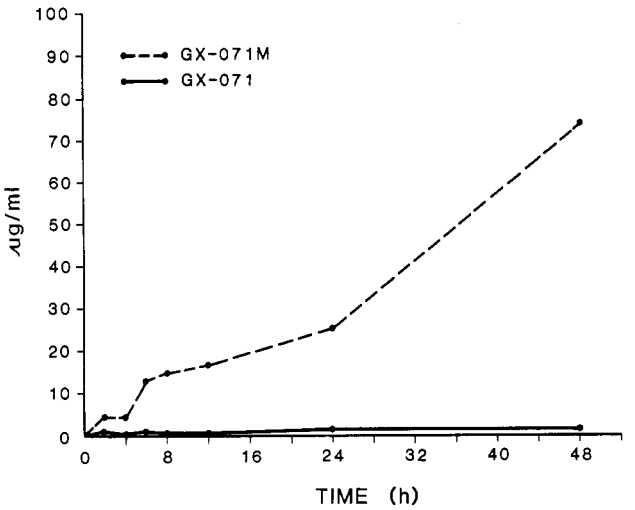

Figure 8. Levels of GX-071 and GX-071M in the blood of a rat after a 150 mg/kg oral dose of GX-071.

poor water solubility, rapid metabolism, and enterohepatic recycling among others resulted in a complex picture for blood levels of these components during the first hour after iv dosage. Analyses of a 40-μL sample of blood taken from a rat 6 h after administration of a 10 mg/kg iv dose of GX-071 are shown in Figure 7. The presence of GX-071 and GX-071M (PFB), in a 40-μL sample of blood taken from a rat 15 min after administration of an iv dose of 25 mg/kg GX-071, was confirmed by cold on-column injection capillary GC/MS by comparison of retention times and mass fragmentation patterns with those of authentic standards. A graph showing the levels of GX-071 and GX-071M in the blood of a rat after a single 150 mg/kg oral dose of GX-071 again indicated that GX-071 was extensively metabolized to GX-071M (Figure 8). These data suggest that GX-071 undergoes a significant hepatic metabolism (first-pass effect) prior to entry into the systematic circulation.

Registry No. GX-071, 4151-50-2; GX-071M, 754-91-6.

LITERATURE CITED

Anand, N. Sulfonamides and Sulfones. In *Medicinal Chemistry*, 4th ed., Part II; Wolff, B., Ed.; Wiley: Chichester, 1979; pp 1–40.

Arrendale, R. F.; Chortyk, O. T. Construction and Application of a Cold On-Column Injection System for Capillary Gas Chromatography. *HRC CC, J. High Resolut. Chromatogr. Chromatogr. Commun.* **1985**, *8*, 62–68.

Arrendale, R. F.; Martin, R. M. The Preparation of Immobilized Stationary Phase Fused Silica Capillary Columns with OV-1701-Vinyl Deactivation. *HRC CC J. High Resolut. Chromatogr. Chromatogr. Commun.* **1988**, *11*, 157–161.

Arrendale, R. F.; Severson, R. F.; Chortyk, O. T. Open Split Interface for Capillary Gas Chromatography/Mass Spectrometry. *Anal. Chem.* **1984**, *56*, 1533–1537.

Brandstrom, A.; Junggren, U. Ion Pair Extraction in Preparative Organic Chemistry - III. Alkylation of Methyl Cyanoacetate and Related Components. *Acta Chem. Scand.* **1969**, *23*, 2203–2204.

Ehrsson, H. Quantitative Gas Chromatographic Determination of Carboxylic Acids and Phenols After Derivatization With Pentafluorobenzyl Bromide. *Acta Pharm. Suec.* **1971**, *8*, 113–118.

Garrett, E. R. Classical Pharmacokinetics to the Frontier. In *Pharmacology and Pharmacokinetics*; Terrell, et al., Eds.; Plenum Press: New York, 1974; pp 3–25.

Grob, K.; Grob, K., Jr. On-column Injection on to Glass Capillary Columns. *J. Chromatogr.* **1978**, *151*, 311–320.

Gyllenhaal, O.; Ehrsson, H. Preparation and Properties of Perfluoroacyl and Pentafluorobenzyl Derivatives. *J. Chromatogr.* **1975**, *107*, 327–333.

Hekster, C. A.; Vree, T. B. Clinical Pharmacokinetics of Sulphonamides and Their N4-Acetyl Derivatives. In *Pharmacokinetics II*; Schonfeld, H., Ed.; S. Karger: Bazel, 1982; pp 22–118.

Metcalf, R. L. Fluorine-containing Insecticides. In *Pharmacology of Fluoride*, Part 1; Smith, F. A., Ed.; Springer-Verlag: New York, 1970; pp 355–386.

Schnellmann, R. G. The Mechanism of Toxicity of a Unique Pesticide (N-Ethylperfluorooctane Sulfonamide) to Rabbit Renal Proximal Tubules. *Tox. In Vitro.* **1989**, in press.

Smith, F. A. Biological Properties of Selected Fluorine-Containing Organic Compounds. In *Pharmacology of Fluorine*, Part 2; Smith, F. A., Ed.; Springer-Verlag: New York, 1970; pp 253–408.

Smith, R. V.; Stewart, J. T. Separatory and Purification Measures. In *Textbook of Biopharmaceutic Analysis*; Lea & Febiger: Philadelphia, 1981; pp 47–50.

Yakatan, G. J.; Iglesia, F. A. Toxicokinetics - A Role in Drug Discovery and Development. In *Experimental and Clinical Toxicokinetics*; Yacobi, A., Barry, H., Eds.; American Pharmaceutical Association: Washington, DC, 1984; pp 9–26.

Received for review June 24, 1988. Accepted March 24, 1989.

Distribution of Tolclofos-methyl in Potatoes Grown on Soil Treated with Rizolex

Ebbe Smith and Kommer Brunt*

Tolclofos-methyl residue levels in potato tubers are affected both by the applied dosage of the fungicide Rizolex-50 WP and by the type of soil on which the potatoes were raised. All the tolclofos-methyl present in the tuber accumulates in the peels wherein about 70% is located in the fiber fraction and about 20% in the juice fraction. No tolclofos-methyl could be detected in the starch fraction. Applying the recommended amount of Rizolex-50 WP (15 kg/ha), the tolclofos-methyl residue level in the unpeeled potatoes never exceeded 0.05 ppm.

Potato disease caused by infection of *Rhizoctonia solani* (e.g., black scurf and stem canker) are serious agricultural and economical problems in the production of potato crops. It is known that, after desinfection of the seed potatoes, in many cases the potato crop will still be affected by *R. solani* from the soil. Many chemicals have been used in an attempt to control these diseases. In the past several years a new fungicide, with the trade name Rizolex, with

Netherlands Institute for Carbohydrate Research TNO, Rouaanstraat 27, 9723 CC Groningen, The Netherlands.

0021-8561/89/1437-1135$01.50/0

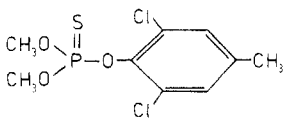

Figure 1. Chemical structure of the fungicide tolclofos-methyl.

the active compound tolclofos-methyl [chemical name *O,O*-dimethyl *O*-(2,6-dichloro-4-methylphenyl)-phosphorothioate], developed by Sumitomo Chemical Co. Ltd. (Ohtsuki and Fujinami, 1982), is being used for the chemical control of *R. solani*. The chemical structure is given in Figure 1.

In recent years several investigations have been published that were performed in order to estimate the effects of application of this fungicide against *R. solani*. Soil treatment (Mulder and Roosjen, 1982, 1984) as well as applications of Rizolex upon storage (Elenwa et al., 1983) or during planting (Barnes et al., 1983; Harris and Rea, 1984; Harris et al., 1986; Oxley and Lang, 1987) of seed potatoes were carried out. Both dust applications (Barnes et al., 1983; Harris and Rea, 1984; Oxley and Lang, 1987) as sprayable formulations (Elenwa et al., 1983; Mulder and Roosjen, 1984; Harris et al., 1986; Oxley and Lang, 1987) have also been tested. In general Rizolex applications gave good control of stem canker in early and main crop potatoes. Moreover, it was effective in controlling *Phoma exigua* foveate in wounded stored seed potatoes (Elenwa et al., 1983), and laboratory tests showed that it also killed sclerotia of *R. solani* on potato tubers (Barnes et al., 1983).

No information is available concerning the residue levels in potato crops raised after a treatment with Rizolex. Information provided by the manufacturer indicates that the tolclofos-methyl levels are low (Ohtsuki and Fujinami, 1982). In The Netherlands experiments were carried out in several consecutive years to investigate the possibilities of chemical control of *R. solani* in seed and starch potatoes by soil treatments with Rizolex (Mulder and Roosjen, 1982, 1984). In the harvested potatoes, among others the tolclofos-methyl residue levels were measured. This paper describes the extent to which the residue level was affected by the Rizolex dosage and the soil type. The potatoes were raised on sandy and on peaty soil. The present paper deals also with the distribution of the tolclofos-methyl residue in the potato. Residue levels have been measured in whole potatoes, peeled potatoes, and potato peels. Moreover, the levels were determined in the starch, juice, and fiber fractions because these are the main product and byproducts of the potato starch industry.

EXPERIMENTAL SECTION

Potato Samples. The potatoes (1981, 1983 variety Prominent, 1984 variety Elkana) were raised at two different experimental farms in the northern part of The Netherlands. One is located at Rolde on a sandy soil (3.6% organic matter); the other is located at Emmercompascuum on a peaty soil (16.1% organic matter). The experimental plots were used in a randomized block design in three replicates. Prior to planting, the soils were treated with different amounts of Rizolex-50 WP (containing 50% of the active compound tolclofos-methyl) by spraying and incorporating as described before (Mulder and Roosjen,1982, 1984). The applied dosages varied from 0.5 to 4 times the recommended normal dose of 15 kg/ha Rizolex-50 WP. The potatoes originating of each triplicate experimental plots were pooled at harvesting. An average sample of 15–20 kg of mature potatoes was taken from each pool for analysis.

Potato Sample Preparation and Fractionation. Immediately upon receipt of the samples the potatoes were carefully washed with water to remove all the adherent ground. Then, 1 g of sodium pyrosulfite/kg of potatoes was added, and the potatoes were disintegrated with use of an industrial rotary sawblade rasp, type Holthuis.

With some of the samples, the potato pulp was fractionated into a starch, a juice, and a fiber fraction. The juice was prepared by squeezing out the pulp in a filter cloth, followed by centrifuging the juice to remove all solids.

The fiber fraction was separated from the pulp by a standard wet sieving procedure with a sieve width of about 0.16 mm. The obtained fraction was washed extensively with water to remove adherent juice.

The starch granules passed the sieve, and fine fiber particles were removed by sedimentation in water. Also the starch fraction was washed repeatedly to remove adherent juice. The prepared potato pulp, the fiber fractions, and the potato juice fractions were stored at −18 °C up until analysis. The starch fractions were first dried at about 40 °C and then stored at about 10 °C.

Analytical Procedures. *Tolclofos-methyl.* When potato pulp, juice, or fiber was analyzed, first a sample of about 500 g was well homogenized by using a Waring Blendor. Then, 50 g of the homogenized sample was taken for extraction. For analyzing starch, two different methods of sample pretreatment were used: First, a suspension of 20 g of starch in 40 mL of distilled water was taken for extraction. Second, a suspension of 20 g of starch in 40 mL of 0.025 M acetate buffer (pH 5.5) was hydrolyzed enzymatically with BAN-L (bacterial amylase obtained from NOVO, Bagsvaerd, Denmark) for 1 h at 70 °C and then taken for extraction.

The tolclofos-methyl was extracted from the sample matrix by stirring the prepared sample slurry for 20 min in a 250-mL beaker with 10 mL of distilled water and 150 mL of methanol/acetonitrile (1/4, v/v). After stirring and sedimentation, the upper layer was filtered under suction with aid of Celite Hyflo Super-Cel (thickness about 1 cm). The insoluble residue in the beaker was stirred once more with 60 mL of methanol/acetonitrile (1/4, v/v) for 5 min and filtered. Both filtrates were combined in a 500-mL separatory funnel, and 100 mL of an aqueous solution of 10% g/v sodium chloride and 120 mL of dichloromethane were added.

After the mixture was shaken for 10 min, the lower dichloromethane layer was dried over anhydrous sodium sulfate (about 30 g) and brought into a round-bottom flask. The aqueous residue in the separatory funnel was extracted once more with 80 mL of dichloromethane. This dichloromethane layer was treated in the same way, and both quantities were combined and evaporated to dryness at a temperature of about 50 °C on a rotary film evaporator (Buchi Rotavapor EL). The residue was transferred and eluted through a column packed with 15 g of activated silica (overnight activated at 130 °C in a Heraeus drying oven with air circulation) and with 1 g of anhydrous sodium sulfate on top by washing the round-bottom flask with eight portions of 10 mL of toluene. The collected eluate was evaporated to dryness at 50 °C. The residue was dissolved in 2 mL of acetone. Then, 2 mL of the parathion internal standard solution in acetone (2.1 μg/mL) was added, and an aliquot of 2 μL of the homogenized solution was injected in the gas chromatograph. A Tracor 550 gas chromatograph equipped with a flame photometric detector with a phosphorus filter (526 nm) and a glass column (length 2.40 m, internal diameter 2.7 mm) packed with 3% OV 225 on Gaschrom Q 100/120 was used. The oven temperature was 230 °C, and both the injector and the detector temperatures were 240 °C. The applied gas flows were as follows: nitrogen, 40 mL/min; hydrogen, 50 mL/min; air, 80 mL/min. Peak areas measured with a CRS 204 integrator were used for quantifying the compounds of interest. All samples were analyzed in duplicate.

Dry Matter. The dry matter contents of the pulp, fiber, and juice fractions were calculated by their loss in weight after 8 h of drying at 45–50 °C followed by $1^1/_2$-h drying at 130 °C (pulp and fiber) or 2 h at 130 °C (juice).

Starch. The starch contents in the pulp, peel, and fiber fractions were determined polarimetrically according to Ewers (Willigen, 1974). Thus, the Kohlraus flasks, containing the samples suspended in 0.1 N HCl, were heated for 30 min in boiling water, followed by filtration and measurement of the optical rotation of the filtrate. The starch content was calculated by comparing the optical rotation with the specific rotation of hydrolyzed pure potato starch.

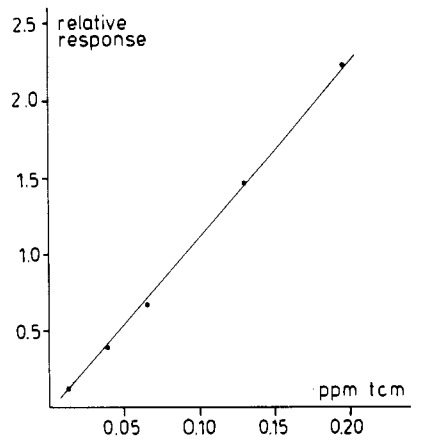

Figure 2. Tolclofos-methyl calibration curve with parathion as an internal standard.

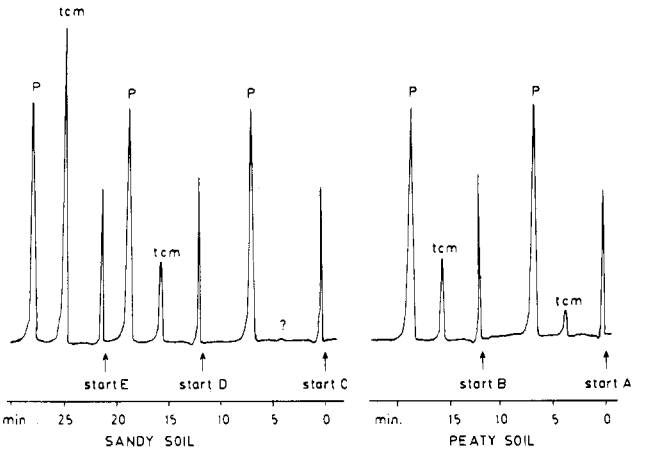

Figure 3. Chromatograms of tuber extracts of potatoes raised on sandy and peaty soils treated with different dosages of Rizolex-50 WP: A = 30 kg/ha; B = 60 kg/ha; C = 0 kg/ha; D = 30 kg/ha; E = 60 kg/ha.

Table I. Average Tolclofos-methyl Recoveries (%) and the Coefficient of Variation in the Tolclofos-methyl Residue Determinations in Macerated Whole Potatoes and in Fractions of the Macerated Whole Potatoes

sample matrix	recovery	no. of determin	coeff of var in determin
potato pulp	75	8	11
potato juice	94	5	5
potato fiber	82	12	5
potato starch	61	12	15

RESULTS AND DISCUSSION

There was a good linear relationship between the concentration of tolclofos-methyl in the standard solutions and the ratio of the peak areas of tolclofos-methyl and the internal standard parathion added in the analytical procedure (Figure 2). Figure 3 shows typical gas chromatograms of some tuber extracts of potatoes raised on soil treated with Rizolex-50 WP. The tolclofos-methyl and the parathion peaks in the chromatograms were identified by their retention times and by standard addition experiments. Due to the selectivity of the flame photometric detector with phosphor filter, no interfering peaks occur in the chromatograms.

The tolclofos-methyl recoveries in the potato pulp and in the other isolated potato fractions were determined by standard addition experiments at different concentration levels (0.013, 0.026, 0.039, or 0.052 ppm tolclofos-methyl was added). In Table I both the average recoveries and the coefficients of variation in the analyses are presented for the different sample matrices. All tolclofos-methyl levels reported in this paper have been corrected for these recovery values.

Table II. Effect of Soil Type and Applied Rizolex-50 WP Dosage on Tolclofos-methyl Levels in Potatoes

harvesting year	Rizolex-50 WP dosage,[a] kg/ha	measd tolcolofos-methyl levels in mg/kg potatoes (ppm): sandy soil (3.6% org matter)	peaty soil (16.1% org matter)
1981	0	0.001	0.002
	30	0.031	0.009
	60	0.129	0.032
1983	0	0.002	0.000
	7.5	0.005	
	15	0.016	
	30	0.071	0.010
1984	0	0.000	
	15	0.019	
	30	0.029	
	60	0.086	

[a] Recommended normal dosage: 15 kg/ha Rizolex-50 WP.

Table III. Composition of the Unpeeled Potatoes and Tolclofos-Methyl Levels in the Isolated Potato Fractions as a Function of the Applied Dosage of Rizolex-50 WP to the Sandy Soil (Experimental Farm in Rolde, Crop 1984)

	dosage of Rizolex-50 WP,[a] kg/ha: 0	15	30	60
dry matter content, %				
potato pulp	24.4	23.9	23.9	24.1
juice	5.9	6.1	6.2	6.3
fiber fraction	10.9	8.4	8.2	8.8
yield fiber fractions (as is) from potato pulp, %		62.2	49.3	52.3
starch content, %				
potato pulp	16.4	16.6	16.8	17.3
dry matter fiber fraction	61.2	52.7	51.6	45.0
tolclofos-methyl content, ppm				
potato pulp	<0.001	0.019	0.029	0.086
juice	<0.001	0.004	0.006	0.013
starch	<0.001	<0.001	<0.001	<0.001
fiber fraction (as is)	<0.001	0.031	0.049	0.124

[a] Recommended dosage: 15 kg/ha Rizolex-50 WP.

For several years the tolclofos-methyl levels have been measured in potatoes raised on sandy and on peaty soils that were treated with different dosages of Rizolex-50 WP. In this study unrealistically high dosages were applied in order to facilitate investigations into the presence of tolclofos-methyl and its distribution in the potato tuber. In The Netherlands the recommended dosage of Rizolex-50 WP is 15 kg/ha. According to Dutch law concerning the use of pesticides (Bestrijdingsmiddelenwet, 1983), the maximum acceptable level of tolclofos-methyl in washed, unpeeled potatoes is 0.05 ppm. As can be seen in Table II, the tolclofos-methyl levels in the potatoes are affected both by the type of soil and by the dosage of Rizolex-50 WP. When no more than the recommended amount of 15 kg/ha was applied, the maximum acceptable level of 0.05 ppm tolclofos-methyl in the potatoes was never exceeded (Table II). The low levels in the potatoes raised on peaty soil compared with those raised on sandy soil confirm the assumption that tolclofos-methyl adsorbs on organic matter in the soil (Mulder and Roosjen, 1982, 1984).

The potatoes raised in 1984 on sandy soil treated with different dosages of Rizolex-50 WP were investigated in more detail. In this crop the tolclofos-methyl levels were determined in unpeeled and in peeled potatoes as well as

Table IV. Composition of the Potato Peels and Tolclofos-methyl Levels in the Isolated Fractions of the Potato Peels as a function of the Applied Dosages of Rizolex-50 WP to the Sandy Soil (Experimental Farm in Rolde, Crop 1984)

	dosage of Rizolex-50 WP,[a] kg/ha			
	0	15	30	60
peel yield, %	16.5	18.7	21.1	20.9
dry matter content, %				
potato peel pulp	17.4	18.6	18.6	18.7
potato peel juice	5.5	5.7	5.8	6.1
potato peel fiber fraction	11.8	13.0	12.1	13.0
yield fiber fraction (as is) from potato peel pulp, %		59.0	62.8	61.6
starch content, %				
potato peel pulp	10.2	11.7	11.7	11.3
dry matter potato peel fiber fraction	61.2	62.4	65.7	62.9
tolclofos-methyl content, ppm				
potato pulp	0.011	0.083	0.152	0.383
juice	0.002	0.019	0.036	0.089
starch	<0.001	<0.001	<0.001	<0.001
fiber fraction (as is)	0.007	0.095	0.156	0.453

[a] Recommended dosage: 15 kg/ha Rizolex-50 WP.

Table V. Tolclofos-methyl Levels (ppm) in Unpeeled Potatoes as a Function of the Applied Rizolex-50 WP Dosage in the Sandy Soil and the Calculated Residue Levels Using the Measured Levels in the Corresponding Peels and the Potato Peel Yield

Rizolex dosage,[a] kg/ha	tolclofos-methyl level in potato peels	yield potato peels, %	tolclofos-methyl level in unpeeled potatoes	
			measd	calcd
0	0.011	16.5	0.000	0.002
15	0.083	18.7	0.019	0.016
30	0.153	21.1	0.029	0.032
60	0.383	20.9	0.086	0.080

[a] Recommended dosage: 15 kg/ha Rizolex-50 WP.

in the potato peels. Also the tolclofos-methyl levels were measured in the isolated juice, starch, and fiber fractions of the unpeeled potatoes and of the potato peels itself (Tables III and IV).

In the peeled potatoes no tolclofos-methyl could be found regardless of the applied Rizolex-50 WP dosages. This indicates that all the tolclofos-methyl present in the potatoes accumulates in the peels, as confirmed by the data in Tables III and IV. Table V demonstrates that it is possible to calculate the residue levels in unpeeled potatoes by using the measured tolclofos-methyl levels in the potato peels and the weight percentages of peels removed by peeling as given in Table IV.

As shown in Tables III and IV tolclofos-methyl was mainly present in the fiber fractions and to a lesser extent in the juice fractions. No tolclofos-methyl could be detected in the starch fractions.

So far, the tolclofos-methyl concentrations in the different isolated fractions have been discussed. On the basis of the dry matter data in Tables III and IV, it is also possible to calculate the starch, fiber, and juice contents of the unpeeled potatoes and of the potato peels. In combination with the measured tolclofos-methyl concentrations in the starch, fiber, and juice fractions, these data can be used to calculate the distribution of tolclofos-methyl in the fractions originating of the unpeeled potatoes and the potato peels. The results are given in Table VI.

As can be seen, the major part of the tolclofos-methyl is concentrated in the fiber fractions while the juice fractions contain only a minor portion. As mentioned before, no tolclofos-methyl could be detected in the starch. When

Table VI. Tolclofos-methyl Balance in Microgram Absolute and Percentage Distributions over the Starch, Juice, and Fiber Fractions Originating from 100 g of Unpeeled Potatoes and of 100 g of Potato Peels as a Function of the Applied Dosage Rizolex-50 WP to Sandy Soil (Crop 1984)[a]

Rizolex-50 WP dosage, kg/ha		unpeeled potatoes		potato peels	
		a	b	a	b
15	pulp	1.90	100	8.30	100
	starch	<0.02	<1	<0.01	<1
	juice	0.32	17	1.64	20
	fiber	1.93	101	5.61	68
30	pulp	2.90	100	15.2	100
	starch	<0.02	<1	<0.01	<1
	juice	0.49	17	3.11	20
	fiber	2.42	83	9.80	64
60	pulp	8.60	100	38.3	100
	starch	<0.02	<1	<0.01	<1
	juice	1.05	12	7.70	20
	fiber	6.49	76	27.9	73

[a] Key: (a) Micrograms of absolute tolclofos-methyl. In isolated fractions. (b) Distribution over isolated fractions (%).

the measured amount of tolclofos-methyl present in 100 g of pulp is compared with the sum of the contributions of the different isolated fractions originating from 100 g of pulp, it seems that some tolclofos-methyl is missing. Taking into account the very low concentration levels (in the ppb range) and the coefficients of variation presented in Table I for the analysis, the differences in the calculated tolclofos-methyl mass balance (the content in the pulp versus the sum of the contents in the fiber and juice) are hardly statistically significant. Probably a very small part of the tolclofos-methyl was lost during the extensive washing procedures to which the fiber and starch fractions were subjected.

CONCLUSIONS

Tolclofos-methyl residues present in potatoes raised on soil treated with Rizolex-50 WP accumulate entirely in the potato peels. About 70% of the total amount tolclofos-methyl present in the peels is found in the fiber fractions and 20% in the juice; in the starch it was not detectable. The missing 10% is probably lost during the extensive washing of the fiber and starch fractions. With the recommended dosage of Rizolex-50 WP, the maximum acceptable level of 0.05 ppm tolclofos-methyl in the unpeeled potatoes was never exceeded.

ACKNOWLEDGMENT

We are indebted to H. J. Brinkema and J. Zinsmeester for their technical assistance. Also, we are indebted to Schering-Aagrunol Co. (Haren, The Netherlands) who informed us about the analytical method for the determination of tolcofos-methyl, which, after some modification, was very useful to us.

Registry No. Tolclofos-methyl, 57018-04-9.

LITERATURE CITED

Barnes, G.; Harris, R. I.; Russell, P. E. Tolclofos-methyl, a new fungicide for the control of *Rhizoctonia solani*. Proceedings, 10th International Congress of Plant Protection, 1983; pp 460–464.

Bestrijdingsmiddelenwet, Uitvoeringsvoorschriften (C II-4) Residubeschikking, 1983.

Elenwa, E. N.; Holgate, M. E.; Clifford, D. R.; Chemical control of gangrene in potatoes. In Test of agrochemicals and cultivars no 4. *Ann. Appl. Biol.* **1983**, *102* (Supplement), 70–71.

Harris, R. I.; Rea, B. L. Methods of applications of tolclofos-methyl for the control of stem canker and black scurf of potatoes. Proceedings, British Crop Protection Conference - Pests and Diseases, 1984; pp 767–771.

Harris, R. I.; Thompson, P. J.; Atkinson, R. J.; Control of stem canker and black scurf in potatoes with sprayable formulation of tolclofos-methyl. Proceedings, British Crop Protection Conference - Pests and Diseases, 1986; pp 1071–1076.
Mulder, A.; Roosjen, J. Bestrijding van Rhizoctonia in aardappelen door middel van grondbehandeling I. *Onderzoek* **1982**, 178–187.
Mulder, A.; Roosjen, J. Control of *Rhizoctonia solani* in seed potato crops by soil treatments with tolclofos-methyl, furmecyclox and pecycuron. Proceedings, 9th Triennial Conference of the European Association for Potato Research, Interlaken, 1–6 July 1984; pp 107–108.
Ohtsuki, S.; Fujinami, A. Rizolex (tolclofos-methyl) *Jpn. Pestic. Inf.* **1982**, *No. 41*, 21–25.
Oxley, S. J. P.; Lang, R. W. The control of potato stem canker and black scurf (*Rhizoctonia solani*) in Scotland using fungicide seed treatment. Proceedings, 10th Triennial Conference of the European Association for Potato Research, Aalborg, 26–31 July 1987; pp 331–332.
Willigen, A. H. A. de. Die Bestimmung der Stärke, Verlag Paul Parey, Berlin, Hamburg, 1974, pp 21–25.

Received for review July 7, 1988. Accepted February 27, 1989.

Poor Correlation between the Levels of Proteinase Inhibitors Found in Seeds of Different Cultivars of Cowpea (*Vigna unguiculata*) and the Resistance/Susceptibility to Predation by *Callosobruchus maculatus*

J. Xavier-Filho,* F. A. P. Campos, Maria B. Ary, C. Peres Silva, Marta M. M. Carvalho, Maria Ligia R. Macedo, F. J. A. Lemos, and G. Grant

Larvae of *Callosobruchus maculatus* have poor performance in seeds of the cowpea (*Vigna unguiculata*) cultivar TVu 2027, and of its progenies IT81D-1045 and IT81D-1064, when compared to cultivars CE-31, CE-11, and CE-524. We did not find any correlation between proteinase (trypsin, chymotrypsin, Subtilisin BPN′, papain) inhibitory activity and the resistance shown by cultivars TVu 2027, IT81D-1045, and IT81D-1064. Similarly, there was no obvious relationship between the levels of lectin (hemagglutination activity) or tannins and the resistance or susceptibility of the seeds to predation by *C. maculatus*. The *C. maculatus* resistant cowpea seeds (IT81D-1045, IT81D-1064) support the development of *Zabrotes subfasciatus*, a bruchid that infests seeds of both *Phaseolus vulgaris* and *Vigna unguiculata*.

Lectins, tannins, proteinase inhibitors, and trypsin inhibitors, in particular, and other compounds found in large amounts in seeds of many plants are thought to be part of an array of constitutive defenses that plants utilize against attacking microorganisms and insects (Janzen et al., 1986; Xavier-Filho and Campos, 1989).

There are reports in the literature suggesting that proteinase inhibitors may also be involved in the resistance that some seed varieties present to their common predators (Xavier-Filho and Campos, 1989). For example, Gatehouse et al. (1979) suggested that the resistance of seeds of a cowpea (*Vigna unguiculata*) cultivar (TVu 2027) toward its bruchid pest *Callosobruchus maculatus* was due to the high levels of trypsin inhibitors found in these seeds.

However, we have found other cowpea cultivars whose seeds have trypsin inhibitory activity as high as the seeds of the cultivar TVu 2027, which are susceptible to *C. maculatus* (Xavier-Filho and Campos, 1984).

In addition, several observations show that the larvae of *C. maculatus* utilize mainly cysteine proteinases for protein digestion (Gatehouse et al., 1985; Kitch and Murdock, 1986; Campos et al., 1989) rather than serine proteinases, like trypsin.

These observations and the availability of seeds of different cowpea cultivars led us to investigate the proteinase inhibitor levels in seeds of this legume in relationship to the resistance to *C. maculatus*. Two other groups of compounds possibly implicated with the resistance, namely, tannins and lectins, were also investigated.

Departamento de Bioquímica e Biologia Molecular, Universidade Federal do Ceará, Caixa Postal 1065, 60001 Fortaleza, Ceará, Brazil (J.X.-F., F.A.P.C., M.B.A., C.P.S., M.M.M.C., M.L.R.M., F.J.A.L.), and The Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen AB2 9SB, Scotland (G.G.).

METHODS AND MATERIALS

Rearing of Insects. The *C. maculatus* used in this work were supplied by Dr. J. H. R. Santos, Centro de Ciências Agrárias, Universidade Federal do Ceará, Fortaleza, Brazil. Permanent colonies of the beetles were established on *V. unguiculata* (cv. Pitiuba, CE-31) seeds in this department since 1978. The insects were reared at 29 ± 5 °C and relative humidity 65% ± 5%. A *Zabrotes subfasciatus* culture was also established in 1987 from insects supplied by Prof. F. M. Wiendl of the Centro de Energia Nuclear na Agricultura, Piracicaba, São Paulo, Brazil. The insects were reared on *V. unguiculata* (cv. Pitiuba, CE-31) under the same conditions as for *C. maculatus*.

Cowpea Seeds. Six *V. unguiculata* cultivars were utilized in this study. Seeds of the cultivars CE-11 (Quebra-cadeira), CE-31 (Pitiuba), and CE-524 were supplied by J. B. Paiva of the Centro de Ciências Agrárias, Universidade Federal do Ceará, Fortaleza, Brazil. These were local cultivars. Seeds of the cultivars IT81D-1045, IT81D-1064, and TVu 2027 were obtained through the Centro Nacional de Pesquisa Arroz-Feijão (EMBRAPA), Goiânia, Brazil; the cultivars IT81D-1045 and IT81D-1064 were bred from TVu 2027 at IITA (International Institute for Tropical Agriculture, Ibadan, Nigeria).

Performance of *C. maculatus* and *Z. subfasciatus* on Cowpea Seeds. To measure the performance of *C. maculatus* and *Z. subsciatus* on cowpea seeds, three replicates of 20 seeds of each cultivar were used; two fertilized females (2–3 days old) were put in each glass bottle containing 20 cowpea seeds and left to oviposit for 24 h. After that period, the insects were removed and the eggs laid in excess of two were scrapped. The number of emerged adults was counted so that we could calculate the percentage survival to adult emergence (S); the days in which

0021-8561/89/1437-1139$01.50/0