A Soil-free System for Assaying Nematicidal Activity of Chemicals

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Abstract: A biological assay system for studying the nematicidal activity of chemicals has been devised using a model consisting of cucumber (*Cucumis sativus* L. cv. Long Marketer) seedlings growing in the diSPo® growth-pouch apparatus. *Meloidogyne incognita* was used as the test organism. The response was quantified in terms of the numbers of galls produced. Statistical procedures were applied to estimate the ED_{50} values of currently available nematicides. This system permits accurate quantification of galling and requires much less space and effort than the currently used methods. *Key words:* nematicides, *Meloidogyne,* assay, screening, and growth-pouch.

The methods for evaluating the nematicidal activity of experimental compounds have been reviewed by Bunt (1). Among these, a biological assay using the root-knot nematodes is widely used to test candidate nematicides. Test chemicals are added directly to nematode-infested soil, and after an interval a susceptible host plant is transplanted into this soil. Nematicidal efficacy is then evaluated by rating the extent of root galling (2). This procedure simulates field conditions. The use of soil, however, presents numerous problems for a rapid bioassay: soil preparation is time consuming and evaluation of efficacy by means of the galling index is not precise. Measuring the host response to infection by counting the numbers of galls produced gives a more precise galling index for estimating the efficacy of test compounds. A testing system that can be used as an indicator of nematicidal activity is needed; this system should be speedy, require comparatively little effort and space, and permit precise quantification of host root galling. We report here such a system and the test results using several nematicides.

METHODS AND MATERIALS

Meloidogyne incognita Kofoid and White (Chitwood) were maintained for inoculum on tomato (*Lycopersicon esculentum Mill.* cv. Rutgers) in the greenhouse. Second stage larvae were obtained by the method of Thirugnanam (4).

Cucumber (*Cucumis sativus* L. cv. Long Marketer) seeds were individually germinated in diSPo growth-pouches (3) moistened with 9 ml water and held in a growth chamber maintained at 90% RH, 24 C, and 16-h photoperiod. A 5-mm incision was made in the center of the paper trough to facilitate penetration by the emerging radicle.

At 0, 24, 48, 72, 96, or 144 h after seeding, the pouches were inoculated with 1 ml of water containing 200 larvae. The tops of the pouches were sealed with transparent tape, leaving enough unsealed space for the plumule to grow through. One milliliter of inoculum containing ca. 50, 100, 200, or 400 larvae was applied to each root system 96 h after the pouches had been seeded and moistened with 9 ml of water. After the inoculation the tops of the pouches were resealed, leaving room around the stem of the seedling.

Test chemicals (Vydate-L, 24% methyl N'N'-dimethyl-N-[(methylcarbamoxyl)oxy]-1-thiooxamimidate; Nem-A-Tak 4L, 40% 2-(diethoxy-phosphinylimino)-1,3-dithietane; and Furadan 4F, 40% 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) were dissolved in ethanol so that the final

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solutions were in 0.25% ± 0.02 aqueous ethanol. Each of the pouches was moistened with 9 ml of the respective solution by means of a pipetting syringe. Aqueous ethanol (0.25%) was used as the control. The pouches were seeded immediately after adding the test solutions. Ninety-six hours later the tap root lengths were measured and the roots inoculated with ca. 200 larvae in 1 ml water.

Galls were counted under a dissecting microscope 6 d after inoculation. The individual gall counts were square root transformed to stabilize the variances and establish a linear relationship with reference to log-doses. The estimated concentrations to obtain a 50% reduction in gall counts as compared to the control (ED_{50}), the corresponding 95% confidence intervals, and the slopes of the regression lines for each chemical were calculated. A regression model for the transformed gall counts vs. tap root lengths at the time of inoculation was formulated.

RESULTS AND DISCUSSION

Galls could be discerned 48 h after inoculation. Five days after inoculation the galls were large enough to be counted under a dissecting microscope. Beyond this time the confluence of contiguous galls made accurate counting difficult. Under the experimental conditions used, the optimum time to inoculate the plants, as determined from the maximum numbers of discrete galls, was 4–6 d after seeding the pouches (Table 1). Earlier inoculations result in more massive and concentrated infection but fewer discrete galls.

When plants were inoculated with 50, 100, 200, or 400 larvae/pouch, the slopes of the regression lines of square root transformed gall counts and logs of the inoculum levels were similar, but the intercepts were significantly ($P = 0.05$) different (Fig. 1). The similarity of the slopes indicates the reliability of using gall counts as a measure of response, even though the actual gall counts varied among the experiments. In bioassay methods, heterogeneity in responses, in this case the gall counts, is consistently present; such heterogeneity in this model was stabilized by using the square root transformation of the responses.

Table 1. Mean numbers of galls and lengths of tap roots of cucumbers seedlings inoculated with 200 larvae at different times after seeding the pouches.

Time of inoculation (days after seeding)	Length of tap root (cm)*	Gall counts†
0	0 a	0.9 a
1	0.3 b	2.5 b
2	2.5 c	4.4 c
3	6.0 d	4.8 c
4	8.6 e	6.1 d
6	10.3 f	6.2 d

*Mean of 20 replicates.

†Mean of square root transformed data. Means followed by the same letter(s) are not statistically significantly different ($P = 0.05$) using Duncan's new multiple-range test.

The mean gall counts under different treatments and the relationship between root lengths at the time of inoculation and subsequent gall counts are shown in Table 2. There are significant differences in gall counts among different concentrations of each of the chemicals tested. It is also seen that there is no consistently significant relationship between the responses expressed as transformed gall counts and the respective tap root lengths because, in a majority of the cases, the regression slopes are not significant. It appears that variation in the

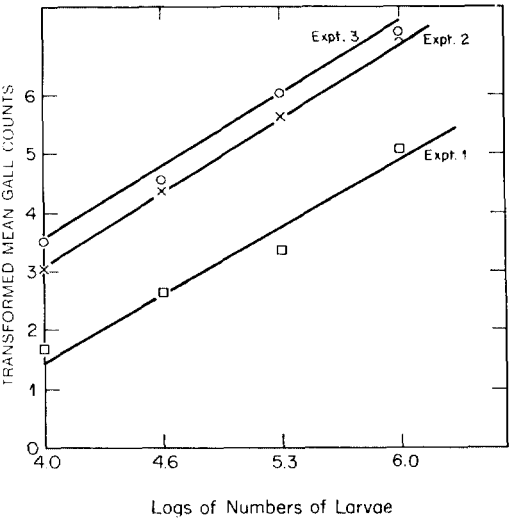

Fig. 1. The relationship between the means of square root transformed gall counts on *Cucumis sativus* and the logs of numbers of *Meloidogyne incognita* larvae used as inoculum in three experiments.

Table 2. The effect of various nematicides tested at different concentrations on the mean gall count and the mean gall count as a function of root length.

Chemical	Concentration (ppm)	Mean gall counts†	Slope of root length vs. gall count
Vydate	1	4.8 b	0.56
	3	2.9 d	0.02
	10	0.5 fg	−0.50
Nem-A-Tak	1	4.1 c	0.73*
	3	0.3 fg	0.15
	10	0 g	—
Furadan	5	3.2 d	0.22
	10	1.7 e	−0.92
	20	0.8 f	−0.36
Control 0.25% aqueous ethanol	—	6.1 a	−0.53**

†Means of 10 replicates; gall counts were square root transformed. Means followed by the same letter(s) are not statistically significantly different ($P = 0.05$) accordiing to Duncan's new multiple-range test.

*, **Significant at 0.05 and 0.01, respectively.

root systems does not have a significant effect on the galling response.

The ED_{50} values and the corresponding 95% confidence intervals for the three chemicals tested are given in Table 3. Nem-A-Tak showed a high level of activity as compared to Furadan and Vydate. The slopes of the log-dose vs. response regression lines are of dissimilar magnitude among the chemicals tested in this model. As a result, no relative potency estimates could be obtained.

This system can be used as an elimination or screening test by which test compounds with little or no activity can be separated from those that appear to merit further observations. It is possible that a chemical which shows nematicidal activity in this soil-free system may not do so under the complex interactive conditions of a field. Since such interactive factors are more numerous in a soil-based system, it is more likely that more potential control chemicals will be missed in a soil test. In addition, preliminary tests are done using high concentrations of test chemicals that generally result in an all-or-none response in terms of infection. Under these conditions the soil-free system can be used to screen a large number of chemicals speedily, conveniently, and with a high level of probability of detecting nematicidal activity.

Table 3. ED_{50} values, 95% confidence intervals (C.I.), and the slopes of log-dose vs. response regression lines for three chemicals tested.

Chemical	ED_{50} (ppm)	95% C.I.	Log-dose vs. response slope
Furadan	5.11	3.43, 6.64	−4.08
Nem-A-Tak	1.33†	1.17, 1.50	−7.86
Vydate	2.58	2.02, 3.25	−4.34

†Highest dose was omitted in calculating the ED_{50}.

This system is useful in many other aspects of our nematicidal investigations. Phytotoxicity in terms of aborted germination of seeds, arrested root growth in the treated pouches, and infection as it develops can be readily observed. It can be determined whether the chemical prevented penetration or arrested the further development of the larvae after penetration of the root. Infected roots in the pouches are also excellent sources of inoculum that is free of masses of root tissue and free living nematodes seen in cultures grown in soil.

There is a considerable need in nematicidal investigations for quantification that enables the use of biometric analysis of the assay results. As a result of subjectivity involved in conventional root-knot indexing, an accurate comparison of different dosages and chemicals is difficult. This soil-free system offers a means of establishing precise dose-response relationships for comparing the nematicidal activity of chemicals.

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