants throughout the world. By its enzymatic activities, *A. mellea* is generally considered a white-rot fungus, capable of decomposing lignin as well as cellulose and hemicellulose (2). However, its parasitic behavior is quite different from typical white-rot fungi. It grows beneath the bark in direct contact with the cambium. When *A. mellea* encounters a living root, it generally attacks the bark (20), where TA is ubiquitously present. The infection process is accomplished by the action of rhizomorphs. Therefore, the promotion of rhizomorph development by TA may contribute to infection.

A. mellea is a slow-growing fungus. In competition with other saprophytic fungi in the vicinity of a weakened root system, this slow growth rate is disadvantageous to parasitism. However, the tolerance (with beneficial stimulatory consequence) of *A. mellea* to many phenolic compounds may serve as a distinct countermeasure that allows *A. mellea* to gain advantage over competitive microorganisms. Therefore, the observation that TA and other naturally occurring phenolic compounds are stimulatory to growth and rhizomorph production in *A. mellea* while inhibitory to more rapid growing saprophytic fungi has implications for its ability to compete in nature.

TABLE 2. Effect of a range of glucose concentrations in a basal medium with and without addition of tannic acid on thallus growth of *Armillaria mellea* (21 days of incubation)

Glucose concentration in medium (%)	Average dry weight (mg)[a]					
	Without TA			With TA at 0.5%		
	Mycelium	Rhizomorph	Total	Mycelium	Rhizomorph	Total
0.25	4.34 ± 1.9	0.0	4.34	12.72 ± 2.4	4.52 ± 1.8	17.24 ± 1.6
0.5	5.32 ± 0.8	0.0	5.32	9.18 ± 3.7	13.56 ± 3.3	22.74 ± 1.6
1.0	9.02 ± 2.1	0.0	9.02	29.72 ± 4.9	17.88 ± 5.7	47.60 ± 3.2
2.0	18.20 ± 5.2	0.0	18.20	36.88 ± 4.9	6.26 ± 2.2	43.14 ± 5.6
4.0	27.81 ± 4.1	0.22	28.03	40.88 ± 11.2	2.28 ± 1.2	43.16 ± 10.2

[a] Mean values of standard deviation(s).

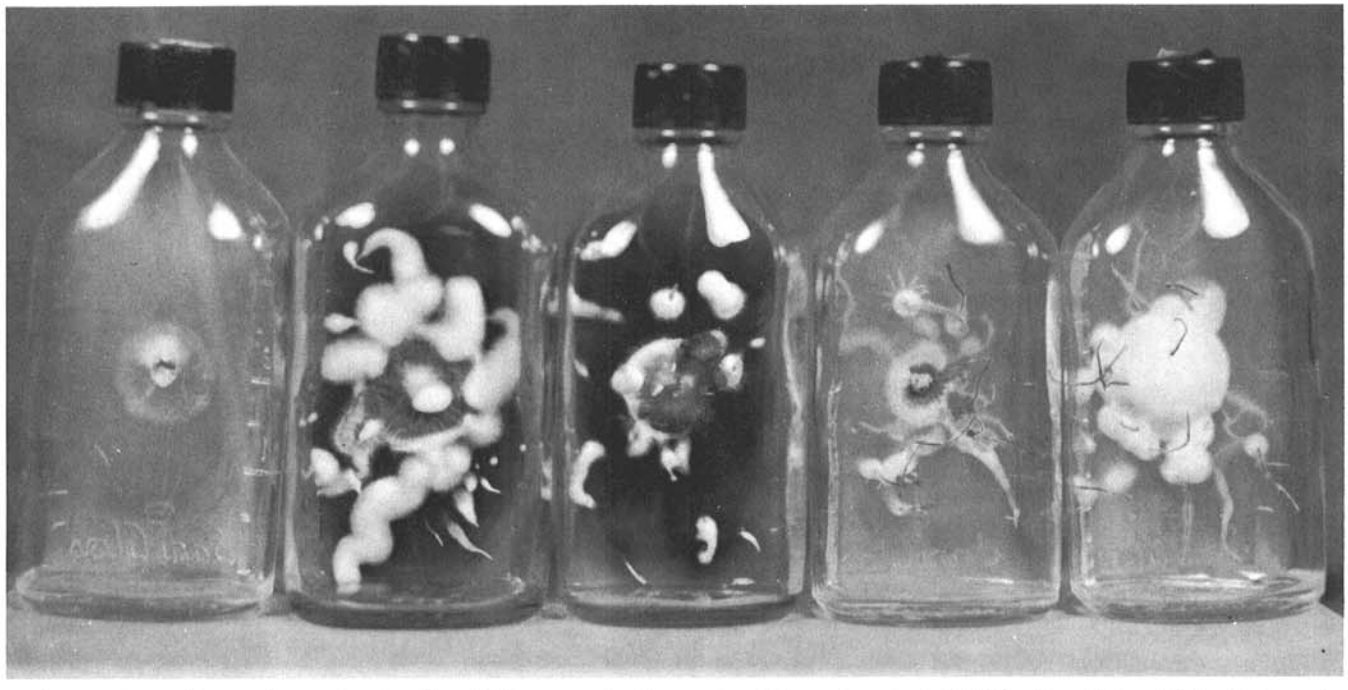

Fig. 2. Growth of *Armillaria mellea* on a basal medium (BM) compared with growth on BM supplemented with TA or ethanol. From left to right: growth on BM, BM + 0.3% TA, BM + 0.6% TA, BM + 0.025% ethanol and BM + 0.05% ethanol. Picture taken after 21 days of incubation at 23 C.

TABLE 3. Growth of some common plant pathogenic and wood-rotting fungi on a basal medium containing different concentrations of tannic acid

Fungi	Average dry weight (mg) of thalli developed in cultures containing tannic acid (%)[a]				
	0	0.075	0.15	0.3	0.6
Fusarium roseum	13.5 ± 1.33	13.2 ± 2.47	7.9 ± 2.04	0.5 ± 0.3	S[b]
Verticillium dahliae	25.8 ± 1.95	4.8 ± 0.7	0	0	0
Alternaria solani	20.3 ± 2.4	24.0 ± 4.7	24.3 ± 4.4	20.2 ± 3.4	16.7 ± 1.4
Coriolus versicolor	10.1 ± 1.3	10.9 ± 0.8	10.7 ± 1.0	13.7 ± 3.5	5.7 ± 1.2[c]
	27.0 ± 3.3	20.6 ± 2.6	28.5 ± 1.2	10.4 ± 2.1	1.8 ± 0.8
Fomitopsis annosa	18.5 ± 3.1[c]	S	S	S	0
	11.9 ± 2.1	S	S	S	0
Poria placenta	7.3 ± 1.8	5.3 ± 1.4	3.4 ± 0.9	3.1 ± 0.6	3.0 ± 0.8[c]
	7.2 ± 4.1	4.4 ± 0.7	2.2 ± 1.2	3.9 ± 0.7	2.9 ± 0.4

[a]Mean values with standard deviation(s).
[b]Slight growth from the site of inoculum.
[c]Two separate experiments.

LITERATURE CITED

1. Bavendamn, W. 1928. Über das Vorkommen und den Nachweis von Oxydasen bei Holzzepstorenden Pilzen. Z. Pflanzenkrankh. Pflanzenschutz 38:257-276.
2. Boyce, J. S. 1961. Forest Pathology. McGraw-Hill, New York.
3. Cook, M. T., and Taubenhaus, J. J. 1911. The relation of parasitic fungi to the content of the cells of the host plants. I. The toxicity of tannin. Del. Agric. Exp. Stn. Bull. 91:3-77.
4. Cruickshank, I. A. M., and Perrin D. R. 1964. Pathological function of phenolic compounds in plants. Pages 511–544 in: J. B. Harborne, ed. Biochemistry of Phenolic Compounds. Academic Press, New York.
5. Day, W. R. 1927. The parasitism of *Armillaria mellea* in relation to conifers. Quart. J. For. 21:9-21.
6. Fawcett, C. H., and Spencer, D. M. 1970. Plant chemotherapy with natural products. Annu. Rev. Phytopathol. 8:403-418.
7. Garraway, M. O. 1970. Rhizomorph initiation and growth in *Armillaria mellea* promoted by *o*-amino-benzoic and *p*-amino-benzoic acids. Phytopathology 60:861-865.
8. Garrett, S. B. 1956. Rhizomorph behavior in *Armillaria mellea* (Vahl.) Quel. Logistics of infection. Ann. Bot. (N.S.) 20:194-209.
9. Kuć, J. 1963. The role of phenolic compounds in disease resistance. Perspectives of biochemical plant pathology. Conn. Agric. Exp. Stn. Bull. 662:20-25.
10. Leach, R. 1937. Observations on the parasitism and control of *Armillaria mellea*. Proc. R. Soc. Lond., Ser. B, 121:561-572.
11. Moody, A. R., Garraway, M. O., and Weinhold, A. R. 1968. Stimulation of rhizomorph production in *Armillaria mellea* with oils and fatty acids. Phytopathology 58:1060-1061.
12. Moody, A. R., and Weinhold, A. R. 1970. Unsaturated fatty acids as natural stimulants of rhizomorph production by *Armillaria mellea*. Phytopathology 60:1305.
13. Raabe, R. D. 1962. Wood-based culture media for growing *Armillaria mellea*. Phytopathology 52:364.
14. Raabe, R. D. 1966. Variation of *Armillaria mellea* in culture. Phytopathology 57:1241-1244.
15. Rubin, B. A., and Artsickhovskaya, Y. V. 1963. Biochemistry and physiology of plant immunity. Pergamon. London. 358 pp.
16. Thomas, H. E. 1934. Studies in *Armillaria* (Vahl.) Quel.: Infection, parasitism, and host resistance. J. Agric. Res. 48:187-218.
17. Weinhold, A. R. 1963. Rhizomorph production by *Armillaria mellea* induced by ethanol and related compounds. Science 142:1065-1066.
18. Weinhold, A. R., Hendrix, F. F., and Raabe, R. D. 1962. Stimulation of rhizomorph growth of *Armillaria mellea* by indole-3-acetic acid and figwood extract. Phytopathology 52:757.
19. Weinhold, A. R., and Garraway, M. O. 1966. Nitrogen and carbon nutrition of *Armillaria mellea* in relation to growth-promoting effects of ethanol. Phytopathology 56:108-112.
20. Woeste, U. 1956. Anatomische Untersuchungen über die Infektionswege einiger Wurzelpilze. Phytopathol. Z. 26:225-272.