

Istituto di Nematologia Agraria - C.N.R., 70126 Bari, Italy

MODELLING OF THE *IN VITRO* EFFECT OF CADUSAFOS ON *MELOIDOGYNE INCOGNITA*

by

N. SASANELLI and T. D'ADDABBO

Summary. The nematicidal effect of cadusafos on eggs of *Meloidogyne incognita* was tested in two *in vitro* experiments. Final hatch was significantly reduced by four weeks incubation at concentrations ≥ 20 mg/ml. A 15 $\mu\text{g/ml}$ dilution x 768 hour incubation periods was required to obtain a 90% mortality. The linear relationship between log time of exposure to different concentrations and probit hatchability, fitted to the observed final hatch percentages, was: probit $b = b_{Cr} - m \log_{10} t_C d^{(Cr-C)}$. Cr is a reference concentration and for $Cr = 25$ $\mu\text{g/ml}$, $b_{Cr} = 10.82$, $m = 2.3$ and $d = 0.9528$. On a log scale, the above relationship is estimated by the general equation $\log_{10} b = b' - m_{Cr} t d^{(Cr-C)}$, with $b' = 2.1377$, and $m_{Cr} = 0.0013$.

Root-knot nematodes, *Meloidogyne* spp., are widespread and can cause substantial yield losses to numerous crops (Di Vito *et al.*, 1980; Sasanelli *et al.*, 1992; Sasanelli, 1996). Control of these phytonematodes is mainly based on the use of chemicals, but there is a lack of analytical models which can predict the efficacy of nematicide application by describing the relationship between nematode survival and chemical dosage. According to Finney's (1971) theory on the relationship between dosage and effect of an active principle on organisms, the relationship is linear between nematicide dosage and probit decrease of a nematode population.

Cadusafos (S, S-di-sec-buthyl O-ethyl phosphorodithioate) is an organophosphate cholinesterase-inhibiting pesticide, available both in granular and emulsifiable formulation. It has been used to control nematodes affecting annual and tree crops (Sasanelli *et al.*, 1995; 1996; McClure and Schmitt, 1996). Nevertheless, investigations have not been undertaken to relate rates of the nematicide with nematode mortal-

ity. Therefore, two *in vitro* experiments were undertaken with the aim of modelling the nematicidal activity of cadusafos on *Meloidogyne incognita* (Kofoid *et White*) Chitw. as related to dosage rates and exposure times.

Materials and methods

Batches of thirty egg masses (averaging 4,500 eggs per batch) of an Italian population of *M. incognita*, host race 1 (Taylor and Sasser, 1978), were collected from infested roots of tomato (*Lycopersicon esculentum* Mill.) cv. Roma VF. The batches were placed on 2 cm diam sieves (215 μm aperture) in a 3.5 cm diam Petri dish.

In the first experiment 3 ml of 5, 10, 15, 20 and 25 $\mu\text{g/ml}$ aqueous solutions of cadusafos were added to the batches of egg masses, using distilled water and a 5 $\mu\text{g/ml}$ aqueous solution of fenamiphos as controls (Greco and Thomason, 1980). The dishes were in a complete randomized block design with six replicates of

each treatment, and were incubated in a growth cabinet at 20 °C (Ekanayake and Di Vito, 1984).

Emerging juveniles were removed and counted at weekly intervals, and the test solutions and the control solutions were renewed at the same time for the first four weeks. From the fourth week and for five further weeks the incubation continued only in distilled water, as done in previous experiments (Sasanelli and Di Vito, 1991; Sasanelli and D'Addabbo, 1992).

In the second experiment egg masses were maintained in the same test solutions over a 24, 48, 96, 192, 384 and 768 hour periods at 8 °C temperature to avoid egg hatch. Distilled water only was used as a control. There were six replicates for each combination of concentration and exposure time. At the end of each incubation period the batches were transferred to distilled water at 20 °C and a hatching test was conducted over a seven week period.

At the end of both experiments egg masses were shaken in a 1% sodium hypochlorite aqueous solution (Hussey and Barker, 1973) and the unhatched eggs were counted. Number of juveniles emerging weekly were expressed as the cumulative percentage of the total initial population.

Data from experiment one were subjected to

analysis of variance and comparisons made with LSD's, whereas final hatch percentages from the second experiment were fitted to the proposed mathematical model derived from Finney's theory (1971).

Results

Experiment 1. Final hatch percentages of eggs in 20 and 25 µg/ml concentrations were significantly lower than those in other test concentrations and a distilled water control ($P=0.01$), whereas no significant difference from hatching in water was found at 5-15 µg/ml concentrations of cadusafos (Table I). Incubation in fenamiphos reduced final hatch by 48.5%, compared to those in distilled water and the two lower concentrations of cadusafos. Nematode hatching behaviour in the tested concentrations was similar over the nine week period in comparison with the water control, whereas differences between the two highest concentrations and that of fenamiphos were shown only from the sixth week. Hatching in water and in 5 and 10 µg/ml concentrations occurred mostly (95-99% of the final hatch) during the first four weeks. Hatch percentage increased significantly only after remov-

TABLE I - Effect of cadusafos at different concentrations on the cumulative percentage hatch of *Meloidogyne incognita*.

Treatment	Incubation period (weeks)									LSD	
	In test solutions				In distilled water					0.05	0.01
	1	2	3	4	5	6	7	8	9		
5 µg/ml	47.9	67.8	71.9	73.4	75.5	76.4	76.7	76.7	76.8	14.8	19.9
10 µg/ml	54.9	69.9	72.5	73.2	75.3	76.2	76.5	76.7	76.8	7.6	10.3
15 µg/ml	39.9	45.5	46.8	47.9	54.1	58.3	58.8	58.9	59.1	17.7	23.8
20 µg/ml	13.6	15.3	16.1	16.4	20.0	23.8	24.2	24.6	24.8	13.6	18.3
25 µg/ml	18.0	19.5	20.5	20.8	24.7	26.8	27.4	28.8	30.3	8.8	9.2
Fenamiphos	26.8	28.0	28.8	29.1	37.6	45.1	46.5	47.6	48.5	14.4	15.3
Distilled water	45.1	67.3	69.7	69.9	70.2	70.4	70.4	70.5	70.6	21.0	28.3
LSD: 0.05	11.4	14.6	14.6	14.8	13.8	14.1	14.2	14.0	14.2	-	-
0.01	15.5	19.9	20.3	20.6	19.2	19.6	19.8	19.6	19.7	-	-

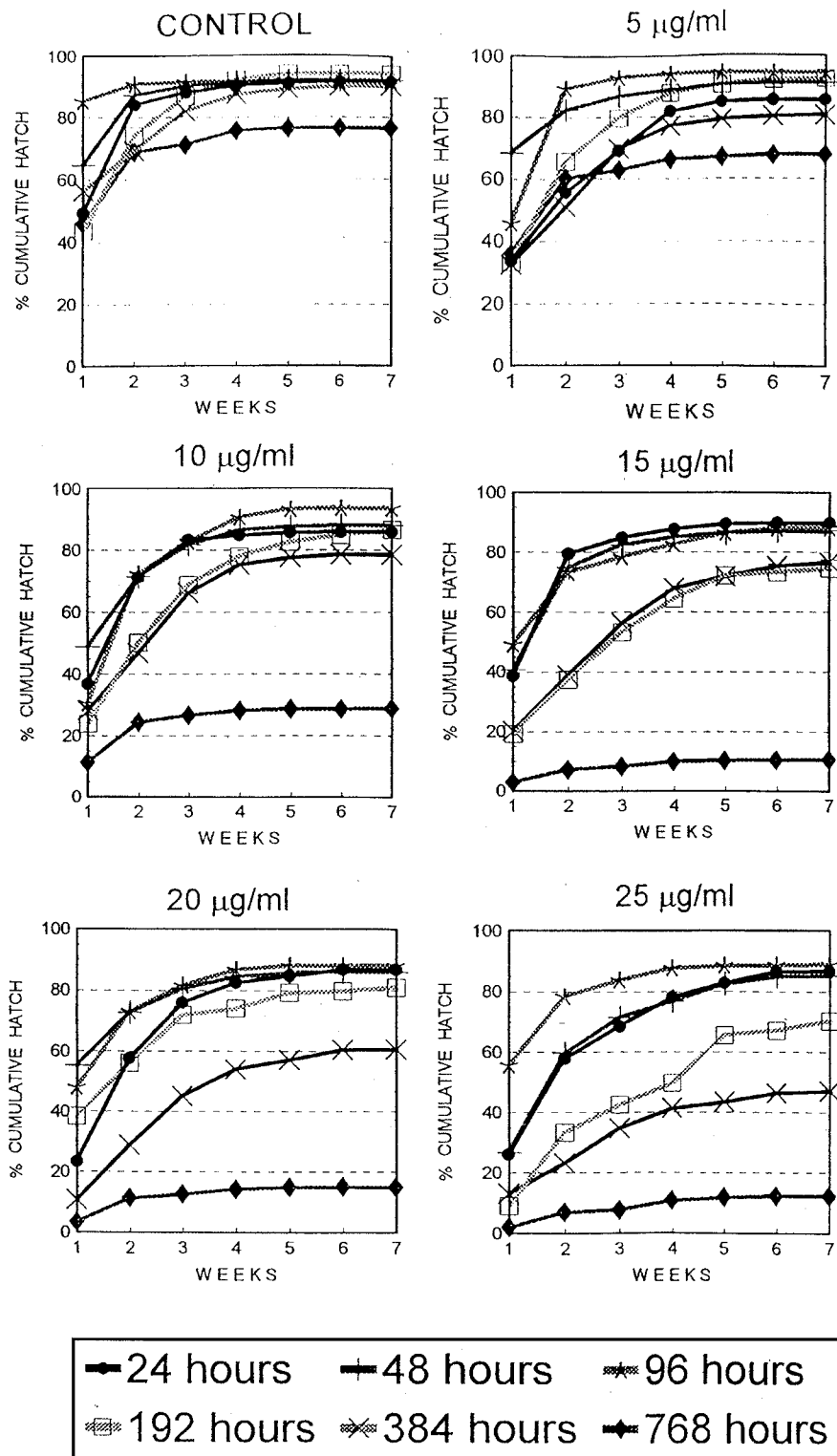


Fig. 1 - Effect of concentrations of cadusafos and different exposure durations on the cumulative percentage hatch of *Meloidogyne incognita* eggs.

ing fenamiphos, but not when the different concentrations of cadusafos were removed.

Experiment 2. There was no nematicidal activity in any of the cadusafos concentrations before 96 hour exposure time (Fig. 1). Percentage hatch decreased to 70% after 192 hours in the 25 µg/ml concentration. Incubation in 20 µg/ml x 384 hours gave a 61% hatch, while a 768 hour incubation period reduced hatch to 10% in the 15 µg/ml concentrations of cadusafos.

A model describing the decrease of hatchability of *M. incognita* eggs, as affected by concentrations of cadusafos at different exposure times, was derived from data of the final hatch percentage (Table II). The model is based on Finney's theory (1971) on the relation between dosage and effect of active principles on mortality of organisms.

Assumptions made with this model are that the maximum hatchability of the population of *M. incognita* was 92% (average of the final hatch in the control at different exposure times), and that the relation between treatment concentration and probit decrease at any duration is linear (parallel equidistant curves in Fig. 2).

With these assumptions, "best fit" curves were constructed to the observed hatchability data (Fig. 2). The decreasing effect of the various

treatments was represented by the estimated hatch of the hatchable eggs and this effect was estimated by dividing the observed per cent hatches by the maximum hatchability (Table III).

Probits of percentages (Table III) were plotted against log time to determine the best fitting linear relation between the two variables (Fig. 3). According to Fig. 3 probit hatchability (*b*) decreased by one unit if the concentration of the chemical was increased by 20 µg/ml, or the exposure time was multiplied by 2.7213. Increasing treatment duration to 1.2734 times, or treatment concentration by 1 µg/ml, resulted in a 0.05 unit decrease of probit *b*.

The relation in Fig. 3 can be described by the generic linear equation:

$$\text{probit } b = b - m \log_{10} t \quad (1),$$

where *b* = probit *b* at $\log_{10} t = 0$, *m* = the tangent of the angle between the regression line and abscissa, and *t* = the duration of the exposure to a certain dilution.

Probit *b* differs for different concentrations *C* because the relations between $\log_{10} t$ and *b*, for different concentrations *C*, are represented by parallel lines (Fig. 2), whereas *m* is the same for all concentrations. At a 25 µg/ml reference concentration (*C_r*) eq. (1) is:

probit *b* = 10.82 - 2.3 $\log_{10} t$, or more generally:

$$\text{probit } b = b_{C_r} - m \log_{10} t \quad (2).$$

TABLE II - Cumulative percentage hatch of *M. incognita* eggs in a seven week hatching test in distilled water after previous exposure for different times to different concentrations of cadusafos.

Exposure time (hours)	Concentrations (µg/ml)					
	0	5	10	15	20	25
24	91.6	85.5	85.9	89.7	86.7	86.6
48	92.0	91.0	88.0	87.2	85.9	85.1
96	91.7	94.4	93.5	88.1	88.0	88.7
192	94.2	92.5	86.5	74.5	80.9	70.3
384	90.2	80.5	78.4	76.4	60.5	47.0
768	76.4	67.6	28.7	10.4	14.9	12.2

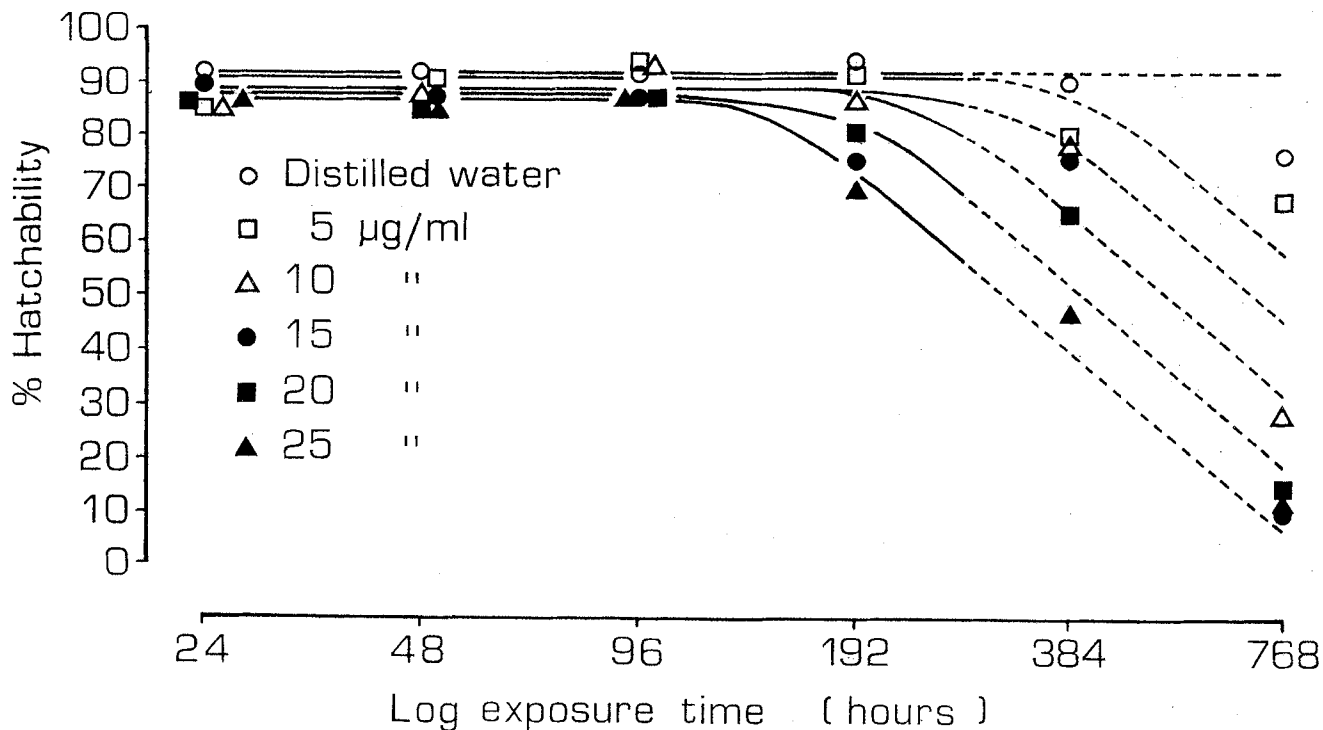


Fig. 2 - Decrease of hatchability of *M. incognita* eggs as affected by exposure of different durations to different concentrations of cadusafos.

TABLE III - Hatch percentages of Table II divided by maximum percentage hatchability of a *M. incognita* population.

Exposure time (hours)	Concentrations (µg/ml)					
	0	5	10	15	20	25
24	99.6	92.9	93.4	98.6	94.2	94.1
48	100.0	98.9	95.6	94.8	93.2	92.5
96	99.7	102.6	101.6	95.8	95.6	96.4
192	102.4	100.5	94.0	81.0	87.9	76.4
384	98.0	87.5	85.2	83.0	65.8	51.1
768	83.0	73.5	31.2	11.3	16.2	13.3

As the relation between probit b and C for a given exposure time is linear, decreasing treatment concentration by 1 µg/ml probit b increases by 0.05 unit. Therefore, for a x variation of concentration:

$$b_{C-r-x} = b_{C-r} + 0.05 (C_r - C) \quad (3).$$

According to Figs. 2 and 3:

$$t_{C-5} = 1.2734 t_C \text{ and then}$$

$$t_{C-1} = 1.2734^{1/5} t_C = 1.0495 t_{C_r}$$

and more generally

$$t_{C-x} = 1.0495^x t_C \quad (4) \text{ and}$$

$$t_C = 0.9528^x t_{C-x} \quad (5). \text{ Then:}$$

$$\text{probit } b = 10.82 - 2.3 \log_{10} t - 0.9528^{(25-C)} \quad (6),$$

$$\text{or more generally}$$

$$\text{probit } b = b_{C-r} - m \log_{10} t_{C-d}^{(C_r-C)} \quad (7).$$

From eq. (7) it is possible to derive probit b

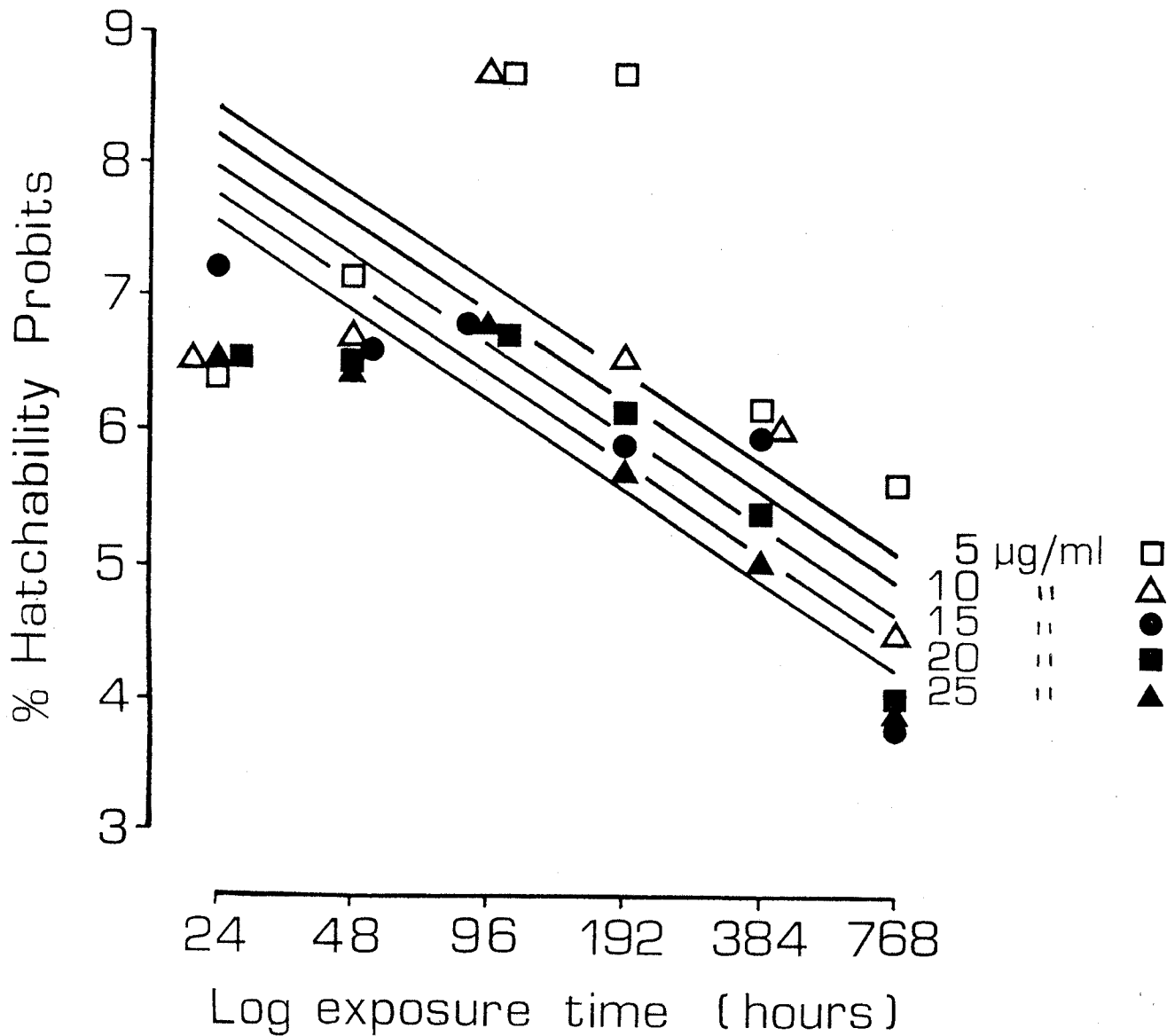


Fig. 3 - Relationship between log duration of exposure to different concentrations of cadusafos and probit hatchability of *M. incognita* eggs according to eq. (6).

for any other considered concentration by using a correction factor $d^{(Cr-C)}$ or, graphically, by shifting the straight line relative to C_r by the same factor.

Using a log scale for hatchabilities b (survival), the relation between $\log_{10} b$ at a given concentration C and the duration of exposure t is linear and is described by the general equation:

$$\log_{10} b = b' - m't \quad (8) \quad (\text{Fig. 4}).$$

According to the straight lines in Fig. 4, for

$C_r = 25 \mu\text{g/ml}$, $b' = 2.1377$ (survival rate = 137%) and $m' = 0.0013$.

For other concentrations we can adapt eq. (8), analogously to eq. (7), by multiplying for a factor $0.9528^{(25-C)}$, and then:

$\log_{10} b = 2.1377 - 0.0013 t_C 0.9528^{(25-C)}$ (9), or more generally

$$\log_{10} b = b' - m_{Cr} t_C d^{(Cr-C)} \quad (10), \text{ and}$$

$$t_C = (b' - \log_{10} b) [m_{Cr} d^{(Cr-C)}]^{-1} \quad (11).$$

From eq. (11) the relation between concentrations of the chemical and log exposure time necessary to reduce a *M. incognita* population to a predetermined level can be drawn (Fig. 5). This relation, as at any given percentage reduction of hatchability, is linear and its general equation is:

$$\log_{10} t_C = k - 0.021 C \quad (12),$$

and then

$$t_C = 10^{(k-0.021 C)}$$

in which $k = \log_{10} t_C$ at $C=0$, and C the considered concentrations of the active principle.

Discussion

In the first experiment, hatchability was not affected by the chemical up to 15 $\mu\text{g/ml}$ dilu-

tion and was strongly reduced only by concentrations $\geq 20 \mu\text{g/ml}$, at which concentration the nematocidal efficacy of cadusafos was greater than that of 5 $\mu\text{g/ml}$ of fenamiphos. At the highest concentrations, hatching was slower, demonstrating a nematostatic effect on the remaining viable eggs, similar to that of fenamiphos. In a previous glasshouse experiment reproduction of the population of *M. incognita* on tomato was strongly suppressed by cadusafos at rates of 2.26-9.04 mg a.i./500 cm^3 soil, with no difference from 16.95 mg a.i./500 cm^3 soil of fenamiphos (Sasanelli *et al.*, 1996).

Data from the second experiment confirmed the above results and demonstrated that at least 192 hours exposure time is required to kill 25% of hatchable eggs at 25 $\mu\text{g/ml}$ concentration.

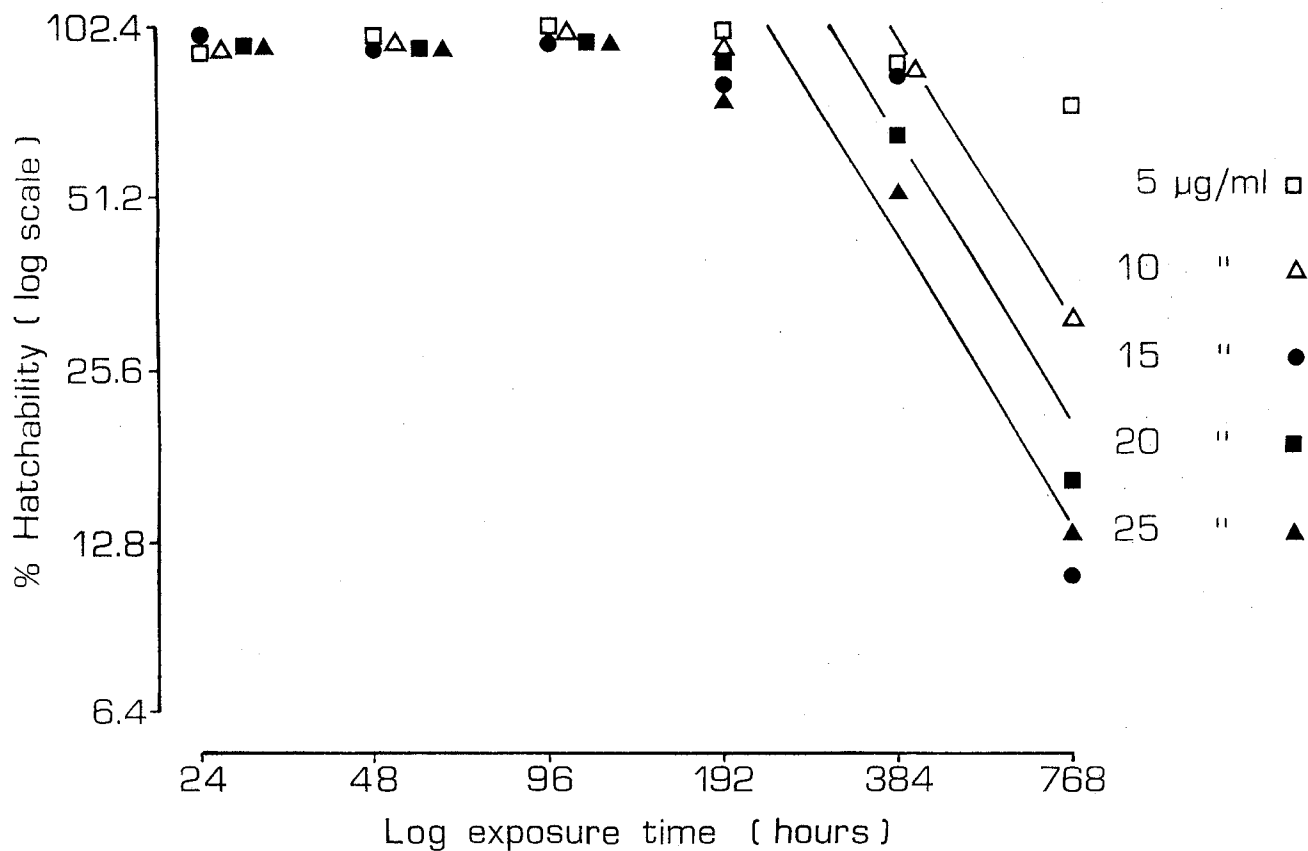


Fig. 4 - Linear relation between duration of exposure to different concentrations of cadusafos and log percentage hatchability of *M. incognita* eggs according to eq. (9).

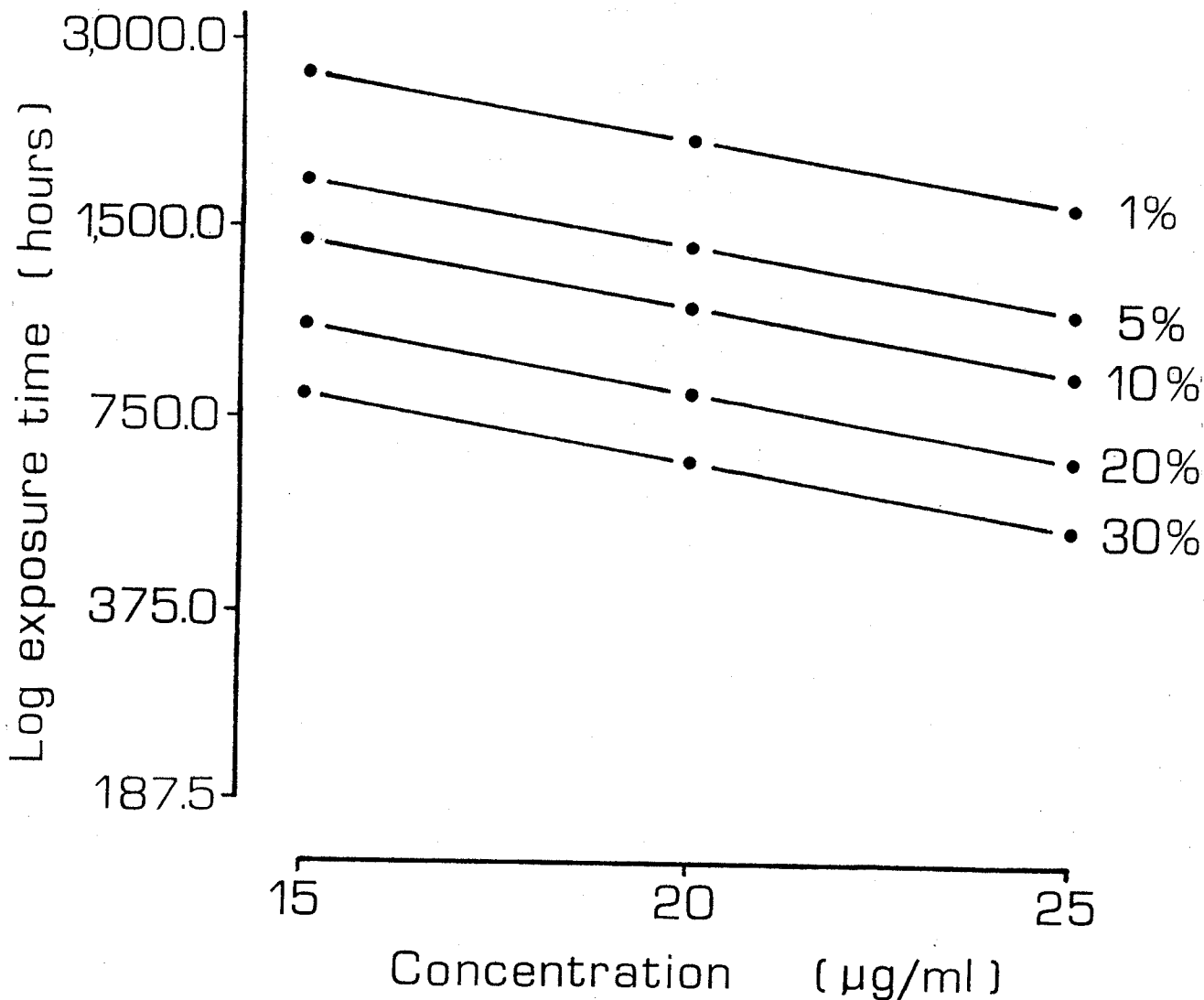


Fig. 5 - Linear relation between concentrations of cadusafos and log exposure time at different levels of survival of *M. incognita* eggs according to eq. (12).

However, a reduction of hatchability to 10-15% of the initial population was possible only with a 768 hours exposure to concentrations ≥ 15 µg/ml.

The relation between probit hatchability and log exposure time and that between concentration and log exposure time, at any given percentage reduction of hatchability, are linear.

According to the observed relationship between exposure time to different chemical di-

lutions and nematode percentage hatch, a decrease of treatment concentration by 1 µg/ml at a duration of exposure t was equivalent to a decrease of duration of exposure to $0.9528 t$ without varying the concentration.

The model can be applied to find graphically, or by computation, the duration of exposure to a certain concentration needed to reduce an egg population of *M. incognita* to a required level: e.g., to obtain a 90% mortality of egg

population, from eq. (11) an exposure of 875 hours at a 25 µg/ml concentration is required.

The same result can be obtained at 20 µg/ml with 1,114 hour exposure, or at 15 µg/ml after a 1,419 hour incubation.

The maximum solubility of cadusafos in water is 248 µg/ml, therefore the model can be applied for concentrations higher than those tested. For example, to achieve a 90% mortality, the exposure duration is reduced to 261 hours for a 50 µg/ml concentration, and to 78 hours for a 75 µg/ml concentration.

The model is based on data from *in vitro* experiments and therefore further investigations are required to ascertain its applicability in glasshouse and field conditions where concentrations decrease as a consequence of biotic and abiotic factors.

Acknowledgement. We thank Mr R. Lerario for preparing the Figures.

Literature cited

- DI VITO M., VOVLAS N. and INSERRA R. N., 1980. Influence of *Meloidogyne incognita* on growth of corn in pots. *Plant Dis.*, 64: 1025-1026.
- EKANAYAKE H. M. R. K. and DI VITO M., 1984. Influence of root leachates and temperatures on egg hatch of *Meloidogyne* species. *Nematol. medit.*, 12: 119-127.
- FINNEY D. J., 1971. *Probit analysis*. Third Edn., Cambridge University Press, 324 pp.
- GRECO N. and THOMASON I. J., 1980. Effect of fenamiphos on *Heterodera schachtii* and *Meloidogyne javanica*. *J. Nematol.*, 12: 91-96.
- HUSSEY R. S. and BARKER K. R., 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp. including a new technique. *Plant Dis. Repr.*, 57: 1025-1028.
- MCCLURE M. A. and SCHMITT M. E., 1996. Control of Citrus Nematode, *Tylenchulus semipenetrans*, with Cadusafos. *J. Nematol.*, 28 (4S): 624-628.
- SASANELLI N., 1996. Importanza economica di nematodi fitoparassiti e valutazione delle perdite di produzione. *Nematol. medit.*, 23 (Suppl. 1995): 5-13.
- SASANELLI N. and D'ADDABBO T., 1992. The effect of *Cineraria maritima*, *Ruta graveolens* and *Tagetes erecta* extracts on the hatching of *Heterodera schachtii*. *Nematol. medit.*, 20: 49-51.
- SASANELLI N., D'ADDABBO T., BASILE M. and CARELLA A., 1996. Effect of cadusafos on the reproduction of *Meloidogyne incognita*. *Afro-Asian J. Nematol.*, 6: 36-39.
- SASANELLI N., D'ALOISIO V., BASILE M. and RANA G. L., 1995. Control of *Ditylenchus dipsaci* on Onion by Chemical Treatments with Cadusafos, Fenamiphos and Aldicarb. *Afro-Asian J. Nematol.*, 5: 24-27.
- SASANELLI N. and DI VITO M., 1991. The effect of *Tagetes* extracts on the hatching of an Italian population of *Globodera rostochiensis*. *Nematol. medit.*, 19: 135-137.
- SASANELLI N., VOVLAS N. and D'ADDABBO T., 1992. Influence of *Meloidogyne javanica* on growth of sunflower. *Afro-Asian J. Nematol.*, 2: 84-88.
- TAYLOR A. L. and SASSER J. N., 1978. *Biology, Identification and control of Root-knot Nematodes* (*Meloidogyne species*). North Carolina State University Graphic, Raleigh NC, U.S.A., 111 pp.