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Field evaluations of *Romanomermis yunanensis* (Nematoda: Mermithidae) for control of Culicinae mosquitoes in China [1]

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Summary – The use of *Romanomermis yunanensis* for the control of Culicinae mosquitoes was field tested in five Chinese provinces from 1986 to 1995. About 2000-4000/m^2 preparasitic larvae of *R. yunanensis* were released in rice paddies, ponds, and streams. This resulted in parasitism rates of 52.2-96.7 % for native *Culex tritaeniorhynchus*, 35.5-89.5 % for *C. quinquefasciatus*, 90.1 % for *C. shebbearei*, 78.3 % for *C. pseudovishnui*, and 53.7-92.8 % for *C. theileri*. In addition, preparasitic larvae of *R. yunanensis* were added to small water sites in several towns. This resulted in parasitism rates of 53.0-100 % for *Aedes albopictus*, 90.1 % for *Ae. pseudalbopictus*, and 70.9 % for *C. tritaeniorhynchus*, but only 28.3-32.6 % for *Ae. albopictus* in sites with oily water surface and 6.7-34.2 % for *C. theileri* in streams with turbid water or with organic matter in suspension. These results suggest that under appropriate conditions *R. yunanensis* should be very effective for controlling Culicinae mosquitoes both in the country side and within city limits. Preliminary observations on the establishment of *R. yunanensis* were made at natural mosquito breeding sites where preparasitic larvae had been released 1-3 years previously. Some factors determining the effectiveness of field application are discussed. © Orstom/Elsevier, Paris

Résumé – ***Évaluation au champ de* Romanomermis yunanensis *(Nematoda : Mermithidae) pour le contrôle des moustiques Culicinae en Chine*** – L'utilisation de *Romanomermis yunanensis* en vue du contrôle de moustiques Culicinae a été testée au champs dans cinq provinces chinoises de 1986 à 1995. Environ 2000 à 4000 larves préparasites de *R. yunanensis* par m^2 ont été dispersées dans des rizières inondées, des mares et des ruisseaux. Il en est résulté des taux de parasitisme de 52,2–96,7 % pour *Culex tritaeniorhynchus* (espèce indigène), 35,5–89,5 % pour *C. quinquefasciatus*, 90,1 % pour *C. shebbearei*, 78,3 % pour *C. pseudovishnui* et 53,7–92,8 % pour *C. theileri*. De plus, des larves préparasites de *R. yunanensis* ont été dispersées dans différents sites aquatiques de surface restreinte dans plusieurs villes. Cela a conduit à des taux de parasitisme de 53,0–100 % pour *Aedes albopictus*, 90,1 % pour *Ae. pseudalbopictus* et 70,9 % pour *C. tritaeniorhynchus*, mais de 28,3–32,6 % seulement pour *Ae. albopictus* dans les sites où l'eau était couverte d'un film huileux et de 6,7-34,2 % seulement pour *C. theileri* dans des ruisseaux où l'eau était turbide ou chargée en matières organiques. Ces résultats suggèrent que, dans des conditions appropriées, *R. yunanensis* peut se montrer très efficace pour contrôler les moustiques Culicinae dans les zones tant agricoles qu'urbanisées. Des observations ont été effectuées concernant l'établissement durable de *R. yunanensis* dans des sites naturels où les moustiques se reproduisent et où des larves préparasites avaient été dispersées 1 à 3 années auparavant. Quelques facteurs affectant l'efficacité des applications au champ sont discutés. © Orstom/Elsevier, Paris

Keywords: Culicinae, establishment, Mermithidae, mosquito control, nematode, *Romanomermis yunanensis*.

In 1984, *Romanomermis yunanensis* Song & Peng, 1987 was discovered in Henan, China, and has since been maintained for more than 100 generations under laboratory conditions. Until now, no host resistance or decrease in infectivity of the nematode has been observed. Results of experimental infection by *R. yunanensis* of 31 species in six genera of mosquitoes have shown that under laboratory condition Culicinae mosquitoes are highly susceptible to *R. yunanensis* (Peng *et al.*, 1992). With a ratio of mosquito larvae to nematode juveniles of 1:5, parasitism rates of seven species of *Aedes* and two species of *Culesta* tested were always above 95 % and parasitism rates of seven species of *Culex* were above 85 %. In addition, in another experimental infection test (unpubl.) with a 5:1 infection ratio, parasitism rates of *Culex pseudovishnui*, *C. halifaxia*, *C. shebbearai*, and *Aedes pseudalbopictus* were 93.3, 0, 91.3, and 91.0 %, respectively, whereas

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many *Anopheles* were not susceptible to the nematode. Parasitism rates of eleven species of *Anopheles* except *A. dirus* and *A. freeborni* were 0-50 %. The parasitism rate of *A. dirus* was 89.7 %, but most (88.8 %) parasitizing mermithids underwent melanization and died. In *A. maculatus,* retarded development of *R. yunanensis* was observed as the nematode remained at the parasitic stage in both larvae and pupae of the mosquito. However, some medically important vectors such as *Aedes aegypti* (98.3 %), *Ae. albopictus* (98.5 %), *Culex tritaeniorhynchus* (97.1 %), and *C. quinquefasciatus* (98.6 %) were very susceptible to *R. yunanensis,* which indicates that this mermithid may be a good prospect for the control of these vector mosquitoes. Here, we report the results of field tests with *R. yunanensis* for the control of Culicinae mosquitoes.

Materials and methods

A laboratory strain of *R. yunanensis* was established from one female and five males collected from Henan, China, in 1984 and maintained at the laboratory of the Department of Parasitology, West China University of Medical Sciences.

Based on the principles developed by Petersen and Willis (1972) and Platzer and Stirling (1978), mass rearing of *R. yunanensis* was established (Xue *et al.*, 1992) with *C. quinquefasciatus* as host to support field applications.

Sand cultures containing *R. yunanensis* eggs were flooded for 2 h with distilled water. Preparasitic juveniles (infective stage) of *R. yunanensis* hatched within 8 h and were released to the water surface using a compressed air spray. Rates varied from 2000 to 4000 juveniles per m^2 in rice paddies, ponds, and streams, depending on the density of the target mosquito. A set number of preparasitic juveniles (Table 1) were distributed by ladle to the surface of standing water sites (pots, vats, and tyres). Field sites in ecologically diverse regions with Culicinae mosquitoes, with no serious water pollution and proven to be free of mermithid infection by mermithid emergence test and larval mosquitoes dissection, were used for infectivity tests against *Culex* and *Aedes.* Mosquito larvae were sampled 1 or 2 days after application of preparasitic larvae. Parasitism was determined by dissecting mosquito larvae in the laboratory within 24 h after collection. Water temperature was measured and pH and water pollution status were recorded in some sites.

Twenty one permanent or semipermanent sites (Nos. 1–21), *i.e.* rice paddy fields, ponds, and streams in the suburbs of towns, and 40 small-scale water sites (Nos. 22–61), *i.e.* pots, vats, and tyres within city limits, were selected for treatment with *R. yunanensls.*

In seven sites (Nos. 62–68) treated with *R. yunanensis,* larval mosquitoes were also sampled and examined in (1–3) subsequent years to determine whether the mermithid had become established in these natural mosquito breeding sites.

Results

The results of field tests conducted in 21 natural mosquito breeding habitats in the suburbs of five towns from 1986 to 1995 are presented in Table 2.

Parasitism rates of *C. tritaeniorhynchus* in two rice fields (No. 1, 240 m^2; No. 2, 100 m^2) in the suburbs of Wuzhi, Henan province (preparasites released on early July, 1991; water temperature 28-32°C, pH 7.2) were 62.2 and 58.3 %, respectively.

Table 1. *Field tests of* Romanomermis yunanensis *for control of* Aedes *and* Culex *mosquitoes in small water sites in city proper.*

No.	Site	Number of preparasitic larvae	*Aedes albopictus*		*Aedes pseudoalbopictus*		*Culex tritaeniorhynchus*	
			Number of mosquitoes examined	Parasitism (%)	Number of mosquitoes examined	Parasitism (%)	Number of mosquitoes examined	Parasitism (%)
22-30	Pots (9)	400/pot	649	93.5 (91.6-100)	–	–	–	–
31-34	Pots (4)	400-1000/pot	248	29.9* (28.3-32.6)	–	–	–	–
35-57	Tyres (23)	1000/tyre	2215	85.8 (53.0-100)	–	–	–	–
58-59	Flower beds (2)	4000 m^2	–	–	101	90.1	55	70.9
60-61	Vats (2)	3000 m^2	68	89.7	–	–	–	–

* Water surface with oily surface or organic suspended substance.

Table 2. *Field test of* Romanomermis yunanensis *for the control of* Culex *mosquitoes.*

No.	Site	Number of preparasitic larvae per m^2	Percentage of parasitism (number of mosquitoes examined)					
			C. tritaeniorhynchus	*C. quinquefasciatus*	*C. shebbeari*	*C. theileri*	*C. halifaxia*	*C. pseudovishnui*
1	Rice paddy	2000	62.2 (82)	-	-	-	-	-
2	Rice paddy	4000	58.3 (48)	-	-	-	-	-
3	Rice paddy	2000	68.4 (133)	-	-	-	-	-
4	Rice paddy	2000	52.2 (157)	35.5 (76)	-	-	-	-
5	Pond	2000	71.0 (262)	79.5 (254)	-	-	-	-
6	Pool	3000	96.7 (42)	89.5 (277)	-	-	-	-
7-13	Canals (7)	2000-4000	-	-	-	53.7-92.8 (549)	-	-
14-19	Canals (6)	2000-4000	-	-	-	6.7-34.2 (525)	-	-
20	Pool	3000	-	-	90.1 (50)	-	-	-
21	Stream	2500	76.1 (67)	62.5 (56)	-	-	0 (37)	78.3 (42)

In two rice paddies (No. 3, 40 m^2; No. 4, 46 m^2), a pond (No. 5, 60 m^2), and a pool (No. 6, 18 m^2) in the suburbs of Chengdu, Sichuan province, with very low rice plants and no dense vegetation at the water surface of the pond and pool (mermithid application made at the end of June 1986; water temperature 26-36°C; pH 6.5-7), parasitism rates of *C. tritaeniorhynchus* were 68.4, 52.2, 71.0, and 96.7 % in sites No. 3, 4, 5, and 6, and parasitism rates of *C. quinquefasciatus* were 35.5, 79.5, and 89.5 % in sites No. 4, 5, and 6, respectively.

In thirteen 11.7 m^2 irrigation canals in the suburbs of Kunming, Yunnan province (water temperature 20-21°C; pH 6.8-7.6; treated with *R. yunanensis* in July, 1990) parasitism rates of *C. theileri* were 53.7, 92.8, 58.1, 81.0, 60.2, 84.7, and 56.3 % in seven (Nos. 7–13) canals with comparative clean water, whereas the rates were 32.3, 6.7, 33.9, 32.4, 30.4, and 34.2 % in six (Nos. 14–19) canals with turbid water or with organic matter in suspension at the water surface.

In a pool (No. 20, 8 m^2) in the suburbs of Gullin, Guangxi province (application made in July, 1994; water temperature 28-37°C), the parasitism rate of *Culex shebbeari* was 90.1 %.

In a stream (No. 21, 12 m^2) in the suburbs of Guiyang, Guizhou province, with clean water and several species of *Culex* mosquitoes (mermithid released in July, 1994; water temperature 25-31°C), the parasitism rates were 76.1 % for *C. tritaeniorhynchus*, 62.5 % for *C. quinquefasciatus*, 78.3 % for *C. pseudovishnui*, and 0 % for *C. halifaxia*, which is the only species non-susceptible to *R. yunanensis* in the Culicinae mosquitoes tested.

The results of the use of *R. yunanensis* for the control of *Aedes* and *Culex* in small-scale water sites such as pots, vats, tyres, etc., within city limits are presented in Table 1. *Aedes* and *Culex* often breed in flowerpots, pottery basins, and pitchers in Chengdu and the density of larval mosquitoes increases in summer and autumn. In 1987, thirteen pots were treated with 400-1000 mermithids/pot (water temperature 25-32°C; pH 6.3-7.2). Parasitism rate of *Ae. albopictus* was 91.6-100 % in nine pots (Nos. 22–30) but only 28.3-32.6 % in four pots (Nos. 31–34) with oily water surface or with organic matter in suspension.

In autumn 1987, more than 1000 old vehicle tyres were found in an open-air depot. These tyres contained 5–20 L of water with some decomposing leaves or soil. The water was light brown in colour and contained instar larvae of *Ae. albopictus* (water temperature 28-32°C). A total of 820 tyres were treated with 1000 preparasites per tyre. A random sample of 2215 larval mosquitoes collected from 23 tyres (Nos. 35–57) was examined 2 days later. The treatment resulted in 53.0-100 % parasitism rate for *Ae. albopictus*, while natural mortality of larval *Ae. albopictus* in the untreated group was only 10.4 % in 7 days.

Changing bamboo forest, the famous tourist area, is also a breeding site for *Aedes* and *Culex*. Two flower bed (Nos. 58 and 59) in the town of bamboo forest, each 2.8 m^2 in size, with water and a high density of larval mosquitoes, were treated with 4000/m^2 preparasites of *R. yunanensis* (water temperature was 23-29°C). Parasitism rates were 90.1 % for *Aedes pseudalbopictus* and 70.9 % for *C. tritaeniorhynchus* in June 1995.

Two stone vats (Nos. 60 and 61), each 2 m^2 in size with water and larval *Ae. albopictus*, on the shady side of a street inside the town of Guilin were treated with 3000/m^2 preparasites (water temperature 28-37°C). Parasitism of *Ae. albopictus* was 89.7 % in 1994.

The establishment of *R. yunanensis* was observed (Table 3). Two rice paddies (No. 62, 50 m^2, No. 6, 3.50 m^2) in the suburbs of Omeshan, Sichuan province, were treated with 3000/m^2 preparasites of *R. yunanensis* on June 6, 1990. At this time, a heavy rainfall flooded the preparasites and their hosts, so that no infected mosquitoes were collected. On June 16, 1990, a second release was made with 3000/m^2 preparasites. The samples collected from the two paddies showed parasitism rates of 46.2 and 83.3 % for *C. tritaeniorhynchus*. Another paddy (No. 64) treated with 3000/m^2 preparasites in June 1991 had 60.7 % parasitism at that time. In July 1993, we returned to collect and examine the larval mosquitoes from paddy Nos. 63 and 64 (paddy No. 62 was dry at this time) and observed parasitism rates of 50.8 and 32.2 % for *C. tritanieorhynchus*. The water temperature of the paddy was 23-32°C, pH 6.5-7.3. Three old vehicle tyres (Nos. 65–67) treated once in 1987 had parasitism rates of 71.4, 0, and 30.5 % for *Ae. albopictus* in 1988. The temperature of the water in the tyres was 28-33°C. Unexpectedly, we found that many postparasitic mermithids emerged in the course of larval *Ae. albopictus* rearing in 1988. These *Aedes* larvae were taken from a stone pot (No. 68) in the air-open, near our laboratory. This pot had received 400 preparasites of *R. yunanensis* in 1987. Dissection of larval mosquitoes taken from this pot showed 73.1 % parasitism of *Ae. albopictus* (Table 3).

Discussion

Field tests have been reported for *Romanomermis jingdeensis* (Yang *et al.*, 1984) in China. When 2000/m^2 of *R. jingdeensis* were released into rice fields, ponds, and canals in the suburbs of Shanghai, 34.1-95.0 % parasitism of *Anopheles sinensis* were achieved. However, Culicinae mosquitoes are not susceptible to this mermithid. Although *R. culicivorax* has a broad host range and has been demonstrated to be a good biological control agent, it is not highly effective against all species of Culicinae mosquitoes under field conditions (Petersen *et al.*, 1972; Mitchell *et al.*, 1974; Brown *et al.*, 1977); in comparison to that of *R. culicivorax*, the infectivity of *R. yunanensis* on mosquitoes important for Culicinae mosquitoes under field conditions (Tables 1 and 2).

Two years after introduction of *Diximermis petersini* Nickle, 1972 into a natural site in 1971, 88 % of the *Anopheles* spp. were parasitized (Petersen & Willis, 1974). Based on periodical and continuous observation from 1971 to 1974, Petersen and Willis (1975) confirmed that *R. culicivorax* can become established and reproduce in both permanent and semipermanent water sites where it had been previously released. Establishment of *R. yunanensis* was observed at the natural mosquito breeding sites where preparasitic larvae had been released 1-3 years before (Table 3). However, regular and continuous surveys will be necessary to determine the fluctuation of mermithid infections and the effects of several environmental parameters on the establishment of *R. yunanensis*

Table 3. *Establishment of* Romanomermis yunanensis *in natural breeding habitat of mosquitoes.*

No.	Site	Number of preparasitic larvae	*Culex tritaeniorhynchus*				*Aedes albopictus*			
			Number of mosquitoes examined	% of immediate action	Number of mosquitoes examined	Parasitism due to establishment (%)	Number of mosquitoes examined	% of immediate action	Number of mosquitoes examined	Parasitism due to establishment (%)
62	Rice paddy	3000/m^2	52	46.2	–	–	–	–	–	–
63	Rice paddy	3000/m^2	114	83.3	65	50.8	–	–	–	–
64	Rice paddy	3000/m^2	56	60.7	62	32.2	–	–	–	–
65	Vehicle tyres	1000/tyre	–	–	–	–	91	53.1	105	71.4
66	Vehicle tyres	1000/tyre	–	–	–	–	77	89.3	47	0
67	Vehicle tyres	1000/tyre	–	–	–	–	109	70.7	82	30.5
68	Pot	400/pot	–	–	–	–	–	–	130	73.1

in sites where preparasitic larvae were previously released.

Several authors (Petersen & Willis 1970, 1971; Calloway & Brust, 1977; Platzer, 1981) considered that infection dosage, water temperature, pH, duration of drought, and water pollution were major factors for the effectiveness of field application of mermithids for mosquito control. Brown and Platzer (1977) showed that infection of mosquito larvae could occur at 12°C. Levy and Miller (1977) concluded that it had little value in habitats with temperature above 40°C. However, the optimum temperature was in the 21-33°C range (Platzer, 1981). Chen (1976) stated that optimal pH for parasitism was in the range 6.7-7.2 and no parasitism was observed at values lower than 5.7 or higher than 8.7. Petersen (1979) found that *R. culicivorax* was fully infective at pH 5.4-7.9. In this study, the value of pH in the natural sites tested was 6.3-7.6 and the water temperature 20-36°C, which did not adversely affect the ability of *R. yunanensis* to infect mosquito hosts. Hence, pH and temperature of most natural water sites during the mosquito season should not be a limiting factor. The present field tests also showed that the infection dosage of 2000-4000 *R. yunanensis*/m^2 may be appropriate for the control of mosquitoes.

The present studies showed that heavy rainfall can wash away the preparasites and their mosquito hosts and that water pollution had a negative influence on the effectiveness of field applications *of R. yunanensis.* Serious water pollution would restrict the use of this mermithid.

Petersen and Willis (1971) reported that *R. culicivorax* seems to be adapted to both sites that dry up periodically and sites that are more permanently flooded. Also it can persist for long periods in a particular habitat and give continuous partial control of mosquitoes. Peng *et al.* (1993) noted that *R. sichuanensis* can persist in some natural breeding sites that remain dry for more than 6 months. The environmental conditions of most of the sites tested for this study appeared similar to those in sites found naturally infested by *R. yunanensis.* This nematode occurs naturally in rice paddies and streams in Xinyung, Henan province. These semipermanent flooded sites always dry out after the rice harvest. A survey of natural breeding sites of *R. yunanensis* showed that, in 1984-1991, the natural parasitism rate of *Culex* remained in the 1.18-7.04 % range (Song *et al.*, 1993). The relative stability of prevalence of *R. yunanensis* during these years suggested that *R. yunanensis* was adapted to periods of drought. A study of the life cycle of *R. yunanensis* showed that most adults and eggs survived in moist sand culture for over 1 year and 6 months, respectively, but that they died immediately after the sand dried. Biological and ecological data on *R. yunanensis* indicated that the duration of drought at mermithid breeding sites should not exceed a specified limit for the soil moisture to maintain a level where adult mermithids and eggs remain viable.

It will be necessary to understand and master the factors determining the effectiveness of field application and the establishment of mermithid to reach the full potential of the biocontrol of mosquitoes with mermithid nematodes.

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