Biodegradation of Metalaxyl in Avocado Soils

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ABSTRACT

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Using a sensitive bioassay involving *Phytophthora boehmeriae* as the test organism, biodegradation of metalaxyl was detected in three of five avocado soils that had received repeated applications of the fungicide over a period of 2–5 yr. The average half-life of metalaxyl in these soils was 28 days, and in the most active soils the half-life was 14 days. The composition and levels of the microbial populations (bacteria, fungi, and actinomycetes) of similar soils, either active or inactive in the breakdown of metalaxyl, did not differ. Active microbial populations were recovered both from soils with a history of prior metalaxyl treatment and from similar untreated soils with no ability to degrade the fungicide. Fungal and bacterial microflora were recovered from these two soils by using either selective media or filtration techniques and were capable of degrading metalaxyl over a 45-day period. One soil capable of degrading metalaxyl (50% over 14 days) did not promote degradation of either the acylanilide fungicides RE 26745 and oxadixyl, or the chemically related herbicide metolachlor.

In a previous study, a sensitive bioassay was developed for detecting low concentrations of metalaxyl (*N*-[2,6-dimethylphenyl]-*N*-[methoxyacetyl]-alanine methyl ester), in soils (1). Metalaxyl, a systemic fungicide active specifically against plant pathogens of the order Peronosporales (2–5,11), has been studied in plants (8–10). Very few studies have evaluated its longevity and biodegradation in soils (1,7,12). Due to its low adsorption and high mobility, metalaxyl can be rapidly leached from sandy soils which are low in organic matter (12). In certain cases it also may be subject to microbial degradation (1,12). Under severe disease pressure, extensive biodegradation of metalaxyl could lead to a significant or premature loss of fungicidal efficacy.

The purpose of this study was to obtain basic information on the longevity of metalaxyl in avocado soils that had received repeated applications of the fungicide over a period of several years as well as in soils not previously treated with the fungicide.

MATERIALS AND METHODS

Soils. Five sandy loam and sandy clay loam soils with a history of metalaxyl treatment, and typical of those in which avocado (*Persea americana* Mill.) is cultivated in southern California, were selected to study biodegradation of metalaxyl (Table 1). Prior to selection of the samples, soils A, B, C, and D received metalaxyl for 2 yr at a rate of 2.5 g a.i./m^2 per application, with three applications per year. Soil E received 30 applications over a period of 5 yr at rates of 2.05 or 4.10 g a.i./m^2. Samples of similar soils that had not received metalaxyl were selected as controls.

Coefficients for metalaxyl soil adsorption. Samples of a sandy loam soil taken from an avocado grove in Fallbrook, San Diego County, CA (Table 1, soils E5-9) were used to determine the adsorption of metalaxyl. Soil samples were treated with 5, 10, 15, and 20 μg of metalaxyl (technical grade, 94.3% a.i.) per milliliter, and adsorption isotherms were obtained by using the method described by Sharom and Edgington (12). Concentrations of metalaxyl were determined by using the bioassay described by Bailey and Coffey (1).

Biodegradation of metalaxyl in soils. The soils (Table 1) with a history of metalaxyl treatment were studied to determine their ability to biodegrade that fungicide. Soil moisture was adjusted to field capacity, and each sample, equivalent to 200 g of oven-dried soil, was thoroughly mixed with 40 mg a.i. of metalaxyl dissolved in 2 ml of methanol by using mechanical agitation, to give a final concentration of 200 μg/g dry weight of soil. For each soil, two samples were incubated in the dark at 23 C in separate 500-ml Mason jars. A control sample, to which 2 ml of methanol was added, was included for each soil. Four samples, each consisting of about 5 g of soil, were removed from each jar at 0, 14, 28, 42, 56, and 70 days. The samples were treated and bioassayed as described previously (1).

Microbial populations. The microbial population of each soil was determined before the addition of metalaxyl by using a combination of the dilution-plate technique (6) and different selective media for the major components of the microflora. Glucose peptone agar was used to isolate bacteria, water agar for actinomycetes, and Martin's rose-bengal agar for fungi.

Biological degradation by different soil microflora. Two soil samples were collected from the same avocado grove: one, E9, was active in degrading metalaxyl and originated from a fungicide-treated site; the other, E7, originated from an untreated site, and showed no capacity to degrade the fungicide. The moisture content of each soil was adjusted to field capacity in 500-ml Mason jars and a sample, consisting of 200 g of soil (dry weight equivalent), was sterilized in a saturated chloroform atmosphere inside a dessicator for 2 days at 23 C. The soils were then aerated and reinfested with portions of the natural microflora isolated previously from these same soils. Two treatments consisting of a 5-ml aqueous suspension from three plates of the appropriate selective medium for fungi, using the 10^2 dilution, and bacteria using the 10^5 dilution, were added to the soils. Additionally, 5-ml soil extracts (1%, w/v) consisting of either bacterial or fungal suspensions obtained using Millipore membranes, a SC 8.0-μm pore size for separation of fungi from bacteria, or an HA 0.45-μm pore size for bacteria, were added to soils. Soils treated with microbial suspensions were amended with metalaxyl at 200 μg a.i./g dry weight and incubated in the dark at 23 C. Controls consisted of chloroform-treated soils with and without 200 μg a.i. of metalaxyl per milliliter. Soil samples were removed from each jar at 0, 14, 28, and 45 days and the concentration of metalaxyl present was determined by using the bioassay (1).

Biodegradation of different acylanilide pesticides. The persistence of metalaxyl in soil was compared to that of several other acylanilide compounds. Soil E9, active in degrading metalaxyl and maintained at field capacity, was treated with either

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200 μg of metalaxyl, 500 μg of oxadixyl (2-methoxy-*N*-[2-oxo-1,3-oxazalidin-3-yl]-acet-2′,6′-xylidine), 200 μg of RE 26745 (2-methoxyl-*N*-[2,6-dimethylphenyl]-*N*-[tetrahydro-2-oxo-3-furanyl]-acetamide), or 1000 μg metolachlor (2-chloro-*N*-[2-ethyl-6methylphenyl]-*N*-[2-methoxy-1-methylethyl] acetamide) per gram dry weight of soil. The samples were bioassayed at 0, 14, 28, 42, 56, and 70 days for the level of phenylamide compound present. Actual concentrations of the acylanilides present in soils were calculated by reference to standard curves of the growth responses of an isolate (P 1257) of *Phytophthora boehmeriae* Sawada that were determined for each chemical.

RESULTS

Coefficients for metalaxyl soil adsorption. The *K* values (relative measure of adsorption) for metalaxyl of five different soil samples (E5, E6, E7, E8, and E9) from the same avocado grove were 0.20, 0.28, 0.36, 0.43, and 0.60 nmoles/g, respectively.

Biodegradation of metalaxyl. With the exception of one soil (D1), most of the original 200 μg of metalaxyl added was still detected by the bioassay after 70 days in soils with no prior history of metalaxyl treatment. In contrast, in three out of five of the soils with a history of metalaxyl treatment, the fungicide could not be detected after 70 days (Figs. 1 and 2). The soil samples most active

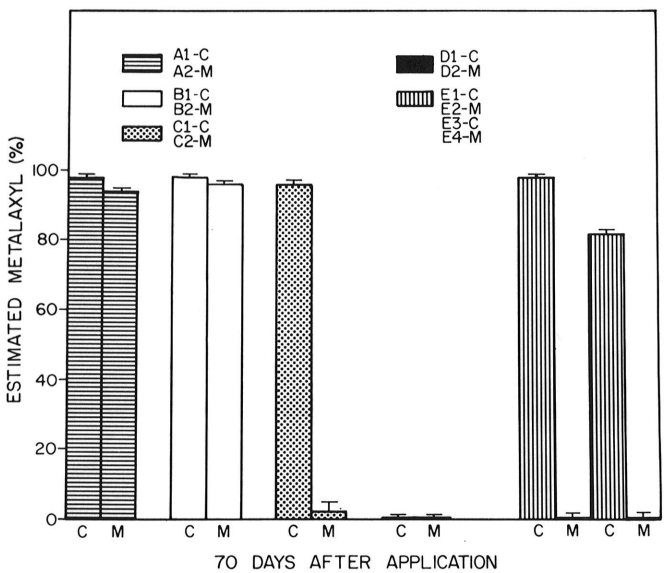

Fig. 1. Concentrations of metalaxyl estimated in four sandy loam soils (A, B, C, and E) and a sandy clay loam (D) (see Table 1 for details of their physical composition) with the bioassay procedure at 70 days after application. The terminating letter C indicates a control sample without a history of metalaxyl treatment and the similarly placed letter M a sample with a history of metalaxyl application. Bar lines represent the standard error of the mean based on three replicate assays for each sample ($P = 0.05$).

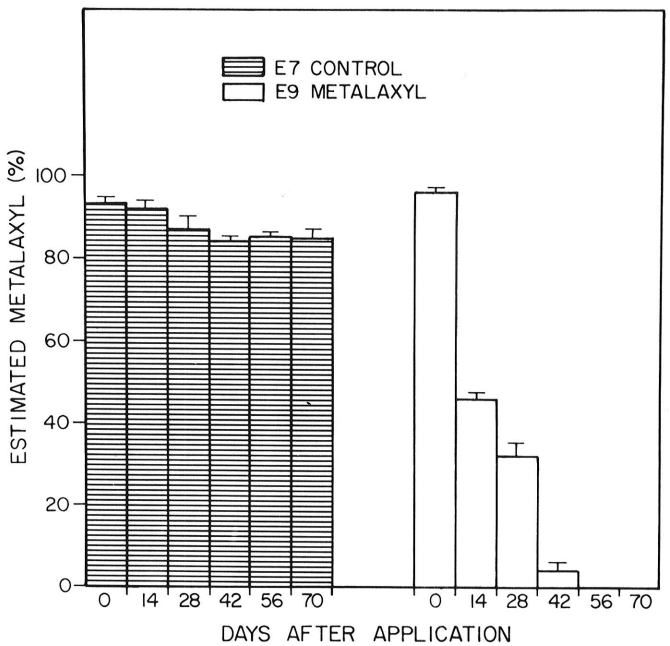

Fig. 2. Concentrations of metalaxyl estimated for two sandy loam soils up to 70 days after initial application. E7 is a control soil without any history of prior metalaxyl treatment, and E9 is a soil with a history of prior metalaxyl applications. Bar lines represent the standard errors ($P = 0.05$) based on three replicate assays for each sample ($P = 0.05$).

TABLE 1. Physical and biological properties of soils used in studies of metalaxyl degradation[a]

Soil sample	Clay (%)	Silt (%)	Sand (%)	pH	Microbial population levels before application of metalaxyl (per gram of soil)[b]		
					Bacteria (10^6)	Fungi (10^2)	Actinomycetes (10^4)
A1-C[c]	12.4	16.4	71.2	7.7	252	40	5
A2-M[c]	10.7	15.9	73.4	7.5	378	50	13
B1-C	16.8	20.0	63.2	6.9	189	91	2
B2-M	17.4	18.7	63.9	6.7	315	70	2
C1-C	12.3	10.3	77.4	6.9	315	29	...
C2-M	12.3	8.9	78.8	7.0	430	50	...
D1-C	23.0	20.9	56.1	7.4	378	30	3
D2-M	23.0	21.2	55.8	7.1	252	27	5
E1-C	8.4	14.6	77.0	7.2	789	27	3
E2-M	9.2	16.0	74.8	7.1	756	53	9
E3-C	7.7	11.7	80.6	7.0	273	44	4
E4-M	9.2	14.3	76.5	6.8	630	19	9
E5-C	7.0	12.9	80.1	7.1	750	50	5
E6-M	8.2	13.1	78.7	6.8	756	27	4
E7-C	8.4	15.9	75.7	6.9	399	23	5
E8-M	7.5	13.3	79.2	7.0	588	23	2
E9-M	8.4	12.2	79.4	6.9	1134	150	3

[a] The percentage composition of the soils was determined by mechanical analysis using the hydrometric method. Soil pH was measured using a saturated soil paste. All soils are sandy loams except D1 and D2 which are sandy clay loams.

[b] The microbial population numbers were obtained using the dilution plate technique and selective media. Glucose peptone agar was used for bacterial isolation, water agar for actinomycetes, and Martin's rose-bengal agar for fungi.

[c] The terminating letter C indicates that the soil has no prior history of treatment with metalaxyl; the similarly placed M indicates that the soil has a history of 2–5 yr of treatment with metalaxyl.

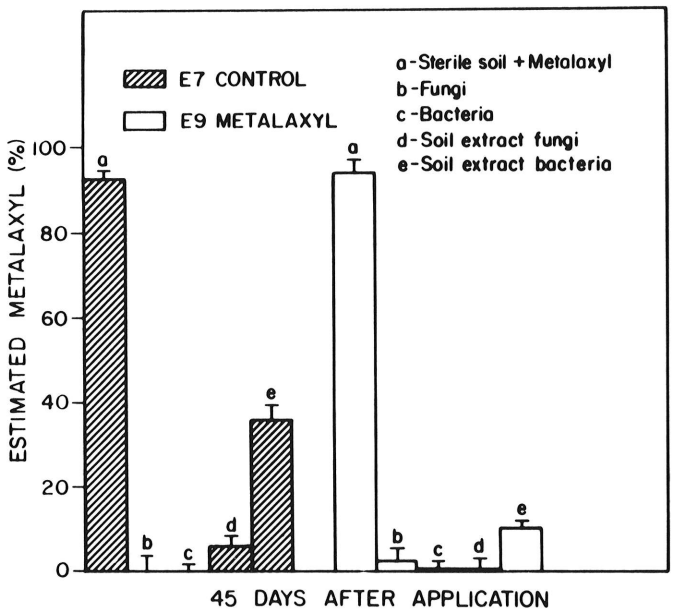

Fig. 3. Estimated metalaxyl concentrations using the bioassay at 45 days after application for original control (E7) and metalaxyl-treated (E9) sandy loam soils reinfested with various microflora isolated from each soil. Bar lines represent the standard errors ($P = 0.05$) based on three replicate assays for each sample ($P = 0.05$).

in biodegradation were E4 and E9, with 47.5 and 46% breakdown of detectable metalaxyl after 28 and 14 days, respectively. No difference was found between soils treated previously with either 2.05 or 4.1 g a.i./m^2.

Microbial populations and degradation of metalaxyl by soil microflora. Using analysis of variance, no differences were detected between the total recovered microbial population levels of the various soil samples, or between their proportions of total bacteria, fungi, or actinomycetes (Table 1), and the degradation of metalaxyl in the soils (Fig. 1). Metalaxyl was rapidly degraded by various portions of microflora isolated from the two soil samples E7 and E9, even though metalaxyl was not degraded in soil E7 (Fig. 3). Biodegradation of metalaxyl was nearly complete with fractions containing either fungi or bacteria from these soils. In the chloroform-sterilized soil, however, even after 45 days metalaxyl was still assayed at its original level.

Biodegradation of different acylanilides. By using the bioassay for *P. boehmeriae* (1), soil samples were compared for their ability to degrade the fungicides metalaxyl, RE 26745, or oxadixyl, and the herbicide metolachlor. Metalaxyl was completely degraded by 70 days. There was no significant degradation of the other acylanilide pesticides over the same period.

DISCUSSION

The adsorption coefficients determined for metalaxyl in this study were low, and this confirms previous reports on the adsorption and mobility of this fungicide in soils (7,12). Metalaxyl was very stable in chemically sterilized soils, with >90% of the initial concentration remaining after 70 days. However, metalaxyl was degraded in some unsterilized soils which indicates that biological degradation was responsible for its loss. In three of five soils with a history of metalaxyl treatment, there was much more rapid degradation of the fungicide compared with similar soils that had not received the chemical. However, one untreated soil with no history of prior exposure to metalaxyl also was capable of degrading it. The average half-life of metalaxyl in soils capable of degrading it was 28 days. The most active soil, E9 had a half-life of only 14 days. The levels of total recovered microbial populations of the different soils were not related to previous metalaxyl applications, which suggests that changes in total microbial numbers were not correlated with biodegradation of metalaxyl.

The results obtained following application of portions of the microflora from two soil sites to the same sterile soil indicated that diverse components of the microflora were probably involved in the degradation process. Microflora capable of degrading metalaxyl were isolated from soils which in their natural state (without sterilization) were both active and inactive in this capacity. Apparently, both bacteria and fungi that are capable of degrading metalaxyl are present in soils with no history of metalaxyl usage, but the capacity to degrade the fungicide is not expressed in these soils. With the one soil tested, there was no capacity to degrade three other acylanilide pesticides. This may indicate that among the acylanilides, different chemical structures vary in resistance to biodegradation. However, this hypothesis requires testing with more soils that demonstrate rapid degradation of metalaxyl. Additional research also will be necessary to identify the specific components of the soil microflora involved in the phenomenon and to determine mechanisms and pathways involved in the breakdown of metalaxyl.

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