Degradation of isoxaflutole and metabolites in surface and subsoils under field conditions

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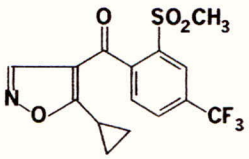
ABSTRACT

Field studies conducted with isoxaflutole in 1999 and 2000 included soil sampling at five locations in the United States. Parent isoxaflutole degraded rapidly with a half-life less than 4 days. The biologically active metabolite RPA 202248 degraded with a half-life of 1 to 3 weeks. Degradation rates for the biologically inactive metabolite RPA 203328 were not determined but appeared to be somewhat faster than for RPA 202248. Degradation rates measured in these studies were similar to those observed in previous field dissipation studies conducted in the U.S. and Europe. At two of the sites, heavy rainfall following application resulted in small amounts of the two metabolites moving via preferential flow into subsoils. Since no further movement of surface residues into subsoils occurred, degradation rates in surface and subsoils could be compared. At both locations degradation of the two metabolites in the subsoils continued at a rate comparable to the degradation of the metabolites remaining in the surface soil.

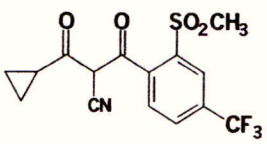
INTRODUCTION

Isoxaflutole is the active ingredient in Balance herbicide, which is applied prior to emergence to control weeds in maize. Degradation of isoxaflutole and its two principal soil metabolites (RPA 202248, a diketonitrile metabolite and RPA 203328, a benzoic acid metabolite) is primarily by soil organisms (Figure 1 presents chemical structures) although parent can also degrade by hydrolysis. Isoxaflutole rapidly degrades to RPA 202248, which in turn degrades to RPA 203328, which finally degrades to carbon dioxide. RPA 202248 is biologically active while RPA 203328 in biologically inactive. All three compounds are relatively mobile in soil (Koc values of 122, 92, and 69 mL/g for parent, RPA 202248, and RPA 203328, respectively), are essentially non-volatile, and do not degrade by photolysis in soil.

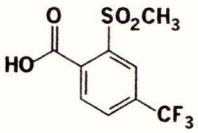
Figure 1. Chemical structures of isoxaflutole and its two principal metabolites.



Isoxaflutole



RPA 202248



RPA 203328

Field studies in the United States in 1999 and 2000 included soil sampling following carefully controlled applications at five test sites. This paper reports the dissipation rates measured in these studies. Since losses by other mechanisms such as runoff, leaching, drainage, photolysis, and volatilization were minor compared to degradation, the dissipation rates measured in these studies are close to the actual degradation rates in soil.

MATERIALS AND METHODS

Applications of isoxaflutole at a rate of 157 g/ha were made to two 1.8 ha test plots in Nebraska and Iowa in spring 1999. In spring 2000 isoxaflutole was applied at a rate of 102 g/ha to a 1.8 ha test plot in La Porte County, Indiana and at a rate of 157 g/ha to two larger plots of 30 and 11 ha in Allen and Owen Counties in Indiana. Table 1 provides a brief description of the properties of the surface soil at each of the five locations. The application rate was confirmed by analysis of 16 filter paper samples collected immediately after application. At each site soil cores were also collected immediately after application and 0.25, 0.5, 1, 2, 4, and 6 months after application. At each sampling interval, 16 soil cores were collected (four from each of four subplots) in 0.15 m depth increments. The depth of the core varied from 0.15 m for the samples collected immediately after application to a depth of up to 1.2 m depending on the position of residues in the soil profile. Cores taken immediately after application were collected by pushing a 75 mm diameter tube into the soil. The remaining samples were collected by a 83 mm bucket auger with appropriate procedures to avoid contamination during the raising and lowering of the auger. All samples were thoroughly mixed in the field and subsampled to provide the necessary volume for analysis. At the Nebraska, Iowa, and La Porte, Indiana sites, samples collected 2 months and later were composited to provide one sample per depth per subplot. At the other two Indiana locations, samples were similarly composited except for those collected immediately after application.

Table 1. Surface soil properties in the five U.S. studies conducted in 1999 and 2000.

Location	Properties of the Surface Soil (0-0.3 m)		
	Soil Texture	Organic Matter (%)	pH
Merrick County, Nebraska	loam	2.0	5.8
Sioux County, Iowa	loam	5.7	6.7
La Porte County, Indiana	sandy loam	3.5	5.8
Allen County, Indiana	silty clay	3.0	7.4
Owen County, Indiana	silt loam	1.6	6.2

Residues of isoxaflutole and its two principal metabolites were extracted from soil samples by shaking in an acetonitrile: 0.8 % formic acid solution for fifteen minutes. The mixture was centrifuged, and the supernatant diluted to the desired concentration for analysis by LC/MS/MS using a C-8 column and ^{13}C internal standards. The limit of quantification was

0.4 ng/g for parent isoxaflutole and RPA 202248 and 2 ng/g for RPA 203328. The limit of detection was 0.11 ng/g for parent isoxaflutole, 0.04 ng/g for RPA 202248, and 0.29 ng/g for RPA 203328.

The amount of parent and the two metabolites remaining in the soil as a function of depth was calculated from the soil concentrations, bulk density, and the depth increment. Model Manager Version 1.1, (Cherwell Scientific) was used to determine the dissipation rates of parent and RPA 202248.

RESULTS AND DISCUSSION

The degradation rates from Model Manager expressed as half-lives from the five test sites are shown in Table 2. Parent degraded quite rapidly at all locations with a half-life of 2-4 days and the biologically active metabolite RPA 202248 degraded with a half-life of about 1-3 weeks. The shorter half- life at the Allen County, Indiana location was due mainly to a low value obtained at one sampling interval so the actual degradation rate at this location was probably not significantly different from the other four locations. Figure 2 shows the disappearance of parent isoxaflutole and the rise and decline of RPA 202248 for the La Porte County, Indiana site. The pattern was similar at the other four sites.

Table 2. Degradation rates of isoxaflutole and RPA 202248 observed in the five U.S. studies conducted in 1999 and 2000 (from Model Manager).

Location	Half-Life (days) for Specified Compound	
	Isoxaflutole	RPA 202248
Merrick County, Nebraska	2.6	14
Sioux County, Iowa	2.3	19
La Porte County, Indiana	3.7	20
Allen County, Indiana	NC	14*
Owen County, Indiana	NC	7*

NC Not calculated due to high amounts of RPA 202248 in initial samples.
*Calculated from total parent isoxaflutole plus RPA 202248 residues

The degradation rates observed in the 1999 and 2000 studies are similar to the degradation rates observed in the field dissipation studies previously conducted in the United States and Europe (Table 3). The slow degradation rate at the California site was probably the result of the extremely dry soil conditions for several months prior to the start of the study. The half-lives for RPA 202248 in Table 3 are not directly comparable with the half-lives in Table 2. The half-lives for RPA 202248 in Table 3 were calculated with a simple regression using only the data RPA 202248 after the peak concentration was reached, ignoring the degradation in the first few days of the study as well as the formation of RPA 202248 after the peak concentration was reached. Thus, the half-lives of RPA 202248 in Table 3 represent an upper

bound on the actual half-life values. For example, the half-lives of RPA 202248 for the Goch and Manningtree locations calculated using Model Manager (which includes degradation of RPA 202248 occurring at the start of the study as well as the effect of formation of RPA 202248 throughout the study) were 17 and 10 days, respectively.

Figure 2. Residues of isoxaflutole and RPA 202248 as a function of time in days at the La Porte County, Indiana site. The fitted lines are those for the half-life values reported in Table 2 (this figure was generated by Model Manager).

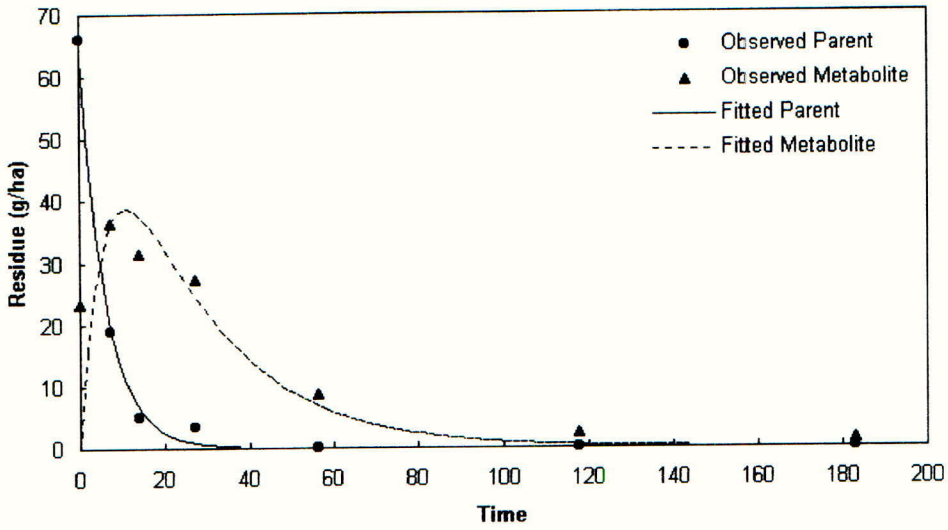


Table 3. Degradation rates observed in previous field dissipation studies conducted with isoxaflutole in the United States and Europe.

Location	Half-Life (days) for Specified Compound	
	Isoxaflutole	RPA 202248
U.S Field Dissipation Studies		
San Juan Baustista, California	1.4	125
York, Nebraska	1.5	8
Clayton, North Carolina	3.0	16
Euphrata, Washington	2.2	13
European Field Dissipation Studies		
Mereville, France	0.7	16
Goch, Germany	1.7	24
Bologna, Italy	0.5	22
Manningtree, United Kingdom	0.9	17

The degradation rates of the non-biologically active metabolite RPA 203328 at each of the test sites were not determined because of the relatively small amount formed and its continuous formation and degradation. Residues of RPA 203328 peaked at 10-22 percent of applied in the 0.5 to 1 month samples and appeared to degrade at a faster rate than RPA 202248, although its continuous formation meant that concentrations of this metabolite were present when RPA 202248 was also present.

At two of the sites, Merrick County, Nebraska, and Sioux County, Iowa, heavy rainfall during the month following application resulted in standing water in the corn fields and a small amount of the two metabolites moving into subsoils via preferential flow. Since little movement of residues in the soil profile occurred after one month following application, this provided an opportunity to compare degradation rates observed in subsoils and surface soils. Given the limitations imposed by the variability of the data, this examination indicates that the degradation rates in surface soils and subsoils were approximately the same. This is illustrated by Figures 3 and 4, which show the dissipation of residues at the surface and below 30 cm at the Nebraska and Iowa test sites. At the Nebraska site, the dissipation of both RPA 202248 and RPA 203328 below 0.3 m was as fast as in the surface soils. At the Iowa site, the degradation rates in surface and subsoils were similar through about four months. The variability in the six month data was probably associated with the low amount of residues present at this time interval (only about 0.1 to 1 percent of applied). With such small amounts of material remaining the increased variability would be expected due to local heterogeneity in degradation rates as well as lack of precision in analytical results since concentrations are below the limit of quantification. Based on the rainfall occurring between four and six months, the increase in RPA 202248 levels during this time period was not due to movement from surface soils to subsoils.

Figure 3. Effect of depth on degradation of RPA 202248 (DKN) and RPA 203328 (BA) at the Nebraska test site. The lines represent the amount of residues remaining from 1-6 months after application at the soil surface and below 0.3 m.

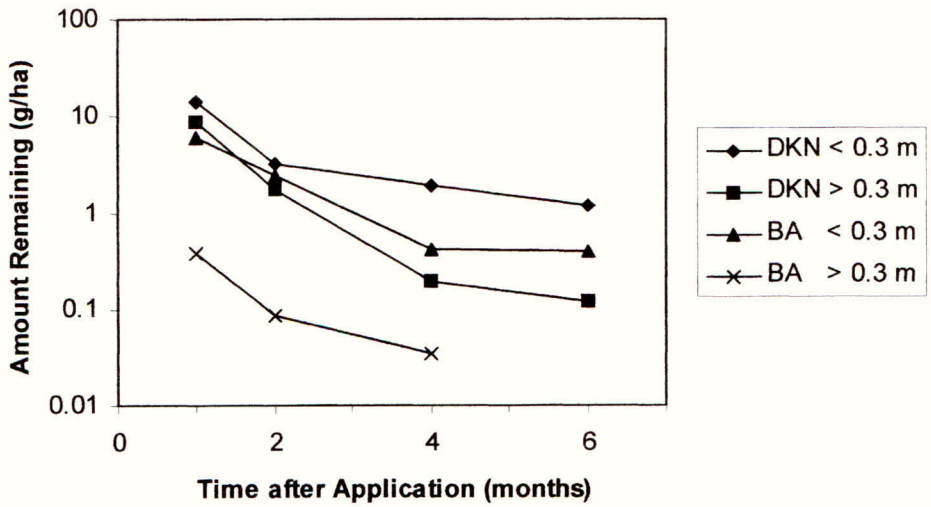
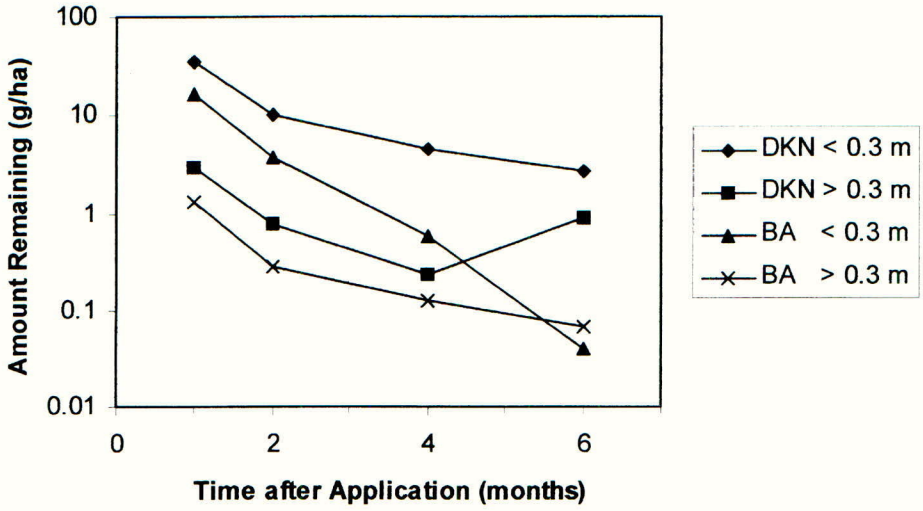


Figure 4. Effect of depth on degradation of RPA 202248 (DKN) and RPA 203328 (BA) at the Iowa test site. The lines represent the amount of residues remaining from 1-6 months after application at the surface and below 0.3 m.



CONCLUSIONS

Field studies conducted at five U.S. locations with isoxaflutole in 1999 and 2000 showed that parent isoxaflutole degraded rapidly with a half-life of under 4 days and the biologically active metabolite RPA 202248 degraded with a half-life of 1 to 3 weeks. These degradation rates were similar to those observed in previous field dissipation studies conducted in the U.S. and Europe. At two of the sites, where heavy rainfall following application resulted in small amounts of the two metabolites moving via preferential flow into subsoils, degradation continued at a rate comparable to the degradation of the metabolites remaining in the surface soils.

Evidence for the enhanced degradation of metalaxyl in UK carrot soils

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ABSTRACT

Laboratory studies were carried out to determine the rates of degradation of metalaxyl in soils from nine fields in which metalaxyl had been used extensively as a soil treatment for the control of cavity spot disease in carrots. In all these fields, the fungicide had failed to control the disease in recent years. A further carrot field was sampled to which metalaxyl had never been applied. Soil samples were taken from 10 stations within each field and each sample was processed individually. Sub-samples of the 10 were also bulked to produce a composite sample for each field. The time taken for 50 % of the fungicide to be degraded (DT_{50}) was calculated using GENSTAT 5, fitting Gompertz or linear regressions to the data. Comparisons were made between the regressions fitted for the composite samples and the average of those fitted for the 10 stations. In fields where the fungicide failed, the DT_{50} values varied from 4 to 14 days. The composite samples produced DT_{50} values that were comparable (4 to 15 days). In the field where no metalaxyl had been applied the average DT_{50} for the 10 stations was 46 days compared with 43 days from the composite sample. A second study was carried out with soil samples from two fields adjacent to one another. One field had no previous history of metalaxyl application whilst the other had a history of application and failure in the control of cavity spot. The DT_{50} values for metalaxyl degradation in soils from these fields were 39.3 and 13.2 days, respectively.

INTRODUCTION

Cavity spot disease of carrots in the UK is largely due to the metalaxyl-sensitive fungus *Pythium violae*. It produces sunken lesions on the carrot root, and is particularly damaging in years of high rainfall. Control of the disease has relied on the use of metalaxyl and, more recently, metalaxyl-M, applied between drilling and first true leaf stage. In recent years deterioration in the performance of metalaxyl has been observed in some fields, both by growers on their own field sites and by scientists during cavity spot field experiments (McPherson, pers. comm.). Populations of the pathogen have been continuously monitored for metalaxyl resistance, using the method of White *et al.*,(1988), but no resistance to metalaxyl has been found.

Various studies have established that metalaxyl is subject to degradation by soil microorganisms (Bailey & Coffey 1985; Droby & Coffey 1991). Recent studies in Western Australia have shown that reduced persistence of metalaxyl in fields used for carrot production is associated with previous metalaxyl use (Davison & McKay 1999). This study aims to examine the persistence of metalaxyl in fields used for carrot

production in the UK, and to compare persistence in two adjacent fields with different metalaxyl treatment histories.

MATERIALS AND METHODS

Site selection and sampling methods

Nine fields were identified by UK carrot growers, as having received metalaxyl applications over several years, and recent crop failure due to cavity spot despite metalaxyl use. One field was also identified at HRI Wellesbourne as having no previous history of metalaxyl use.

For each field, approximately 1 kg of top soil was collected from each of 10 stations within the field, along a 250 – 300 m transect. The trowels used for sampling were washed and disinfected between stations and fields to prevent cross contamination between samples. A composite sample was produced for each field by bulking together an equal quantity of soil from each of the 10 station samples, and mixing well. All samples were stored at 5°C prior to the laboratory incubations.

Control soils were difficult to obtain, and despite collecting soil samples from untreated areas like headlands, comparison of these soils with the field samples often revealed considerable differences in characteristics such as pH, making the soils unsuitable as controls.

To address this, a second study was carried out with soil from a further two fields located adjacent to one another. One field had received a number of metalaxyl applications together with a recent metalaxyl-treated carrot crop with cavity spot; the other field had no metalaxyl pre-treatment history. Soil samples were collected from 5 stations within the treated field. These samples were processed separately and bulked to produce a composite sample. Soil was also collected from 5 stations in the untreated field and processed as one bulked sample which was divided to produce 2 replicate subsamples.

Sample preparation and residue analysis

When handling soil, new or autoclaved equipment was used for each sample, and the bench was sprayed with industrial methylated spirit between samples. All soils were sieved to 3mm, and the maximum water holding capacity (MWHC) of the soil from each field was assessed using soil from the composite samples. In addition the moisture content of individual samples was determined and, where necessary, they were air-dried to reduce the water content to below 40 % of the MWHC.

Analytical (99.6 %, Novartis) and technical (97.4 %, Novartis) grade metalaxyl were used throughout the study. A solution of technical metalaxyl (0.5 g/l in water) was pipetted onto the soil sample to give a concentration of 10 mg/kg dry soil and the samples were thoroughly mixed and then transferred to 500 ml pots. For fields 1-4 there were two replicate pots per soil sample. For fields 5-10 this was reduced to one pot per sample. Sterilised distilled water (SDW) was pipetted around the edge of the pots to increase the moisture level to 40 % of the MWHC. The lids were replaced loosely and pots were

incubated at 15°C. Sub-samples of soil (15 g) were taken on d 0 and at regular intervals thereafter. On each sampling occasion any water lost from pots was replaced with SDW.

Metalaxyl was extracted from each 15 g sub-sample by shaking with 20 ml methanol for 50 min on a wrist-action shaker. The soil samples were allowed to settle for at least 10 min. Samples of clear supernatant were removed and analysed by hplc using a LiChrospher-RP18 (5μm) column and acetonitrile: water: orthophosphoric acid (70: 30: 0.25 by volume) eluant at a flow rate of 1 ml min^{-1}; detection was by UV absorbance at 210 nm. The retention time of metalaxyl was 3.5 min. The response on hplc was calibrated against a 5 mg/l analytical grade metalaxyl standard.

RESULTS AND DISCUSSION

Examples of the soil residue data are shown in Figures 1, 2 and 3 for fields 1, 5, and 10 respectively. They illustrate the results from the individual soils and those from the composite samples for each field. The times taken for 50 % loss of the metalaxyl (DT_{50}) were determined by fitting either a Gompertz curve or a linear regression as appropriate, using GENSTAT 5. This was carried out for each of the composite samples and by grouping data for each of the 10 stations within the field. The estimated DT_{50} values are listed in Table 1.

Table 1. Comparison of the DT_{50} (d) derived from fitting of the Gompertz equations or linear regressions to data from the composite sample and 10-station samples.

	DT_{50} (d) of fitted curves / linear regressions	
Field identification number	10 stations processed individually	Composite sample
1	14.4	14.9
2	8.1	8.8
3	8.2	9.0
4	9.2	8.9
5	9.7	8.3
6	3.7	3.6
7	4.0	3.5
8	6.8	5.5
9	7.5	7.1
10	45.7	42.7

Considerable variation in the rate of degradation of metalaxyl was seen between soils from the different fields. The highest DT_{50} value (45.7 d) was recorded in soil from the field (number 10) with no history of metalaxyl treatment, with metalaxyl persisting in all samples for 72 d or more. Despite the variable degradation rate within this field (Figure 3), the DT_{50} of metalaxyl in the composite sample was similar to that based on the regression for the data from the 10 stations.

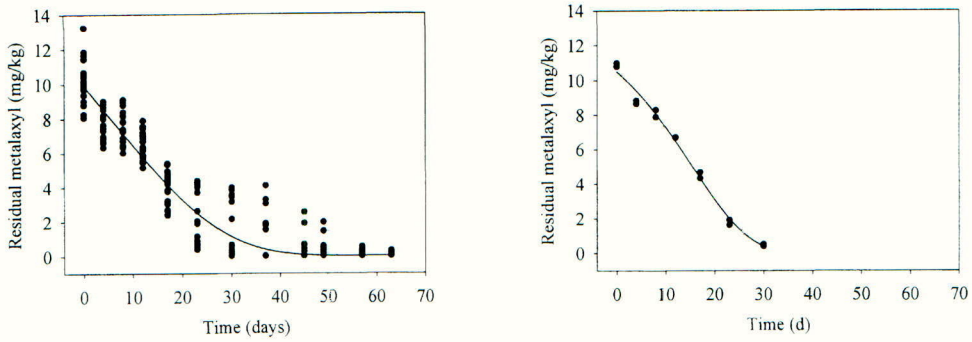

Figure 1. Comparison of metalaxyl degradation between the 10-station (left) and composite (right) soil samples from field 1.

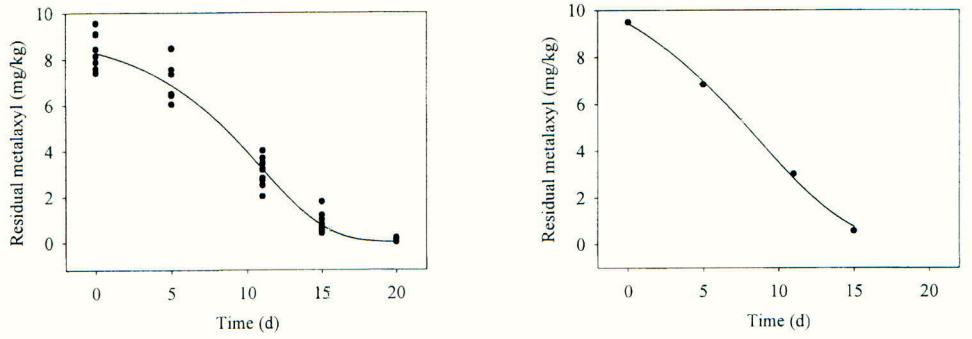

Figure 2. Comparison of metalaxyl degradation between the 10-station (left) and composite (right) soil samples from field 5.

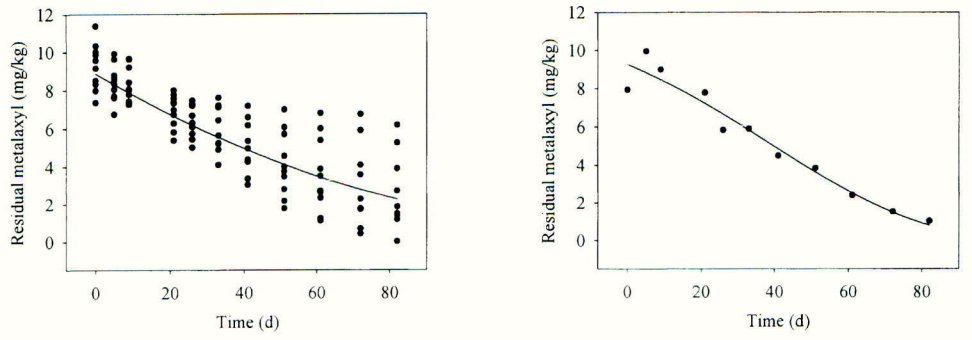

Figure 3. Comparison of metalaxyl degradation between the 10-station (left) and composite (right) soil samples from field 10.

In soil from nine fields with metalaxyl treatment histories, eight (fields 2-9) had half-lives of less than 10 d (e.g. Figure 2). This parallels the work of Davison & McKay (1999) in Western Australia where half-lives in soil from 3 fields, each with a metalaxyl treatment history and a failed crop, was 10 d or less. In soil from their field with no previous metalaxyl use and successful control of cavity spot, the half-life was 82 d.

Field 1 exhibited the greatest variability in degradation rates between soil samples from the different stations, with metalaxyl persisting in soil samples from 3 stations for over 60 d but at other stations disappearing within 30 d (Figure 1). In soil from all nine fields where performance of the chemical had been poor and disease levels were at or near crop write-off, the DT_{50} was between 4 and 14 d. By bulking together soil samples from individual stations within a field to produce a composite sample, a good indication of metalaxyl performance was still achieved (DT_{50} values of 4 to 15 d). This would appear to be a good approach for soil sampling to predict the behaviour of the fungicide in a particular field.

In the second study with soil from two adjacent fields with similar properties, the degradation rate of metalaxyl was considerably faster in soil from the treated field compared to the untreated field (Figure 4), with DT_{50} values of the bulked composite samples of 13.2 and 39.3 d respectively. Since these fields were located next to each other, were of the same pH, and other properties, yet differed in their metalaxyl treatment histories, this would suggest enhanced microbial degradation of the fungicide was occuring at this site.

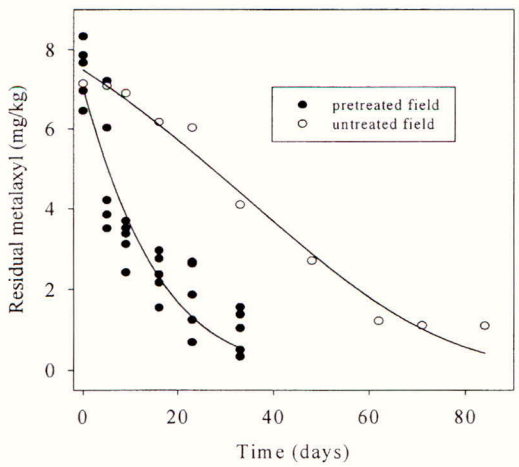

Figure 4. Comparison of metalaxyl degradation between soil samples from fields with and without pretreatment histories.

Studies have shown that the timing of metalaxyl application is an important factor in the control of cavity spot disease of carrots, and there appears to be a crucial time early in the life of a crop when protection from the pathogen is essential. Gladders & McPherson (1986) found the best control was achieved with metalaxyl (+ mancozeb) applications made

between sowing and four weeks post-crop emergence. Clearly, if the fungicide persists for less time in the soil, crop protection will be reduced.

Metalaxyl, and the recently introduced metalaxyl-M, are the only reliable fungicides for the control of cavity spot and are still effective in the vast majority of carrot production areas. Reports of failure of the fungicide on a small number of fields is a cause for concern and these experiments show that enhanced biodegradation could be factor at some of these sites.

ACKNOWLEDGEMENTS

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