SUSCEPTIBILITY OF *ROMANOMERMIS CULICIVORAX* (NEMATODA:MERMITHIDAE) POSTPARASITES TO AGRICHEMICALS USED IN LOUISIANA RICE PRODUCTION[1]

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ABSTRACT. Susceptibility of *Romanomermis culicivorax* postparasites to riceland agrichemicals was evaluated during 1983. Nematode mortalities for all agrichemicals at 2 days posttreatment ranged from 0 to 11.4% with fentin hydroxide + urea responsible for the highest mortality. Fertilizers were significantly more toxic to nematodes at 2 and 25 days posttreatment than were insecticides. Carbofuran and methyl parathion produced <1% mortalities at 2 days and <5% mortalities at 25 days posttreatment. Approximately 80–84% of the surviving females exposed to insecticides as postparasites became gravid. Infectivity of the subsequent F_1 generation preparasites resulted in >92% parasitism in *Culex quinquefasciatus* larvae, except those produced by nematodes exposed to carbofuran (76.4%), 2,4–D (70.9%) and fentin hydroxide (67.4%).

INTRODUCTION

The successful incorporation of the mermithid nematode *Romanomermis culicivorax* Ross and Smith, a parasite of mosquito larvae, into an integrated pest management program for riceland mosquitoes depends on its ability to complete its life cycle and reproduce without continual reapplication. One factor that poses a threat to the natural cycling of this nematode is the continual application of pesticides and fertilizers to the riceland agroecosystem. Research data are available on the susceptibility of *R. culicivorax* preparasites to various agrichemicals (Mitchell et al. 1974, Levy and Miller 1977, Brown and Platzer 1978, Winner and Steelman 1978). In contrast, there is only limited research on the susceptibility of other stages of this nematode species. Dhillon and Platzer (1978) reported benomyl[5] appeared to have nematicidal properties against the postparasitic stage of *R. culicivorax* and Washino and Kerwin (1983) indicated that molinate had adverse effects on the postparasites.

The susceptibility of soil-inhabiting nematodes to several herbicides and fertilizers has been investigated. Ishibashi et al. (1983) evaluated the herbicides, chlormethoxynil (=chlomethoxyfen) and thiobencarb + simetryn, and found that they decreased populations of soil surface monochid nematodes in rice fields. Walker (1971) reported that populations of *Pratylenchus penetrans* (Coff) Filipjev and Shuurmans-Stekhoven, a plant lesion nematode, decreased after applications of nitrate, nitrite, organic nitrogen and ammonium compounds. In comparison, Brown and Platzer (1978) reported that adults of *R. culicivorax* were more tolerant of inorganic ions, such as potassium, sulfate, nitrate, nitrite and phosphate, than preparasites.

This study was designed to assess the feasibility of using *R. culicivorax* as a component of an integrated pest management program in the riceland agroecosystem. Postparasites of *R. culicivorax* were exposed to commercially-applied rice field agrichemicals in Louisiana to determine their susceptibility to these chemicals. An assessment of the infectivity of their F_1 progeny into *Culex quinquefasciatus* Say larvae was also performed.

MATERIALS AND METHODS

To provide a pragmatic evaluation of the toxicological effects of rice field agrichemicals on *R. culicivorax* postparasites, tests were conducted during routine applications in commercial rice fields in Louisiana. Under these circumstances it was not always possible to evaluate each pesticide or fertilizer. Rice farmers often combine agrichemicals into one application to economize rice production costs.

During the 1983 rice growing season, 11 aerial applications were conducted involving individual or mixed agrichemicals. Refer to Table 1 for chemicals and application rates.

Twenty-four hours prior to each test 5-day

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[5] Common names of agrichemicals listed herein are found in C. R. Worthing (ed.) 1983. The pesticide manual, a world compendium (7th ed.). Lavenham Press Ltd. Lavenham, U. K.

old nematode postparasites were obtained from the USDA-ARS Gulf Coast Mosquito Research Laboratory in Lake Charles, LA. Aliquots of 100 postparasites (50♂♂:50♀♀) were placed in twelve 600 ml glass beakers containing 2 cm of moist medium grade blasting sand approximately 12 hr prior to treatment to allow time for the nematodes to penetrate into the substrate. The beakers were covered with aluminum foil and placed in 0.95 liter plastic soft drink cartons. The cartons were transferred to and from the field tests in a 45.5 liter insulated chest. Immediately preceding the individual field tests, each beaker was filled with 350 ml of well water. Two beakers were withheld from the agrichemical application as controls. Ten beakers were placed in styrofoam (expanded polystyrene) floats (27 × 40 × 2.5 cm) and randomly placed throughout the rice field. Aerial applications of the riceland agrichemicals were made from Grumman AgCat® aircraft. Each pesticide and fertilizer was applied according to rates and specifications printed on the label.

Approximately 24 hr posttreatment, the beakers were collected from the fields and excess surface water removed from the beakers prior to transport to the laboratory. At 2 days posttreatment the nematodes were separated from the sand using a procedure modified from Seinhorst (1956).

The contents of a beaker were flushed into a 2 liter stainless steel pitcher filled with tapwater and gently agitated to suspend the nematodes in the water. The nematodes were collected on a no. 320 U.S. Standard sieve (45 μm openings). This procedure was repeated 3 times to enhance recovery of the nematodes from the sand. The nematodes were then separated by sex and percent mortalities were recorded.

In order to evaluate long term toxicological effects on *R. culicivorax*, the recovered nematodes from each beaker were placed in 29.6 ml plastic jelly cups that contained 1 cm of moist blasting sand. The cups were sealed with paper caps and Parafilm® and incubated at 28 ± 05°C. Five of the 10 treated nematode cups and one of the 2 untreated check cups were randomly selected to monitor nematode mortality over a 25-day period. Intermediate readings of mortality were made at approximately 5-day intervals beginning with the 2 days posttreatment count. Data were kept of the number of males and females, percentage mortality (dead nematodes were removed), number of nematodes per developmental stage and percentage of gravid females. The surviving nematodes were placed in new cups with fresh seals as previously described, and stored in an incubator.

Data were also collected to assess the effects of riceland agrichemical treatments on the ability of the *R. culicivorax* F_1 progeny to infect *Cx. quinquefasciatus* larvae. The remaining 6 cups of nematodes were held undisturbed for 12 wk to allow sufficient time for egg deposition and maturation (Petersen 1975). At the end of the incubation period, the contents of each cup were placed in a separate enameled pan (18 × 30 × 5 cm) with 1 liter of well water and 200 first instar *Cx. quinquefasciatus*. After 24 hr post-exposure the mosquito larvae were removed by pouring the contents of each pan through a no. 100 mesh U.S. Standard sieve (150 μm opening) to prevent further nematode infection. The pan was thoroughly washed, refilled with 1 liter of well water, and the mosquito larvae were replaced. Mosquito larvae were reared to the fourth instar and parasitism was determined by dissection. Data were subjected to analyses of variance using SAS general linar models procedure (SAS 1982).

RESULTS AND DISCUSSION

Results of field tests to determine percent mortalities of *R. culicivorax* postparasites exposed to riceland agrichemicals are provided in Table 1. At 2 days posttreatment, the agrichemicals produced low levels of mortalities for postparasitic males and females. Mortalities ranged from 0 to 11.4% with fentin hydroxide + urea responsible for the highest rate. The controls had mortality of <1%. Mortalities in treatments with carbofuran and methyl parathion at 0.6 kg AI/ha were <1%. For each application of a pesticide and/or fertilizer, the mortalities of male and female nematodes were generally similar with the only exceptions being 2,4-D, fentin hydroxide + sulfur, and fentin hydroxide + urea. The herbicide 2,4-D induced a higher female percent (2.5%) than male (0.4%) mortality. Fentin hydroxide + sulfur produced a higher male (2.2%) than female (0.2%) mortality. Similarly, fentin hydroxide + urea application resulted in a 11.4% male mortality compared to 1.6% for females.

Analyses of variance evaluating the susceptibilities of *R. culicivorax* postparasites to the agrichemicals are given in Table 2. *Romanomermis culicivorax* was more susceptible to fertilizer applications than the pesticides at 2 days posttreatment. There was a highly significant difference ($P<0.01$) between fertilizer and insecticide percent mortalities. When administered individually, insecticide, herbicide and fungicide applications had little or no effect on the postparasitic nematodes. The percent mortalities resulting from carbofuran and methyl parathion were not significantly different

Table 1. Susceptibility of *Romanomermis culicivorax* treated as postparasites to commercially applied Lousiana rice field agrichemicals at 2 and 25 days posttreatment, 1983.

Agrichemicals	AI (kg/ha)	2 days posttreatment[1] % female mortality	2 days posttreatment[1] % male mortality	25 days posttreatment[2] % female mortality	25 days posttreatment[2] % gravid females	25 days posttreatment[2] % male mortality
$(NH_4)_2SO_4$, P_2O_5, K_2O	36.3, 36.3, 36.3	1.2	1.2	10.5	79.0	9.0
$CO(NH_2)_2+NH_4NO_3$, P_2O_5, K_2O	50.4 + 33.6, 67.2, 33.6	9.5	9.6	33.4	—	35.1
$(NH_4)_2SO_4$	23.5	4.4	5.2	24.3	87.6	20.7
$CO(NH_2)_2$	50.4	5.3	4.9	11.8	98.5	9.6
Carbofuran	0.6	0	0	4.8	79.6	4.1
Methyl parathion	0.6	0.2	0.6	2.5	83.8	4.6
2,4-D	0.5	2.5	0.4	9.0	97.4	2.3
Benomyl	0.6	0.4	0.4	9.7	97.7	5.7
Fentin hydroxide	0.5	0	0	4.3	100.0	0.4
Fentin hydroxide + Sulfur	0.3 + 0.4	0.2	2.2	9.6	100.0	20.6
Fentin hydroxide + $CO(NH_2)_2$	0.3 + 105.4	1.6	11.4	6.9	100.0	27.5
Control	—	0.4[3]	0.8[3]	5.2[4]	92.5[4]	5.8[4]

[1] Values were back-transformed from transformed data representing the mean of 10 replications for each treatment.
[2] Values were back-transformed from transformed data representing the mean of 5 replicates for each treatment.
[3] The control represents the mean of all treatment controls (2/treatment).
[4] The control represents the mean of all treatment controls (1/treatment).

($P>0.05$) from each other. Mortalities associated with applications of benomyl and fentin hydroxide at 0.6 and 0.5 kg AI/ha, respectively, were not significantly different ($P>0.05$) from each other. Fentin hydroxide and benomyl applied alone resulted in no mortality for both females and males relative to the control.

At 25 days posttreatment riceland agrichemicals continued to exert toxicological effects on *R. culicivorax* nematodes (Table 1). Percent mortality for each agrichemical increased from the day 2 evaluations, however, the relative toxicity of each chemical remained the same as at 2 days posttreatment.

The highest mortalities for female and male nematodes occurred in treatments with a mixture of urea + N-P-K fertilizer (33.4% for females and 35.1% for males) (Table 1). When ammonium sulfate was individually applied to the rice field, the female mortality was 24.3% and the male was 20.7%. Fentin hydroxide + sulfur and fentin hydroxide + urea caused 20.6 and 27.5% mortalities, respectively, among the male nematodes. The same two agrichemicals

Table 2. Analyses of variance evaluating the mortality of *Romanomermis culicivorax* treated as postparasites exposed to Lousiana rice field agrichemicals at 2 days and 25 days posttreatment, 1983.[1]

Source	2 days posttreatment F value Female	2 days posttreatment F value Male	25 days posttreatment F value Female	25 days posttreatment F value Male
Agrichemicals	10.36**	15.60**	42.53**	5.35**
Contrast				
Control vs. agrichemicals	7.35**	8.21**	4.58*	5.96*
Fertilizer vs. insecticides	41.68**	44.51**	18.25**	8.80**
Carbofuran vs. methyl parathion	0.11	1.18	0.29	0.01
$(NH_4)_2SO_4$ vs. $CO(NH_2)_2$	4.88*	0.00	2.56	2.19
$CO(NH_2)_2$ vs. $CO(HN_2)_2$ + NH_4NO_3, P_2O_5, K_2O	5.17*	5.19*	7.85**	9.64**
Fentin hydroxide vs. fentin hydroxide + $CO(NH_2)_2$	5.78*	63.86**	0.14	20.92**
Sulfur chemicals vs. Fentin hydroxide and $CO(NH_2)_2$ + NH_4NO_3, P_2O_5, K_2O	9.89**	0.29	0.65	0.86
Benomyl vs. fentin hydroxide	0.25	0.50	0.82	2.54

[1] Analyses conducted on transformed values. * $P<0.05$; ** $P<0.01$.

caused female mortalities of 9.6 and 6.9%, respectively. The insecticides, carbofuran and methyl parathion, caused no mortality to the nematodes at 25 days posttreatment relative to the control.

Thus fertilizers in general remained significantly ($P<0.01$) more toxic to the nematodes at 25 days posttreatment than were the insecticides. As previously mentioned, nitrates, nitrites, phosphates and potassium ions of riceland fertilizers have been incriminated as causing mortality in certain soil inhabiting nematodes (Walker 1971, Brown and Platzer 1978). These may have contributed to the comparatively high mortality of *R. culicivorax* following fertilizer applications. Unlike the day 2 reading, there were no significant differences ($P>0.05$) at 25 days posttreatment for females relative to ammonium sulfate vs. urea, fentin hydroxide vs. fentin hydroxide + urea, and sulfur vs. fentin hydroxide and urea + N-P-K fertilizer (Table 2).

The percentages of surviving nematode females developing to a gravid state by 25 days after exposure to riceland agrichemicals as postparasites are shown in Table 1. All of the surviving female nematodes treated with the fungicide fentin hydroxide alone or in combination with sulfur or urea were able to produce eggs. Approximately 80-84% of the females exposed to insecticide applications of carbofuran and methyl parathion, respectively, also developed eggs. Similarly, herbicides did not inhibit egg development. The percentage gravid females exposed as postparasites to benomyl and 2,4-D were 97.7 and 97.4%, respectively. Gravid females that matured from fertilizer treated postparasites ranged from 79.0% for ammonium sulfate-P-K fertilizer [$(NH_4)_2SO_4$] to 98.5% for urea [$CO(NH_2)_2$] alone.

Analyses of variance evaluating the effects of rice field agrichemicals after 25 days on the maturation of *R. culicivorax* females to the gravid state are shown in Table 3. Insecticides were significantly different ($P<0.05$) in terms of having lower percentages of female nematodes developing eggs when compared to fertilizers. There was also a significant difference ($P<0.01$) between ammonium sulfate and urea fertilizers.

Parasitism of *Cx. quinquefasciatus* larvae by F_1 progeny of *R. culicivorax* adults, previously exposed as postparasites to commercially applied riceland agrichemicals, is shown in Table 4. The degree of parasitism ranged from 25.9% for the urea application to 100% for ammonium sulfate-P-K fertilizer. The pesticides did not appreciably inhibit the infectivity of F_1 preparasites relative to the untreated control. The control had a 84.4% level of parasitism. With the exceptions of urea + N-P-K fertilizer (72.8%), urea (25.9%), carbofuran (76.4%), 2,4-D (70.9%), and fentin hydroxide (67.4%), the preparasites induced >84% parasitism in *Cx. quinquefasciatus* larvae.

Table 3. Analyses of variance evaluating the effects of Louisiana rice field agrichemicals on the maturation of female *Romanomermis culicivorax* to the gravid state when treated as postparasites 25 days posttreatment, 1983.[1]

Source	df	MS	F
Agrichemicals	10	0.1725	10.47**
Contrast			
Control vs. agrichemicals	1	0.0004	0.02
Fertilizers vs. insecticides	1	0.0900	5.46*
Carbofuran vs. methyl parathion	1	0.0015	0.09
$(NH_4)_2SO_4$ vs. $CO(NH_2)_2$	1	0.1905	11.56**
Fentin hydroxide vs. fentin hydroxide + $CO(NH_2)_2$	1	0.0000	0.00
Benomyl vs. fentin hydroxide	1	0.0335	2.04
Error	49	0.0165	

[1] Analyses conducted on transformed values. * $P<0.05$; ** $P<0.01$.

Nematodes treated with fertilizers exhibited a greater range of variability in their infectivity of mosquito larvae. Those exposed to urea alone resulted in the lowest level of percent parasitism (25.9%). However, when urea was applied in combination with a N-P-K fertilizer and with fentin hydroxide, the percent parasitism increased to 72.8% and 94.6%, respectively (Table 4). In contrast, the ammonium sulfate-P-K fertilizer treatment resulted in no observable inhibition to preparasitic infection (100%) in this test. When ammonium sulfate was applied individually some reduction in parasitism (87.7%) was recorded compared to the mixture.

Parasitism by F_1 progeny of *R. culicivorax* was not seriously inhibited when the postparasites had been exposed to rice field applications of the fungicides, fentin hydroxide and benomyl. Excluding the application of fentin hydroxide alone, percent parasitism by F_1 progeny ranged from 92.9% for fentin hydroxide + sulfur to 97.0% for benomyl (Table 4).

In summary, little mortality occurred in postparasitic males and females of *R. culicivorax* at 2 days posttreatment following exposure to field applications of riceland agrichemicals. Maximum mortality rates were <11% and most agrichemicals caused <1% nematode mortality relative to the control. Insecticides had minimal deleterious effects on postparasites of *R. culicivorax*. Generally, when the mortality of nematodes exceeded 4%, it was associated with fertilizer applications. Comparable results were obtained for mortality at 25 days posttreatment and the viability of F_1 preparasites was similar.

Table 4. Parasitism of *Culex quinquefasciatus* larvae by F_1 progeny of *Romanomermis culicivorax* adults, previously treated as postparasites, to commercially applied Louisiana rice field agrichemicals, 1983.[1]

Agrichemicals	AI (kg/ha)	Sample replications	% parasitism
$(NH_4)_2SO_4$, P_2O_5, K_2O	36.3, 36.3, 36.3	5	100.0
$CO(NH_2)_2$ + NH_4NO_3, P_2O_5, K_2O	50.4 + 33.6, 67.2, 33.6	4	72.8
$(NH_4)_2SO_4$	23.5	4	87.7
$CO(NH_2)_2$	50.4	5	25.9
Carbofuran	0.6	5	76.4
Methyl parathion	0.6	5	98.4
2,4-D	0.5	5	70.9
Benomyl	0.6	5	97.0
Fentin hydroxide	0.5	5	67.4
Fentin hydroxide + Sulfur	0.3 + 0.4	5	92.9
Fentin hydroxide + $CO(NH_2)_2$	0.3 + 105.4	4	94.6
Control	—	10	84.4

[1] Approximately 200 *Cx. quinquefasciatus* were added to each flooded sample.

These data provide a better insight into the use of *R. culicivorax* as a biocontrol agent for managing larval mosquito populations in the riceland agroecosystem. Even though some of the pesticides and fertilizers evaluated in this study showed deleterious effects on the nematode, the impact was considered minimal and at least for the postparasite stage, agrichemicals would not seriously jeopardize its role in an integrated pest management program. Additional information including qualitative laboratory tests and subsequent field evaluations, is required to assess the effects of these and other pesticides and fertilizers on adults of *R. culicivorax* before their use as a component of riceland mosquito control can be optimized.

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References Cited

Brown, B. J. and E. G. Platzer. 1978. Salts and the infectivity of *Romanomermis culicivorax*. J. Nematol. 10:53–61.

Dhillon, M. S. and E. G. Platzer. 1978. Laboratory evaluation of fungicide for the control of *Catenaria anguillulae,* a fungus parasite of *Romanomermis culicivorax* (Nematoda: Mermithidae). Proc. Annu. Conf. Calif. Mosq. Vector Control Assoc. 46:92–93.

Ishibashi, N., E. Kondo and S. Ito. 1983. Effects of application of certain herbicides on soil nematodes and aquatic invertebrates in rice paddy fields in Japan. Crop Protection 2:289–304.

Levy, R. and T. W. Miller, Jr. 1977. Susceptibility of the mosquito nematode *Romanomermis culicivorax* (Mermithidae) to pesticides and growth regulators. Environ. Entomol. 6:447–448.

Mitchell, C. J., P. Chen and H. C. Chapman. 1974. Exploratory trials utilizing a mermithid nematode as a control agent for *Culex* mosquitoes in Taiwan. J. Formosan Med. Assoc. 73:241–254.

Petersen, J. J. 1975. Development and fecundity of *Reesimermis nielseni,* a nematode parasite of mosquitoes. J. Nematol. 7:211–214.

SAS Institute Inc. 1982. SAS user's guide:Statistics, 1982 edition. SAS Institute Inc., Cary, NC.

Seinhorst, J. W. 1956. The quantitative extraction of nematodes from soil. Nematologica 1:249–267.

Walker, J. T. 1971. Populations of *Pratylenchus penetrans* relative to decomposing nitrogenous soil amendments. J. Nematol. 3:43–49.

Washino, R. K. and J. L. Kerwin. 1983. Establishment and overwintering of postparasitic *Romanomermis culicivorax* in rice fields. Annu. Rep. Univ. Calif. Mosq. Control Res. 1983:66–67.

Winner, R. A. and C. D. Steelman. 1978. Effects of selected insecticides on *Romanomermis culicivorax,* a mermithid nematode parasite of mosquito larvae. Mosq. News 38:546–553.