Rishitin a natural plant product with nematicidal activity

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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

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.

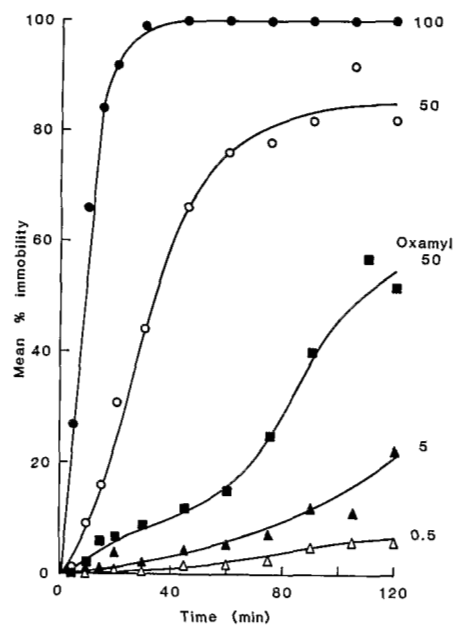

Fig. 1. Graph showing loss of mobility with respect to time in solutions of rishitin : ●, 100 µg ml^{-1}; ○, 50 µg ml^{-1}; ▲, 5 µg ml^{-1}, △, 0.5 µg ml^{-1} and oxamyl; ■, 50 µg ml^{-1}.

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.

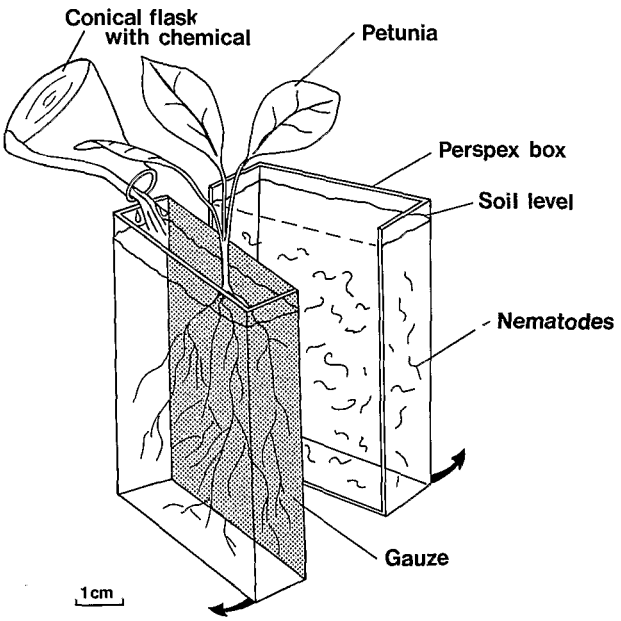

Fig. 2. Split-pot showing the two halves divided by a nylon gauze which confines the roots of a seedling petunia plant to one side. The two halves of the box are taped together during

ed 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),

[redacted]

at preventing root galling, even though 2-6 times more active ingredient was applied. The application rate of

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