Biological Control of the Brown Planthopper by a Mermithid Nematode

Mermithid 선충을 이용한 벼멸구의 생물적방제

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ABSTRACT The brown planthopper (BPH), Nilaparvata lugens, is the major pest of rice in Asia. Current control tactics rely primarily on chemical insecticides and resistant rice varieties. In Korea, the most important biological control agent appears to be the naturally-occurring, mermithid nematode, Agamermis unka. Although parasitism of BPH is highly variable from place to place and from year to year, the mermithid is a promising biological control agent because it reduces the fecundity of the host and ultimately causes its death. The mermithid has only one generation per year compared to the three to four generations of BPH, but the mermithid females stagger their egg production so that many individuals in all BPH generations are parasitized. Augmentation of this mermithid into BPH populations is only possible on a limited scale because it is an obligate parasite and mass production technology has yet to be developed, Conservation of naturally-occurring populations through cultural techniques and the use of compatible resistant rice varieties and chemical insecticides may lead to an effective integrated pest management program for BPH.

> KEY WORDS Agamermis unko, Nilaparvata lugens, brown planthopper, Mermithidae, Rice, Entomogenous nematode, Augmentation and conservation

초

- 벼띨구(Nilaparvata lugens)는 아시아의 중요한 벼해충이다. 현재의 방제법은 주로 살충제와 저항성 품종에 의존하고 있다 한국에서의 가장 중요한 생물적 방제인자는 자연 발생하고 있는 mermithid 선충의 항종인 Agamermis unka로 생각된다. 벼멸구에의 기생율이 비록 장소와 해에 따라 변이가 높다 하더라도 mermithid 선충은 기주의 생식력을 감소할 뿐만 아니라 결과적으로는 기주치사를 가져오기 때문에 전망이 밝은 생물적 방제인자이다 Mermithid 선충은 벼멸구가 년 3~4세대를 경 과하는데 비하여 년 1세대를 나나 mermithid 선충 암컷은 일시에 산란을 하지 않기 때문에 전세대의 많은 벼멸구가 기생된다 본 mermithud 선충은 완전기생충으로서 대량생산 기술이 아직 개발되지 않아 개체군 억제를 위한 증대법은 단지 제한적인 범위에서 가능하다. 그러나 재배적 방법이나 저 항성품종의 이용, 살충재와의 혼용을 통한 자연발생 선충 개체군을 보존함으로서 효과적인 벼멸구의 종합방제를 이룰 수 있다.

검색어 Agamermis unka, Nilaparvata lugens, 벼덜구, Mermithidae, 벼, 곤충기생성선충, 증대와 보존

The brown planthopper (BPH), Nilaparvata lugens, a serious pest of rice, is widely distributed in tropical, subtropical and temperate regions of the Far East (Kuno 1979). In Korea and other temperate countries, BPH cannot survive the harsh winters and reinfections are dependent on adult dispersal by tropical

storms (Kuno 1979, Choo et al. 1989). Its reproductive rate is high on modern, susceptible rice varieties (Kisimoto 1979), which contributes to its status as one of the major, if not the most important, pests of rice today. BPH causes annual losses worth millions of dollars through its feeding activity resulting

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in hopperburn and reduction in yield (Dyck & Thomas 1979) For example, in Korea BPH damaged 2.4×10^8 and 1.0×10^8 hectares of rice in 1991 and 1992, respectively, and ca. 90,000 metric tons of insecticides valued at \$116 million were applied to suppress insect pests in 1991 with most of them used for BPH control(Anonymous 1992).

In some countries, cultural methods have been developed to reduce damage by BPH (Oka 1979). but its suppression is still dependent on the application of chemical insecticides and the use of resistant rice varieties. Unfortunately, the continuous use of chemical insecticides has led to the development of BPH resistance (Nagata & Morita 1974) as well as other undesirable side effects Moreover, outbreaks of BPH have been associated with insecticide use caused, in part, by reproductive stimulation and the loss of natural enemies (Kiritani et al. 1971, Reissig et al. 1982). Although resistant rice vaneties have been successfully developed, the resistance in these parts has tended to break down relatively quickly through biotype selection in populations of BPH resulting in further outbreaks of this insect (Pathak & Heinrichs 1982).

Biological control offers an alternative tactic. Yet, because of the temporary nature of the rice habitat, no insect pest of rice has ever been consistently controlled solely by natural enemies. Perhaps, the most acceptable method to suppress BPH is to have biological control serve as a cornerstone of an integrated pest management (IPM) program (Chiu 1979). In this paper, we evaluate the biological control of BPH in relation to an IPM program with particular attention to the mermithid mematode, Agamermis unka.

NATURAL ENEMIES AND IPM (OTHER THAN AGAMERMIS)

A number of natural enemies have been isolated from BPH including 37 species of predators and 34 species of parasitoids (Chiu 1979), and several entomopathogenic fungi (Rombach et al. 1987). Insect predators, particularly the mirid Cyrtorhinus lividipennis (Chiu 1979. Reissig et al. 1982) and the veliid Microvelia atrolineata (Reissig et al. 1982),

and spiders(Chiu 1979, Reissig et al. 1982) have been documented to be effective biological control agents of BPH in Asian rice paddies

In Korea, the effect of insect predators on BPH populations has not been adequately evaluated, but the predatory habits of spiders have been studied. The dominant spider is *Pirata subpiraticus*, followed by *Pachygnatha clercki* and *Gnathonarium denatatum* (Paik & Park 1983). For example, *P. subpiraticus* can prey upon 5.3 BPH/day, whereas *P. clercki* and *G. denatatum* can consume 2.0 and 1.1 BPH/day, respectively.

Drawing upon work by Reissig et al (1982) and Fabellar and Heinrichs (1984), these predators, especially the spiders and the velid, may be compatible with many insecticides. Most insecticides did not significantly reduce field populations of spiders and the velid, but the mirid was adversely affected (Reissig et al. 1982), whereas some synthetic pyrethroids (i.e., cypermethrin and deltamethrin) were toxic to the mirid, the velid, and the spider. Lycosa pseudoannulata, upon contact (Fabellar & Heinrichs 1984). Selective insecticide use may lead to the development of an effective IPM program with these predators.

In Korea, parasitoids appear to be ineffective as biological control agents. Egg parasitism by three mymarid egg parasitoids, Anagrus flaveolus, A. incarnatus and A. optabilis, has been low (Kim 1991). At four insecticide-free rice paddles in Gyeongnam Province, parasitism ranged from 3 to 25% for A. flaveolus, 0 to 2% for A. incarnatus, and 1 to 4% for A. optabilis. Similarly, numphal parasitism by the dryinid parasitoid, Pseudogonatopus flavifemur, was also low and ranged from 0.5 to 2.5% (Kim 1991). Percent parasitism presents only one of several parameters to determine effective biological control. and long-term studies to assess host densities in the presence of the parasitoids are needed. In terms of integrating chemical pesticides and parasitoids, the parasitoids are generally believed to be poorly preadapted for detoxifying pesticides because they are specialized camivores and do not have to deal with plant toxicants (Roush 1990). The extensive use of insecticides may warrant the search for or development of parasitoids resistant to these chemicals. Before such an attempt is made, however, the parasitoid should be an effective biological control agent of BPH. Two examples using this approach are available. Selection for resistance to azinphosmethyl by *Trioxys pallidus*. a parasitoid of walnut aphid, *Chromaphis juglandicola*, in walnuts (Hoy & Cave 1988, 1991) and for resistance to carbaryl by *Aphytis melinus*. a parasitoid of the California red scale, *Aonidiella aurantu*, in citrus (Rosenheim & Hoy 1988, Spollen & Hoy 1992) has been accomplished. An effective parasitoid for such an approach in a highly disruptive rice cropping system needs to be found.

Another possible approach is to find an alternative host for the parasitoids. For example, the effectiveness of the egg parasitoid, Anagrus epos, against the grape leafhopper, Erythroneura elegantula a pest of grapes, was enhanced by the parasitoid's ability to use another leafhopper, Dikrella cruentata on blackberry (Doutt & Nakata 1973) However, the intensive monocultural cropping system of rice does not leave much opportunity for an alternate host approach as demonstrated for the grape-blackberry system. Considerable changes in agricultural practices including polycultures will be needed if an alternate host is found for the parasitoids of rice pests.

Entomopathogenic hyphomycetous fungi have shown promise for biological control for BPH when they have been applied as biological insecticides (Rombach et al. 1986, Aguda et al. 1987), Suspensions of conidia of Metarhizium anisopliae. M. flavoviridae, Beauveria bassiana, and Hirsutella citriformis or dry mycelia of M. anisopliae and Paecilomyces lilacinus, all applied separately during the rainy season in the Philippines, produced fungal infections from 63 to 98% (Rombach et al. 1986). Aguda et al. (1987) reported similar results with Beauveria and Metarhizium species in Korea when the fungi were applied as a conidial suspension or as dry mycelia. Although a high concentration of a fungal species (220 to 2,000g/ha for dry mycelia or 4 to $7.5 \times$ 10¹²conidia/ha) was needed to obtain efficacious results, Rombach et al. (1986) and Aquda et al. (1987) suggested that lower fungal concentrations may also be effective. If entomopathogenic fungi are developed as biological insecticides, their effect on

other biological control agents and control tactics needs to be evaluated

THE MERMITHID, AGAMERMIS UNKA. IN IPM

The mermithid, A. unka, has received considerable attention as a biological control agent of BPH in Korea because it (1) occurs naturally in rice paddies, (2) can parasitize a high percentage of BPH in some situations, (3) has long-term control potential, (4) is specific to the leafhopper complex including another serious pest of rice, the whitebacked planthopper, Sogatella furcifera, and is harmless to non target organisms, (5) is environmentally safe, and (6) appears to be compatible with insecticides. Despite these positive attributes, it is an obligate parasite and cannot be produced in vitro and has a one year life cycle under field conditions. Therefore, augmentation through inoculative releases will be a limited approach to BPH suppression until such time that it can be mass-produced cheapty. Conservation, where management systems enhance and conserve existing naturally-occurring mermithid populations, may be a more practical approach. To achieve this goal, it is imperative that the bionomics of this nematode be understood.

Bionomics

The life cycle of A. unka is similar to other mermithids. The eggs generally take 17 to 25 days to hatch. The newly-hatched juvenile (preparasite) is very active and crawls up the rice stem and parasitizes BPH on the lower part of the rice stem where most of them are found. The preparasite penetrates directly into the host's hemocoel. Two days after penetration, the mermithid molts to the third stage juvenile (Choo, unpublished data). It molts to the fourth stage in the host, but exactly when this molt occurs is not known. Two to three weeks after parasitization, the fourth stage mermithid exits the host as a postparasite, burrows into the soil, molts to the adult stage, and overwinters as an adult. According to Kaburaki and Imamura (1932), mating occurs the following spring and oviposition occurs from June to late autumn. Our data indicate that the female

Table 1. Fecundity of Agamermis unka collected from the field at various times of the year

Month	Total females ^b	Mean number of eggs(± SE)	Mean oviposition period(days) (± SE)
January	45	1466± 155	37± 22
March	37	950± 879	22 ± 7.4
April	50	543± 557	$17 \!\pm 10.6$
May	52	1851 ± 1167	$30\!\pm11.8$
September	r 19	3013± 366	49± 3.1

^aFemales were collected from Chinyan and Tongyoung in Gyeongnam Province

may be mated in fall or winter or may be parthenogenetic because females collected in January will oviposit viable eggs in the laboratory (Table 1). This aspect of the life cycle needs to be clarified.

Agamermis has only one generation per year (Kaburaki & Imamura 1932, Zhao et al. 1987), whereas BPH has three to four generations per year (Park 1987) The female mermithid begins to lay eggs from the end of June to early autumn with the highest production occurring in early August (Kaburaki & Imamura 1932). The average number of eggs laid by females collected in May averaged 1,851 (range $231 \sim 4385$, n=52), whereas those collected in September averaged 3,013 (range 500~8197, n=19) (Table 1). The females from May oviposited an average of 30 days, but the females collected in September oviposited for 49 days. It appears that some females begin to lay eggs early whereas others delay their egg-laying activity so that parasitism of BPH occurs throughout summer and fall. Because BPH adults are dispersed by tropical storms each summer into Korea, the initial populations are small and fewer hosts are available until later in the growing season.

The size of the female mermithids did not seem to adversely affect egg production except for those <2.0 cm in length (Table 2) The high fecundity of the mermithid suggests that even if a few mermithids occur in a field, substantial progeny can be produced to parasitized BPH. Under favourable conditions (25°C), 95 to 100% of the eggs hatched in 29 days with initial hatch observed at 14 days after being

Table 2. Fecundity of Agamermis unka according to the length of the field-collected female^a

Length (cm)	Total females ^b	Mean number of eggs(± SE)	Mean oviposition period(days) (± SE)
<2.0	10	1434±148	29± 2.5
2.1~25	22	1791 ± 261	35 ± 3.0
$2.6 \sim 3.0$	19	1584 ± 281	40 ± 3.2
>3.0	10	1879±387	39±4.4

^aFemales were collected from Chinyang and Tongyoung in Gyeongnam Province

laid. No eggs hatched at 15° C after 52 days, but they were still viable 96% hatched at 20° C over 42 days, and 63% hatched at 30° C over 31 days. A dark environment was more favorable to egg-laying and egg hatch than a lighted environment Over 29 days, 98% of the eggs hatched in the dark compared with 68% in the light. Egg survival at 50° C was $95.8 \pm 0.04\%$ at 15 days but gradually declined to $80.4 \pm 4.0\%$ at 30 days and $65.9 \pm 2.4\%$ at 90 days. Survival was only $16.6 \pm 2.1\%$ at 180 days. More temperature studies to determine the optimal storage conditions are in order and may lead to the development of an inoculative release program for this mermithid.

Mermithid parasitism results in nutritional depletion, retarded growth, organ disruption, reduced fecundity or sterility and death. The amount of protein in parasitized BPH was less than in unparasitized BPH (Choo, unpublished data) and followed a similar pattern as reported for locusts parasitized by Mermis nigrescens (Gordon et al. 1973). In addition, parasitized BPH appeared smaller and produced less honeydew than unparasitized ones suggesting reduced feeding activity by the parasitized BPH. Upon dissection, the alimentary canal, Malpighian tubules and reproductive systems of parasitized BPH showed obvious signs of disruption. Only 4% contained eggs in the ovaries of parasitized BPH females compared with 86% in unparasitized females (Choo & Kaya 1990). More recently, 100% of parasitized females (n=382) collected from the field contained no eggs in their ovaries.

^bFemales held individually in petri dishes

^bFemales held individually in petn dishes

Table 3. Parasitism by Agamermis unka of brown planthopper(BPH) from rice paddies in Gyeongnam Province, Korea in August and September. The parasitism data have been combined for all collection dates

Site	Year	Rice variety	%(n)BPH parasitized	Mean number of mermithids in soil°±SE
Chinju	1990	Palgong	3.6(390)	3.0 ± 0.3
Chinyang	1990	Nagdong	80.8(303)	20.1 ± 61
Sacheon	1990	Nampoong	85(364)	37 ± 0.2
Goseong	1990	Nagdong	22.3(421)	67.0 ± 3.8
-	1991	Nagdong	12.7(584)	_
Tongyoung	1990	Dongjin	23 6(389)	_
, in the second second	1991	Dongjin	20.6(427)	_
	1992	Dongjin	48.8(422)	_
Namhae	1990	Hwaseong	238(467)	130± 1.9
	1991	Nagdong	26.1(639)	_

 $^{^{}a}$ Mean number of postparasites/20×20 cm plots from the same rice paddies after harvest. Six samples were taken per rice paddy.

Prevalence of Parasitism

In Japan, Esaki and Hashimoto(1931) reported Agamermis parasitism of 41% in BPH and 71% in the whitebacked planthopper. In Hunan Province, China, no control measures are needed when the population density is under 2,000/100 rice plants. provided the parasitizm by the mermithid is above 75% (Wang & Li 1987). In Korea, Agamermis parasitism varies from place to place and year to year (Table 3). Although there is variability in the Korean populations, the high prevalence of parasitism by Agamermis suggests that it has potential to effect biological control. For example, parasitism of first and second generation BPH was 56% for each generation (Choo et al. 1989). Both the short-winged (brachypterous form) and the long-winged (macropterous form) adults are susceptible to mermithid parasitism, but the brachypterous form had higher parasitism (57%) than the macropterous form (8%) (Choo et al. 1989). The difference in parasitism between the two forms is probably related to the behavior of the adults rather than host suitability. The brachypterous form is usually found lower on the rice plant where the mermithid is more likely to encounter it. Difference in parasitism was also observed between female and male BPH with higher parasitism detected in the female (48%) vs. the male (4%), The higher parasitism of the females contributes to reduce BPH populations in subsequent generations.

However, the reason for the difference in parasitism between the sexes is unknown.

Augmentation

In experiments conducted in 1200 cm² plastic boxes, the release of preparasites of A. unka at a mermithid to BPH ratio of 10:1 or 20:1 resulted in 44% (range 40 to 49%) and 40% (range 34 to 52%) parasitism of BPH, respectively. These data suggest that inoculative releases into areas where the mermithid population is low or nonexistent is feasible. In field trials with Romanomermis culicivorax, releases have been successful against mosquito larvae (Petersen & Willis 1972, 1975; Willis et al. 1980). When the preparasites were released at 10 different sites, the mean level of parasitism was 65%, 58%. and 33% for second-, third-, and fourth-stage larvae, respectively (Petersen & Willis 1972). This mermithid often became established in many semi-permanent and permanent water sites and produced from 2 to 100% parasitism of Anopheles mosquitoes (Petersen & Willis 1975).

Conservation

Soil Factors and Cropping Systems. The physical-chemical properties of soil appear to be important factors in the distribution of the mermithid. In China, the postparasites and adults were most likely to be found in light to medium clay soils with a

pH of 5.6 to 6.5 (Zhao et al. 1987).

Parasitism of BPH by Agamermis may be influenced by cultivation methods. For example, significantly greater parasitism of BPH occurred in tilled (78±9%) compared with untilled (39±9%) soil (Choo & Kaya 1990). The difference in parasitism between tilled and untilled soils may be the redistribution of the mermithid adults. They are normally found 1 to 10 cm below the soil surface (Choo et al. 1987, Zhao et al. 1987), and tilling probably brings them nearer the surface Upon flooding the rice paddies, the preparasites emerge from the eggs directly into the water or do not have to migrate far through the soil. Moreover, tilling makes the soil less compact and allows greater numbers of the preparasites to migrate through the soil and parasitize the BPH.

Because nematodes usually require moisture for survival, the rice paddy should be kept moist when it is fallow. Agamermis (=Amphimermis sp.) numbers decreased 71% after rice harvest when the soil was exposed to the sun for 10days compared with the number before harvest (Yan et al. 1986). In addition, the type of cover crop may influence the mermithid's survival. The number of Agamermis in fields with the Chinese milkvetch, Astragalus sinicus, was 6 times higher than in fields with rape, Brassica napus (Zhao et al. 1987). Accordingly, cultivation of a suitable cover crop will help conserve the natural population of this mermithid during the nonrice growing period.

Irrigation. Proper water management can contribute to the success of the preparasite finding its BPH host. As expected, Agamermis parasitism was higher in irrigated plots (35%) compared with nonirrigated plots (17%). During this time, the nonimigated plots received very little rain and the soil was dry. However, in two other tests, when rainfall was high, there was no difference in parasitism between irrigated (7 and 44% for tests 1 and 2, respectively) or nonimigated plots (6 and 59% for tests 1 and 2, respectively) (Choo, unpublished data). In addition, frequent and shallow irrigation shortened by 20 days the peak hatching rate for the mermithid compared with paddies flooded continually (Yan et al. 1986). If the phenology of the mermithid and BPH is

known, imigation practices may be manipulated to initiate peak egg hatch to coincide with the most susceptible stage of BPH.

Water Temperature. Water temperature can influence parasitism of BPH. Parasitism was highest at $25\,^{\circ}$ C ($37\pm1\%$) followed by $30\,^{\circ}$ C($21\pm1\%$) and $20\,^{\circ}$ C ($9\pm1\%$) (Choo, unpublished data). No parasitism occurred at $15\,^{\circ}$ C.In general, water temperature from the middle of July to middle of September is above $20\,^{\circ}$ C allowing the preparasites to parasitize BPH. In the irrigation test that received substantial rain, we noted that parasitism was higher in the nonirrigated plots than the irrigated ones. Perhaps, the results may be related to water temperature. The unirrigated plots that received rain water were shallow and warmed by the sun, whereas the irrigated plots contained significantly more water and were cooler affecting the behavior of the preparasites.

Compatibility with Some Insecticides. Agamermis adults are compatible with chemical insecticides used for insect pest control in rice. Choo et al. (1986) reported that the untreated control plots had a lower mean number of mermithid adults than in three of the four insecticide treatment plots. However, BPH in the carbofuran-treated plots had a significantly lower percentage of parasitism than did the cartap-treated or BPMC-treated plots. Carbofuran and cartap were applied as granules to soil and may have adversely affected the eggs, the preparasites or both stages thus reducing parasitism in these plots. We have not tested the compatibility of carbofuran against Agamermis, but Yan et al. (1986) observed that this insecticide adversely affected the mermithid juveniles and adults. Based on these observation, carbofuran should not be applied during the peak time of Agamermis hatching (August and September). A replacement for this insecticide may be a lower concentration of a wettable powder formulation of cartap+buprofezin which had no the preparasites (Choo, unpublished data).

Interactions with Beneficial Organisms. Agamermis is known to be host specific to planthoppers and leafhoppers (Esaki & Hashimoto 1931, Kaburaki & Imamura 1932, Kuno 1968, Otake 1979,

Choo et al. 1987, 1989; Choo & Kaya 1990), but it may still have a negative impact upon beneficial orgaisms. To data, there is no evidence to suggest that there is a negative effect. For example, in the field, multiple parasitism between the dryinid parasitoid and Agamermis was observed in <1% of the samples, and both natural enemies appeared to be unharmed (Choo & Kaya 1990).

In laboratory tests, the spider *P. subpiraticus*, was placed with BPH adults parasitized by *Agamermis*. The spider readily attacked and sucked body fluids from the parasitized BPH. If the postparasite emerged from the BPH and contacted the body of the spider, the spider stopped feeding and attempted to remove the mermithid with its legs. It is not known whether premature emergence of the mermithid adversely affects its further development.

FUTURE RESEARCH DIRECTIONS

Agamermis appears to be the major biological control agent of BPH in Korean rice paddies. It persists from year to year in the soil and although it only has one generation per year, individual females produce progeny throughout the summer and early fall to parasitize the three to four generations of BPH. Much information has been obtained about the biology and ecology of this mermithid, but more basic studies on the behavior of the preparasites and postparasites are needed. Long-term studies to determine whether the mermithid is effective at low and high host densities are needed. That is, we do not know whether it can regulate BPH populations.

Increasing the effectiveness of the mermithid against the first generation of BPH may reduce subsequent generations. Shifting the peak egg-laying cycle of the mermithid from early August to early July to parasitize the first BPH generation may be sufficient to reduce later generations. The concept of frequent and shallow irrigation management which warms the water and hastens the peak egg-laying and hatching of the mermithid needs to be pursued. In addition, very little is known about the effect of resistant rice varieties on the fitness of the mermithid. Moreover, some rice varieties seemed to have some effect on percent parasitism (Table 3), and

the suitability of BPH as hosts for these mermithids when they feed on these varieties needs to be examined. In vitro production of a mermithid from the banded cucumber beetle. Diabrotica balteata, has been successfully reported (Fassuliotis & Creighton 1982), but this production could not be sustained (Kaya 1993). Thus, pursuing this aspect with our current state of knowledge of mermithid physiology, particularly of A. unka, does not seem feasible. Perhaps, if BPH can be mass produced easily, in vivo production of the mermithid may be used to augment natural populations. In vivo production has been accomplished with the mermithids from the banded cucumber beetle (Creighton & Fassuliotis 1982) and from mosquitoes (Petersen 1984). In addition to production, however, methods to store the eggs or adults and timing of introduction into BPH populations need to developed. For the present, the primary approach will be conservation of the naturally-occurring populations of A. unka. Using this conservation approach with the mermithid, there is a need to implement an effective IPM program combining all the control tactics including biological control, chemical insecticides, resistant varieties, and cultural techniques to suppress BPH rather than relving only on insecticides and resistant varieties.

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