Population Development and Reproduction of Fungus- and Bacterium-Feeding Nematodes in the Presence of Insect Growth Regulators

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Abstract: The insect growth regulators (IGRs), diflubenzuron and BAY SIR 8514, at 300 and 1,000 ppm a.i. in potato dextrose agar (PDA) inhibited the radial growth of the fungus *Rhizoctonia solani* host of *Aphelenchus avenae*. The IGRs had no effect on the growth of the bacterium *Pseudomonas pseudoalcaligenes* host of *Acrobeloides nanus* and *Diplogaster iheritieri*. At 50 ppm a.i., neither IGR inhibited the population development of *A. nanus* and *D. iheritieri* on *P. pseudoalcaligenes;* however, diflubenzuron stimulated the population development of *D. iheritieri*. At 300 ppm, both IGRs inhibited the population development of *A. nanus* and *D. iheritieri;* however, BAY SIR 8514 was more effective than diflubenzuron except on *A. nanus* L_4's. At 300 ppm, only BAY SIR 8514 affected the population development of *A. avenae,* except the L_4's. At 1,000 ppm, both IGRs inhibited development, except diflubenzuron for L_2 and L_3's. Again, BAY SIR 8514 was more effective than diflubenzuron. With single females of *A. nanus* and *D. iheritieri,* both IGRs at 300 ppm reduced egg laying, inhibited embryonation, and slowed larval development.

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Diflubenzuron, a substituted phenyl urea insecticide with nematicidal activity (15,16,17), is classified as an insect growth regulator (IGR) and has been shown to disrupt moulting processes in insect larvae (12). Furthermore, ovicidal effects have been demonstrated in Coleoptera (11), Diptera (18), Lepidoptera (1), and Nematoda (16). Recently BAY SIR 8514, another IGR with greater biological activity than diflubenzuron (Broadbent, unpublished data), has become available. This paper presents results of tests on the effects of diflubenzuron and BAY SIR 8514 on the population development and reproduction of three species of nematodes and their respective hosts.

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MATERIALS AND METHODS

Aphelenchus avenae Bastian was isolated from tobacco soil obtained in Norfolk County, Ontario. This fungus-feeding nematode was reared on *Rhizoctonia solani* Kuhn grown on potato dextrose agar (PDA).

Diplogaster iheritieri Maupas and *Acrobeloides nanus* de Man were isolated from alfalfa soil obtained in Wellington County, Ontario. A baiting technique using alfalfa seedlings was used to isolate the nematodes and the bacteria on which they fed. The two nematodes were reared on *Pseudomonas pseudoalcaligenes* Stanser which also grew well on PDA, the common medium for all experiments.

Three sets of experiments were performed with diflubenzuron 25 WP (1-[4-chlorophenyl]-3-[2,6-difluorobenzoyl]urea) and BAY SIR 8514 25 WP (N - [([4 - (trifluoromethoxy)phenyl] - amino) carbonyl]-2-chlorobenzamide). In all tests,

IGRs were added to the medium immediately after sterilization. In the first set of tests, the effect of IGRs on the radial growth of the fungus, *R. solani,* and on the growth of the bacterium, *P. pseudoalcaligenes,* was measured. Petri dishes (100 × 20 mm) containing 300 ppm a.i. or 1,000 ppm a.i. of the IGRs in PDA were each inoculated with a 6-mm plug of the fungus. Diameters of the fungal colonies were measured every 12 hours until the mycelium reached the margin of the 10 control dishes. In the bacterial growth experiment, Erlenmeyer flasks (125 ml) containing 50 ppm a.i. or 10 flasks containing 300 ppm a.i. of the IGRs, in 25 ml of Potato Dextrose Broth (PDB), were inoculated with 25 × 10^4 bacteria and placed on a shaker. Controls consisted of IGR-free media. After 4 days the number of bacteria per milliliter was determined with a haemocytomiter. All tests were conducted at 18–20 C, and each treatment was replicated 10 times.

In the second set of tests, the effects of IGRs on population development of the fungus-feeding nematode, *A. avenae,* and the bacterium-feeding nematodes, *D. iheritieri and A. nanus,* were determined at concentrations of 300 and 1,000 ppm and 50 and 300 ppm, respectively. The fungus and bacterium were allowed to overgrow the petri dishes (100 × 20 mm) of PDA containing the IGRs before inoculating with nematodes. Fifty to one hundred second- and third-stage larvae were used to inoculate each replicate. Nematodes were extracted from stock cultures in miniature Baermann pans (13). The nematode suspension was poured onto a fine screen (44-um openings) through which only the second- and third-stage larvae passed. The larval suspension was pipetted into the petri dishes and the inoculated dishes were incubated 2 wk.

In the third set of tests, the effects of 300 ppm of IGRs on the development of *A. nanus* and *D. iheritieri* were determined. A colony of *P. pseudoalcaligenes* (1-cm-d) developed in 4–6 days in petri dishes (60 × 15 mm) of PDA containing the IGRs before inoculation. A single gravid female was transferred to each Petri dish. The numbers of various life stages were observed and recorded every 2 days from 10 days for *A. nanus* and for 2, 4, and 8 days for *D. iheritieri.*

Data from the first two sets of tests were subjected to an analysis of variance and LSDs calculated at the 5% level.

RESULTS

Both IGRs inhibited the radial growth of the fungus, *R. solani,* (Table 1) but BAY SIR 8514 had a greater effect. Inhibition of growth was apparent at 36 hours, and by 84 hours diflubenzuron and BAY SIR 8514 at 300 ppm had reduced fungal growth 20% and 26%, respectively. Growth inhibition of *R. solani* was apparent at 24 hours, and by 72 hours diflubenzuron and BAY SIR 8514 at 1,000 ppm had inhibited growth of *R. solani* by 22% and 30% respectively.

The IGRs did not inhibit growth of *P. pseudoalcaligenes;* bacterial counts averaged 81 × 10^6/ml at 96 hours in the control and chemically treated flasks.

Both diflubenzuron and BAY SIR 8514 reduced development of populations of *A. avenae* (Table 2). BAY SIR 8514 was more

Table 1. Effect of insect growth regulators (IGRs) on the radial growth of *Rhizoctonia solani.*

IGRs	Rate (ppm a.i.)	Radial growth (mm) 0 h	12 h	24 h	36 h	48 h	60 h	72 h	84 h	L.S.D.$_{5\%}$
Control	0	7	14	24	34	42	53	65	80	3
Diflubenzuron*	300	7	13	21	28	37	46	57	64	
Bay SIR 8514†	300	7	12	21	29	34	43	56	59	
Control	0	8	...	32	45	59	74	86	...	1
Diflubenzuron	1,000	8	...	23	32	44	55	67	...	
Bay SIR 8514	1,000	8	...	19	29	39	49	60	...	

*1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)urea.

†N-[[[4-(trifluoromethoxy)phenyl]-amino]carbonyl]-2-chlorobenzamide.

Table 2. Effect of insect growth regulators (IGRs) on the population development of *Aphelenchus avenae* on *Rhizoctonia solani* on PDA.

IGRs	Rate (ppm a.i.)	Number/plate at 2 wk			
		Adults	L_4	$L_3 + L_2$	Total
Control	0	3,180	3,660	38,580	45,420
Diflubenzuron	300	4,020 (126)*	4,240 (116)	43,480 (113)	51,740 (114)
Bay SIR 8514	300	1,780 (56)	3,430 (94)	16,870 (44)	22,080 (49)
$LSD_5\%$		1,193	1,199	8,967	10,271
Control	0	5,100	7,950	23,800	36,850
Diflubenzuron	1,000	3,300 (65)	4,900 (62)	26,000 (109)	34,200 (93)
Bay SIR 8514	1,000	265 (5)	355 (4)	1,330 (6)	1,950 (5)
$LSD_5\%$		1,223	2,123	6,686	7,524

*Values in parentheses are percent of control

effective, reducing total population development (at 1,000 ppm) by 95%. At 300 ppm, only BAY SIR 8514 reduced populations of *A. avenae*. At 300 and 1,000 ppm, BAY SIR 8514 reduced the numbers of all stages except L_4 of *A. avenae*, whereas diflubenzuron at 1,000 ppm reduced only the numbers of L_4 larvae and adults.

Diflubenzuron and BAY SIR 8514 also reduced population development of *D. iheritieri* (Table 3) at 300 ppm. BAY SIR 8514 was more effective, causing 92% reduction in total population development vs. 33% reduction with diflubenzuron. At 50 ppm, no overall reductions in population growth occurred, but numbers of L_4 larvae and adults were reduced by BAY SIR 8514. Although not significant, diflubenzuron appeared to stimulate total population growth, particularly the numbers of L_2's, L_3's, and adults.

Total populations of *A. nanus* were reduced 62% and 89% by 300 ppm diflubenzuron and BAY SIR 8514, respectively (Table 4). Populations in media treated with BAY SIR 8514 had only 6% of L_2 and L_3 larvae compared to the control population. At 50 ppm, BAY SIR 8514 had no effect on *A. nanus* development, whereas diflubenzuron stimulated an increase in all life stages.

Exposure of single gravid females of *A. nanus* or *D. iheritieri* to media containing diflubenzuron or BAY SIR 8514 at 300 ppm resulted in decreased egg production and decreased egg hatch (Tables 5, 6). The rate of embryonation was inhibited, with many eggs remaining in the two-cell stage longer than normal. Also the rate of larval development lagged. Consequently the numbers of larvae (L_2–L_4) were progressively fewer and adults absent or rare in treated plates.

Table 3. Effect of insect growth regulators (IGRs) on the population development of *Diplogaster iheritieri* on *Pseudomonas pseudoalcaligenes* on PDA.

IGRs	Rate (ppm a.i.)	Number/plate at 2 wk			
		Adults	L_4	$L_3 + L_2$	Total
Control	0	1,670	5,440	38,560	45,670
Diflubenzuron	50	2,100 (126)*	3,330 (61)	52,500 (136)	57,900 (127)
Bay SIR 8514	50	900 (54)	2,600 (48)	39,000 (101)	42,500 (93)
$LSD_5\%$		677	2,060	n.s.	n.s.
Control	0	4,700	10,200	33,200	48,100
Diflubenzuron	300	2,600 (55)	4,700 (46)	24,900 (75)	32,200 (67)
Bay SIR 8514	300	515 (11)	1,120 (11)	2,450 (7)	4,080 (8)
$LSD_5\%$		1,480	2,260	4,950	5,800

*Value in parentheses are percent of control.

Table 4. Effect of insect growth regulators (IGRs) on the population development of *Acrobeloides nanus on Pseudomonas pseudocaligenes* on PDA.

IGRs	Rate (ppm a.i.)	Number/plate at 2 wk			
		Adults	L_4	$L_3 + L_2$	Total
Control	0	1,200	2,250	9,550	13,000
Diflubenzuron	50	2,250 (188)*	3,050 (136)	17,500 (183)	22,800 (175)
Bay SIR 8514	50	1,100 (92)	1,750 (78)	4,300 (45)	7,150 (55)
$LSD_5\%$		666	967	5,380	6,315
Control	0	4,000	4,300	39,900	48,200
Diflubenzuron	300	2,100 (53)	2,300 (53)	13,700 (34)	18,100 (38)
Bay SIR 8514	300	950 (24)	2,100 (49)	2,450 (6)	5,500 (11)
$LSD_5\%$		1,240	1,028	7,000	8,014

*Values in parentheses are percent of control.

In the controls the life cycle of *A. nanus* was ca. 10 days, whereas that of *D. iheritieri* was 4 days or less.

DISCUSSION

Our studies showed that BAY SIR 8514 inhibits population growth more effectively than diflubenzuron. However, our studies on the effect of IGRs on nematode development itself indicated that diflubenzuron was more effective because *D. iheritieri* did not produce L_4 and adult stages in the presence of diflubenzuron but it did in the presence of BAY SIR 8514. In the population studies, inoculum was composed only of larvae which developed into females. These young females may have been less sensitive to diflubenzuron than to BAY SIR 8514. In the development studies, inoculum was composed only of females, many of which were likely of advanced maturity. These females may have been more sensitive to diflubenzuron than to BAY SIR 8514. The concentrations tested were comparable to those used by Veech

Table 5. Effect of insect growth regulators (IGRs) on the development of *Acrobeloides nanus** on *Pseudomonas pseudoalcaligenes* on PDA.

IGRs	Rate (ppm a.i.)	Time (day)	Number/plate				
			Eggs	L_2	L_3	L_4	Adults
Control	0	0	0				
		2	3				
		4	16				
		6	31	7	1		
		8	30	9	6	3	
		10	26	9	13	7	2
Diflubenzuron	300	0	0				
		2	0.1				
		4	0.1				
		6	3	0.3			
		8	2	2			
		10	4	2	2	0.1	0
Bay SIR 8514	300	0	0				
		2	1				
		4	4				
		6	3	1			
		8	11	1	0.2		
		10	8	2	1	1	0

*Inoculum single female/dish.

Table 6. Effect of insect growth regulators (IGRs) on the development of *Diplogaster iheritieri** on *Pseudomonas pseudoalcaligenes* on PDA.

IGRs	Rate (ppm a.i.)	Time (day)	Number/plate				
			Eggs	L_2	L_3	L_4	Adults
Control	0	0					
		2	0.2	0.2	0.2		
		4	0.2	1.0	1.0	1.0	1.0
		8	86.0	20.0	31.0	15.0	15.0
Diflubenzuron	300	0					
		2	1.4	0.1			
		4	1.7	0.1	0.1		
		8	1.8	0.3	0.2	0	0
Bay SIR 8514	300	0					
		2	1.1	0.7	0.4		
		4	1.3	1.4	1.0		
		8	14.0	1.4	0	0.9	1.0

*Inoculum single female/dish.

(17) to suppress the population development of *Acrobeloides* sp. and *Panagrellus* sp. Furthermore, he found *Pelodera* sp. to be more sensitive, requiring only 10 ppm a.i. of diflubenzuron to inhibit population development.

The mode of action of these IGRs is uncertain. Most authors agree that IGRs of the diflubenzuron and BAY SIR 8514 type disrupt the formation of insect cuticle. Chitin synthetase has been suggested as the target by several investigators (6,7,14). However, Mayer *et al.* (9,10) and Cohen and Casida (5) recently have shown that neither of these IGRs directly affect chitin synthetase. Another recent study (8) indicated that IGRs are direct-acting serine protease inhibitors that block the conversion of chitin synthetase zymogen into active enzyme. However, chitin has not been isolated from larvae or adults (2,3,4) of nematodes, although it does occur in the eggs of several species (3), and Veech (16) has observed ovicidal effects of diflubenzuron on other nematode species. Effects shown here on eggs may be consistent with the inhibition of chitin synthesis observed with insects, but effects on larvae, as shown with BAY SIR 8514 on *A. avenae* and *D. iheritieri,* are not as readily explained. Mayer et al. (10 have suggested that IGRs may inhibit N-acetylglucosamine transferases or glucosyltransferases which may, at least in nematodes, be important in gut or other surface membrane synthesis.

The lower susceptibility of *A. avenae* to these IGRs, as compared to *D. iheritieri* and *A. nanus,* may be related to the mode of feeding by these nematodes. Both *D. iheritieri* and *A. nanus* are particle-feeding nematodes, whereas *A. avenae* pierces and feeds on the contents of fungal hyphae. The IGRs have a relatively low water solubility and are nonsystemic. Generally these chemicals must be ingested by insects to have an effect (12). It is possible that uptake and/or exposure by *A. avenae* was less than that of the other two species.

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