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ENTOMOGENOUS NEMATODE RESEARCH IN CHINA

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Almost eight hundred years ago, there were records of insects infected by nematodes in China. One of the ancient descriptions *(Gaoyou Zhouzhi)* wrote " year of chengyuan (1196), many grasshoppers died grasping weeds; there was one worm inside every insect eating its brain ". According to further investigations, it is thought that this is an accurate observation of these insects killed by nematodes (Peng, 1983). More recently, particularly in the last twenty or thirty years, intensive research on nematode parasites has been conducted all over the world and this field has become a scientific discipline : entomohelminthology. Although entomogenous nematode research in China may not be quite as advanced as in some other countries, it should be pointed out that research on this field in China, especially on practical utilization of nematodes to control insect pests has achieved some very promising results. The present paper gives a review of entomohelminthological research in China.

The Mermithidae

Five genera and eleven species of Mermithidae have been described from China. The insect hosts of these nematodes are included in seven orders, nineteen families, and 38 species. One of them is a resource insect, seven of them are natural enemies of insects, and all the others are agricultural and medical insect pests (Wang & Xu, 1984).

MERMITHIDS OF THE OAK-SILKWORM

The oak-silkworm *Antheraea pernyi* Guérin-Meneville, is a very important natural resource insect which is reared in the wild in northeast China. In 1937, a Japanese scientist collected a parasitic nematode from oak-silkworm in Liaoning Province in China, and named it *Pernyimermis* sp. From 1949, large scale surveys and investigations on the nematodes that infect the oak-silkworm have been conducted. As a result of wide collection and taxonomical investigations, two genera and six species of Mermithidae have been identified as the parasites of the oak-silkworm. They are *Amphimermis elegans* Hagmeier, 1912, *Amphimermis* sp., *Hexamermis micromphidis* Steiner, 1925, *H. arsenoidea* Hagmeier, 1912, *H. kirjanovae* Pologentsev & Artyuhovsky, 1958 and *H. brevis* Hagmeier 1912. Many nematode specimens were collected in the area where the Japanese found the nematode later named *Pernymermis* sp., but it was not possible to rediscover this; all nematodes from that area were *A. elegans* (Wei, 1982; Wu, Yan & Qian, 1983).

The mermithid nematodes have one generation a year in the outdoor rearing areas of the oak-silkworm in Liaoning Province. Their eggs, larvae and adults occur in the soil in autumn and winter and the mating period is in the middle of May. Eggs are laid from the middle of June to early July. The free-living infective larvae hatch from the eggs in late June. When it is raining or misty in the morning, and the trees are wet, they move up the tree trunks and migrate to the branches and leaves at a speed measured at 73 mm per minute. When they encounter the oak-silkworms, they enter the coeloms of the insects, mainly by penetrating the intersegmental membrane of the insect's abdomen. The infected oak-silkworms show symptoms of parasitism after one week. They become sluggish; feeding is reduced resulting in malnutrition; the intestine becomes thin and fragile; there is a lack of fat, and glands develop poorly or not at all. The nematodes grow quickly inside the silkworm larvae and after fifteen to 23 days of parasitism, they leave the insects, generally in the early morning (before the sun rises). The exit of the parasite results in death of the host oak-silkworm. This causes a high loss in the yield of oak-silkworm cocoons in Liaoning, Jiling, Shandong, Henan and Guizhou Provinces (Wei & Wu, 1981).

Satisfactory methods of controlling mermithids of the oak-silkworms have been worked out. Spraying 0.02 to 0.03 % Phenamiphos No. 1 or 0.005 to 0.01 % Phenamiphos No. 2 onto the oak tree leaves, on which the oak-silkworms feed, can successfully kill the nematodes inside the insects. Field trials resulted in 100 % mortality of the nematodes, and in large scale applications, mortalities were above 95 %. Phenamiphos retains its activity on the leaves for up to 28 days, and it does no harm to the silkworm (Wei, Qi & Wu, 1979).

It is of interest that researchers are now working on using these oak-silkworm parasitic nematodes to control orchard insect pests. Susceptibility tests show that *A. elegans*, *Amphimermis* sp., *H. micromphidis* and *H. arse-*

noidea infect nine families and 28 species of Lepidopterous pests of orchards. It was found that in the area where oak-silkworm were heavily infected, natural infection rates of mermithids in some other insect pests were quite high. There was 67.2 % natural infection of *Parasa consocia*, 66.7 % of *Nadata cristata* But. and 37.5 % of *Phalea assimilis* Brem. By rearing and artificially introducing the nematodes *A. elegans* and *H. micromphidis* to the non-nematode infected areas, there was a 53 % mortality rate in *N. cristata*, 45 % in *P. consocia*, 30 % in *Dendrolimus spectabilis* Butl and 27.7 % in *Hyphantria cunea* Drury (Wei & Wu, 1984).

MERMITHIDS OF THE ARMYWORM

The mermithid *Hexamermis* sp. parasitizing the armyworm *Pseudaletia separata* Walker appears in the wheat fields in Hebei, Henan and Shandong Provinces. Its natural infection rate is up to 70 %. The infection rate in wheat fields where cotton was cultivated earlier is ten times higher than that where rice was planted previously (52.6 % and 5.17 %). Adults of *Hexamermis* sp. mate and oviposit in March. The first stage nematodes emerge from the eggs in 30 to 40 days at 20°, and become second (infective) stages soon afterwards. The infective stages enter the insects by cuticular penetration using their mouth spines. Then they moult and develop into parasitic juveniles. The parasitic juveniles leave their insect hosts after two or three weeks and spend a free-living period (about a month) in the soil; they then moult for the last time into adult stages in the soil in June or July (Li, Jin & Pang, 1981; Wang, Bao & Xu, 1981; 1982).

In 1979, particular measures for the protection and utilization of the *Hexamermis* sp. to control the armyworm were taken over an area of more than 2 000 hectares of severely infested wheat fields in Henan Province. Careful surveys and further investigations based on the field observations proved that the natural infection rate of armyworm by the mermithid was as high as 80 %. Therefore, instead of applying insecticides or other chemicals to the fields, special action such as irrigating the fields to increase humidity was taken to improve nematode infection. Examinations in late May showed that in the fields where the original armyworm population density was 57.5 larvae per square meter, only six insects per square meter survived; where population was originally of 53.5/m^2 it dropped to 3.5, and no armyworm was found in the fields where there was originally 24.5 insects/m^2 (Chen, 1982; 1983; 1984). It is believed that as a result of mermithid infection, damage by armyworm was reduced to well below the economic threshold.

It was found in laboratory tests that the armyworm mermithid, *Hexamermis* sp. can also infect the larvae of *Agrotis ypsilon* Rottemberg, *Pieris rapae* Linnaeus, *Heliothis armigera* Hubner and *Spodoptera litura* Fabricius. Using the armyworm larvae as hosts it was possible to successfully subculture the mermithid and its reproductivity and infectivity were normal after three years successive rearing (Chen *et al.*, 1983).

MERMITHIDS OF THE BROWN PLANTHOPPER

There are two species of mermithids parasitizing brown planthopper *Nilaparvata lugens* : *Amphimermis* sp. and *Agamermis unka*. The former has an elongated body, and the spicules are long and screwed in the middle. The latter is short and has short spicules which are not curved (Yan, 1979; Xiao, 1982; Wan, 1983).

During field observations, it was found that more than 50 % of brown planthoppers were infected by mermithids in rice paddies in Southern China, with the highest levels (95 %) found in Hunan province. The eggs of *Amphimermis* sp. appear in the field from June to September each year. They hatch in fourteen days at 28°. The young juveniles enter their hosts one or two days after hatching. They spend their parasitic lives inside planthoppers for two to three weeks, and then exit from the hosts. *Nilaparvata lugens*, infected by the mermithid in its early stage, dies before it reaches its adult stage; those infected in the late stage may develop into adults, but the adult fecundity is greatly reduced (93.8 %), or it totally supressed. The host never survives after the exit of the nematode. The pre-adult mermithid lives in the soil at depths of 0 to 20 cm. Over the winter it moults and becomes a mature adult, and copulation occurs in the next May (Li, 1981; Chen, Yang & Shen, 1982).

There is a close correlation between natural infection rates of *Nilaparvata lugens* by the mermithids and the amount of rain or humidity in the field. In the high rainfall years, high infection rates were observed (Chen & Yang, 1980; 1985). Investigations in Hunan province show that no control measures are needed when the population density of the brown planthoppers is under 2 000 insects in one hundred paddy plants, provided the natural infection rate by the mermithids is above 75 %. By introducing the mermithids (500 infected brown planthoppers) into a rice field where no mermithids were found, 21.9 % control of the planthoppers by the nematodes was achieved in the next year, and the mermithid population in the field was found to be 147 500; in the following year, the infection rate increased to 30.4 %, and the nematode population reached 234 300. In the fields where planting and irrigation were conducted during the winter, the mermithids survived much better. Application of insecticides was found to be harmful to the mermithids (Li, 1979; 1981).

MERMITHIDS OF MOSQUITOES

Isomermis sp. of *Culex triaeniorhynchus* (Bao, Wang & Wu, 1981; Bao, 1984).

The mosquito *Culex triaeniorhynchus* is known to be an important disease vector of epidemic encephalitis B and filariasis. This mosquito is found in Jilin, Liaoning, Hubei and Fujian Provinces. The mermithid *Isomermis* sp. was found in larvae of *Culex triaeniorhynchus* collected in the suburbs of Wuhan City in 1981. The length of female adult *Isomermis* sp. is between 17 and 28 mm, averaging 21.6 mm; the body width is 0.10 to 0.14; the length of male is between 8 and 13 mm, averaging 11.1 mm; the width is 0.06 to 0.10. Many generations of the nematode *per annum* in the field were observed. It completes one life cycle in 22 days at 30° : nematodes which emerged from mosquito larvae become adults after four days, and after another four days mate. Oviposition commences three days later; five days afterward, pre-parasitic juveniles hatch from the eggs and they enter insect hosts within one day. The parasitic stage lasts five days before the nematode emerges.

Surveys in natural environments indicate that the mermithid *Isomermis* sp. parasitizes *Culex triaeniorhynchus* from April to November each year. The natural infection rate is from 5 to 48 %. The post-parasitic stage of the mermithid which emerges from the host in November moults into an adult in seven to ten days. The adults overwinter in the soil between 10 to 40 centimeters under the water.

Romanomermis jingdeensis of *Anopheles sinensis* Wiederman (Yang, Qian & Wu, 1980; Yang, 1983; Yang & Chen, 1983).

This nematode is found in the mosquito larvae of *Anopheles sinensis* collected from Jingde county of Anhui Province in 1979-1980, and has been identified as *Romanomermis jingdeensis.* It is readly distinguished from the other species of the genus with the exception of *R. culicivorax* and *R. iyengari* by its vagina which has a distinct flexure. In comparison with *R. culicivorax* and *R. iyengari*, the new species has a vagina with a more bent canal anterior to the midregion and relatively shorter spicules; the ratio of body length to spicule length (37.8) differs significantly ($P < 0.01$) from those in *R. culicivorax* (25.0) and *R. iyengari* (26.2). *R. jingdeensis* completes each generation in 21 to 36 days at 25-27°. A motile larva forms within ten days after the egg is laid, and the first moult takes place inside the egg. The preparasitic juvenile, which is short-lived, enters the host by cuticular penetration. The second moult occurs three or four days after it enters the insect. Its parasitic stage inside the insect lasts six to ten days. The postparasite kills the mosquito when it exists. The third and fourth moults occur simultaneously about seven days after it leaves its host, and after the moulting, adults usually copulate immediately. Oviposition periods range from 13-31 days and the number of eggs laid by each female varies from 725 to 3 791.

For evaluating the host range of the mermithid, eleven species of mosquitoes were tested as target organisms. Among the species tested, anophelines were found to be more susceptible than culicines. The infection rate of anopheline species decreases in the following order : *A. sinensis* > *A. lesteri anthropophagus* > *A. minimum* and *A. stephensi* > *A. dirus, Aedes aegypti, A. albopictus* and *Culex P. quinquefasciatus* are much less susceptible to the mermithid, whereas *C. tritaeniorhynchus, C. P. pallens* and *A. togoi* are not infected at all. *R. jingdeensis* has a short life cycle, and it parasitizes its natural mosquito hosts from April to September. It is thought to be a promising biological control agent against anopheline mosquitoes.

Another mermithid was found in the *Culicoides riethi* Kieffer from Chungking in 1977. The nematode causes ovarian degeneration and sterilization of its host (Jeu, 1977).

Steinernematidae and Heterorhabditidae

Steinernematids were first introduced into China in 1950s. From the late 1970s, various species or strains of Steinernematidae and Heterorhabditidae have been introduced into China from Australia, the United States and other parts of the world. In the recent years, *Steinernema* and *Heterorhabditis* species have been isolated from the soil and insect hosts in China (Liu & Zhang, 1982; Dai, 1984; Wang, 1984; Liu, 1985). Some of these nematodes are being used in biological control programs (Anon., 1983; Sha, 1985).

Nematode culture

A number of different species of insects that are easy to rear artificially in the laboratory such as *Galleria mellonella*, or to collect in the fields such as *Heliothis zeae* and *Chilo suppressalis* have been used for culturing nematodes of Steinernematidae and Heterorhabditidae. Old instar larvae of these insects are infected with the infective juveniles of these nematodes, and the resulting insect cadavers are put in collecting trays where emergent nematodes are collected in water. Autoclaved *Galleria* larvae placed on nutrient agar slants are used to keep successive monoxenic stock cultures of *Steinernema* spp. and most of them do not degenerate even after many years culture (Li *et al.*, 1981; Anon., 1983; Yu & Liang, 1983).

Bedding's (1976; 1981; 1984) three dimensional monoxenic culture methods for mass-rearing *Steinernema* and *Heterorhabditid* species have been used by many researchers. To solve problems concerned with availability of medium resources in China, investigations have been made into using plant materials such as wheat flour, corn flour, etc. together with some animal proteins and fats to make up artificial complex media for culturing the nematodes. Some promising results have been achieved, but the yields of nematodes produced by these media were not as high as that of Bedding's chicken offal medium.

Host range, resistance to chemicals and safety to vertebrates

In order to determine potential targets for *Steinernema* and *Heterorhabditis* species, the infectivities of these nematodes to various agricultural, forestry and medical insect pests have been tested. So far, of the more than 60 species of insect pests in ten orders that have been tested, 90 % were found to be susceptible. The infectivities of these nematodes to Lepidoptera, Hymenoptera and Coleoptera are particularly high, but they are very low, or the nematodes are non-infective to Homoptera, Hemiptera and Thysanoptera (Anon., 1982; Xu, 1983).

Ten different insecticides and six fungicides that were commercially used in agricultural practice were applied to *Steinernema feltiae* DD-136, *S. feltiae* Agriotos, *S. glaseri* and the steinernematid QI in various dosages. Results show that these nematodes are resistant to most of the chemicals in certain dosages. Their resistance to microbial insecticides is high, and that to fungicides and organic phosphorus insecticides is lower. DDVP (0,0-dimethyl-0-2, 2-dichlorovinylphosphate) and dichlor-diphenyl-trichlorethane are most harmful to the nematodes. Different nematode species or strains were found to show different resistances to chemicals. Among the above *Steinernema* species, *S. feltiae* has the highest resistance, *S. glaseri* next, and the steinernematid QI the lowest. When *S. feltiae* was put in 800 ppm solution of DDD (2, 2-bis-1, 1-dichloroethane) for 30 days, a 37.7 % survival rate was observed; no survivals were found with the other species placed in the same solution for three days (Qin, 1984; Xu, 1983).

A series of safety tests was made on *S. glaseri* since it has been found to be a potential agent for controlling surgarcane beetles. Rats, rabbits and monkeys were exposed to intranasal, intraperitioneal, per os, and dermally administered nematodes. Detailed analysis on the blood cells, haemoglobin and glutamic-pyruvic transaminase (GPT) revealed no symptoms in any of the animals tested (Wang & Liu, 1983; Wang, Qiu & Liu, 1983; Wang, Chen & Huang, 1984).

Utilization of nematodes for control

Peach fruit moth

The peach fruit moth *Carposina nipponensis* Walsingham is one of the most important insect pests in production of fruits such as apple, pear and date (jujube) in China. Because of the long term and high dosage application of chemicals to control this pest, the orchard ecosystem has been damaged and serious environmental pollution caused. Therefore, an integrated pest management program based on biological control of this insect is urgent. Since larvae of *C. nipponensis* enter the soil and spend several months there, it was considered more favourable to use nematodes to control the pest in the soil environment. It was found in laboratory tests that *C. nipponensis* is most susceptible to the nematode *Steinernema feltiae* Agriotos. The nematode infects the larvae, pupae and adults of the insect, and kills them in a very short time. In September, just before *C. nipponensis* larvae enter the soil, nematode suspensions (0.7 million nematodes per square meter) were sprayed onto the ground around the fruit trees; 98.4 % of the insect larvae, which dropped from the trees and burrowed into the soil to form their overwinter cocoons, were killed by the nematodes; 61.4 to 73.7 % of these died before their cocoons were formed. Following application of *S. feltiae* (0.5-0.9 million nematodes/m^2) to the soil to control the peach fruit moth larvae, which emerge from the overwinter cocoons in May or June, 91.8-95 % mortality was achieved. It was found that temperature and humidity conditions that are suitable for the larvae to leave their cocoons were also favourable for nematode infection. Dense orchards were found to be good for nematode survival. *S. feltiae* can overwinter and survive for over a year in the field after its application. In sandy soil, it has better migrating ability and was able to kill insect larvae three to five meters away from the nematode application position (Anon., 1983; Li *et al.*, 1984*a*).

In 1984, a *Heterorhabditis* species (8406) was isolated from the soil and from *C. nipponensis* larvae in Shandong Province. Preliminary studies on its morphology and life history were carried out. It was shown that *Heterorhabditis* sp. (8 406) is more likely to be able to penetrate the cocoon and kill the insect inside. The mortality rate of *C. nipponensis* larvae inside their cocoons caused by *Heterorhabditis* sp. (8 406) infection (85.1 %) was much higher than that of *S. feltiae* (37.1 %) (Li *et al.*, 1985). However the nematode 8 406 did not survive as well as *S. feltiae.*

Sugarcane beetle

In the sugarcane production areas in Southern China, one of the most harmful insect pests is the sugarcane beetle *Alissonotum impressicolle* Arrow. It has caused great losses to the sugarcane industry, particularly in recent years. The insect has one generation each year, and spends most of its life in the soil. It is very difficult to control it by applying chemical insecticides onto the ground, because the insect remains deep under the ground or drills into the sugarcane stem. Investigations on using nematodes to control the pest have been carried out since 1982. It has been shown that nine species or strains of *Steinernema* and *Heterorhabditis* can infect and kill the insect, but their infectivities vary significantly. At a dosage of 2 500 infectives for each scarab, the mortality resulting after one week was as follows : *S. glaseri* 100 %; *Heterorhabditis* sp. (8 406) 100 %; *H. heliothidis* (NZ) 82.6 %; *S. bibionis* 65.3 %; *Heterorhabditis* sp. (8 404) 56.5 %; *Heterorhabditis* sp. (C 1)

43.5 %; *Heterorhabditis* sp. (8 405) 34.75 %; *S. feltiae* 30.4 % and *Heterorhabditis* sp. (8 401) 4.3 % (Wang, Qiu & Lian, in press).

Field applications of *S. glaseri* to control the third instar scarabs in early spring achieved promising results. Nematode suspensions were sprayed onto the ground along the base of sugarcane plants (1 500 million nematodes per hectare), and 71.2 % mortality of the scarab resulted fifteen days after the application. At a later date, a more than 80 % reduction of the pest population was found compared to the control fields. The result of using *Heterorhabditis* sp. (8 406) against the sugarcane beetle in field applications was not as good as that of *S. glaseri*. In sandy soil, nematodes were found to have better migrating abilities, and caused much higher mortalities of the insect pest than in clay soil (Li *et al.*, 1983; Wang, Qiu & Lian, in press).

Army-worm

In laboratory tests, *S. feltiae* has a high infectivity to the armyworm, *Mythimna separatia* Walker. Since the armyworm lives and feeds on plants above the ground, enviromental factors such temperature and humidity are particularly significant. At 16°, *S. feltiae* is infective to the insect but the nematode has its highest infectivity when the temperature is between 25° and 28°; at 31°, its infectivity to the pest decreases, and it loses its infectivity at 34°. High humidity is another important factor for the nematode infection. When a high humidity was maintained for more than 12 hours in the laboratory, *S. feltiae* (0.3 million per m^2) produced over 90 % mortality of the armyworm. In the field environment, at least 24 hours of wet foliage is essential to obtain good results. In field trials, 80.5 % mortality was achieved when the suspension of 3 500 nematodes/m^2 was sprayed and 89.5 % mortality resulted when the dosage was 7 000 nematodes/m^2. A model which describes the nematode infection of the armyworm under various conditions has been constructed following the laboratory and field experiments. Using computer analysis, factors affecting the result of field application of the nematodes against armyworm can be predicted (Xia; 1984; Qin & Zhou, 1984).

Cabbage-worm

All of the *Steinernema* species infect the cabbageworm, *Pieris rapae* Linnaeus in laboratory tests, but *S. feltiae* DD-136 gives the best result. At a dosage of 50 infective juveniles to one cabbageworm, DD-136 produced 100 % mortality of the insect pest in 48 hours; 89.4 % mortality of the pest was obtained in 72 hours in field trials. The infection rate varies greatly with the time when the nematode is applied. Generally, it was found preferable to spray nematode suspension onto plants after the sun sets. Maintenance of a 15 hour or longer period at high humidity after nematode application resulted in higher mortality of the pest. Adding effective viscous material greatly assisted the nematode to attach onto the foliage, and therefore increased the resulting mortality (Anon., 1983; Li *et al.*, 1984*b*).

Mango branch borer

The mango branch borer, *Rhytidodera bowrigii* White, significantly affects mango production. *S. feltiae* DD-136 infects the larvae and pupae of this borer. Infected hosts are dead in three days, and thousands of infective nematodes come out from the cadaver after fifteen days. By injecting nematode suspension (2 ml with 22 000 infective juveniles) into branches infected with the pest, more than 80 % mortality resulted (Shen & Han, 1985).

Acknowledgements

The authors are grateful to Dr. R. A. Bedding and Ms. M. Tomlinson, CSIRO Division of Entomology, Australia for their advice and correction of the manuscript which makes the English publication of this article possible.

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Accepté pour publication le 16 janvier 1987.

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