Effects of quassinoids extracted from *Hannoa undulata* seed on the penetration and reproduction of *Meloidogyne javanica* on tomato

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SUMMARY

The quassinoid fraction extracted from seeds of *Hannoa undulata* is composed of a mixture of three polycyclic lactones : chaparrinone, glaucarubolone and klaineanone. Their effects on the penetration and the reproduction of *Meloidogyne javanica* on tomato was studied in pots. Quassinoids prevent the penetration of juveniles of *M. javanica* into tomato roots. Full inhibition of penetration occured during three days of continuous exposure to a 5 ppm quassinoid solution in the soil water. Quassinoids had a direct action on the juveniles, which were not able to recover and penetrate the roots after exposure for 4 days at concentration of 10 ppm or for days at a concentration of 5 ppm. Soil amendment of crude powder of *H. undulata* seeds fully inhibited the reproduction of *M. javanica* on tomato roots. Quassinoid extracts at concentration of 1 ppm or higher in the soil water significantly reduced reproduction, although complete reproductive inhibition was not obtained even when the concentration of quassinoids in the soil water was 10 ppm.

RÉSUMÉ

Effet des quassinoïdes extraits de Hannoa undulata *sur la pénétration et la reproduction des juvéniles de* Meloidogyne javanica *dans les racines de tomate*

La fraction quassinoïde extraite des graines de *Hannoa undulata* est constituée d'un mélange de trois lactones polycycliques : chaparrinone, glaucarubolone et klaineanone. Ces quassinoïdes inhibent la pénétration des juvéniles de *Meloidogyne javanica* dans les racines de plants de tomate. Une inhibition totale de la pénétration est obtenue lorsque leur concentration dans la solution de sol est égale ou supérieure à 5 ppm. Ces produits ont une action directe sur les nématodes; ils n'agissent pas comme des endothérapiques. Leur effet sur les juvéniles est irréversible lorsque ces derniers ont été exposés pendant quatre jours à une concentration de 10 ppm ou sept jours à une concentration de 5 ppm. L'addition de poudre de graines de *H. undulata* dans le sol inhibe totalement la reproduction de *M. javanica* sur les racines de tomate. Les quassinoïdes extraits de ces graines réduisent significativement la reproduction lorsque leur concentration dans la solution de sol est égale ou supérieure à 1 ppm. Aucune inhibition totale de la reproduction n'a été observée, même lorsque la concentration en quassinoïdes dans la solution de sol était de 10 ppm au début de l'expérience. Cette différence d'efficacité entre la poudre brute et la fraction quassinoïdes est peut être due à une dégradation rapide de cette dernière dans le sol, dégradation qui, dans le cas de la poudre brute, serait palliée par un relâchement constant de quassinoïdes dû à la décomposition de la poudre.

It has been recently shown that crude and delipidified seed extracts of *Hannoa undulata* (Guill. Perr.) Planch. and quassinoid extract from seed of *Hannoa klaineana* Planch. are able to reduce the penetration of *Meloidogyne javanica* (Treub) juveniles into tomato roots (Prot & Kornprobst, 1983). Four root penetration experiments and three experiments to measure nematode reproduction were realized in pots in order to verify that the quassinoids contained in *Hannoa* seeds were responsible for the reduction of the penetration, to characterize their mode of action and to test the duration of their effects.

Materials and methods

All experiments were conducted using autoclaved (120° for 30 mn) sandy soil. Tomato cv. Roma seedlings were prepared in plastic tubs and plants were utilized two weeks after germination. Seedlings were irrigated with water as needed and with half-strength Hoagland's nutrient solution added weekly.

Juveniles of *M. javanica* used in these experiments were derived from a culture maintained on kenaf (*Hibiscus cannabinus* L.) in the greenhouse. Second-stage juveniles were extracted from galled roots using a mist

chamber (Seinhorst, 1950) and only individuals collected within a 24 h period were used.

H. undulata seeds were ground in a Waring Blender into a crude powder.

Crude quassinoids of *H. undulata* were crystallised according to Polonsky and Bourguignon-Zylber's (1965) procedure. The crude quassinoid extract was a mixture of three polycyclic lactones : chaparrinone, glaucarubolone and klaineanone as determined by Polonsky and Bourguignon-Zylber's method. Solutions in distilled water were prepared in concentrations of 1, 5, 10, 20, 50 and 100 ppm.

PENETRATION

Four experiments to test penetration were conducted at a constant temperature of 28° in a controlled temperature humidity chamber. Tomato roots were washed free of soil and stained with cold cotton blue lactophenol (de Guiran, 1966) and juveniles in the roots were counted using a dissecting microscope.

In the first experiment batches of 100 juveniles were put into Syracuse watch glasses (B.P.I.) containing 0.2 cm^3 of distilled water or quassinoid solution (0, 1, 5, 10, 20, 50 and 100 ppm) for 24 h at 22°. The suspension of juveniles in the pretreatment solution was then inoculated on tomato seedlings 24 h after transplantation to glass pots containing 20 g soil watered with 3.5 cm^3 of distilled water. Seventy-two hours after inoculation tomato roots were stained and juveniles in the roots counted. Eighteen replications were realized for each concentration.

In the second experiment lots of 100 juveniles were put in each Syracuse watch glass containing 0.2 cm^3 of distilled water or quassinoid solution (0, 1, 5, 10 and 20 ppm) and maintained at 22°for 2, 4, 7 or 10 days. Following treatment, each lot of juveniles was washed with 10 cm^3 of distilled water on a Millipore filter (0.45 µm) and inoculated as previously described. After three days tomato roots were stained and nematodes counted. Treatments were replicated 18 times.

In the third experiment batches of 100 juveniles were inoculated in glass pots containing 20 g of soil moistened with 3.5 cm^3 of distilled water or quassinoid solution (0.1, 0.5, 1, 5, 10 and 20 ppm). Pots were placed in a humidity chamber at 28° and a tomato plant was transplanted in each pot after 24 h. Seventy-two hours following transplantation, roots were stained and the juveniles counted. The experiment was repeated 18 times for each concentration.

In the fourth experiment , two-week old tomato seedlings were pretreated with quassinoid solution by dipping the roots of each seedling in a test tube containing 5 cm^3 of solution (0, 5, 10, 20, 50 and 100 ppm) until each plant had absorbed 3.5 cm^3 of solution. Seedlings were transplanted in glass pots containing 20 g of soil moistened with 3.5 cm^3 of distilled water, and 100 juveniles of *M. javanica.* Seventy-two hours later, the juveniles in the roots were counted. The test had twelve replications at each concentration.

REPRODUCTION

Three experiments on reproduction were conducted in plastic pots containing 1.5 kg of soil premoistened with either 225 cm^3 of water or quassinoid solutions. During the experiments, plants were watered as needed and half-strength Hoagland's nutrient solution was added weekly.

Pots were maintained in a sand bed and the soil temperature at the center of a check pot was continuously recorded. Plants were harvested approximately 5 weeks after transplantation, when Heat Units (Hu) exceeded 400 units ($Hu = \Sigma \frac{(H + l)}{2} - 15$, with H = highest daily temperature and l = lowest daily temperature and 15° as the basal temperature). Washed root-systems were incubated for three weeks in a mist chamber (Seinhorst, 1950) and the juveniles collected and counted after 1, 2, 4, 7, 10, 14, 17 and 21 days.

The effect of crude powder of *H. undulata* seeds on the reproduction of *M. javanica* on tomato cv. Roma was studied by mixing 0.3, 4.5, 7.5 or 10 g of crude powder with the soil contained in each pot. At the same time, 3,000 juveniles of *M. javanica* were inoculated at the center of each pot. Two week-old tomato seedlings were transplanted in each pot after 7 days. Plants were harvested 37 days after transplantation (Hu = 440). Treatments were replicated 18 times.

The effect of different concentrations of *H. undulata* quassinoid solutions on the reproduction of *M. javanica* on tomato cv. Roma was studied by premoistening the soil contained in each pot with 225 cm^3 of solution containing 0, 1, 5 or 10 ppm of quassinoids. At the same time 500 second stage juveniles of *M. javanica* were inoculated at the center of each pot. A two week-old tomato seedling was transplanted in each pot one day later. Plants were harvested after 40 days (Hu = 482). Eighteen replications of each treatment were conducted.

The effect of a 3 days pretreatment of the juveniles of *M. javanica* by a solution of 10 ppm quassinoids of *H. undulata* was studied by premoistening the soil in each pot with 225 cm^3 of a solution of 10 ppm quassinoids in distilled water and by inoculating 800 juveniles of *M. javanica* at the center of each pot at the same time; 225 cm^3 of distilled water were added in each check pot. Three days later, a two-week old tomato seedling was transplanted in each pot. Plants were harvested after 37 days (Hu = 410). Treatments were replicated eight times.

Results

Penetration

Inhibition of juvenile penetration increased with the concentration of quassinoids in pretreatment solutions (Fig. 1). A 24 h pretreatment with a solution of 5 ppm of quassinoids significantly reduced root penetration and full inhibition of penetration was obtained with 100 ppm. Since, after the pretreatment, juveniles were inoculated in suspension in the pretreatment solution, the concentrations of quassinoids in the soil water during the penetration phase were respectively 0.28 ppm for a significant reduction of the penetration and 5.7 ppm for a full inhibition.

Inhibition of penetration increased with the length of the pretreatment (Fig. 2). A significant reduction of the penetration was observed after four days of pretreatment with a concentration of 1 ppm of quassinoids and a full inhibition of penetration after either 7 days of pretreatment with a concentration of 5 ppm or four days of pretreatment with 10 ppm. After two days of pretreatment some juveniles incubated in quassinoid solutions of 10 and 20 ppm were able to recover and penetrate the roots. After ten days of incubation the capacity of penetration of the check lots was significantly reduced.

After a 24 h pretreatment in the soil and with a continuous exposure during the penetration period a significant reduction of the penetration was induced by a quassinoid concentration of 1 ppm (Fig. 3) and a full inhibition of the penetration was observed with 10 ppm.

Pretreatment of tomato seedlings (fourth experiment) with concentration of 20 or 50 ppm of quassinoids proved phytotoxic and plants were killed in a 100 ppm solution. Moreover, no significant reduction of root penetration was observed in any treatment.

Reproduction

All results are expressed in terms of a reproductivity factor, R = Pf/Pi (Oostenbrink, 1966) where Pf is the number of juveniles recovered from the roots in the mist chamber at the end of the experiments and Pi the initial inoculum.

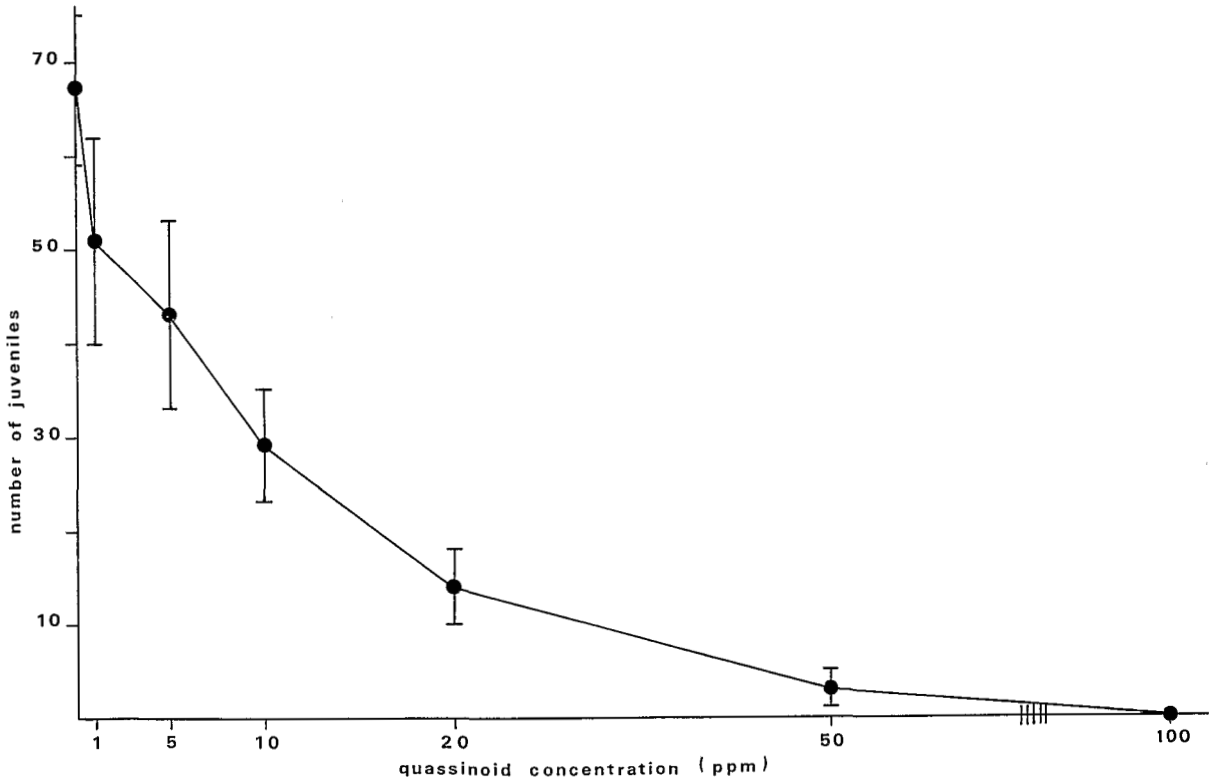

Fig. 1. Effect of a 24 h pretreatment with *H. undulata* quassinoids seed extract on the penetration of *M. javanica* juveniles into tomato roots. Confidence intervals (P = 0.05) are indicated.

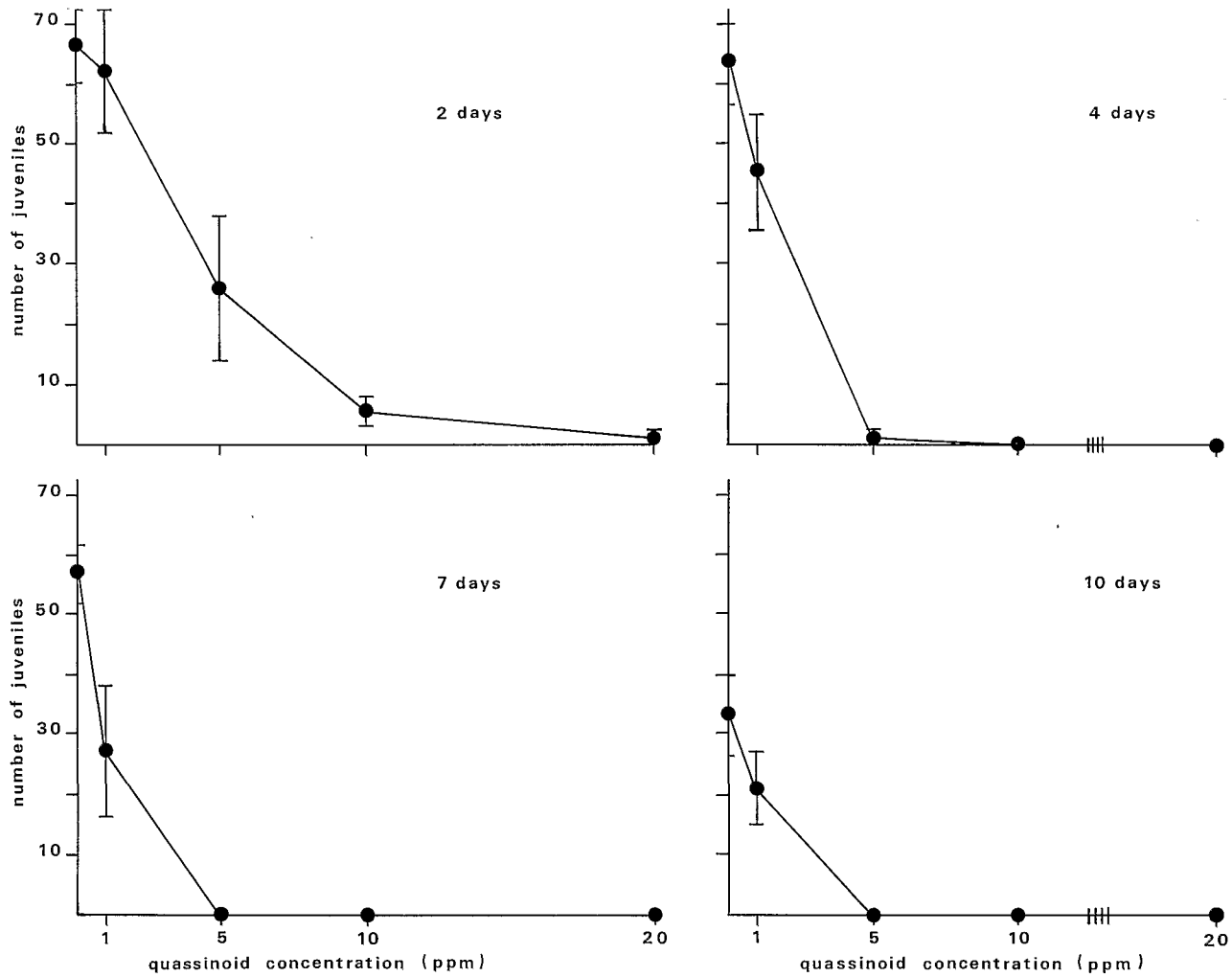

Fig. 2. Effect of different times and concentrations of *H. undulata* quassinoids pretreatment on the penetration of *M. javanica* juveniles into tomato. Confidence intervals (P = 0.05) are indicated.

The addition of crude powder of *H. undulata* to the soil inhibited the reproduction of *M. javanica* juveniles on tomato. On the check plants the number of juveniles recovered after 37 days of growth and 21 days in the mist chamber was 39 times greater than the initial inoculum. The addition of 3 g of crude powder of *H. undulata* per pot reduced the reproductivity factor to 0.2 and no juveniles were recovered when 4.5, 7.5 or 10 g of powder were used. However phytoxicity occured at a dose of 10 g of crude powder.

The reproductivity factor decreased as the concentration of the quassinoid solutions used to premoisten the soil increased (Fig. 4). In the control lot the average reproductivity factor after 40 days growth was 164. The addition of quassinoids at a concentration of 1 ppm in the soil water significantly reduced this factor and at a concentration of 10 ppm it was reduced to 7 % of that on control plants.

A three days pretreatment of the juveniles in the soil with a concentration of 10 ppm of quassinoids before planting reduced the reproductivity factor five fold by the 21st day (Fig. 5).

Discussion

Results obtained in these experiments confirm previous observations (Prot & Kornprobst, 1983) that quassinoids extracted from *H. undulata* seeds are capable of inhibiting *M. javanica* penetration into tomato roots. These polycyclic lactones probably act directly on the juveniles since a pretreatment of the seedlings failed to inhibit penetration.

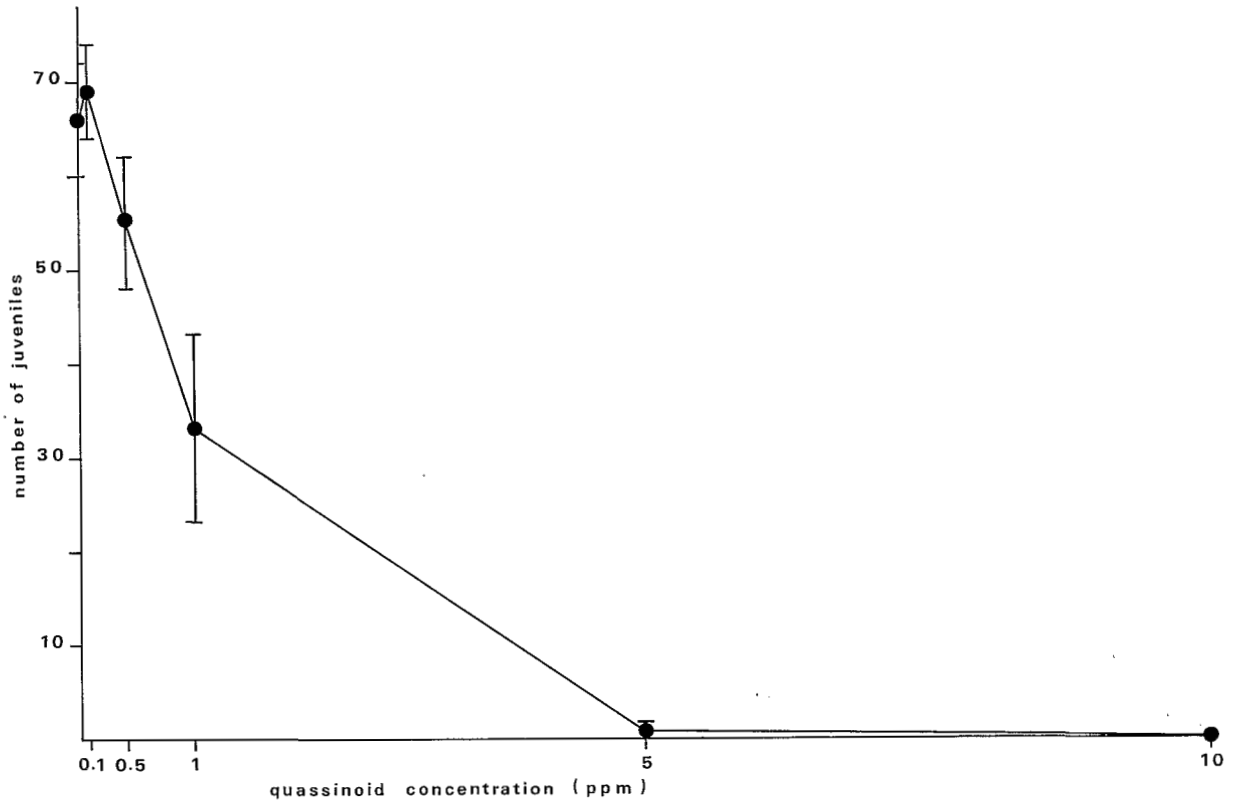

Fig. 3. Effect of a 24 h pretreatment in soil and a continuous exposure during the penetration phase to different concentrations of *H. undulata* quassinoids on the penetration of *M. javanica* juveniles into tomato roots. Confidence intervals (P = 0.05) are indicated.

Several observations have bearing on the control potential of these compounds. A 24 h pretreatment of juveniles in the soil before planting with a concentration of 5 ppm of quassinoids in the soil water fully inhibited the penetration during three days. In order for the effect of quassinoids on the juveniles to become irreversible it was necessary to incubate juveniles at 5 ppm for seven days or 10 ppm for four days.

The reproduction experiments show that quassinoids introduced in the soil at the beginning of the phase of contact between juveniles and roots, are capable of reducing reproduction when the concentration of quassinoids in the soil water is equal or superior to 1 ppm although full inhibition of the reproduction was not obtained even at concentration of 10 ppm.

The experiments on reproduction do not isolate penetration, development and reproduction; the reduction of the reproductivity factor could be a consequence of reduced penetration. The fact that a full inhibition of the reproduction was not obtained at concentration of 10 ppm indicates that the penetration was not fully inhibited by the quassinoid solutions in these experiments.

Full inhibition of reproduction was obtained however when the crude powder of *H. undulata* seeds was used. It is possible that another compound contained in these seeds has an effect on the nematodes. It is also possible that the active compounds (quassinoids) are rapidely degraded in the soil or absorbed by the plants and that degradation or absorbtion is mitigated by slow release of the quassinoids from the seed powder.

Thus in order to obtain effective protection of the plants it may be necessary to introduce quassinoids in the soil before planting and to maintain the quassinoid concentration in the soil water during the penetration phase by multiple application in irrigation water or by introducing them in the soil in slow release granules. Based on these experiments the concentration of quassinoids necessary to obtain good control is 5 ppm of the soil solution. To provide such a concentration in the top

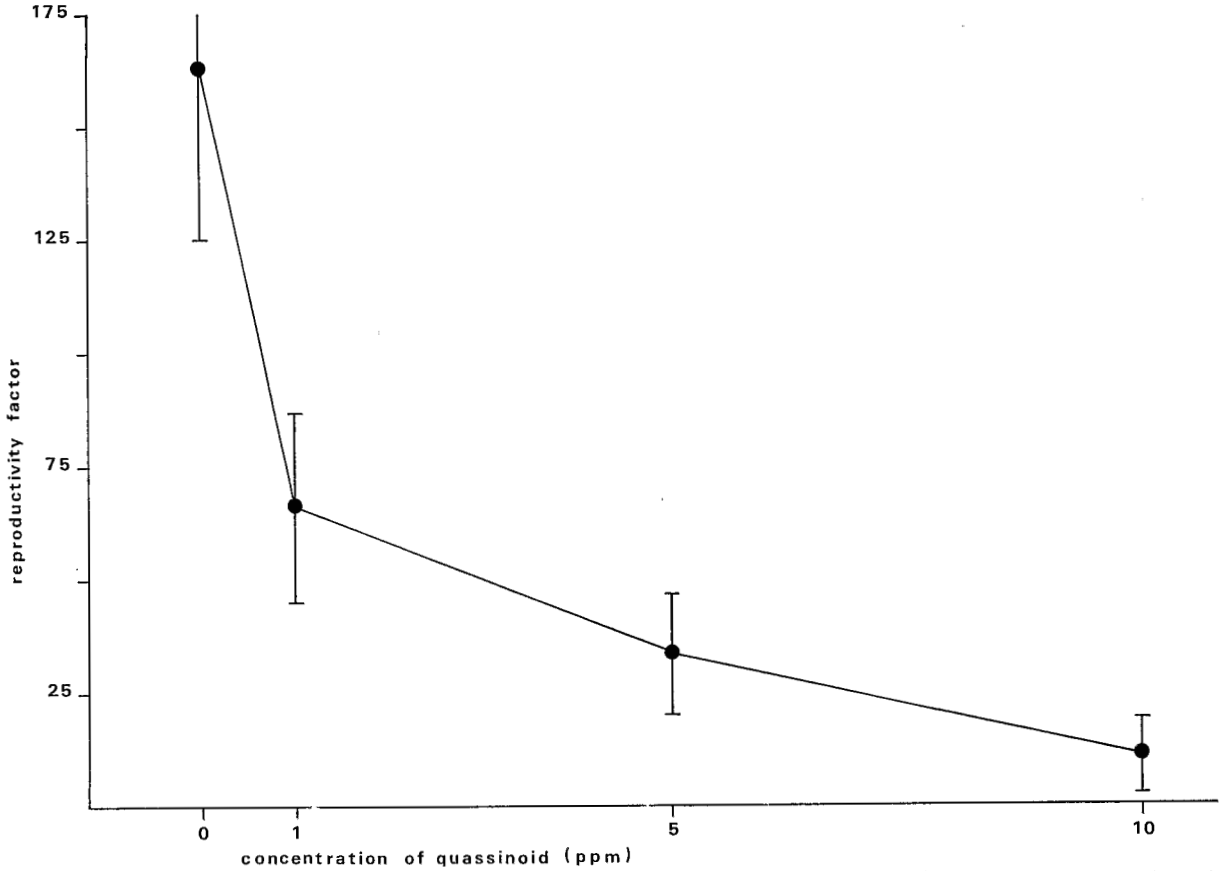

Fig. 4. Effect of different concentration of *H. undulata* quassinoids in the soil water on the reproduction of *M. javanica* juveniles on tomato plants. Confidence intervals (P = 0.05) are indicated.

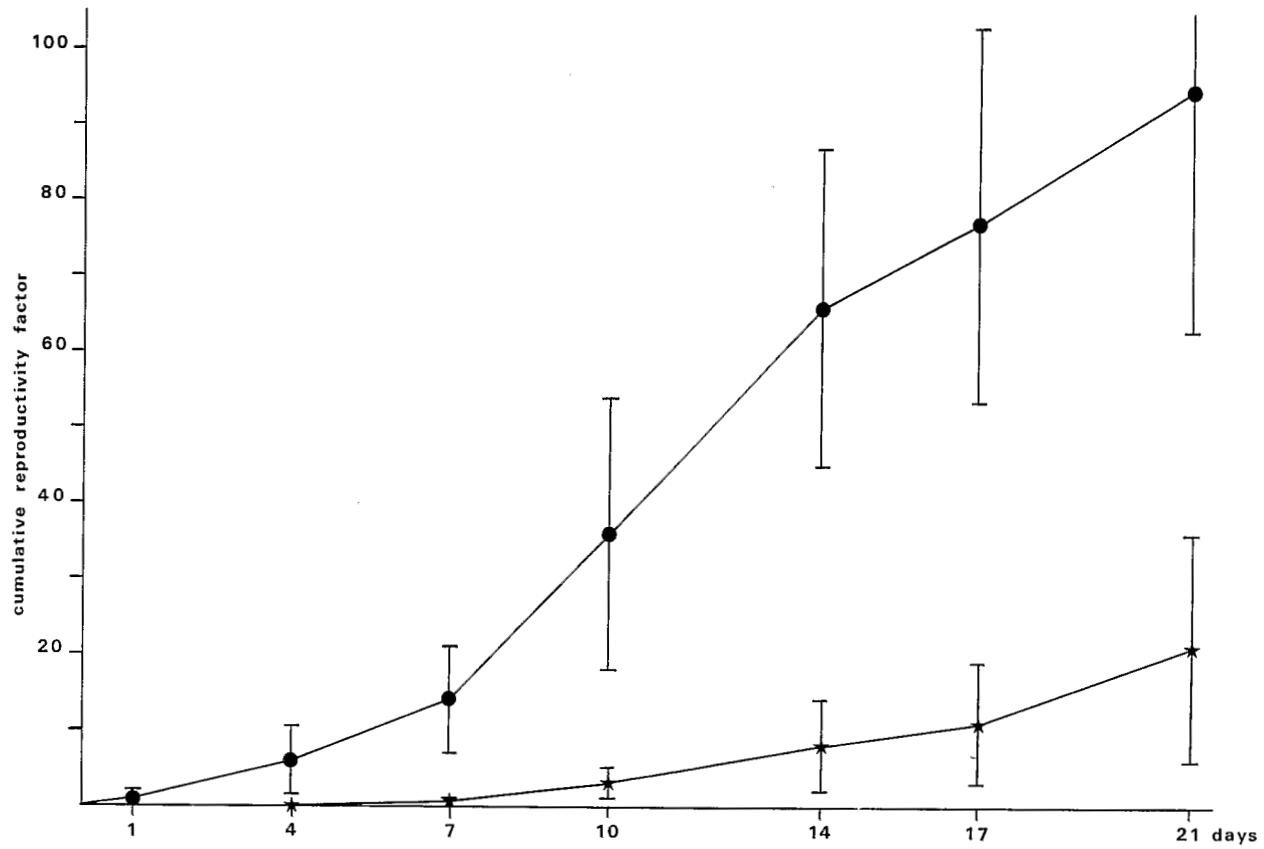

Fig. 5. Cumulative reproductivity factor during 21 days in mist chamber of *M. javanica* juveniles grown for 37 days on control plants (circles) and on plants cultivated with 10 ppm of *H. undulata* quassinoids in the soil water (stars). Confidence intervals (P = 0.05) are indicated.

40 cm of a soil containing 15 % water would require 3 kg of quassinoids per hectare.

It has been shown that avermectins which are macrocyclic lactones effectively control root infection by root-knot nematodes (Sasser, Kirkpatrick & Dybas, 1982; Garabedian & Van Gundy, 1983). Because quassinoids are polycyclic lactones it is possible that the two classes of compounds have the same action on nematodes.

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