Rishitin a natural plant product with nematicidal activity

Thomas J. W. ALPHEY, Walter M. ROBERTSON and Gary D. LYON

Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, UK.

SUMMARY

The phytoalexin rishitin produced in potato tissue challenged by the bacterium *Erwinia carotovora* was shown to have nematode repellant and nematicidal properties. A number of *in vitro* and small-pot studies were made to demonstrate the effect of rishitin on nematodes. On agar *Xiphinema diversicaudatum* became agitated and were repelled from point sources of 20 µg or more of rishitin. They became inactive within 10 min and died within 2 h when immersed in a 200 µg ml^{-1} solution of rishitin. Rishitin at 0.5 mg, 1.0 mg and 1.5 mg per 74 ml soil was added with a seedling of *Petunia hybrida* to one side of a split-pot and a known number of *X. diversicaudatum* to the other. In pots treated with 1.5 mg rishiting, 83 % of the nematodes, of which 93 % were inactive, remained at the inoculated side 18 days after treatment and in those containing 0.5 mg rishitin, 53 % remained on the inoculated side. At the two higher rates of treatment many nematodes were immobilised in the soil at the inoculation site and therefore prevented from feeding on roots and causing damage. At the lowest rate of treatment, and in untreated soils, nematodes migrated throughout the soil, fed on the seedling roots and caused damage to the plants.

RÉSUMÉ

La rishitine, substance végétale douée d'activité nématicide

La rishitine, une phytoalexine produite dans les tissus de pomme de terre infectés par la bactérie *Erwinia carotovora*, possède des propriétés répulsives vis-à-vis des nématodes, ainsi que des propriétés nématicides. Des expériences *in vitro* et en pots ont permis d'étudier l'effet de la rishitine sur les nématodes. Les études en boîtes de Petri, sur agar, ont montré que *Xiphinema diversicaudatum* est stimulé puis repoussé hors de la zone de dépôt de 20 µg, ou plus, de rishitine. Les nématodes deviennent inactifs en 10 minutes et meurent en 2 heures après avoir été immergés dans une solution de 200 µg ml^{-1} de rishitine. Les études dans le sol, réalisées en « split-pot », révèlent des effets similaires. Des concentrations de 0,5 mg, 1,0 mg et 1,5 mg de rishitine pour 74 ml de sol ont été ajoutées à un semis de pétunia d'un côté du « split-pot »; de l'autre côté, un nombre connu de *X. diversicaudatum* a été mis en place. Après 18 jours, dans les pots traités avec 1,5 mg de rishitine 83 % des nématodes (dont 93 % inactifs) sont demeurés du côté inoculé. Dans les pots traités avec 0,5 mg de rishitine, 53 % des nématodes seulement sont restés du côté inoculé. Pour les deux plus fortes concentrations du traitement, la plupart des nématodes sont immobilisés dans le sol au point d'inoculation et ne peuvent donc se nourrir sur les racines et causer des dommages. Pour la plus faible concentration, et chez le témoin non traité, les nématodes migrent à travers tout le pot, se nourrissant sur les racines et causant des dommages aux plants.

The first chemicals to effectively control plant parasitic nematodes were fumigants. Following the work of Kühn (1881) (cited in Bunt, 1975) which showed that carbon bisulphide could be used to control *Heterodera schachtii*, several other fumigants were tested. The modes of action of soil fumigants have been reviewed by Wright (1981). The actions are varied and include alkylation (Castro & Belser, 1978) and oxidation (Castro & Thomason, 1971) reactions. Therefore, such materials have broad biocidal activity. More recently, non-fumigant organophosphate and carbamoyl oxime nematicide/insecticides have become widely used. These compounds act by inhibiting acetylcholinesterase and therefore affect a more limited range of organisms. Even though the non-fumigant nematicides have been formulated to have limited persistence their widespread use and detection of residues in soil, soil water and edible crops has caused concern. Consequently, there is a need for less toxic and environmentally more acceptable nematicides. Compounds from healthy plant tissues which exhibit nematicidal or nematostatic properties have been reviewed by Gommers (1981). The use of natural plant products to control nematode pests has a number of possible advantages over synthetic products. Such compounds, being biodegradable, are less likely to cause environmental problems than conventional pesticides, and they may be more readily available and therefore less costly in developing countries.

In their review on mechanisms conferring plant incompatibility to nematodes, Kaplan and Keen (1980) cite phytoalexins as the most likely chemicals responsible for the repulsion of nematodes from plants. More recently, Veech (1982) reviewed the role of phytoalexins in the resistance of plants to nematodes and also concluded that they may serve as effective mechanisms of resistance to nematodes. Phytoalexins are low molecular weight antimicrobial compounds that are synthesised by, and accumulate in, plants after infection by patho-

gens (Paxton, 1980). Rishitin is a phytoalexin which accumulates in potato tuber tissue in response to infection by certain fungi (Tomiyama *et al.*, 1968) and bacteria (Lyon, 1972). *In vitro* tests have shown that rishitin is toxic to some bacteria (Lyon & Baylis, 1975) and fungi (Harris & Dennis, 1976). Zinovyeva and Chalova (1986) found that rishitin accumulated in potato tissue in response to invasion by *Ditylenchus destructor* and *D. dipsaci* and that some nematodes were inactivated. They also demonstrated the ability of rishitin to immobilise nematodes in *in vitro* tests.

In this paper the effects of rishitin on the behaviour and control of two dorylaimoid plant parasitic nematodes on agar plates and in soil are reported. The potential of rishitin as a nematicide is discussed.

Materials and methods

Populations of *Xiphinema diversicaudatum* (Micoletzky, 1927) Thorne, 1939 and *Longidorus elongatus* (de Man, 1876) Thorne & Swanger, 1936, originally obtained from field sites in Angus, Scotland were maintained in soils under ryegrass (*Lolium perenne*). Nematodes were extracted from the soils by sieving and decanting over water (Flegg, 1967).

Rishitin was purified from potato tubers inoculated with *Erwinia carotovora pv atroseptica* as described by Lyon (1972). Rishitin is a sesquiterpene whose structure was elucidated by Katsui *et al.* (1968) and the chemical and physical data have been reviewed by Stoessl, Stothers and Ward (1976). Although it has a melting point of 65-67° rishitin is difficult to crystalise and it was always obtained in the form of an oil in these studies. Its solubility in water is approximately 500 µg ml^{-1} at 20° but it is freely soluble in organic solvents. Rishitin was stored in ethanol solution (33 mg ml^{-1}) which was diluted with distilled water to produce the experimental concentrations required.

The carbamoyl oxime nematicide oxamyl (Vydate L Du Pont®; 24 % oxamyl in methanol) was used as a comparative treatment.

Time-lapse studies

X. diversicaudatum were extracted from soil, washed twice in distilled water and approximately 100 specimens hand-picked and placed onto 0.5 % Davis standard agar (12 ml in a 9 cm diameter Petri dish). The dishes were stored at *c.* 22° for 3-4 h during which time the nematodes distributed themselves randomly on and in the agar. The movement of the nematodes was observed by time-lapse photography (2 frames mn^{-1}) for 2 h using dark field illumination. A rishitin-impregnated paper was then placed into the agar at the centre of the dish. Papers were prepared by applying various amounts of rishitin from 20 µg to 250 µg in ethanol to small strips of filter paper (4 mm × 1.5 mm) and the ethanol evaporated in a stream of warm air. Control dishes received papers treated with ethanol alone. The response of the nematodes was filmed for a further three days. The experiment was repeated on several occasions using both *X. diversicaudatum* and *L. elongatus*.

In vitro studies

Within 3 h of extraction from soil, batches of ten adult *X. diversicaudatum* were hand-picked into tap-water and transferred, all at the same time, into clean dishes containing 1 ml of water or a solution of rishitin or oxamyl. Rishitin was used at 100, 50, 5 and 0.5 µg ml^{-1}; oxamyl at 50 µg ml^{-1}; the water control contained ethanol at the same concentration as that used in the most concentrated rishitin solution. There were four replicates of each treatment and the experiment was repeated on four occasions. At various time intervals (Fig. 1) the numbers of immobile nematodes in each treatment were recorded. Nematodes were considered immobile if they failed to respond to stimulation with a bristle.

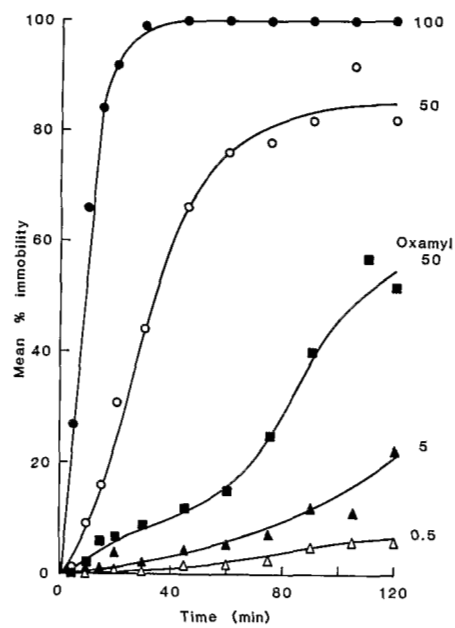

Fig. 1. Graph showing loss of mobility with respect to time in solutions of rishitin : ●, 100 µg ml^{-1}; ○, 50 µg ml^{-1}; ▲, 5 µg ml^{-1}, △, 0.5 µg ml^{-1} and oxamyl; ■, 50 µg ml^{-1}.

A separate test was made to determine the recovery of nematodes after exposure to rishitin. Batches of ten adult nematodes were hand-picked, checked for mobility, and transferred to dishes containing 1 ml 200 or 100 µg ml^{-1} rishitin, or water-ethanol controls. Nematode activity was noted over 5 h and at hourly intervals, batches of nematodes were transferred back into distil-

led water and the numbers recovering mobility within 1 h noted. There were four replicates of each concentration tested.

SPLIT POT BIOASSAY

A pot which could be split into two was devised to test the efficacy of rishitin to repel nematodes from plant roots in soil. The pots (Fig. 2) comprised two perspex boxes (capacity 2 × 37 ml) bound together by waterproof-tape but whose contents were separated from each other by nylon gauze (pore size 95 µm). The boxes were filled with dry soil made by mixing sand with sterilised loam 3:1 (v/v). A single petunia seedling *(Petunia hybrida)* was planted in one side.

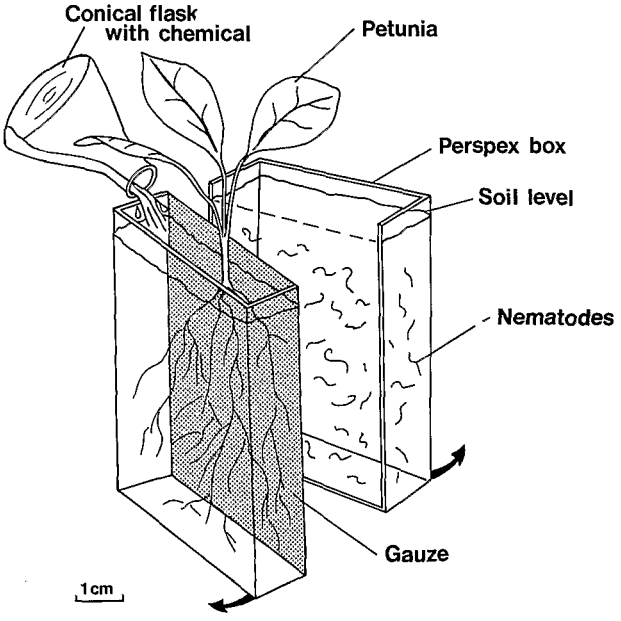

Fig. 2. Split-pot showing the two halves divided by a nylon gauze which confines the roots of a seedling petunia plant to one side. The two halves of the box are taped together during use.

Quantities of 0.5 mg, 1.0 mg and 1.5 mg rishitin were applied in 8 ml water to the planted side of the split-pot and equivalent volumes of water containing 100 *X. diversicaudatum* were added to the opposite sides. Oxamyl at 0.25 mg was also added to each pot and water-ethanol was applied to control pots. Each treatment was replicated at least eight times. All nematodes were hand-picked and used within 3 h of extraction from soil. All pots were placed, in random order, in a propagator maintained at 20-22°. The pots were lightly watered with tap-water when necessary. After eighteen days the two halves were separated and nematodes extracted from each side of the pots by washing and decanting. The numbers of mobile and immobile nematodes in each half were recorded. The roots of the seedling, which had been confined, by the gauze, to one half of the pot, were washed and examined for galls.

Results

TIME-LAPSE STUDIES

X. diversicaudatum migrated randomly on agar plates which received filter paper treated with ethanol throughout the three day study period. Filter papers containing 20 µg rishitin, repelled nematodes and the diameter of the zone of repulsion increased with time (Fig. 3). Papers treated with 50 µg rishitin caused nematodes *c.* 1 cm away to migrate towards the edge of the Petri dish within 2-3 h, and those 2.5 cm away responded within 12 h. Some nematodes, particularly those near the rishitin source at the time of application, were immobilised. Filter papers containing 250 µg rishitin immobilised all nematodes on the plate within six days. The effect on *L. elongatus* was similar to that on *X. diversicaudatum.*

IN VITRO STUDIES

X. diversicaudatum remained mobile making undulating and coiling movements in water-ethanol throughout the observation period and only occasional individuals became immobile. However, in rishitin solutions nematodes initially became agitated, making erratic movements, and subsequently died. Increasing concentrations of rishitin caused mobility to be lost more rapidly (Fig. 1). Rishitin also caused some nematodes to protract their stylets. In oxamyl many nematodes protracted their stylets and rapidly became immobile.

The rates at which *X. diversicaudatum* were immobilised by several concentrations of rishitin was analysed by probit analysis using the Maximum Likelihood Program (Ross, 1980). The EC 50s (median effective concentration) for nematodes after 10 mn, 30 mn, and 60 mn were 97.9 µg ml^{-1} (fiducial limits 83.2-120.5), 33.2 µg ml^{-1} (28.2-38.7) and 18.0 µg ml^{-1} (15.1-21.2) respectively.

In the recovery test, all nematodes placed in the 200 µg ml^{-1} and 100 µg ml^{-1} rishitin solutions lost activity within 15 min and 1 h respectively. Nematodes in the control remained active throughout the test. On transfer to water following exposure to rishitin many nematodes were able to recover mobility within 1 h. From batches of 10 nematodes the mean number of nematodes recovering activity after exposure to 100 µg ml^{-1} rishitin for 3 h was 9 (SE = 1.2); after 4 h exposure 7.5 (SE = 2.0); and after 5 h exposure 8.5 (SE = 1.5). After exposure to 200 µg ml^{-1} rishitin for 1 h only 4 nematodes regained mobility and following 2 h or longer no recovery was observed.

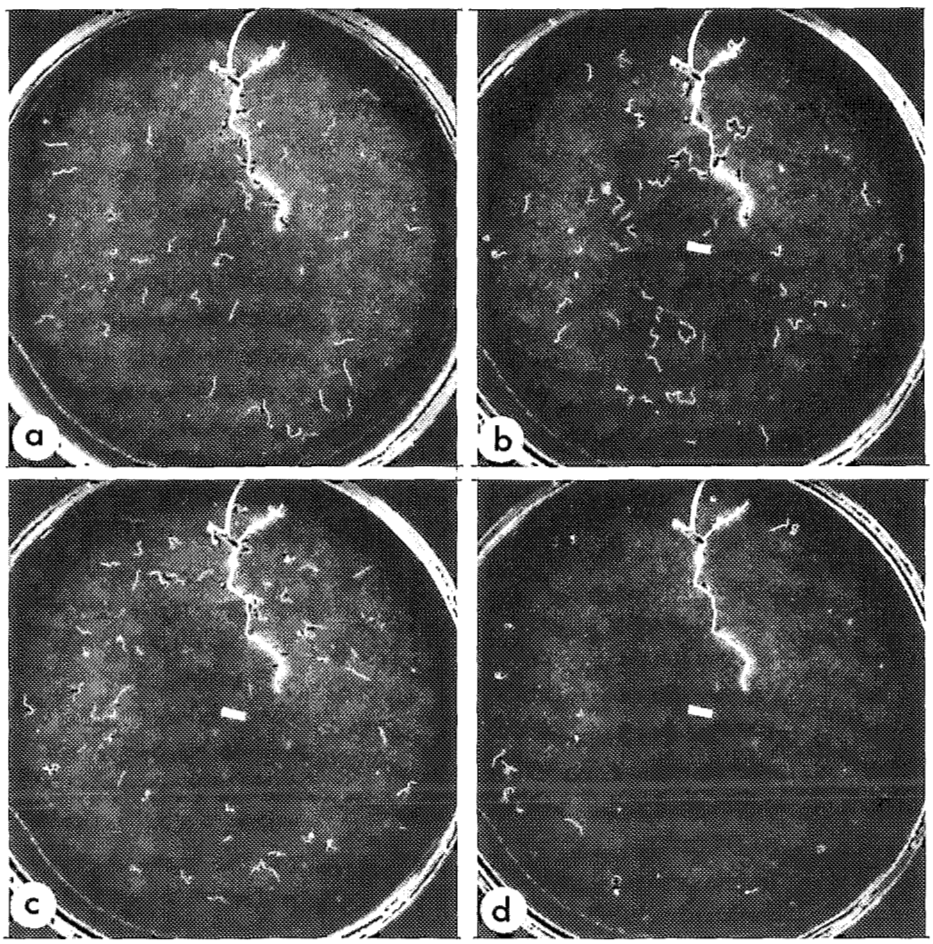

Fig. 3. Example of the distribution of *X. diversicaudatum* on an agar plate which also contains a grass seedling root : (a) with nematodes randomly distributed at the start of the experiment; (b) 4 h after adding 250 µg rishitin on filter paper with zone-of-repulsion beginning to form; (c) zone-of-repulsion increasing in size 12 h after adding rishitin; and (d) zone-of-repulsion almost filling agar plate 96 h after adding rishitin.

SPLIT POT BIOASSAY

After eighteen days in the water-ethanol control pots a mean of 70.3 % of the *X. diversicaudatum* initially added were recovered of which *c.* 10 % were inactive (Tab. 1). Although the total numbers of nematodes extracted per pot was not affected by treatment, the numbers of active nematodes and their distribution were affected by oxamyl and rishitin. The numbers of active nematodes following application of 1.5 mg, 1.0 mg and 0.5 mg rishitin per pot were reduced by 86 %, 69 % and 27 % respectively in comparison to the untreated controls and by 41 % after application of 0.25 mg oxamyl.

In the water-ethanol control pots more than 60 % of the nematodes had migrated from the inoculation site through the gauze to the planted side and more than 90 % of those recovered from the planted side were alive and active. In oxamyl similar numbers of nematodes were found on either side of the split pot and between 51 % and 73 % of those on the plant side were active. In pots treated with 1.5, 1.0 and 0.5 mg rishitin per pot, 83 %, 66 % and 53 % of the nematodes were in the inoculated side and of these 93 %, 78 % and 29 % respectively were immobile. Examination of the plant root systems indicated that the numbers of root galls resulting from nematode feeding had been significantly decreased by both oxamyl and rishitin (Tab. 2).

Table 1

Mean numbers of nematodes recovered after 18 days in the planted and unplanted sides of the split pot

*Experiment**		*Concentration*	*Total nematodes*		*Mobile nematodes*		*Immobile nematodes*	
		mg per pot	*Plant*	*No plant*	*Plant*	*No plant*	*Plant*	*No plant*
Rishitin	1	1.5	9.6	49.3	3.9	3.5	5.7	45.7
	2	1.0	22.8	52.6	7.8	11.6	15.0	41.0
	3	1.0	29.5	51.2	10.2	11.1	19.2	40.1
	4	0.5	26.7	29.9	24.3	21.3	2.5	8.6
Oxamyl	1	0.25	33.1	28.4	21.6	15.5	11.5	12.9
	2	0.25	31.8	38.8	23.2	22.3	8.6	16.5
	3	0.25	35.5	35.2	20.2	19.4	15.2	15.9
	4	0.25	20.4	28.2	10.4	11.1	10.0	17.1
Control	1	—	45.9	17.0	42.6	9.1	3.2	7.9
	2	—	52.8	24.0	47.6	18.2	5.2	5.9
	3	—	50.9	23.6	46.9	17.7	3.9	5.9
	4	—	41.0	26.0	39.1	23.4	1.9	2.6
SED	1	DF (21)	4.29/4.19		3.07/2.92		3.34/2.76	
	2	DF (26)	6.08/6.04		4.61/4.74		4.57/4.29	
	3	DF (21)	4.70/4.70		4.20/3.87		3.19/2.99	
	4	DF (21)	5.69/6.17		5.14/5.04		2.45/2.57	

DF : Degrees of Freedom in parenthesis.
SED : Standard error of the difference between means : numerator is the SED between rishitin, oxamyl and control treatments of the same number and the denominator is the SED between " plant " and " no plant " treatments of the same number.
* Experimental runs 1, 3 and 4 each contained eight replicates of each treatment; experimental run 2 contained ten replicates of each treatment.

Table 2

Mean numbers of galls produced 18 days after treatment by nematodes feeding on Petunia roots

Experiment	*Rishitin**	*Oxamyl*	*Control*	*SED*	*DF*
1	1.87	1.62	4.29	0.808	21
2	4.67	5.90	10.50	1.936	26
3	6.25	5.38	16.25	1.954	21
4	8.00	6.25	12.75	1.492	21

* Rishitin 1 = 1.5 mg per pot; 2 & 3 = 1.0 mg pot; 4 = 0.5 mg per pot; Oxamyl 1-4 = 0.25 mg per pot.
SED Standard error of the difference between means.
DF Degrees of Freedom.

Discussion

The role of phytoalexins as resistance mechanisms of plants to nematodes has been reviewed by Kaplan and Keen (1980) and Veech (1982). The action of the sesquiterpenoid phytoalexin rishitin against *Ditylenchus destructor* and *D. dipsaci* has been demonstrated by Zinovyeva and Chalova (1986). Mahajan *et al.* (1986) investigated the nematicidal activity of several sesquiterpenoids against the root knot nematode *Meloidogyne incognita* and identified several which exhibited good biological activity.

In our studies rishitin produced behavioural responses in *X. diversicaudatum* and *L. elongatus* under soil-free conditions. Low doses (20 µg rishitin as a point source in 12 ml agar) caused nematodes to become agitated, make erratic movements, and then migrate away from the source. Nematodes in close proximity to the point source in a Petri dish rapidly lost mobility and appeared dead. The effects of rishitin increased with concentration. There was a linear relationship between concentration and duration of treatment and the EC 50 values decreased from 97.9 µg ml^{-1} to 33.2 µg ml^{-1} and then to 18.0 µg ml^{-1} as treatment time was increased from 10 min to 30 min and 60 min. Consequently, low concentrations of rishitin in soil over long periods of time may be sufficient to control nematodes and protect crops. Zinovyeva and Chalova (1986) calculated the ED 50 of rishitin to inactivate *D. dipsaci* to be 100 µg ml^{-1}, but they did not specify the duration of exposure. In our studies nematodes remained inactive whilst maintained in the toxic environment, they were however, not necessarily dead. Even after 5 h in a 100 µg ml^{-1} rishitin solution many nematodes rapidly recovered mobility on transfer to water. However, at the higher concentration of 200 µg ml^{-1} all nematodes became irreverisbly damaged after treatment for 2 h.

In the series of split-pot experiments, all three rates of rishitin decreased migration and galling caused by feeding. However, rishitin was less effective than oxamyl

at preventing root galling, even though 2-6 times more active ingredient was applied. The application rate of oxamyl was at a rate higher than that normally used in the field. Field equivalent rates calculated from the volume of soil in the pots and assuming incorporation to a depth of 20 cm in the field were 13.5, 27 and 40.5 kg ha^{-1} for the 0.5, 1.0 and 1.5 mg per pot rishitin rates and 6.75 kg ha^{-1} for the 0.25 mg per pot oxamyl. The potency of rishitin was therefore considerably less than that of oxamyl in soil. As with oxamyl, the short term effects of low rates of rishitin on nematodes are reversible and the effectiveness to control is therefore dependant on the persistence of the chemicals in soil. The half-life of oxamyl has been reported to be between 6 and 21 days in moist soils at 15° (Bromilow *et al.*, 1980). Although rishitin is known to be stable to high temperatures under laboratory conditions (it can be identified and measured by GLC at 200°) its persistence in the soil, where it will be degraded by microorganisms, has not yet been determined. Rishitin is toxic to fungi and bacteria at high concentrations (Harris & Dennis, 1976; Lyon & Bayliss, 1975) but it has been shown to be metabolised at low concentrations by some fungi *in vitro* (Lyon, 1976). As rishitin is biodegradable the risk of environmental pollution is minimised and mammalian toxicity is likely to be low as no toxic effects were observed when it was fed to marmosets *(Callithrix jacchus)* (Poswillo *et al.*, 1973). Its activity against other pests has yet to be investigated.

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REFERENCES

BROMILOW, R. H., BAKER, R. J., FREEMAN, M. A. H. & GOROG, K. (1980). The degradation of aldicarb and oxamyl in soil. *Pestic. Sci.*, 11 : 371-378.

BUNT, J. A. (1975). Effect and mode of action of some systemic nematicides. *Meded. LandbHoogesch. Wageningen*, 75 : 1-127.

CASTRO, C. E. & BELSER, N. O. (1978). Intoxication of *Aphelenchus avenae* by ethylene dibromide. *Nematologica*, 24 : 37-44.

CASTRO, C. E. & THOMASON, I. J. (1971). Mode of action of nematicides. In : Zuckerman, B. M., Mai, W. F. & Rohde, R. A. (Eds) *Plant Parasitic Nematodes, Vol. II.* New York & London, Academic Press : 289-296.

FLEGG, J. (1967). Extraction of *Xiphinema* and *Longidorus* species from soil by a modification of Cobb's decanting and sieving technique. *Ann. appl. Biol.*, 60 : 429-437.

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GOMMERS, F. J. (1981). Biochemical interactions between nematodes and plants and their relevance to control. *Helminth. Abstr., Ser. B*, 50 : 9-24.

HARRIS, J. E. & DENNIS, C. (1976). Antifungal activity of post-infectional metabolites from potato tubers. *Physiol. Pl. Pathol.*, 9 : 155-165.

KAPLAN, D. T. & KEEN, N. T. (1980). Mechanisms conferring plant incompatibility to nematodes. *Revue Nématol.*, 3 : 123-134.

KATSUI, N., MURAI, A., TAKASUGI, M., IMAIZUMI, K. & MASAMUNE, T. (1968). The structure of rishitin, a new antifungal compound from diseased potato tubers. *J. Chem. Soc., Chem. Commun.*, 43-44.

KÜHN, J. (1881). Bericht über die Ergebnisse der im Auftrage des Vereins fur Rübenzucker-Industrie des Deutschen Reiches aufgeführten Versuche zur Ermittelung der Ursache der Rübenmüdigkeit des Bodens und zur Erforschung der Natur der Nematoden. *Bericht. Physiol. Lab. Landwirts. Inst. Univ. Halle*, 3 : 153 p.

LYON, G. D. (1972). Occurrence of rishitin and phytuberin in potato tubers inoculated with *Erwinia carotovora var. atroseptica. Physiol. Pl. Pathol.*, 2 : 411-416.

LYON, G. D. (1976). Metabolism of the phytoalexin rishitin by *Botrytis* spp. *J. gen. Microbiol.*, 96 : 225-226.

LYON, G. D. & BAYLISS, C. E. (1975). The effect of rishitin on *Erwinia carotovora* var. *atroseptica* and other bacteria. *Physiol. Pl. Pathol.*, 6 : 177-186.

MAHAJAN, R., PRABHSARAN S., BAJAJ, K. L. & KALSI, P. S. (1986). Nematicidal activity of some sesquiterpenoids against rootknot nematode *(Meloidogyne incognita). Nematologica*, 32 : 119-123.

PAXTON, J. (1980). A new working definition of the term " phytoalexin ". *Plant Dis.*, 64 : 734.

POSWILLO, D. E., SOPHER, D., MITCHELL, S. J., COXON, D. T., CURTIS, R. F. & PRICE, K. R. (1973). Further investigations into the teratogenic potential of imperfect potatoes. *Nature, London*, 244 : 367-368.

ROSS, G. J. S. (1980). *MLP. Maximum Likelihood Probability Program.* Statistics Dept., Rothamsted Exper. Statn, England., 242 p.

STOESSL, A., STOTHERS, J. B. & WARD, E. W. B. (1976). Sesquiterpenoid stress compounds of the Solanaceae. *Phytochemistry*, 15 : 855-872.

TOMIYAMA, K., SAKUMA, T., ISHIZAKA, N., SATO, N., KATSUI, N., TAKASUGI, M. & MASAMUNE, T. (1968). A new antifungal substance isolated from resistant potato tuber tissue infected by pathogens. *Phytopathology*, 58 : 115-116.

VEECH, J. A. (1982). Phytoalexins and their role in the resistance of plants to nematodes. *J. Nematol.*, 14 : 2-9.

WRIGHT, D. J. (1981). Nematicides : Mode of action and new approaches to chemical control. In : Zuckerman, B. M. & Rohde, R. A. (Eds) *Plant Parasitic Nematodes, Vol. III* New York & London, Academic Press : 421-449.

ZINOVYEVA, S. V. & CHALOVA, L. I. (1986). [The formation of phytoalexins on invasion of potatoes by stem nematodes.] *Dokl. Akad. Nauk SSSR*, 288 : 1277-1280.