Journal of Nematology 31(2):201–206. 1999.

Cadusafos Inhibits Hatching, Invasion, and Movement of the Potato Cyst Nematode *Globodera pallida*

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Abstract: The effect of the nematicide cadusafos on the hatching of the potato cyst nematode *Globodera pallida* in potato root diffusate, soil leachate, and distilled water was investigated. Cadusafos had a significant effect on the hatching, migration, movement, and root invasion by the second-stage juveniles. Hatching was completely inhibited at low concentrations of cadusafos (0.002–0.004 μg/ml), but hatching resumed a week after removing the nematicide. At concentrations of 0.05 μg/ml and higher of analytical-grade cadusafos, the inhibition of hatching was permanent.

Key words: cadusafos, *Globodera pallida,* hatching, nematicide, nematode, potato cyst nematode.

The potato cyst nematodes *Globodera pallida* (Stone) and *G. rostochiensis* (Wollenweber) are major pests of potato in the United Kingdom. The estimated annual yield loss that they cause to potato production in the European Community is valued at 300M ECU ($342 million) (Mulholland et al., 1996). *Globodera pallida* is difficult to control because there are no fully resistant potato cultivars and non-fumigant nematicides may be less effective against this species when compared with *G. rostochiensis* (Whitehead et al., 1994). Cysts can survive in soil for several years in the absence of a host crop, and highly infested land can be unusable for potato cropping for many years.

Hatching of the invasive second-stage juveniles (J2) from encysted eggs is initiated mainly by exposure of the cysts to potato root diffusate (PRD) (Perry, 1989). The dependency on host diffusates ensures that the majority of juveniles will hatch only in the presence of a suitable host; the ability to survive between host crops is due to the protection against environmental extremes provided by the eggshell and the cyst wall.

Cadusafos (O-ethy S, S-di-sec-butyl l phosphorodithioate) is an organophosphorus nematicide discovered and manufactured by FMC Corporation. Marketed under the trade name ''Rugby,'' cadusafos is formulated as both an emulsifiable concentrate (10%w/v) (100ME) or granule (10%w/w) (10G). Cadusafos controls a wide range of plant parasitic nematodes, such as *Tylenchulus semipenetrans* (McClure and Schmitt, 1996), *Meloidogyne* spp., and other genera, as well as soil insects such as the larvae of Agriotes spp., Noctuidae, and other insects on citrus, banana, potato, maize, sugarcane, tobacco, and vegetables (Tomlin, 1994). In the present study the effect of cadusafos on the hatching behavior, activity, and invasion of *G. pallida* J2 were examined.

Materials and Methods

Nematicide: Analytical-grade cadusafos (98.1% a.i.) and Rugby 100ME (10% a.i. cadusafos) were supplied by FMC Corporation, Agricultural Chemical Group, Philadelphia, Pennsylvania. Stock solutions of pesticide were prepared and stored at 4 °C.

Nematode and plant cultures. Globodera pallida (IACR-Rothamsted, UK) were collected from several greenhouse pot cultures of potato (*Solanum tuberosum* cv. Maris Piper) and stored at 4 °C. Cysts were soaked in cavity watch glasses with distilled water at 20 °C for 7 days prior to use. Potato plants (cv. Maris Piper) were grown in 14-cm-diam. free-draining plastic pots containing John Innes Number 2 sandy-loam compost in a glasshouse (20–25 °C) with a minimum photoperiod of 10 hours.

Effect of cadusafos, with potato root diffusate, on J2 emergence: Bioassays were carried out as described by Ibrahim et al. (1993), using four batches of 25 cysts in 2 ml of each test solution in cavity watch glasses at 20 °C. The

Received for publication 2 March 1998.

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This paper was edited by S. H. Thomas.
The authors thank FMC Corporation (UK) Ltd. for financial support, J. Beane for supplying cyst nematodes, and S. Woods for help with statistical analyses.

solutions used were distilled water (DW), soil leachate (SL), and potato root diffusate (PRD diluted 1:3) as control treatments and diluted PRD plus various concentrations of technical cadusafos or Rugby 100ME. Potato root diffusate, from 5-week-old plants, was collected using a technique similar to that of Fenwick (1949). Soil leachate was collected from pots containing the same soil but without plants.

Counts of emerged J2 were made weekly for 10 weeks. At each count, the J2 were removed, cysts were rinsed with distilled water, and fresh solutions alone or with PRD plus different concentrations of cadusafos (0.0005, 0.001, 0.002, 0.004, 0.05, 0.1, 0.5, and 1 µg/ml a.i.) and of Rugby 100 ME (0.001, 0.002, 0.005, 0.05, 0.25, 1.0, and 2.0 µg/ml formulated product) were added. At the end of each experiment, cysts were broken open and the number of eggs containing unhatched J2 counted to determine the percent emergence. All treatments were replicated four times, and this experiment was repeated.

Effect of cadusafos on J2 migration: Freshly hatched J2 (approx. 100 nematodes for each watch glass) were placed in a cavity watch glass containing different concentrations (0.0005, 0.001, 0.002, and 0.004 µg/ml a.i.) of cadusafos in 2 ml of distilled water. After 2, 4, 8, 12, and 16 hours the J2 were removed and placed on a sand column (height 1.5 cm, sand particle size 250–500 µm) in plastic tubes (3.0 × 4.0 cm) sealed at the bottom with nylon mesh (53-µm aperture). The column was placed upright in a cavity watch glass containing distilled water and kept at room temperature (20–23 °C). After 24 hours the number of J2 that had migrated through the mesh were recorded. The controls for all experiments without cadusafos were treated identically. This experiment was conducted three times.

Effect of cadusafos on J2 activity: Newly emerged (within 24 hours) J2 (approx. 50 nematodes per watch glass) were placed in cavity watch glasses containing different concentrations (0.0005, 0.001, 0.002, 0.004, and 0.1 µg/ml) of cadusafos in 2 ml of distilled water. After 2, 4, 8, 12, and 16 hours the cadusafos was removed and the J2 placed in distilled water and left to recover. Activity of J2 were recorded after 24 hours had elapsed. This experiment was conducted three times.

Effect of cadusafos on root invasion by J2: Newly hatched J2 (approx. 200 nematodes per watch glass) were placed in cavity watch glasses containing different concentrations (0.0005, 0.001, 0.002, and 0.004 µg/ml) of cadusafos in 2 ml of distilled water. After 2, 4, 8, 12, and 16 hours the J2 were removed and pipetted near the stem of a 2-week-old potato plant. A week later the plant was removed, and the roots were washed and fixed in T.A.F. (Southey, 1970). The roots subsequently were stained in methyl blue (0.1% w/v), destained in boiling lactophenol for 3 minutes, and the nematodes counted. This experiment was conducted twice.

Effect of cadusafos on egg hatch: A batch of 10 cysts was soaked in distilled water for 1 week. Cysts were broken open gently to release the eggs, which were transferred into a cavity watch glass containing potato root diffusate and different concentrations (0.0005, 0.001, 0.002, and 0.004 µg/ml) of cadusafos. After 16 hours of exposure, the solutions were removed and replaced with fresh root diffusate. The numbers of hatched J2 were recorded after 1 week had elapsed. This experiment was conducted twice. Data were subjected to analysis of variance, and the EC_{50} was calculated with probit analysis (Genstat 5™, Version 3.2, Lawes Agricultural Trust, Oxford Press, NY).

Results

Effect of analytical-grade cadusafos and Rugby 100 ME with potato root diffusate on emergence of J2 from cysts: Cadusafos affected the total emergence of J2 at all concentrations used after 10 weeks incubation ($P \leq 0.001$) (Table 1). When cysts were incubated with PRD alone, more than 80% of J2 emerged. But when they were exposed to cadusafos and PRD, for 1 week only, hatching was completely inhibited, even at the lowest concentration. Hatching resumed soon after removing cadusafos at lower concentrations (0.002–0.004 µg/ml a.i.), whereas at 0.05-

TABLE 1. Effect of cadusafos concentrations and subsequent incubation in potato root diffusate on the hatching of second-stage juveniles of *Globodera pallida*.

				PRD + cadusafos treatments										
	Control treatments			Cadusafos (µg/ml a.i)					Rugby 100 ME (µg/ml formulation)					
Exposure (weeks)	DW[a]	SL[a]	PRD[a]	PRD + 0.0005	PRD + 0.001	PRD + 0.002	PRD + 0.004	PRD + 0.05	PRD + 0.001	PRD + 0.002	PRD + 0.005	PRD + 0.05	PRD + 0.25	PRD + 1.0
1	44	3	1,046	5[b]	2[b]	0[b]	0[b]	0[b]	15[b]	0[b]	2[b]	0[b]	0[b]	0[b]
2	27	65	2,400	1,075	265	110	51	0	750	235	1	1	0	0
3	13	15	1,019	81	21	8	15	0	113	14	3	4	8	0
4	15	12	352	1,450	83	29	90	0	1,600	55	51	2	5	0
5	3	4	160	47	5	2	294	0	175	86	43	6	3	0
6	19	24	873	0[c]	0[c]	0[c]	200	0	3[b]	8[b]	394	8	11	0
7	19	24	1,084	49	13	14	202	0	420	306	980	65	75	0
8	7	13	203	3[c]	0[c]	0[c]	107	0	78[b]	20[b]	305	56	11	0
9	0.3	5	84	239	89	0	191	0	253	320	375	146	50	0
10	1	9	187	847	729	150	201	0	1,100	2,025	425	150	64	0
Total	146	170	8,171	3,796	1,207	311	1,353	0	4,507	3,069	2,630	437	227	0
Percent hatch	2.1	2.3	80.1	42.9	16.1	3.3	32.0	0	60.0	45.5	23.1	4.3	1.8	0

Data are means of four replications repeated twice. LSD = 8.17 for cadusafos (analytical-grade a.i) treatment and LSD = 0.624 for Rugby (10% ME) treatments in comparison to control treatments ($p \leq 0.0001$).

[a] DW = distilled water, SL = soil leachate, PRD = potato root diffusate.

[b] Incubated with cadusafos for 1 week only and chemical removed.

[c] Re-incubated with cadusafos for 1 week only and chemical removed.

µg/ml and higher (data not shown) concentrations the inhibition of hatching was permanent (Table 1). Initial treatment of cadusafos with PRD and followed by PRD alone reduced the total hatch by 50% at 0.0005 µg/ml and decreased the hatching to 3.3% when the cadusafos concentration was increased to 0.002 µg/ml. Hatching in DW or SL never exceeded 2.3%.

Immersion of cysts in Rugby 100ME and PRD for 1 week, at low concentrations of cadusafos (0.001 and 0.002 µg/ml, 10% a.i. formulated product), reduced hatching to 60% and 45.5%, respectively; at 0.25 µg/ml, hatch was decreased to 1.8% ($P \leq 0.001$). Concentrations of 1.0 µg/ml and higher (data not shown) completely suppressed J2 emergence (Table 1).

Effect of cadusafos on J2 migration through a sand column: At all concentrations tested (0.0005–0.004 µg/ml), cadusafos reduced the number of J2 migrating through a sand column within 24 hours ($P < 0.01$) (Fig. 1). As the concentration of cadusafos increased, the percentage of successfully migrating individuals decreased. The time of exposure to cadusafos significantly affected J2 migration. For example, when treated with the lowest concentration (0.0005 µg/ml) for 2 hours more than 60% of the J2 migrated through the column, but migration decreased to below 30% after 16-hour exposure to the same concentration. At 0.002 and 0.004 µg/ml, cadusafos almost completely prevented nematode migration. The estimated EC_{50} was 0.0013 µg/ml for a 2-hour exposure.

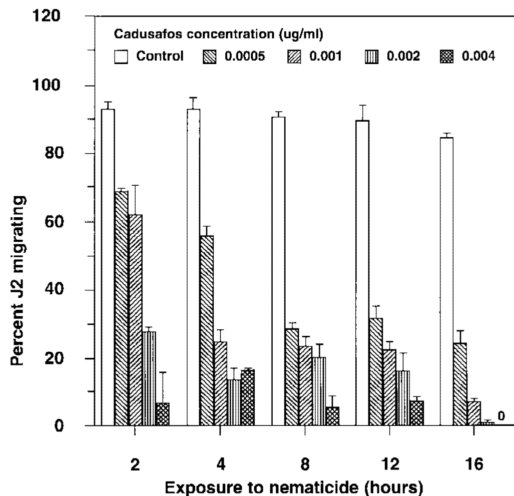

FIG. 1. Effect of cadusafos on migration through a sand column by second-stage juveniles of *G. pallida*. The data points represent the means of three separate experiments (two replicates per treatment). Vertical bars represent standard deviation.

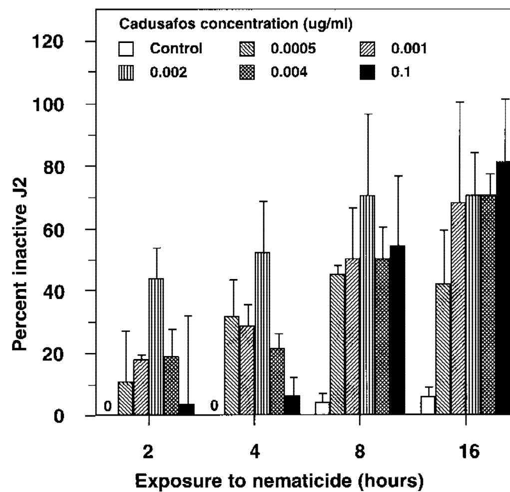

FIG. 2. Effect of cadusafos on the activity of second-stage juveniles of *G. pallida*. The data points represent the means of three separate experiments (two replicates per treatment). Vertical bars represent standard deviation.

Effect of cadusafos on J2 activity: Cadusafos began to affect the J2 within 30 minutes of exposure with slower body movements becoming apparent. As the duration of nematode exposure increased from 2 to 16 hours, there was a corresponding increase in the proportion of inactive nematodes (Fig. 2). There was no consistent effect of cadusafos on nematode activity as the concentration increased, especially after 4 hours of exposure. However, a concentration of 0.002 µg/ml had a marked effect on nematode inactivity, which increased from 45% after 2 hours to 72% after 8 hours, (Fig. 2). The highest concentration of 0.1 µg/ml had little effect on nematode activity after 4 hours of exposure in comparison to the lower concentrations (0.0005–0.004 µg/ml), but a significant effect was shown as the duration of exposure was increased to 8 or 16 hours (Fig. 2). The estimated EC_{50} for 2.9-

hour exposure was a concentration of 0.002 µg/ml.

Effect of cadusafos on root invasion by J2: All the concentrations tested reduced the invasion by J2 from 38% after 2 hours to 7% following 16 hours of exposure to cadusafos at 0.0005 µg/ml ($P < 0.05$). A concentration of 0.004 µg/ml of cadusafos completely prevented root invasion after 8 hours of exposure (Fig. 3). The estimated EC_{50} was 0.0019 µg/ml for a 2-hour exposure.

Effect of cadusafos on egg hatch: Cadusafos had an immediate effect on egg hatch (Fig. 4). Hatching resumed only 2 weeks after removal of cadusafos and its replacement with fresh PRD solution (data not presented). Hatching activity of J2 from individual eggs was reduced as the concentrations (0.0005–0.1 µg/ml) of cadusafos in solution increased ($P \leq 0.01$) (Fig. 4). At a concentration of 0.1 µg/ml, inhibition in the hatching of eggs was permanent. More than 70% of untreated eggs hatched (Fig. 4). The estimated ED_{50} was 0.0008 µg/ml for 16 hours of exposure.

Discussion

Cadusafos affected the emergence of the *G. pallida* J2 from cysts at the lowest concentration (0.0005 µg/ml), and inhibition persisted as long as the cysts were exposed to

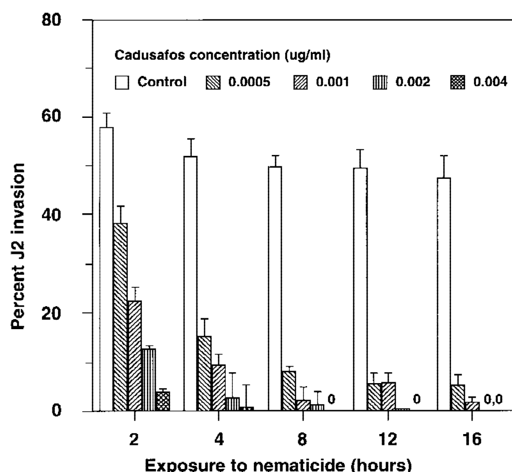

Fig. 3. Effect of cadusafos on root invasion by second-stage juveniles of *G. pallida pallida.* The data points represent the means of two separate experiments (two replicates per treatment). Vertical bars represent standard deviation.

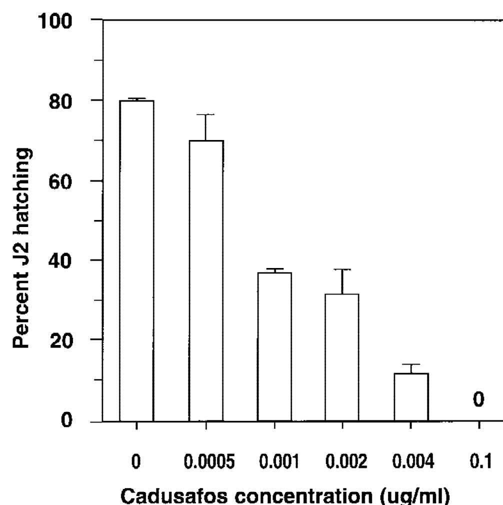

Fig. 4. Effect of cadusafos on the emergence of second-stage juveniles of *G. pallida* from eggs. The data points represent the means of two separate experiments (two replicates per treatment). Vertical bars represent standard deviation.

cadusafos. When the cysts were transferred to root diffusate alone, hatching resumed, but the degree of recovery was affected by the concentration of the initial treatment with cadusafos. A minimum cadusafos concentration of 0.05 µg/ml was required to permanently inhibit hatching. Similar work carried out on other organophosphorus and oxime carbamates (Hague and Pain, 1973; Hough and Thomason, 1975; Mcleod and Khair, 1975) also demonstrated reversible effects on hatching and nematode activity. Osborne (1973) reported that the hatch of *G. rostochiensis* was completely inhibited by 5 to 20 µg/ml aldicarb in the presence of root diffusate following prolonged exposure of up to 12 weeks. Concentrations between 4 and 16 µg/ml of oxamyl were required to suppress the total hatch of the same species (Evans and Wright, 1982). In our experiment, total hatch was suppressed when cysts were incubated with a concentration of cadusafos as low as 0.0005 µg/ml in the presence of root diffusate for only 1 week. Irreversible effects on the hatching of *G. pallida* were observed at a much lower concentration of cadusafos (0.05 µg/ml) than has been reported for oxamyl. While *G. rostochiensis* and *G. pallida* are very similar species and cause the same type of damage to po-

tato crops, they behave differently in the field. *Globodera pallida* is now the predominant species of potato cyst nematode in England (Whitehead, 1991) and is more difficult to control than *G. rostochiensis* due to an extended period of hatching of the J2 from the cyst compared with *G. rostochiensis.* Oxamyl or aldicarb can be less effective at controlling *G. pallida* when compared with *G. rostochiensis.* This may be due to a combination of the relatively short persistence of carbamate nematicides and the prolonged period of emergence of *G. pallida* J2 (Whitehead, 1992).

The estimated EC_{50} value for cadusafos against nematode activity, root invasion, migration, and hatching of *G. pallida* juveniles is very low (0.0005–0.002 µg/ml) when compared to 0.49 µg/ml for oxamyl and 4.62 µg/ml for aldicarb against *G. rostochiensis* (Nelmes, 1970). There is no evidence to suggest any difference in the inherent susceptibility of *G. pallida* and *G. rostochiensis* juveniles to nematicides. Cadusafos markedly affects the ability of *G. pallida* J2 to invade the root system, even at the lowest concentration of 0.001 µg/ml with an exposure of only 2 to 4 hours. On agar plates, concentrations of 0.1 and 0.5 µg/ml oxamyl inhibited the orientation of *Meloidogyne incognita* toward roots (Wright et al., 1980) and affected the orientation of juveniles of *G. rostochiensis* toward potato roots (Evans and Wright, 1982). In the field, the effect of nonfumigant nematicides would appear to prevent invasion of the roots largely by disrupting nematode behavior (Evans and Wright, 1982).

The present study clearly demonstrates that cadusafos affects the hatching, activity, and invasion of roots by *G. pallida* J2. Our data show that cadusafos is a more potent nematicide against potato cyst nematodes than oxamyl and aldicarb, currently the most widely used active ingredients for the control of potato cyst nematodes in the United Kingdom.

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