Biological Control Potential of Neoaplectanid Nematodes[1]

Randy Gaugler[2]

Abstract: The neoaplectanids are among the most studied of all entomogenous nematodes. Because these nematodes kill their insect hosts, they are regarded as having excellent potential as biological control agents. While the host specificity of most entomogenous nematodes tends to limit their potential usefulness, the broad host range and high virulence of neoaplectanids make them attractive candidates for industrial development. Also, recent development of economical mass rearing procedures appears to make production on a commercial basis feasible. Infective stages may be stored for years under various laboratory conditions. Although entomogenous nematodes, as parasites, are exempt from government registration requirements, the mutualistic association of neoaplectanid nematodes with a bacterium will likely necessitate a detailed safety evaluation. Studies conducted to date indicate a lack of pathogenicity to mammals. Field trial success has been limited by the intolerance of infective stages to unfavorable environmental conditions, particularly low moisture. Applications against pests on exposed plant foliage have been especially disappointing. More encouraging and consistent results have been obtained in more favorable environments, including soil and aquatic habitats, but the most promising treatment sites may be cryptic habitats where infective stages are sheltered from environmental extremes. Cryptic habitats also exploit the ability of neoaplectanids to actively seek out hosts in recessed places where conventional insecticide applications are impractical. *Key words: Neoaplectana bibionis, Neoaplectana carpocapsae, Neoaplectana glaseri,* entomogenous nematodes.

Although nematodes are most evident as injurious parasites of man, animals, and plants, those species which attack invertebrates can play a significant role in limiting populations of agriculturally and medically important insect pests. Nematode-insect interactions may range from phoresy to obligate endoparasitism and include host death, sterility, reduced fecundity, delayed development, or aberrant behavior. The majority of these interactions are harmless or mildly debilitative; comparatively few entomogenous nematodes cause host mortality. Among those which do kill their hosts are members of the genus *Neoaplectana*. Reviewers have consistently noted that these nematodes show great promise for use as biological control agents of insect pests (25,57,76). Despite this acclaim, the neoaplectanids have yet to realize their potential.

As many as 20 species have been described within the genus *Neoaplectana;* however, Poinar (59) considers all but seven of these either as strains of earlier described species, as belonging in other genera, or as being insufficiently described. Only three species have been used in insect control attempts: *N. glaseri, N. bibionis,* and *N. carpocapsae*. First reported in 1954, *N. carpocapsae* has been studied more extensively than any other neoaplectanid. Consequently, most of our knowledge concerning the genus has been derived from this single species.

There is little doubt that neoaplectanids show great potential as pest control agents, but several difficulties remain to be fully resolved. This review will examine both the promise and the problems.

BIOLOGY

The ensheathed third-stage juvenile is the neoaplectanid invasive form which locates new hosts and initiates infections. Host finding by these infective-stage juveniles is an active process occurring in response to chemical stimuli provided by the host. *Neoaplectana carpocapsae,* for example, forms aggregations adjacent to insect larvae (63,68) and fecal components (69) and is capable of directed orientation up a gradient of carbon dioxide (18), a compound emitted by insect spiracles. When a suitable host is located, infective juveniles enter via natural body openings (i.e., mouth, anus, or spiracles), exsheath, and penetrate into the hemocoel. Here the nematodes release an associated bacterium from their intestinal lumen into the hemolymph. The bacterium rapidly multiplies and kills the host by septicemia. The nema-

Received for publication 12 December 1980.

[1]Symposium paper presented at the annual meeting of the Society of Nematologists, New Orleans, Louisiana, August 1980. Published by permission of the Director, New York State Museum, State Education Department, Journal Series No. 318.

[2]Senior Scientist, Biological Survey, New York State Museum, State Education Department, Albany, NY 12230. Continuing financial support from the National Institutes of Health (AI-15605) is gratefully acknowledged.

todes feed upon the bacteria and degraded host tissue, mature, mate, and produce two or three generations which emerge as infective-stage juveniles in search of new hosts.

The relationship between the nematode and its associated bacterium, *Xenorhabdus* (= *Achromobacter*) *nematophilus* in the case of *N. carpocapsae* (75), is one of classic mutualism. The bacterium requires the nematode for protection, transport, and penetration into the host hemocoel, while the nematode is dependent upon the bacterium for supplying nutrients essential for successful reproduction. The bacterium also establishes suitable conditions for nematode development by repressing cadaver colonization by other bacteria, thereby allowing the nematode to complete its life cycle without cadaver putrefaction. This bactericidal effect may be the result of defective bacteriophages produced by the bacterium (61). The only other genera of nematodes known to be mutualistically associated wth bacteria, *Heterorhabditis* (62) and *Steinernema* (51), are also parasites of insects.

HOST RANGE

The intense interest in *Neoaplectana* spp. as biological control agents is largely a function of their unusually broad host range. Nearly 250 insect species from 10 orders are reported to serve as hosts for *N. carpocapsae* (59); the host range of other neoaplectanids is probably no less extensive. The basis for this lack of host specificity is the associated bacterium, which kills the host so quickly that the nematodes are not hampered by a defense reaction or required to adapt to the host life cycle. Even in those few insects where invasion elicits a strong host reaction, encapsulation seldom protects the insect since the bacterium is not inactivated (81). Apparently, neoaplectanids kill any insect they penetrate.

Despite the impressive host range attributed to neoaplectanids, it is extremely unlikely that so many insects serve as hosts in nature. Most species described as susceptible were exposed in laboratory petri dishes under optimal conditions where host-parasite contact was assured. In nature, host range may be restricted by ecological and behavioral barriers (67). This point has frequently been illustrated in tests with *N. carpocapsae,* where initial laboratory successes could not be duplicated in the field (32,39,65,66,79).

SAFETY

Insect control agents being considered for wide application must have their safety established. Entomogenous nematodes are regarded as parasites rather than microbial pathogens by the U. S. Environmental Protection Agency (EPA), and as such are not subject to government registration (53). The specific bacterium associated with neoaplectanid nematodes is, however, a microbe and plays a major role in the infection process. Therefore, it is anticipated that registration of the nematode-bacterium complex will be required by EPA.

Although EPA guidelines for safety testing of neoaplectanids are unavailable, some mammalian pathogenicity tests have been conducted. No adverse effects were observed when high dosages of infective-stage juveniles of *N. carpocapsae* were fed to mice (70) and rats (57). In per os and intraperitoneal inoculations (17), infective stages did not cause detectable injury in rats, nor did the nematodes survive: juveniles administered per os were recovered dead in the feces, and intraperitoneally-injected juveniles were encapsulated by rat peritoneal macrophages. Kaya (35) has shown that homoiothermic parasitism is unlikely because growth of both the nematode and bacterium is inhibited at temperatures above 30 C. Thus, safety considerations should not prevent the use of neoaplectanids as agents of pest control.

PROPAGATION

An essential requirement in the utilization of any biological control agent is the capability of propagating large numbers of infective stages at an acceptable cost. Because neoaplectanids can be easily mass produced using either in vivo or in vitro methods, they have been widely available for field use. Dutky et al. (13) used larvae of the greater wax moth, *Galleria mellonella,* to propagate the DD-136 strain of *N. carpocapsae* in vivo and achieved yields of up to 200,000 infective juveniles per host. Al-

though the in vivo technique is simple and in wide usage, it is economically unsuited to large-scale rearing. Efficient mass production for commercial use or large-scale field trials is best accomplished through in vitro techniques. Neoaplectanids can be reared on a wide range of materials, reflecting the ability of the associated bacterium to convert proteinaceous substances into suitable media for nematode development (2,59). An artificial media, dextrose-veal infusion agar, was first used for propagation of *N. glaseri* in 1931 (21); however, the yields (400–800 nematodes/cm^2 of culture area) were inadequate for field tests. Subsequently, yeast-fermented potatoes (46) and ground veal pulp (45) were developed as culture media with progressively greater yields. A dog food based medium devised by House et al. (28) was used on a commercial scale to produce the DD-136 strain of *N. carpocapsae* at a cost of $1 (1971) per million infective juveniles (56). Hara et al. (26) later refined this method, stressing monoxenicity, to produce 125 million nematodes/wk from 100 dog food-agar petri dishes at a cost of $0.28 (1980) per million.

Abandoning petri dish methods as uneconomical, Bedding (2) developed and patented a rearing system which provided a large surface area to volume ratio, rearing the nematode-bacterium complex on Aspen wood wool coated with homogenized chicken heart. In a subsequent improvement (3), shredded plastic foam was soaked in pork kidney-beef fat homogenate and placed in 500-ml flasks. This method was suitable for rearing several species of neoaplectanid and heterorhabditid nematodes, with an average yield of 38 million *N. carpocapsae* juveniles per flask, at a cost of less than $0.02 (1980) per million. One technician can produce 16 million nematodes/wk with this technique (3).

Akhurst (1) has determined that there are two distinct forms of the bacterium mutualistically associated with neoaplectanid and heterorhabditid nematodes. The primary form, found in infective-stage juveniles, is initially produced, but is later displaced by the secondary form. Noting that nematode reproduction is optimal when only the primary form is present, and diminished when the secondary form predominates, Bedding (3) has established that his culture media should be inoculated with the primary form bacteria prior to nematode introduction.

Neoaplectanid infective stages may be stored for prolonged periods (5 yr) without loss of infectivity when kept at low temperatures (7 C) in an aerated aqueous suspension (13), or when stored in water shallow enough to allow oxygen diffusion (42). High survival is also reported when juveniles are held on moist filter paper at 3 C (29). An alternative approach to aqueous storage may be storage in paraffin oils, which hold up to 15 times as much oxygen as water (2).

ENVIRONMENTAL SENSITIVITY

The intolerance of neoaplectanid nematodes to environmental extremes is regarded as the key factor limiting their effectiveness as insect control agents. This sensitivity severely restricts their use as short-term control agents (biological insecticides) and precludes their use for long-term control (colonization) in many habitats.

There is considerable evidence showing that moisture is the most important environmental factor affecting *N. carpocapsae* field persistence. Aqueous spray applications of infective stages onto exposed surfaces quickly result in juvenile desiccation. Neoaplectanids sprayed on plant foliage in the field may survive for less than an hour (79), and even at 85% relative humidity, 98% mortality has occurred after 102 hr (33). Simons and Poinar (72) have indicated that foliar applications are ineffective because evaporation is rapid and, therefore, lethal. They concluded that juvenile survival depended on gradual desiccation and suggested that soil application was, therefore, more practical than application on foliage. In support of this conclusion, Moore (48) reported that infective stages survived 90 min on an exposed leaf surface, but up to 24 d in a quart of slowly dried soil.

Infective stages are sensitive to temperature extremes as well. Their activity decreases at temperatures exceeding 32 C, and 100% juvenile mortality occurs after 16 h of exposure at 37 C (71). At low temperatures (< 15 C), juvenile ability to induce host mortality is reduced (Gaugler, un-

published data), although high rates of host mortality have been achieved at temperatures as low as 11 C (47) and the infective nematodes will survive freezing (71). The optimal temperature for *N. carpocapsae* growth and reproduction is 25 C, with no development occurring at 10 C or above 33 C (35).

Solar radiation, usually regarded as the environmental factor most detrimental to microbial insecticides (31), also adversely affects neoaplectanid nematodes. Exposure of *N. carpocapsae* juveniles to 60 min of direct natural sunlight reduced their pathogenicity to *G. mellonella* larvae by 95%, while nematode development, reproduction, and pathogenicity were severely impaired following 3.5 min of irradiation with short (254 nm) ultraviolet light (15).

Other physical environmental parameters (e.g., pH, photoperiod, oxygen, salinity) are unlikely to be serious limiting factors in most target habitats. Elements of the biotic environment have been almost ignored as factors affecting the survival of neoaplectanids, even though nematodes are known to have many natural enemies (44). The mermithid nematode *R. culicivorax*, for example, is attacked by numerous predators (54,55) and at least one pathogen (73).

FIELD TRIALS

The first attempts to control insects with nematodes used *N. glaseri* against soil-inhabiting pests in the 1930s. Billions of infective stages were applied in these tests, primarily in the eastern United States against the Japanese beetle, *Popillia japonica*. Initial results were encouraging, with high grub mortality and nematode establishment in the test plots (22). Control was not obtained in subsequent applications, although establishment was sometimes recorded (23,24,30). Noting that early workers were unaware of the nematode's association with a symbiotic bacterium, Poinar (59) found it remarkable that establishment occurred at all, since the nematodes were cultured on media containing antimicrobial agents. These agents probably eliminated the associated bacterium, resulting in reduced nematode development, reproduction, and pathogenicity. It was not until 1977, when a natural field population of *N. glaseri* was reisolated, that the long suspected presence of the associated bacterium was finally confirmed (60). Interest in *N. glaseri* declined abruptly with the development in the 1940s of milky disease *(Bacillus popillae)* as an effective control agent of the Japanese beetle. However, the rediscovery of xenic populations of *N. glaseri* should revive interest in this nematode's use against insect pests in the soil.

Neoaplectana bibionis was originally considered to be a parasite of questionable pathogenicity (5) and has only recently found favor with biological control workers. In one of the most notable demonstrations of the biocontrol potential of neoaplectanids, Bedding and Miller (4) applied *N. bibionis* to 50,000 blackcurrant cane cuttings and obtained nearly total suppression of currant borer larvae. They concluded that this nematode could economically disinfest blackcurrant cuttings on a commercial basis.

Most attempts to control field populations of insects with nematodes have used strains of *N. carpocapsae*. Poinar (59) lists 34 field trials conducted up to 1978; at least 10 additional trials have also been reported recently (8,10,12,14,20,37,39,43,64,82), for a minimum of 44 trials in 12 countries. These tests have yielded inconsistent results, producing nearly as many failures as successes, but poor choice of target habitat would appear to account for many of the failures. For example, attempts to control foliage-feeding insects have been generally discouraging, with frequent reports of low mortality, insignificant population reduction, or inadequate crop protection. Interest in such applications has consequently dwindled. Only 6% (1/17) of the field tests reported in the last decade have been foliar applications, compared to 70% (9/13) during the 10-yr period following the nematode's discovery. Not coincidently, the incidence of successful trials also increased.

The use of neoaplectanids appears more promising against soil-inhabiting insects. Soil applications have reduced field populations of wireworms (37), root maggots (9, 80), and root weevils (8,27,74), although population reductions have not always resulted in adequate control. In contrast to foliar applications, where posttreatment

survival has generally been brief, viable juveniles have been recovered from soil treatment plots 2 (39) to 16 (27) months after application. A possible limitation to neoaplectanid use in the soil is that nematode movement may be restricted during periods of low soil moisture (39). Under favorable moisture conditions, infective-stage juveniles of *N. carpocapsae* can migrate in soil up to 14 cm laterally and 12 cm vertically to cause host infection (50).

The aquatic environment provides nearly optimal physical conditions for neoaplectanid survival but remains virtually unexploited as a site for field trials. Briand and Welch (6) briefly noted that applications of *N. carpocapsae* in mosquito pools reduced larval density and adult emergence, although establishment did not result. In a stream trial against blackfly larvae, Gaugler and Molloy (20) obtained 50% mortality of late instar *Simulium vittatum* despite low stream temperatures (9–12 C), but, again, nematode establishment did not occur. The effective use of *N. carpocapsae* against blackflies is restricted to late instar populations, because early instars are resistant and mid instars only moderately susceptible (19).

The most encouraging trials have been conducted against insects in protective, cryptic habitats, where the nematode is sheltered from environmental extremes. Such applications are attractive, not only because survival is enhanced, but also because they capitalize on the nematode's ability to seek out concealed pests, a capability not shared by chemical insecticides. Lindegren et al. (43) reported total suppression of first-instar carpenter worms infesting fig trees after treatment with the Mexican strain of *N. carpocapsae*. These insect pests are nearly invulnerable to conventional control methods because of their cryptic habitat (moist, frass-filled galleries in the tree heartwood). Similarly, larvae of the navel orangeworm, *Amyelois transitella*, a serious pest in almond orchards, are shielded from most insecticides by inhabiting the almond interior. Lindegren et al. (40), noting that the moist almond interior also provides a highly favorable environment for nematode survival, achieved up to 100% mortality of these insects in a 1976 spray application; a larger test the subsequent year resulted in a 55% reduction in the pest population and a 34% reduction in almond damage. Moore (49) demonstrated the nematode's ability to enter and survive in bark beetle tunnels, recording 44% mortality of brood and adults following spray applications on pine bark. In tests against the codling moth, *Carpocapsa pomonella*, on apple trees, Dutky (11) sprayed infective-stage *N. carpocapsae* juveniles onto tree trunks to produce more than 60% mortality among larvae seeking cocooning sites in bark crevices. He found that the nematodes could tolerate even extended periods of drought under the protective canopy of leaves.

PERSPECTIVE

The ultimate objective of most neoaplectanid research is to make these parasites widely available for effective pest control. The most logical step toward the accomplishment of this goal would be commercial production. Commercialization seems justified, since neoaplectanids possess nearly all the attributes of an ideal biological control agent (Table 1). However, several problems remain to be resolved.

The broad host range of neoaplectanids is certainly a desirable attribute. Industry is often reluctant to invest in a control agent attacking only one or two insect pests, because this results in a limited market. On the other hand, a broad host range may also be a liability since nontarget invertebrates, including beneficial insects, could be adversely affected. Although Kaya and Hotchkin (36) emphasize that even a detrimental affect on beneficials by *N. carpocapsae* should not preclude the nematode's

Table 1. Attributes and liabilities of neoaplectanid nematodes as biological control agents of insects.

Attributes	Liabilities
Broad host range	Broad host range
Safety	Registration
Laboratory storage	Commercial storage
High efficacy	Field persistence
Ease of production	
Ease of application	
Power of search	

development as a biological control agent, this potential problem has received scant attention and requires a full assessment based upon the results of both field and laboratory study.

All available evidence indicates that neoaplectanid nematodes are specific for arthropods and not hazardous to man and other vertebrates. Nevertheless, establishing their safety to the satisfaction of government regulatory agencies remains an obstacle to neoaplectanid development.

Laboratory storage of infective-stage juveniles is readily accomplished, but existing procedures appear unsuitable for commercial purposes, where viability must be preserved inexpensively, often under suboptimal conditions. Lyophilization or a related technique would seem ideal, but such methods are unlikely to be developed soon. Only Bedding's (2) work with paraffin oils seems to offer immediate promise, although detailed information on this method is unavailable. Perhaps problems in storage and shipping could be avoided if the nematodes were produced locally by "cottage industries" for immediate application.

Field applications frequently have shown that neoaplectanids possess high efficacy for controlling pest populations when applied under favorable conditions. Several successful attempts have been made to further increase efficacy, including the use of infective stages in conjunction with chemical (64) and microbial (34,38) agents. In novel experiments to exploit the behavioral characteristics of *N. carpocapsae,* Burman and Pye (7) suggest that infective stages might be conditioned to migrate toward the same soil temperature level as the target pest. Enhanced efficacy might also result if efforts were directed toward determining which species or strains are most effective against a particular pest. For example, *N. bibionis* was shown to be far superior to *N. carpocapsae* and *H. heliothidis* in finding and killing currant borers (4), while *N. glaseri* is regarded as best suited for use against soil insects (58).

Instances of low field efficacy have usually been attributed to poor nematode field persistence. Efforts aimed at extending persistence have focused on the development of evaporetardant (2,52,77) and photoprotectant (16) spray additives, but the value of these chemicals under field conditions has been inadequately evaluated. Another approach is nematode application only in selected habitats which provide shelter and moisture, and thus the greatest potential for their survival; e.g., soil, aquatic environment, and cryptic habitats. Soil is an especially attractive site because, as the natural reservoir for neoaplectanids, it offers the possibility of establishment and recycling. Colonization of other habitats seems unlikely; continued pest control in these sites would require repeated inundative applications. Thus, neoaplectanids should be considered as a "specialized tool" (78) and not for use against all pests in all habitats.

Previous field testing has been restricted to small plot treatments because of the relatively inefficient methods used to mass produce neoaplectanids. Advances with in vitro culture now allow large-scale testing at a cost approaching that of many conventional insecticides. It is hoped this breakthrough will induce industry to seriously reconsider commercial production. In the meantime, since the greatest cost associated with production is labor (3,26), the use of neoaplectanids would seem especially appealing in developing countries with inexpensive labor.

Another desirable neoaplectanid characteristic is high virulence. Whereas many microbial agents have only a slow debilitative action, neoaplectanid infection causes host mortality within 24–48 h, thus limiting pest damage. Such rapid host death might be expected to restrict dispersion, but "rapid spread" of *N. glaseri* by infected beetles has been reported (24).

Application technology should not be an impediment to biological control with neoaplectanids. Infective stages are resistant to most agricultural chemicals (3) and may be applied with conventional high-pressure spray equipment, either by ground or air, without loss of viability (11,41).

In contrast to chemical and microbial agents, which must rely on chance contact, neoaplectanid nematodes can actively search for hosts, permitting their use against even well-hidden target pests. Bedding and Miller (4) reported striking behavioral differences between neoaplectanid species in their abil-

ity to locate and parasitize insects inhabiting relatively inaccessible environments. Additional research is needed concerning neoaplectanid behavior if we are to successfully exploit such differences. Selection for strains with even greater searching capabilities also deserves investigation.

CONCLUSIONS

Neoaplectanid nematodes have been evaluated as biological control agents for more than 50 yr, yet none are in general use. Still, tremendous progress has been made in the last 5 yr and interest in their use has accelerated, as indicated by the recent creation of the *Neoaplectana Newsletter* (G. O. Poinar, Jr., ed.). In the United States alone, at least eight states support neoaplectanid research, and worldwide their control potential is being examined in Argentina, Australia, Canada, China, England, France, Italy, New Zealand, and the Soviet Union. In light of this gathering momentum, it seems likely that some of the potential of these nematodes for pest suppression will be realized during the 1980s.

LITERATURE CITED

1. Akhurst, R. J. 1981. Morphological and functional dimorphism in Xenorhabdus spp., bacteria symbiotically associated with the insect pathogenic nematodes Neoaplectana and Heterorhabditis. J. Gen. Microbiol., in press.

2. Bedding, R. A. 1976. New methods increase the feasibility of using Neoaplectana spp. (Nematoda) for the control of insect pests. Pp. 250-254 *in* Proc. Int. Colloq. Invertebr. Pathol., Kingston.

3. Bedding, R. A. 1981. Low cost, in vitro mass production of Neoaplectana and Heterorhabditis species, (Nematoda) for field control of insect pests. Nematologica, in press.

4. Bedding, R. A., and L. A. Miller. 1981. Disinfesting blackcurrant cuttings of Synanthedon tipuliformis using the insect parasitic nematode, Neoaplectana bibionis. Environ. Entomol., in press.

5. Bovien, P. 1937. Some types of association between nematodes and insects. Viden. Meddel. Dansk Naturhist. Foren 101:1-114.

6. Briand, L. J., and H. E. Welch. 1963. Use of entomophilic nematodes for insect pest control. Phytoprotection 44:37-41.

7. Burman, M., and A. E. Pye. 1980. Neoaplectana carpocapsae: Movements of nematode populations on a thermal gradient. Exper. Parasitol. 49: 258-265.

8. Burman, M., A. E. Pye, and N. O. Nöjd. 1979. Preliminary field trial of the nematode Neoaplectana carpocapsae against larvae of the large pine weevil, Hylobius abietus (Coleoptera, Curculionidae). Ann. Entomol. Fennici 45:88.

9. Cheng, H. H., and G. E. Bucher. 1972. Field comparison of the neoaplectanid nematode DD 136 with diazinon for control of Hylemya spp. on tobacco. J. Econ. Entomol. 65:1761-1763.

10. Drooz, A. T. 1960. The larch sawfly: Its biology and control. USDA Tech. Bull. No. 1212.

11. Dutky S. R. 1959. Insect microbiology. Adv. Appl. Microbiol. 1:175-200.

12. Dutky, S. R. 1967. An appraisal of the DD-136 nematode for the control of insect populations and some biochemical aspects of its host-parasite relationships. Proc. Joint US-Japan Seminar on Microbial Control of Insect Pests, Fukuoka, pp. 139-140.

13. Dutky, S. R., J. V. Thompson, and G. E. Cantwell. 1964. A technique for the mass propagation of the DD-136 nematode. J. Insect Pathol. 6: 417-422.

14. Finney, J. R., and C. Walker. 1977. The DD-136 strain of Neoaplectana sp. as a potential control agent for the European elm bark beetle, Scolytus scolytus. J. Invertebr. Pathol. 29:7-9.

15. Gaugler, R., and G. M. Boush. 1978. Effects of ultraviolet radiation and sunlight on the entomogenous nematode, Neoaplectana carpocapsae. J. Invertebr. Pathol. 32:291-296.

16. Gaugler, R., and G. M. Boush. 1979. Laboratory tests on ultraviolet protectants of an entomogenous nematode. Environ. Entomol. 8:810-813.

17. Gaugler, R., and G. M. Boush. 1979. Nonsusceptibility of rats to the entomogenous nematode, Neoaplectana carpocapsae. Environ. Entomol. 8: 658-660.

18. Gaugler, R., L. LeBeck, B. Nakagaki, and G. M. Boush. 1980. Orientation of the entomogenous nematode Neoaplectana carpocapsae to carbon dioxide. Environ. Entomol. 9:649-652.

19. Gaugler, R., and D. Molloy. 1981. Instar susceptibility of Simulium vittatum (Diptera: Simuliidae) to the entomogenous nematode, Neoaplectana carpocapsae. J. Nematol. 13:1-5.

20. Gaugler, R., and D. Molloy. 1981. Field evaluation of the entomogenous nematode, Neoaplectana carpocapsae as a biological control agent of black flies (Diptera: Simuliidae). Mosquito News, in press.

21. Glaser, R. W. 1931. The cultivation of a nematode parasite of an insect. Science 73:614.

22. Glaser, R. W. 1932. Studies on Neoaplectana glaseri, a nematode parasite of the Japanese beetle (Popillia japonica). N. J. Dept. Agric. Circ. 211: 3-34.

23. Glaser, R. W., and C. C. Farrell. 1935. Field experiments with the Japanese beetle and its nematode parasite. J. N. Y. Entomol. Soc. 43:345-371.

24. Glaser, R. W., E. E. McCoy, and H. B. Girth. 1940. The biology and economic importance of a nematode parasitic in insects. J. Parasitol. 26:479-495.

25. Gordon, R., and J. M. Webster. 1974. Biological control of insects by nematodes. Helminthol. Abstr. Ser. A 43:327-347.

26. Hara, A. H., J. E. Lindegren, and H. K. Kaya. 1981. Monoxenic mass-production of the entomogenous nematode, Neoaplectana carpocapsae Weiser on dog food-agar medium. USDA/SEA, Adv. Agric.

Technol. Western Ser., in press.

27. Harlan, D. P., S. R. Dutky, G. R. Padgett, J. A. Mitchell, Z. A. Shaw, and F. J. Bartlett. 1971. Parasitism of Neoaplectana dutkyi in white-fringed beetle larvae. J. Nematol. 3:280-283.

28. House, H. L., H. E. Welch, and T. R. Cleugh. 1965. Food medium of prepared dog biscuit for the mass-production of the nematode DD136 (Nematoda: Steinernematidae). Nature 206:847.

29. Howell, J. F. 1979. New storage methods and improved trapping techniques for the parasitic nematode Neoaplectana carpocapsae. J. Invertebr. Pathol. 33:155-158.

30. Hoy, J. M. 1955. The use of bacteria and nematodes to control insects. N. Z. Sci. Rev. 13:56-58.

31. Ignoffo, C. M., and D. L. Hostetter. 1977. Environmental stability of microbial insecticides. Symposium Entomological Society of America, Dec. 1974, Minneapolis, Minn., Misc. Publ. Entomol. Soc. Amer. 10:1-80.

32. Jaques, R. P. 1967. Mortality of five apple insects induced by the nematode DD-136. J. Econ. Entomol. 60:741-743.

33. Kamionek, M., I. Maslana, and H. Sandner. 1974. The survival of invasive larvae of Neoaplectana carpocapsae Weiser in a waterless environment under various conditions of temperature and humidity. Zesz. Prob. Post. Nauk Roln. 154:409-412.

34. Kamionek, M., H. Sandner, and H. Seryczynska. 1974. Combined action of Paecilomyces farinosus Dicks (Brown et Smith) (Fungi imp: Monaliales) and Neoaplectana carpocapsae Weiser, 1955 (Nematoda: Steinernematidae) on certain insects. Acta Parasitol. Polon. 22:357-363.

35. Kaya, H. K. 1977. Development of the DD-136 strain of Neoaplectana carpocapsae at constant temperatures. J. Nematol. 9:346-349.

36. Kaya, H. K., and P. G. Hotchkin. 1981. The nematode Neoaplectana carpocapsae Weiser and its effect on selected ichneumonid and braconid parasites. Environ. Entomol., in press.

37. Kovacs, A., K. V. Descö, G. Poinar, and A. De Leonardis. 1980. Prove di lotta contro insetti con applicazione di nematodi entomogeni. Pp. 449-456 *in* Atti Gior. Fitopathol.

38. Lam, A. B. Q., and J. M. Webster. 1972. Effect of the DD-136 nematode and of a β-exotoxin preparation of Bacillus thuringiensis var. thuringiensis on leatherjackets, Tipula paludosa larvae. J. Invertebr. Pathol. 20:141-149.

39. Lewis, L. C., and E. S. Raun. 1978. Laboratory and field evaluation of the DD-136 strain of Neoaplectana carpocapsae for control of the European corn borer, Ostrinia nubilalis. Iowa State J. Res. 52:391-396.

40. Lindegren, J. E., C. E. Curtis, and G. O. Poinar, Jr. 1978. Parasitic nematode seeks out navel orangeworm in almond orchards. Calif. Agric. 32: 10-11.

41. Lindegren, J. E., J. E. Dibble, C. E. Curtis, T. T. Yamashita, and E. Romero. 1981. Compatability of NOW parasite with commercial sprayers. Calif. Agric. 35:16-17.

42. Lindegren, J. E., D. F. Hoffman, S. S. Collier, and R. D. Fries. 1979. Propagation and storage of Neoaplectana carpocapsae Weiser using Amyelois transitella (Walker) adults. USDA/SEA, Adv. Agric. Technol., Western Series No. 3.

43. Lindegren, J. E., T. T. Yamashita, and W. W. Barnett. 1981. Parasitic nematode may control carpenter worm in fig trees. Calif. Agric. 35:25-26.

44. Mankau, R. 1980. Biological control of nematode pests by natural enemies. Ann. Rev. Phytopathol. 18:415-440.

45. McCoy, E. E., and H. B. Girth. 1938. The culture of Neoaplectana glaseri on veal pulp. N. J. Dept. Agric. Circ. 285:3-12.

46. McCoy, E. E., and R. W. Glaser. 1936. Nematode culture for Japanese beetle control. N. J. Dept. Agric. Circ. 265:1-10.

47. Molloy, D., R. Gaugler, and H. Jamnback. 1980. The pathogenicity of Neoaplectana carpocapsae to blackfly larvae. J. Invertebr. Pathol. 36: 302-306.

48. Moore, G. E. 1965. The bionomics of an insect-parasitic nematode. J. Kans. Entomol. Soc. 38:101-105.

49. Moore, G. E. 1970. Dendroctonus frontalis infection by the DD-136 strain of Neoaplectana carpocapsae and its bacterium complex. J. Nematol. 2:341-344.

50. Moyle, P. L., and H. K. Kaya. 1981. Dispersal and infectivity of the entomogenous nematode, Neoaplectana carpocapsae Weiser (Rhabditida: Steinernematidae), in soil. J. Nematol., in press.

51. Mrácek, Z. 1977. Steinernema kraussei, a parasite of the body cavity of the sawfly, Cephaleia abietis, in Czechoslovakia. J. Invertebr. Pathol. 30: 87-94.

52. Nash, R. F., and R. C. Fox. 1969. Field control of the Nantucket pine tip moth by the nematode DD-136. J. Econ. Entomol. 62:660-663.

53. Nickle, W. R. 1976. Toward the commercialization of a mosquito mermithid. Proc. First Int. Colloq. Invertebr. Pathol., Kingston 1:241-244.

54. Platzer, E. G., and L. L. MacKenzie-Graham. 1978. Predators of Romanomermis culicivorax. Proc. Calif. Mosq. Vect. Contr. Assoc. 46:93.

55. Platzer, E. G., and L. L. MacKenzie-Graham. 1980. Cyclops vernalis as a predator of the preparasitic stages of Romanomermis culicivorax. Mosquito News 40:252-257.

56. Poinar, G. O., Jr. 1971. Use of nematodes for microbial control of insects. Pp. 181-203 *in* H. D. Burges and H. W. Hussey, eds., Microbial control of insects and mites. New York: Academic Press.

57. Poinar, G. O., Jr. 1972. Nematodes as facultative parasites of insects. Annu. Rev. Entomol. 17: 103-122.

58. Poinar, G. O., Jr. 1978. Generation polymorphism in Neoaplectana glaseri Steiner (Steinernematidae: Nematoda), redescribed from Strigoderma arboricola (Fab.) (Scarabaeidae: Coleoptera) in North Carolina. Nematologica 24:105-114.

59. Poinar, G. O., Jr. 1979. Nematodes for biological control of insects. Boca Raton, Florida: CRC Press.

60. Poinar, G. O., Jr., and W. M. Brooks. 1977. Recovery of the entomogenous nematode, Neoaplectana glaseri Steiner from a native insect in North Carolina. IRCS Med. Sci.: Cell Memb. Biol.; Environ. Biol. Med.; Microbial., Parasitol., Infect.

Dis. 5:473.

61. Poinar, G. O., Jr., R. Hess, and G. Thomas. 1980. Isolation of defective bacteriophages from Xenorhabdus spp. (Enterobacteriaceae). IRCS Med. Sci.: Cell Memb. Biol.; Environ. Biol. Med.; Microbial., Parasitol., Infect. Dis. 8:141.

62. Poinar, G. O., Jr., G. M. Thomas, and R. Hess. 1977. Characteristics of the specific bacterium associated with Heterorhabditis bacteriophora (Heterorhabditidae; Rhabditida). Nematologica 23:97-102.

63. Pye, A. E., and M. Burman. 1978. Neoaplectana carpocapsae: Infection and reproduction in large pine weevil larvae, Hylobius abietis. Exp. Parasitol. 46:1-11.

64. Quattlebaum, E. C. 1980. Evaluation of fungal and nematode pathogens to control the red imported fire ant, Solenopsis invicta Buren. Ph.D. thesis, Clemson University.

65. Reed, E. M., and P. B. Carne. 1967. The suitability of a nematode (DD-136) for the control of some pasture insects. J. Invertebr. Pathol. 9:196-204.

66. Reese, K. M. 1971. Navy fights Formosan termite in Hawaii. Chem. Eng. News, Oct. 11, p. 52.

67. Salt, G. 1970. Cellular defense reactions of insects. London: Cambridge University Press.

68. Schmidt, J., and J. N. All. 1978. Chemical attraction of Neoaplectana carpocapsae (Nematoda: Steinernematidae) to insect larvae. Environ. Entomol. 7:605-607.

69. Schmidt, J., and J. N. All. 1979. Attraction of Neoaplectana carpocapsae (Nematoda: Steinernematidae) to common excretory products of insects. Environ. Entomol. 8:55-61.

70. Schmiege, D. C. 1962. The biology and host-parasite relationships of a neoaplectanid nematode parasitic on some forest insect pests. Ph.D. thesis, University of Minnesota, St. Paul.

71. Schmiege, D. C. 1963. The feasibility of using a neoaplectanid nematode for control of some forest insect pests. J. Econ. Entomol. 56:427-431.

72. Simons, W. R., and G. O. Poinar, Jr. 1973. The ability of Neoaplectana carpocapsae (Steinernematidae: Nematodea) to survive extended periods of desiccation. J. Invertebr. Pathol. 22:228-230.

73. Sterling, A. M., and E. G. Platzer. 1978. Catenaria anguillulae in the mermithid nematode Romanomermis culicivorax. J. Invertebr. Pathol. 32:348-354.

74. Tedders, W. L., D. J. Weaver, and E. J. Wehunt. 1973. Pecan weevil: Suppression of larvae with the fungi Metarrhizium anisopliae and Beauveria bassiana and the nematode Neoaplectana dutkyi. J. Econ. Entomol. 66:723-725.

75. Thomas, G. M., and G. O. Poinar, Jr. 1979. Xenorhabdus gen. nov., a genus of entomopathogenic, nematophilic bacteria of the family Enterobacteriaceae. Int. J. Syst. Bact. 29:352-360.

76. Webster, J. M. 1972. Nematodes and biological control. Pp. 469-495 *in* J. M. Webster, ed., Economic nematology. London: Academic Press.

77. Webster, J. M., and J. F. Bronskill. 1968. Use of Gelgard M and an evaporation retardant to facilitate control of larch sawfly by a nematode-bacterium complex. J. Econ. Entomol. 61:1370-1373.

78. Welch, H. E. 1971. Various target species: Attempts with DD-136. Biological control programmes against insects and weeds in Canada, 1959-1968. Tech. Comm., CIBC 4:62-66.

79. Welch, H. E., and L. J. Briand. 1961. Test of the nematode DD 136 and an associated bacterium for control of the Colorado potato beetle, Leptinotarsa decemlineata (Say). Can. Entomol. 93:759-763.

80. Welch, H. E., and L. J. Briand. 1961. Field experiment on the use of a nematode for the control of vegetable crop insects. Proc. Entomol. Soc. Ont. 91:197-202.

81. Welch, H. E., and J. F. Bronskill. 1962. Parasitism of mosquito larvae by the nematode, DD136 (Nematoda: Neoaplectanidae). Can. J. Zool. 40:1263-1268.

82. York, G. T. 1957. European corn borer research. P. 121 *in* USDA Station annual report, Ankeny, Iowa.