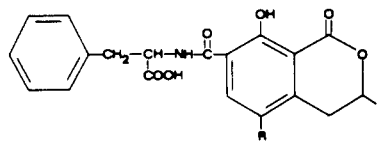

Figure 1. Chemical structure of ochratoxins A (R = Cl) and B (R = H).

nm) for ochratoxin A and B, respectively. Mass spectral data m/e in electron impact mode were 403 ($M^{+\cdot}$), 360, 255, 239, and 221 for ochratoxin A and 369 ($M^{+\cdot}$), 221, and 205 for ochratoxin B. Retention times in HPLC with the fluorometric detector (R_f values in TLC with solvent A) were 8.04 (0.46), 5.53 (0.38), 12.15 (0.75), and 7.80 min (0.51) for ochratoxin A and B and ochratoxin A and B methyl esters, respectively.

The high formation of ochratoxins in such a substrate can be easily explained by considering the storage temperature (25–30 °C) and the high moisture content of bread, which are favorable to the growing of *A. ochraceus* and, consequently, to the ochratoxins' elaboration (Ciegler, 1972; Tyllinen et al., 1977; Haeggblom, 1982).

This is the first report of the natural occurrence of ochratoxin A and B in Italy, and the first time that ochratoxin B is revealed in bread. The presence of ochratoxin A and B in such high concentrations in foodstuffs has never been reported, the highest levels encountered at present being 27.5 ppm of ochratoxin A and traces of ochratoxin B in plant products (Krogh, 1977). The concentration of ochratoxins in the examined sample is remarkably higher than lethal doses for animals affected by the considered mycotoxicosis. In fact, a diet containing less than 10 mg of ochratoxin A/kg of body weight can cause the death of poultry and dogs in a few days (Peckham, 1977; Carlton and Szczech, 1977). Moreover, the translocation of ochratoxins into the meat of animals fed on such contaminated bread is to be expected with a consequent potential risk of intoxication in man.

Although this is a single incident, the high contamination found indicates that the use of moldy bread for animal nutrition represents a very threat. Therefore, it is stressed once more the necessity of the utmost care in diverting into animal feeds waste foods, especially bread, since it has a tendency to become moldy because of its high moisture content.

Registry No. Ochratoxin A, 303-47-9; ochratoxin B, 4825-86-9.

LITERATURE CITED

AOAC "Official Methods of Analysis", 13th ed.; Horwitz, W., Ed.; Association of Official Analytical Chemists: Washington, DC, 1980; p 428.
Bacon, C. W.; Sweeney, J. G.; Robbins, J. D.; Burdick, D. *Appl. Microbiol.* **1973**, *26*, 155.
Bottalico, A.; Lerario, P. *Phytopathol. Mediterr.* **1979**, *18*, 77.
Cantafora, A.; Centi Grossi, M.; Miraglia Cellai, M. "La Contaminazione da Micotossine di Alimenti, Mangimi e Foraggi"; Cirilli, G.; Ligugnana, R., Eds.; Segreteria Simposi: Milano, 1982; p 71.
Carlton, W. W.; Szczech, C. M. "Mycotoxic Fungi, Mycotoxins, Mycotoxicoses"; Willie, T. D.; Morehouse, L. G., Eds.; Marcel Dekker: New York, 1977; Vol. II, p 345.
Ciegler, A. *Can. J. Microbiol.* **1972**, *18*, 631.
Haeggblom, P. *Appl. Environ. Microbiol.* **1982**, *43*, 1205.
Hansen, E.; Jung, M. *Pure Appl. Chem.* **1973**, *35*, 239.
Krogh, P. "Mycotoxins in human and animal health"; Rodricks, J. V.; Hesseltine, C. W.; Mehlman, M. A., Eds.; Pathotox Publishers: Park Forest South, IL, 1977; p 489.
Nelson, T. S.; Beasley, J. N.; Kirby, L. K.; Johnson, Z. B.; Ballam, G. C. *Poult. Sci.* **1980**, *59*, 2055.
Osborne, B. G. *Food Cosmet. Toxicol.* **1980**, *18*, 615.
Peckham, J. C. "Mycotoxic Fungi, Mycotoxins, Mycotoxicoses"; Willie, D. T.; Morehouse, L. G.; Eds.; Marcel Dekker: New York, 1977; Vol. II, p 301.
Pohland, A. E.; Allen, R. *J. Assoc. Off. Anal. Chem.* **1970**, *53*, 686.
Raper, K. B.; Fennell, D. I. "The Genus *Aspergillus*"; Williams & Wilkins Co.: Baltimore, MD, 1965.
Scott, P. M. "Mycotoxic Fungi, Mycotoxins, Mycotoxicoses"; Willie, D. T.; Morehouse, L. G., Eds.; Marcel Dekker: New York, 1977; Vol. I, p 283.
Scott, P. M.; van Walbeek, W.; Kennedy, B.; Anyeti, D. *J. Agric. Food Chem.* **1972**, *20*, 631.
Steyn, P. S. "Microbial Toxins"; Ciegler, A.; Kadis, S.; Ajl, S. J., Eds.; Academic Press: New York, 1971; Vol. VI, p 179.
Tyllinen, H.; Raevuori, M.; Karppanen, E.; Garry-Andersson, A. S. *Nord. Vetinaermed.* **1977**, *29*, 546.
WHO "Environmental Health Criteria: 11, Mycotoxins"; WHO: Geneva, 1979; p 86.

Angelo Visconti
Antonio Bottalico*
Istituto tossine e micotossine da parassiti vegetali
Consiglio Nazionale delle Ricerche
Via Amendola 197/F
70126 Bari, Italy

Received for review February 22, 1983. Accepted May 26, 1983.

Uptake and Persistence of Metalaxyl in Sunflower Plants

In this paper the results of a study of the translocation and persistence of Metalaxyl in sunflower plants grown under controlled conditions are reported. The active principle, which is very effective in the control of *Plasmopara helianti*, was applied both as a seed dressing and by incorporation into the soil. Quantitative analysis were performed on roots, stems, and leaves by extraction of the active principle with methanol, purification by sweep codistillation, and gas chromatography with a nitrogen–phosphorus detector. Mass spectrometry coupled with gas chromatography was used to confirm the identity of the compound. The lowest limit of sensitivity was 0.01 ppm. The results showed rapid translocation of the fungicide toward the upper parts of the plant, as indicated by the high concentration found in the leaves a few days after planting. Although the concentration showed a steady decline after reaching its maximum, Metalaxyl was still present in the leaves 90 days after planting.

New possibilities to control plant diseases have become available with the discovery of systemic fungicides that are active against ficomycetes. Metalaxyl [DL-*N*-(2,6-dimethylphenyl)-*N*-(2-methoxyacetyl)alanine methyl ester] is one of these active principles, which allows the control of one of the more serious sunflower diseases: downy

0021-8561/83/1431-1123$01.50/0

Table I. Mass Fragmentogram Analysis of the Purified Extract of Metalaxyl-Treated Sunflower

	mass	retention time, min	absolute intensity	relative intensity
1	279.0	2.8	5.2	100.0
2	248.0	2.8	21.0	404.5
3	220.0	2.8	9.5	183.0
4	206.0	2.8	21.5	413.0

mildew, caused by *Plasmopara helianthi* Novot.

In a previous paper (Zazzerini et al., 1981) the translocation rate and the high persistence of Metalaxyl in sunflower plants were shown when the active principle was applied either as a seed dressing or into the soil directly. In this paper we present the results of further studies in which the analysis of chemical residues was extended to the entire plant cycle.

EXPERIMENTAL SECTION

Plant Material. The experiments were carried out in a controlled environment (light intensity = 14000 lx, photoperiod = 12 h, temperature = 20 °C, and relative humidity = 80%) with the susceptible cultivar Uniflor 70. Metalaxyl was applied both as a seed dressing and by incorporation into the soil of 2 g/m^2 granular Ridomil (5% of Metalaxyl). Two types of seed dressing were used: Ridomil (25% Metalaxyl as a wettable powder) at the rate of 800 g/100 kg of seeds and Apron (35% Metalaxyl as a slurry) at the rate of 600 g/100 kg of seed. Dressed seeds were planted in 35 cm × 60 cm boxes, each containing 36 kg of soil: pH (H_2O) 7.5; organic matter, 11.1%; sand, silt, and clay, 818, 64, and 118 g/kg, respectively (*Mollic psammaquent*).

Granular Ridomil was broadcasted on the top soil and lightly buried before planting. Boxes were of the same size as for dressed seeds. Each treatment was repeated 4 times, and commencing 4 days after planting, plant material was sampled periodically (after 4, 11, 20, 27, 34, 41, 47, and 90 days).

Analysis of Sunflower Plants. Metalaxyl was extracted from roots, stems, and leaves following the procedure given by Tafuri et al. (1981). The main steps of this procedure were as follows. Metalaxyl was extracted from plant material with methanol and purified by sweep codistillation. The determination of Metalaxyl was done by gas chromatography using a nitrogen–phosphorus detector which allowed measurement of concentrations as low as 0.05 ppm. An additional purification by column chromatography was required to improve the sensitivity; the extract derived from sweep codistillation was dissolved with benzene (3 × 2 mL) and inroduced into a column (1 cm × 6.5 cm) packed with a benzene suspension of Al_2O_3 (activity II or III according to Brockmann).

The column was eluted with 60 mL of benzene, and the eluate was discarded. The column was again eluted with 50 mL of a mixture of chloroform–ethyl ether, 90:10, and the extract was concentrated to 0.5–0.1 mL. Aliquots (1–2 μL) were injected into a Perkin-Elmer Model 900 gas chromatograph equipped with a NPD detector and operating at the following conditions: carrier gas, helium; flow rate, 25 mL/min; injector temperature, 240 °C; hydrogen and air flow rates, 5 and 90 mL/min; a glass column, 2 m × 6 mm, packed with 1.5% cyclohexanedimethanol–succinate on 80–100-mesh Gas-Chrom Q was used at 190 °C. The retention time of Metalaxyl was 7.0 min.

The Metalaxyl was confirmed by GC/MS using a Varian Mat 44 gas chromatograph/quadrupole mass spectrometer equipped with a multiple-ion detector and focused on the following masses: 279, 248, 220, and 206. The instrument was operated in the electron-impact mode (Table I).

The carrier gas was helium at a flow rate of 20 mL/min; the temperature of the injection port was 250 °C; a 1.80 m × 6 mm glass column packed with 3% SE-30 on 100–120-mesh Supelcoport maintained at a temperature of 190 °C was used. The pressure in the mass spectrometer was 4.8 nanobars and the emission current was 1.45 mA.

Table II. Mean Recoveries of Metalaxyl Added to Sunflower before Extraction

amounts added, mg/kg	recovery, %[a]	
	leaves	stems and roots
0.01	87 ± 3.2 (5)	89 ± 2.8 (3)
0.02	92 ± 3.2 (4)	92 ± 3.0 (3)
0.05	89 ± 2.2 (3)	91 ± 2.0 (3)
0.1	98 ± 2.5 (3)	98 ± 2.1 (3)

[a] Mean ± standard error (number of determinations in parentheses).

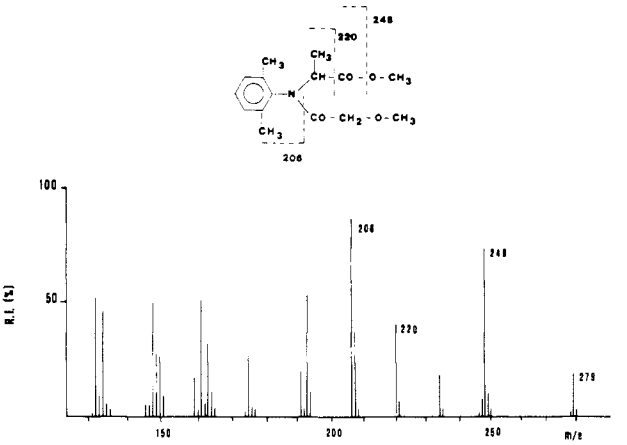

Figure 1. Mass spectrum of Metalaxyl.

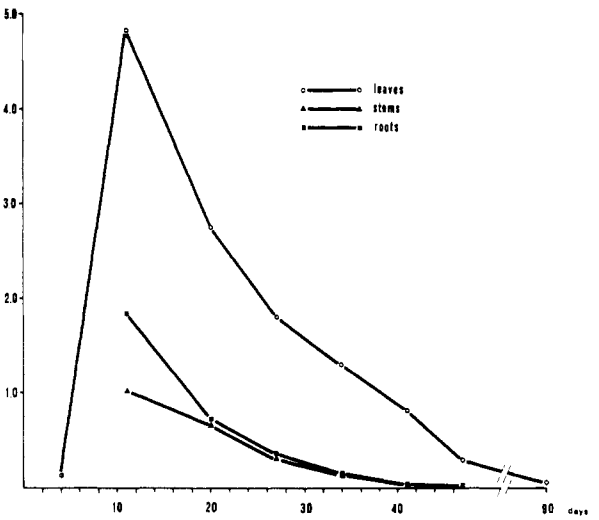

Figure 2. Uptake and persistence of Metalaxyl in sunflower when applied as a seed dressing (Apron: 35% Metalaxyl as a slurry at the rate of 600 g/100 kg of seeds).

RESULTS AND DISCUSSION

The method of analysis was fast and reliable. Recoveries by adding known amounts of Metalaxyl to the plant materials are shown in Table II. Mass fragmentography was used on samples containing small amounts of Metalaxyl to confirm the identity of the gas chromatographic peak. On the basis of the mass spectrum resulting from the injection of a standard solution of Metalaxyl in methanol, the more representative signals were due to the followin

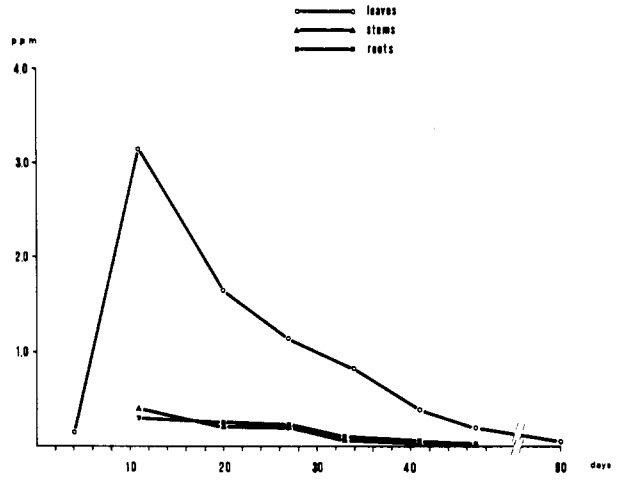

Figure 3. Uptake and persistence of Metalaxyl in sunflower when applied as a seed dressing (Ridomil: 25% Metalaxyl as a wettable powder at the rate of 600 g/100 kg of seeds).

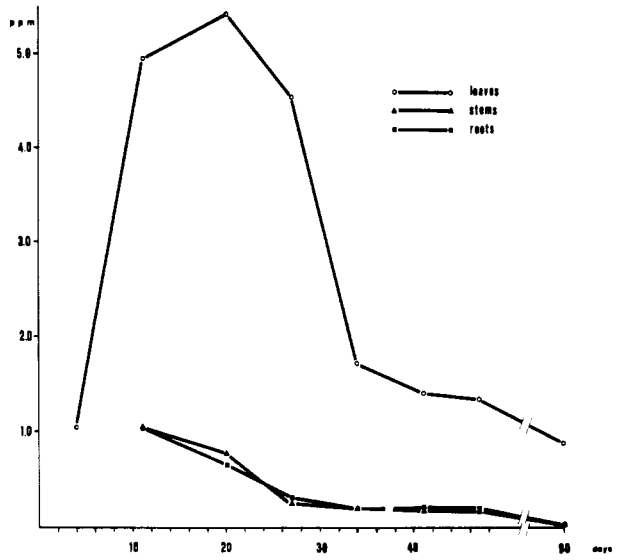

Figure 4. Uptake and persistence of Metalaxyl in sunflower when applied into the soil (granular Ridomil: 5% Metalaxyl at the rate of 2 g/m^2).

masses: 279, 248, 220, and 206 (Figure 1). The fragmentography was therefore focused on these masses to detect the presence of Metalaxyl in the plant extracts.

The mass fragmentograph was especially useful for low active principle concentrations (0.02–0.01 ppm). At these concentrations the NPD detector was not suited for a precise determination because of the interference peaks due to coextracted substances.

The results reported in Figure 2–4 show that Metalaxyl was rapidly absorbed and translocated either when applied as a seed dressing or incorporated into the soil.

The fungicide reached the maximum concentration in the leaves 11 days after planting when applied as a seed dressing (Figures 2 and 3) and 20 days after planting when applied to the soil (Figure 4).

There was then a progressive decrease, and 34 days after planting the concentration of Metalaxyl in the leaves was 70% lower than the maximum concentration, regardless of the treatments (Figures 2–4). Although the concentration of the fungicide was progressively declining, its presence in the leaves was still detected 90 days after planting. The residues detected in the roots and the stems followed a similar pattern, but the concentration was much lower than in the leaves. The presence of Metalaxyl in these organs was detectable for 47 days after planting when applied as a seed dressing and for 90 days when applied into the soil.

The concentration of Metalaxyl in the seeds was always less than the lowest limit of sensitivity of the analysis (0.01 ppm). Figures 2 and 3 show that the Metalaxyl concentration in the leaves decreased from 3–5 to 0.1–0.2 ppm in the range 11–90 days; the weight of leaves however, increased about 25-fold in the same period. For this reason we believe that the decreased concentration of the fungicide in all plant organs has to be attributed to a variation of the ratio between weight of Metalaxyl and weight of either leaves, roots, or stems rather than to a degradation of the active principle (Figures 2 and 3). By comparison of Figures 2 and 3, it can be concluded that, for equal doses of an active ingredient, a higher absorption was found with Apron applied as a seed dressing.

ACKNOWLEDGMENT

We express our thanks to Ciba-Geigy for supplying Metalaxyl as a standard, Ridomil, and Apron.

Registry No. Metalaxyl, 57837-19-1.

LITERATURE CITED

Tafuri, F.; Marucchini, C.; Patumi, M.; Businelli, M. *J. Agric. Food Chem.* **1981**, *29*, 1296.

Zazzerini, A.; Marucchini, C.; Patumi, M., 3rd Symposium "Chemistry of Pesticide", Piacenza, Italy, Feb 26–27, 1981, p 42.

Cesare Marucchini*[1]
Maurizio Patumi[1]
Antonio Zazzerini[2]

[1]Centro di Studio sulla Chimica degli Antiparassitari del C.N.R. Istituto di Chimica Agraria dell'Università
06100 Perugia, Italy
[2]Istituto di Patologia Vegetale dell'Università
Perugia, Italy

Received for review November 11, 1982. Accepted April 11, 1983.

Identification of an Important New Flavor Compound in Concord Grape: Ethyl 3-Mercaptopropionate

Ethyl 3-mercaptopropionate was isolated and identified for the first time in Concord grape by combination gas chromatography–mass spectrometry analysis. At low concentration this compound possesses high-quality Concord grape aroma and flavor.

The volatile components of Concord grape (*Vitus labrusca*) have been studied by Holley et al. (1955), Neudoerffer et al. (1965), Stevens et al. (1965), and Stern et al. (1967). One hundred and fourteen compounds have

0021-8561/83/1431-1125$01.50/0