

The Effect of Chlorophenoxy Acid Herbicides on Growth and Rhizomorph Production of *Armillariella mellea*

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ABSTRACT

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Rhizomorph production by *Armillariella mellea* from dead oak roots in the field was assessed on trees that had been killed 2 to 14 yr before sampling. More rhizomorphs were produced from roots of trees killed by herbicides than from roots of trees killed by hand-girdling. The maximum quantity of rhizomorphs was recovered from root systems 10 yr after trees were treated with herbicides. In culture, 2,4-dichlorophenoxyacetic acid (2,4-D) stimulated the growth rate or the production of rhizomorphs of four *A. mellea* isolates. The range of growth-regulating activity for 2,4-D was between 10 and 250 $\mu\text{g/ml}$. Picloram, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), and 2(2,4,5-trichlorophenoxy)propionic acid (2,4,5-TP) either

had no effect on the fungus at any concentration or were inhibitory at and above 10 $\mu\text{g/ml}$. The addition of 2,4,5-T or 2,4,5-TP to an equal quantity of 2,4-D negated the stimulatory effect of 2,4-D. Gas-liquid chromatography was used to detect 2,4-D and 2,4,5-TP in root-collar phloem of oaks treated with aerial applications of the herbicides. Traces of both compounds were detected as early as 2 days after treatment and as late as 39 days after treatment. In general, less than 2.5 $\mu\text{g/ml}$ of 2,4-D and 0.5 $\mu\text{g/ml}$ 2,4,5-TP were present in analyzed tissue. We concluded that, under field conditions, these herbicides were not present in root phloem at levels high enough to have a direct effect on *A. mellea*.

In Wisconsin, a root rot caused by *Armillariella mellea* (Vahl ex Fr.) Karst. normally causes a low annual amount of pine mortality. Since the early 1970s there has been an increase in the number of young red pine (*Pinus resinosa* Ait.) plantations sustaining losses due to root rot. Mortality surveys showed 12–37% cumulative mortality in three red pine plantations under 10 yr of age (17). Tree losses most often were present in areas where red pine was planted under unproductive oak, and where pine was later released from oak with aerial applications of chlorophenoxy acid herbicides. Before consideration of environmental quality brought restrictions in the use of chemicals in the forest, undesirable oak normally was eliminated with sprays of 1.68 or 2.24 kg/ha of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), 2(2,4,5-trichlorophenoxy)propionic acid (2,4,5-TP), mixtures of 2,4,5-T and 2,4-dichlorophenoxyacetic acid (2,4-D), or mixtures of 2,4,5-TP and 2,4-D. Picloram (4-amino-3,5,6-trichloropicolinic acid) was used occasionally. The sprayed oak stands consisted mainly of northern red (*Quercus rubra* L.), white (*Q. alba* L.), and northern pin (*Q. ellipsoidalis* E. J. Hill) oaks averaging about 15 cm diameter at breast height (dbh).

Pine mortality associated with release operations suggested to us that the herbicides, which have plant growth-regulating properties, directly increased activity of *A. mellea*. Chlorophenoxy acid herbicides are systemic compounds, and it has been demonstrated that 2,4,5-T applied to the foliage of blackjack oak (*Q. marilandica* Muenchh.) could be recovered from the roots after 24 hr (4). In related studies, we showed that epiphytic rhizomorphs commonly are found on roots of most living oaks in sampled stands and are concentrated in the root-collar area of root systems (16). Also, chlorophenoxy acid compounds move almost exclusively via the phloem (2,3), and phloem would be encountered immediately under the rhytidome by a penetrating rhizomorph of *A. mellea*.

Many chemicals have been reported to stimulate growth or rhizomorph formation of *A. mellea* in culture. Among the defined

stimulatory compounds are ethanol, other low molecular weight alcohols, and acetate (1,22,27), ortho- and para-aminobenzoic acids (7,8), lipids and fatty acids (12,13), and indole-3-acetic acid (IAA) (7,8). At the time of writing the effects of 2,4-D or similar herbicides on the growth of *A. mellea* had not been reported. Also, Gruen (9) reported examples of the effects of auxins and other growth-regulating substances on the growth of selected fungi.

The objectives of this study were: (i) to determine if there was any measurable difference in rhizomorph production from root systems of mechanically killed and chemically killed oak trees, (ii) to assess the effect of several chlorophenoxy acids and similar herbicides on growth of *A. mellea* in vitro, and (iii) to determine what quantities of herbicides may be found in oak root-collar tissue after aerial application under field conditions.

MATERIALS AND METHODS

Rhizomorph sampling. By utilizing the planting records for Jackson County forest land and the Black River State Forest in Wisconsin, 12 areas were selected for sampling. All of the areas originally were dominated by stands of oak, had similar well-drained sandy soils, and comparable topography. Oaks in six of the areas had been sprayed once with a chlorophenoxy acid between 1960 and 1971, those in four additional areas had been hand-girdled once between 1963 and 1971, and those in the remaining two areas had not been treated and were used for controls. Eight randomly selected killed oaks were sampled in each area. Each oak chosen was a single-stemmed tree between 12.7 and 15.2 cm dbh.

With a sampling procedure previously described in detail (16), we measured the quantity of *A. mellea* rhizomorphs produced from or present around individual trees at any given time. Rhizomorphs were sampled from an annular zone of soil between 30 and 45 cm from the base of each tree and 15 cm deep. All vegetation and superficial debris first was cleared from around the selected tree. A circle with radius 30 cm greater than that of the base was staked around each tree; this formed the inner margin of a 15-cm-wide zone around the tree from which all of the litter and soil was

removed to a depth of 15 cm. Rhizomorphs were separated from the removed soil and litter, oven dried at 90 C for 12 hr, and weighed to the nearest 0.1 mg.

In vitro herbicide assays. A range of herbicide concentrations was evaluated for effects on *A. mellea* growth. The basal medium described by Weinhold (27), which consisted of 5 g glucose, 2 g L-asparagine, 1.75 g KH_2PO_4 , 0.75 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1 mg thiamine, 20 g agar (Difco Bacto-agar), and 1 L of distilled H_2O , was used for culture tests. The pH of the basal medium was adjusted to 5.5 with NaOH before autoclaving. Glucose was sterilized separately from the rest of the basal medium in order to eliminate the possible formation of toxic or stimulatory materials (24). Stock solutions of each desired herbicide or mixture of herbicides were prepared (pH adjusted to 5.5 with NaOH) and were sterilized by passing through an HA 0.45- μm Millipore filter. The solubility of each herbicide determined the highest concentration level that could be tested. The solubilities in H_2O at 25 C for 2,4-D, 2,4,5-T, 2,4,5-TP, and picloram were 620, 278, 140, and 430 $\mu\text{g}/\text{ml}$, respectively (10). Because the medium had to be prepared in three separate fractions, concentrations of the herbicides for maximum solubility in water could not be achieved in the medium.

The sterilized basal medium, glucose, and proper herbicide stock solution were combined aseptically and dispensed into 214 ml prescription bottles at a rate of 25 ml/bottle. The bottles were laid on a wide side, and after cooling, each bottle was seeded with an inoculum plug cut with a 4 mm diameter (No. 1) cork borer from the margin of a 14- to 21-day-old colony of *A. mellea*. The inoculum source was maintained in petri plates containing 20 ml of basal medium in 2% agar.

Four isolates were used in this study. Isolate P-19 was obtained from roots of a dying red pine, whereas isolates O-20, O-22, and O-23 were obtained from roots of dead oaks. All four isolates were tested with seven concentrations of 2,4-D. Isolates P-19 and O-20 also were tested with seven concentrations of 2,4,5-T, 2,4,5-TP, mixtures of the chlorophenoxy acids, and picloram. Inoculated bottles were stored in the dark at room temperature (20–22 C) for 8 wk. After the incubation period, the bottles were filled with distilled H_2O and steamed for 30 min. The fungal growth then was removed from the bottles, and if rhizomorphs were present, they were separated from undifferentiated mycelium. All fungus material was dried on filter paper in aluminum weighing pans for 12 hr at 90 C and weighed to the nearest 0.1 mg.

Root tissue analysis. We located a stand in Jackson County that was scheduled to be sprayed in August 1972. Control samples were collected 2 days before the area was sprayed. Individual oaks were chosen along randomly selected transects and only living northern red or white oaks between 12.7 and 15.2 cm dbh were used. The forest litter was removed from about 37 cm wide around the base of each tree. An injection ax, which removed a 15-mm diameter cylinder of bark and wood, was used to take five root-collar area samples from each of six trees. On 17 August 1972, the stand was sprayed with 2.24 kg/ha of a 1:1 (w/w) mixture of 2,4-D (propylene glycol butyl ether ester) and 2,4,5-TP (iso-octyl ester). Six additional and different trees were then sampled 2 days, 9 days, 39 days, and 14 mo after spraying.

Gas-liquid chromatography (GLC) employing an electron capture detector was used to analyze the tissues (15). In the field, chlorophenoxy acids usually were applied by aircraft as low-volatile, high-molecular-weight esters. These esters normally are hydrolyzed to the biologically active parent acid as they penetrate foliage and move within the plant (6,14), and therefore, both acid and ester forms may be translocated and detected within the plant (5).

The method described by Baur et al (5) extracts and separates the acid and ester herbicide forms, and we employed their procedures with several alterations. The xylem and rhytidome were removed from each sample core, which left only phloem tissue for GLC analysis. Approximately 2 g, fresh weight, of sample tissue were added to 35 ml acidified acetone (4 ml HCl/L) and homogenized at medium speed for 90 sec with a Sorvall Omni-Mixer. The homogenate was filtered through Whatman No. 1 paper and rinsed with additional acetone. The macerate and filter paper again were

homogenized in another 35 ml acidified acetone. After the second filtering and rinsing, the extracts were combined and slowly reduced to approximately 5 ml in a rotary flash evaporator. The extract was made basic (pH 8.5) with 5 ml of 8% NaHCO_3 , and the high-molecular-weight esters of 2,4-D and 2,4,5-TP were removed with two 25-ml hexane washes. The hexane phase was reduced to near dryness under N_2 and methylated for 6 hr at room temperature in 5 ml of 14% boron trifluoride (BF_3) in methanol (11). An earlier experiment showed that 2,4-D and 2,4,5-TP acids were completely methylated in 1 hr, but that the high-molecular-weight esters required a minimum of 5 hr for complete methylation (16). Therefore, a 6-hr methylation period was used for all analyses. Finally, the resulting methyl esters were extracted with three 5-ml portions of hexane.

The acid form of 2,4-D and 2,4,5-TP remained in the aqueous phase after the esters were removed with hexane. This remaining phase was acidified to a pH of about 2.5 with concentrated HCl and was washed with two 25-ml portions of ether. The two ether extracts were combined and washed three times with 20 ml 0.1 N KOH (pH = 12.5). The aqueous phase was again acidified with HCl and re-extracted four times with 15-ml portions of ether. The combined ether fractions were reduced to near dryness under N_2 , methylated, and taken up into 15 ml of hexane as described above for the esters.

A Model 7620 A Hewlett Packard gas chromatograph with an electron capture detector (Ni^{63}) was used for all analyses. A 1.37-m spiral glass column packed with 177 to 149- μm (80–100 mesh) Gas-Chrom Q coated with 2% SE-30 was used. Argon/methane (95%/5%) was used as the carrier gas with a flow rate of 45 ml/min. The injector, column, and detector temperatures were 270, 115, and 225 C, respectively. The column temperature was held constant for 8 min after each injection and then, in order to purge the column, the oven temperature was increased 15 C/min up to 200 C, held there for 2 min and then allowed to cool. Several compounds were evaluated, and 1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoidene (heptachlor) proved to be the most suitable for an internal standard (23). The heptachlor peak came off the column after 2,4-D and 2,4,5-TP, and did not interfere with any peaks from natural oak wood compounds (16). We constructed two standard curves by comparing ratios of peak areas for various quantities of 2,4-D, 2,4,5-TP, and heptachlor (16). These curves were used to determine herbicide quantities in the field samples by adding a standard amount of heptachlor to each sample extract before analysis (18). We operated the chromatograph with the lower level of reliable detection at 2.5 ng for 2,4-D and at 0.5 ng for 2,4,5-TP.

The efficiency of the entire procedure was tested by extracting oak phloem tissue to which known quantities of pure acids and commercial esters had been added. The recoveries for 2,4,5-TP acid, 2,4-D acid, 2,4,5-TP ester, and 2,4-D ester were 94.2, 90.1, 75.9, and 75.2%, respectively (16). These recovery values were used to correct field sample analysis results.

RESULTS

Rhizomorph sampling. The production of rhizomorphs from roots of oaks killed by herbicides or by hand-girdling for up to 10 yr after treatment is given in Fig. 1. There were no suitable areas with trees that had been hand-girdled more than 10 yr before the time of sampling. In each of the areas sampled at 2, 5, 7, and 10 yr after treatment, more rhizomorphs were recovered from around chemically-killed than mechanically-killed trees. By the *t*-test, treatment means at 2 and 7 yr were significantly different ($P=0.05$). In comparison with the herbicide treatment results, the mechanical treatment results showed a definite lag in rhizomorph production in the 0- to 2- yr period following treatment. This initial divergence appeared to account for a large part of the overall difference between treatments. There was a low (less than 1 g) but measurable quantity of rhizomorphs recovered from around all the living oaks in the untreated areas.

In vitro herbicide assays. The effects of various 2,4-D concentrations on the growth of four *A. mellea* isolates are shown in Table 1. In general, 2,4-D stimulated the growth of mycelia, the

production of rhizomorphs, or both, at concentrations between 10 and 250 $\mu\text{g/ml}$. Isolates demonstrating significant increases in rhizomorph production, however, showed correspondingly significant decreases in mycelial dry weight.

For isolate P-19 there was no significant effect of 2,4-D on total growth, but at 10, 50, and 100 $\mu\text{g/ml}$, rhizomorph initials were formed, which indicates a stimulation of rhizomorph initiation. This stimulation was not very dramatic because no more than four of 10 bottles for any one concentration contained initials. None of the 2,4-D concentrations stimulated rhizomorph initiation or growth of isolate O-20; however, there were significant increases of mycelial growth at 10, 50, and especially at 100 $\mu\text{g/ml}$. The total growth for isolate O-22 also was increased significantly at 10, 50, and 100 $\mu\text{g/ml}$, and in addition, there was a prolific production of

rhizomorphs at all concentrations at and above 10 $\mu\text{g/ml}$. The greatest combined stimulation of mycelium and rhizomorphs for isolate O-22 was at 100 $\mu\text{g/ml}$. Although maximum rhizomorph growth was at 250 $\mu\text{g/ml}$, it also was accompanied by a significant decrease in mycelial growth. Of the four isolates tested, O-23 produced the greatest amounts of rhizomorphs, especially at 100 and 250 $\mu\text{g/ml}$ of 2,4-D. All of the O-23 cultures that produced rhizomorphs showed a steady and significant reduction in mycelial dry weight with increasing concentrations of 2,4-D. The result was that total fungal dry weight remained relatively constant. Representative growth patterns of *A. mellea* isolates in response to 2,4-D are illustrated in Fig. 2.

Table 2 summarizes the growth effects on isolate P-19 of picloram and of several chlorophenoxy acid herbicides, both alone and in mixtures. The herbicide 2,4,5-T was inhibitory at 1 $\mu\text{g/ml}$ and all higher concentrations, and it was one of the more toxic herbicides toward this isolate. Results for 2,4,5-TP at 1 and 10 $\mu\text{g/ml}$ are difficult to interpret. Growth at 1 $\mu\text{g/ml}$ was significantly less than the control, but growth at 10 $\mu\text{g/ml}$ was not significantly different from the control. At 50 and 100 $\mu\text{g/ml}$ growth was drastically below the control level. The lowest concentration at which 2,4,5-TP is actually toxic cannot be determined with certainty from these results.

When equal portions of 2,4-D and 2,4,5-T were mixed at the designated concentrations, the rhizomorph-stimulating effect of 2,4-D alone on isolate P-19 was negated. When 50 $\mu\text{g/ml}$ and 100 $\mu\text{g/ml}$ of each chemical were mixed (100 $\mu\text{g/ml}$ and 200 $\mu\text{g/ml}$ total), growth of isolate P-19 was significantly reduced. Almost identical results were obtained when equal amounts of 2,4-D and 2,4,5-TP were mixed and tested. Picloram was inhibitory to this isolate at all concentrations at and above 10 $\mu\text{g/ml}$. At the highest concentrations, picloram did not appear to be as toxic as 2,4,5-T or 2,4,5-TP.

The same experiment described above for isolate P-19 was repeated with isolate O-20, and the results are tabulated in Table 3. As indicated earlier, isolate O-20 exhibited a significant increase in mycelial growth at 10 and 50 $\mu\text{g/ml}$ of 2,4-D, with maximum growth at 100 $\mu\text{g/ml}$. Also, no rhizomorphs were produced by this isolate at any 2,4-D concentration. The herbicide 2,4,5-T had no detectable effect on isolate O-20 at the concentrations tested. Concentrations of 2,4,5-TP at 10 $\mu\text{g/ml}$ and higher were significantly inhibitory.

When 50 $\mu\text{g/ml}$ of 2,4-D and 2,4,5-T were mixed together, the 2,4,5-T did not cancel the growth promoting effect of 2,4-D alone on isolate O-20. This was the only concentration at which any mixture of herbicides produced a significant increase in fungal growth. One hundred $\mu\text{g/ml}$ either of 2,4-D or 2,4,5-T did not reduce the growth rate of *A. mellea* isolate O-20 effectively. Equal portions of 2,4-D and 2,4,5-TP mixed to total 100 or 200 $\mu\text{g/ml}$ were inhibitory to mycelial growth. All lower concentrations of this mixture had no effect. Finally, picloram reduced growth of this

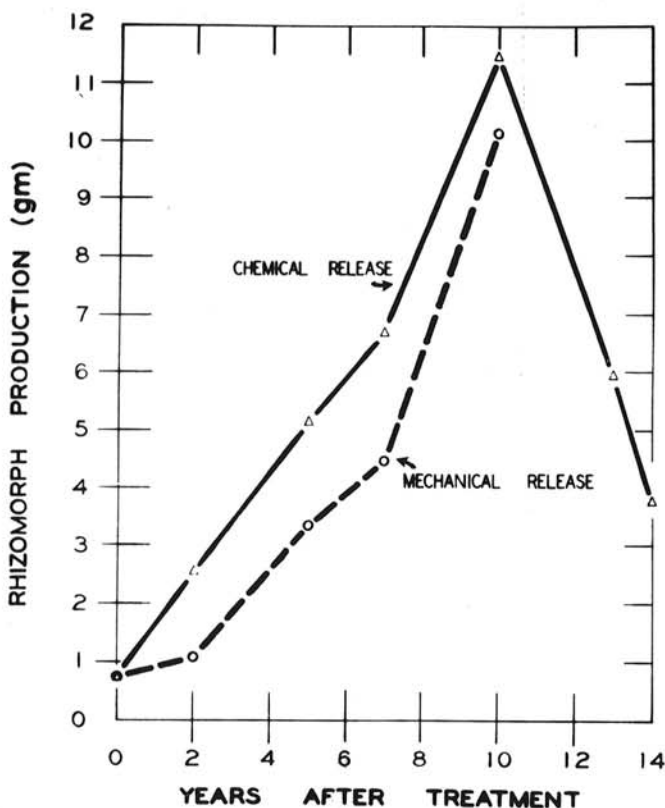


Fig. 1. The quantity of *Armillariella mellea* rhizomorphs recovered from soil around dead oaks that were killed either by herbicides (chemical release) or by hand girdling (mechanical release) at different times prior to sampling. Each point is the mean of eight samples.

TABLE 1. The effect of 2,4-D on the mycelial (M), rhizomorphic (R), and total (T) growth^a of four *Armillariella mellea* isolates in culture on Weinhold's medium

| 2,4-D conc. ($\mu\text{g/ml}$) | Growth (dry weight) of isolate: | | | | | | | | | | | |
|----------------------------------|---------------------------------|-----------------------|---------|---------|--------|---------|--------|------------|---------|---------|------------|--------|
| | P-19 | | | O-20 | | | O-22 | | | O-23 | | |
| | M (mg) | R (mg) | T (mg) | M (mg) | R (mg) | T (mg) | M (mg) | R (mg) | T (mg) | M (mg) | R (mg) | T (mg) |
| 0 | 99.1 A | 0 | 99.1 A | 63.9 A | 0 | 63.9 A | 33.2 A | 0 | 33.2 A | 75.0 A | 0 | 75.0 A |
| 0.1 | 98.8 A | 0 | 98.8 A | 54.3 A | 0 | 54.3 A | 37.9 A | 0 | 37.9 A | 75.3 A | 0 | 75.3 A |
| 1.0 | 103.3 A | 0 | 103.3 A | 75.6 A | 0 | 75.6 A | 37.6 A | 0 | 37.6 A | 67.8 AB | 0 | 67.8 A |
| 10.0 | 96.4 A | 2.4(3) A ^b | 98.8 A | 86.7 B | 0 | 86.7 B | 42.3 A | 11.5(10) A | 53.8 B | 60.8 B | 16.1(10) A | 76.5 A |
| 50.0 | 99.0 A | 2.6(3) A | 101.6 A | 86.2 B | 0 | 86.2 B | 37.4 A | 16.3(9) A | 53.7 B | 53.1 B | 19.0(10) A | 72.1 A |
| 100.0 | 92.9 A | 4.4(4) A | 97.9 A | 102.3 C | 0 | 102.3 C | 31.7 A | 26.8(9) B | 58.6 B | 32.6 C | 34.3(10) B | 66.9 A |
| 250.0 | 93.4 A | 0 | 93.4 A | 77.0 AB | 0 | 77.0 AB | 14.2 B | 30.3(10) B | 44.5 AB | 30.8 C | 35.3(10) B | 66.1 A |

^aIn milligrams dry weight. Cultures grown in 25 ml of medium in the flat side of 214-ml medicine bottles incubated in the dark at 20–22 C for 8 wk. Each value is the mean of 10 observations from two, five-replicate experiments. Means not followed by the same letters within each column are significantly different at $P = 0.05$, as determined by Duncan's multiple-range test.

^bNumbers within parentheses indicate the number of cultures in a total of 10 that contained rhizomorphs or rhizomorph initials.

isolate at $\geq 10 \mu\text{g/ml}$. Overall, this isolate seemed to be inhibited less by the various herbicides tested than isolate P-19.

Root-tissue analysis. In all samples in which the herbicides were detected the amounts of herbicide were below the minimum level for which reliable quantitation was possible. These traces were $< 2.5 \mu\text{g}$ 2,4-D and $< 0.5 \mu\text{g}$ 2,4,5-TP/g (fresh weight) of tissue, well

below the levels required to affect the growth of *A. mellea* (as determined by in vitro assays). The acid form of 2,4-D was detected in samples collected 2 (two samples), 9 (one sample), and 39 (one sample) days after spraying, and the acid form of 2,4,5-TP in samples collected 2 (three samples), 9 (five samples), and 39 (three samples) days after spraying. Neither acid was found in samples

TABLE 2. The total (mycelial and rhizomorphic) growth^a in culture on Weinhold's medium of *Armillariella mellea* isolate P-19 at different concentrations of various chlorophenoxy acids and picloram

| Herbicide conc. ($\mu\text{g/ml}$) | Growth in cultures containing: | | | | | |
|---|--------------------------------|--------------|---------------|-------------------------------------|--------------------------------------|---------------|
| | 2,4-D (mg) | 2,4,5-T (mg) | 2,4,5-TP (mg) | 2,4-D and 2,4,5-T ^b (mg) | 2,4-D and 2,4,5-TP ^b (mg) | Picloram (mg) |
| 0 | 99.1 A | 99.1 A | 108.3 A | 88.8 A | 82.3 A | 83.4 A |
| 0.01 | ... | ... | 103.8 AB | ... | ... | ... |
| 0.1 | 98.8 A | 97.0 AB | 95.1 ABC | 87.3 A | 81.5 A | 87.1 A |
| 1.0 | 103.3 A | 86.6 B | 81.3 C | 87.6 A | 86.3 A | 75.8 A |
| 10.0 | 98.8(3) A ^c | 65.9 C | 87.8 ABC | 81.9 A | 84.2 A | 64.8 B |
| 50.0 | 101.6(3) A | 43.4 D | 49.1 D | 81.3 A | 75.4 A | 59.4 BC |
| 100.0 | 97.9(4) A | 36.1 DE | 39.9 E | 64.4 B | 58.5 B | 55.5 C |
| 150.0 | ... | 27.6 E | ... | ... | ... | 53.4 C |
| 200.0 | ... | ... | ... | 42.1 C | 47.6 B | ... |
| 250.0 | 93.4 A | ... | ... | ... | ... | ... |

^a In milligrams, dry weight. Cultures grown in 25 ml of medium in the flat side of 214-ml medicine bottles incubated in the dark at 20-22 C for 8 wk. Each value is the mean of 10 observations from two, five-replicate experiments. Means not followed by the same letters within each column are significantly different at $P = 0.05$, as determined by Duncan's multiple range test.

^b Mixtures contained equal amounts of both herbicides.

^c Numbers within parentheses indicate the number of cultures in a total of 10 that contained rhizomorph initials.

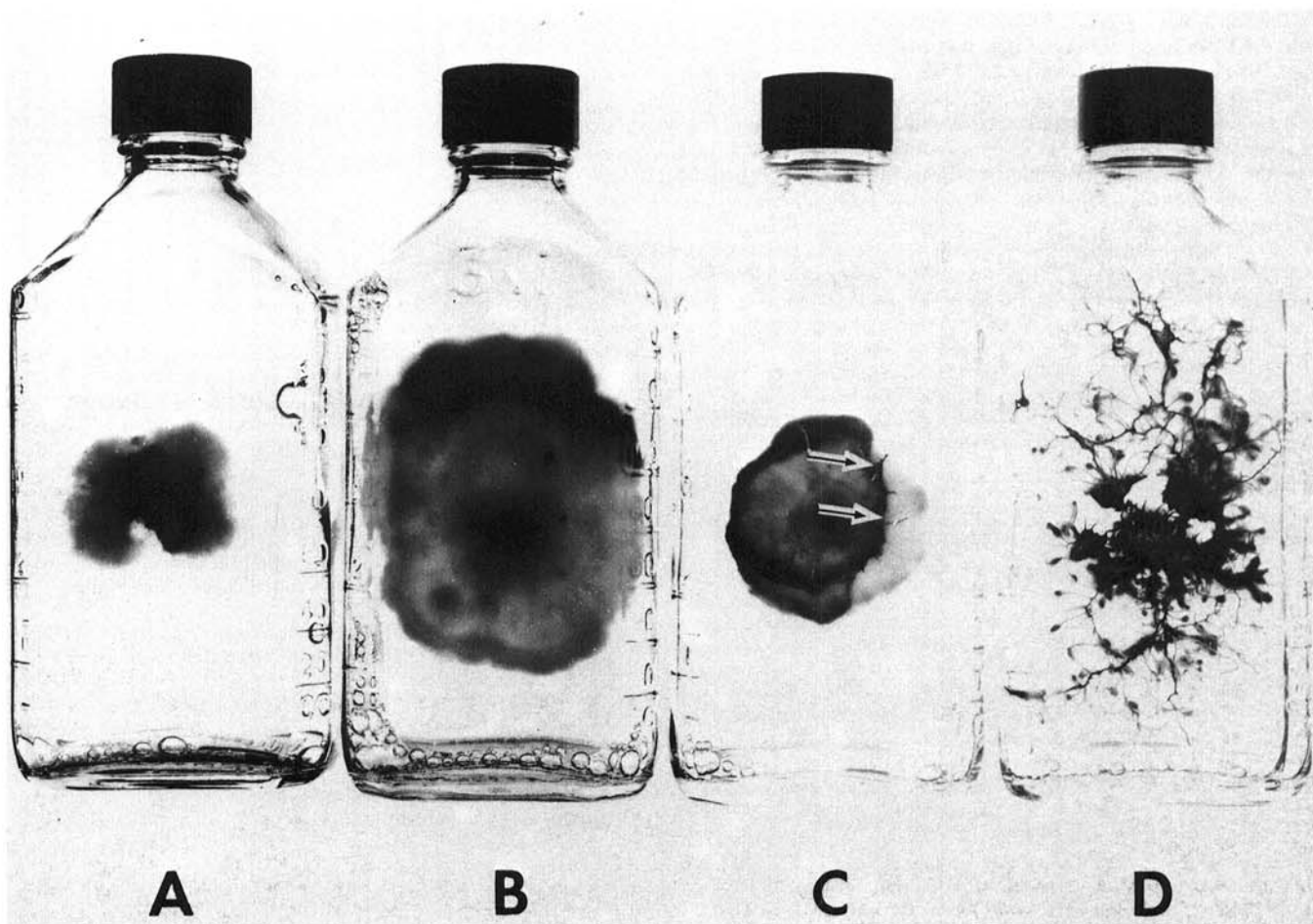


Fig. 2-(A to D). Colony morphology of *Armillariella mellea* isolates on Weinhold's medium without (A) and with (B-D) 2,4-D amendments. A) Control, isolate O-20. B) Increased mycelial growth with no rhizomorph formation by isolate O-20 at 100 $\mu\text{g/ml}$ 2,4-D. C) Rhizomorph initials (arrows) present with no significant increase in mycelial growth by isolate P-19 at 100 $\mu\text{g/ml}$ 2,4-D. D) Prolific rhizomorph production by isolate O-23 at 250 $\mu\text{g/ml}$ 2,4-D. The reaction of isolate O-22 was comparable to isolate O-23.

taken 14 mo after herbicide application, and the long-chain ester forms of these chemicals were not detected in any samples. Detection of the acids in root-collar phloem within 2 days after treatment indicated that these chemicals are readily absorbed and translocated by oaks. It was not surprising that the acid of 2,4,5-TP was detected in almost three times the number of samples as 2,4-D acid, because the chromatograph was five times more sensitive for detection of 2,4,5-TP than for equal quantities of 2,4-D.

DISCUSSION

Possible explanations for the differences in amount and time of rhizomorph production between herbicide-killed and mechanically-killed trees (Fig. 1) were considered. The difference after 2 yr and continuing up to 10 yr was significant, but seemed to be in rate of production rather than total quantity. Unfortunately we could not obtain data from mechanical treatments for the 10- to 14-yr period, but it seemed unlikely that the total quantity of rhizomorphs produced from a root system would be influenced by how the tree was killed. Rather it appeared that the rate of rhizomorph production, which would depend upon the time of initial fungal penetration and subsequent root colonization, could be influenced by the method of killing the tree. Thus, the rate of rhizomorph production might be affected by direct chemical stimulation of *A. mellea* by chlorophenoxy acid compounds or by earlier colonization of roots of herbicide-treated trees.

It was evident that 2,4-D altered the growth pattern or growth rate of all the isolates tested. The stimulatory range of activity for 2,4-D was between 10 and 250 $\mu\text{g/ml}$, with 100 $\mu\text{g/ml}$ the most active concentration. Garraway (8) reported that auxin and auxin-like compounds, which are similar to 2,4-D in plant growth-regulating activity, stimulated rhizomorph growth of *A. mellea* between 10 and 40 $\mu\text{g/ml}$. In forest situations, however, 2,4-D normally is not used alone because it is ineffective against many woody species, and it was not used alone in any of the areas we sampled for rhizomorph production. The rhizomorph production data were obtained from areas sprayed with 2,4,5-T alone or equal mixtures of 2,4-D and 2,4,5-TP. Our assays showed that 2,4,5-T alone had no effect on one isolate and was inhibitory to a second isolate. The mixture of 2,4-D and 2,4,5-TP had no significant effect on *A. mellea* isolates at combined concentrations below 100 $\mu\text{g/ml}$ and was inhibitory at higher levels. Fifty $\mu\text{g/ml}$ each of 2,4-D and 2,4,5-T did stimulate the growth of isolate O-20, but this herbicide combination was not sprayed in any of the field areas studied. The gas chromatography results revealed that 2,4-D and 2,4,5-TP, as

applied under field conditions, were not in the root-collar phloem at levels above 2.5 and 0.5 $\mu\text{g/ml}$, respectively. We concluded that, although 2,4-D has the potential to directly stimulate *A. mellea* in vitro, it was not present in the field samples in the proper quantity to have a direct effect on the fungus and to explain the difference in rhizomorph production between chemically-killed and mechanically-killed oaks.

There are alternate mechanisms which may explain the impact of these chemicals on *A. mellea* in vivo. One is that chemical poisoning killed roots more rapidly than mechanical killing. Redfern (19) reported that roots of both ring-barked (girdled) trees and trees frill-girdled and poisoned with 2,4,5-T were colonized and decomposed faster by *A. mellea* than those of trees felled without any previous treatment. Root invasion seemed to be governed by rapidity of decline in host resistance, and death of roots and consequent decline in host resistance may occur most rapidly in trees killed by herbicide treatment. Rishbeth's (20,21) findings that stump treatment with 2,4,5-T favors colonization by *A. mellea* also tends to support this explanation.

The chlorophenoxy acid herbicides might stimulate *A. mellea* indirectly. Many biochemical changes are induced within plants by 2,4-D and related compounds. One common effect is the alteration of starch and sugar levels (2,3). The exact response of the plant depends upon the time and amount of chemical application. Wargo (25) reported that manual defoliation of sugar maples (*Acer saccharum* Marsh) caused significant increases in the glucose and fructose of the outer root wood, and that extracts of this outer root wood stimulated the growth of *A. mellea* in culture. Glucose and fructose have previously been reported to be excellent carbon sources for the in vitro growth of *A. mellea* (28). Manual defoliation of sugar maples also resulted in increased root infections by this fungus (26). Chemical defoliation or the presence of plant growth-regulating herbicides within oak roots might cause similar changes in root carbohydrates that would be stimulatory to *A. mellea* resulting in rapid colonization of the oak roots.

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TABLE 3. The growth^a in culture on Weinhold's medium of *Armillaria mellea* isolate O-20 at different concentrations of various chlorophenoxy acids and picloram

| Herbicide conc. ($\mu\text{g/ml}$) | Growth in cultures containing: | | | | | |
|--------------------------------------|--------------------------------|--------------|---------------|-------------------------------------|--------------------------------------|---------------|
| | 2,4-D (mg) | 2,4,5-T (mg) | 2,4,5-TP (mg) | 2,4-D and 2,4,5-T ^b (mg) | 2,4-D and 2,4,5-TP ^b (mg) | Picloram (mg) |
| 0 | 63.9 A | 52.7 A | 55.8 A | 56.3 A | 56.8 A | 62.6 A |
| 0.01 | ... | ... | 52.3 A | ... | ... | ... |
| 0.1 | 64.3 A | 43.5 A | 47.2 A | 55.6 A | 53.4 A | 63.0 A |
| 1.0 | 75.6 A | 47.8 A | 52.5 A | 60.0 A | 59.5 A | 64.4 A |
| 10.0 | 86.7 B | 56.9 A | 32.4 B | 64.3 A | 51.6 A | 54.0 B |
| 50.0 | 86.2 B | 46.0 A | 24.9 BC | 62.1 A | 50.4 A | 53.6 B |
| 100.0 | 102.3 C | 51.4 A | 19.4 C | 90.3 B | 35.3 B | 50.5 B |
| 150.0 | ... | 47.1 A | ... | ... | ... | 51.6 B |
| 200.0 | ... | ... | ... | 54.3 A | 32.9 B | ... |
| 250.0 | 77.0 AB | ... | ... | ... | ... | ... |

^aIn milligrams, dry weight. Cultures grown in 25 ml of medium in the flat side of 214-ml medicine bottles incubated in the dark at 20-22 C for 8 wk. Each value is the mean of 10 observations from two, five-replicate experiments. Means not followed by the same letters within each column are significantly different at $P = 0.05$, as determined by Duncan's multiple-range test.

^bMixtures contained equal amounts of both herbicides.

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