

NEMATODE-BASED BIOLOGICAL CONTROL OF GERMAN COCKROACHES

STEPHEN MANWEILER¹, ARTHUR APPEL², AND TERRY WEBER¹

¹biosys, 1057 East Meadow Circle, Palo Alto, CA 94303 USA, ²Auburn University, Department of Entomology, 301 Funchess Hall, Auburn, AL 36849 USA

Abstract—German cockroaches (*Blattella germanica* (L.)) are known to be susceptible to infection by the entomopathogenic nematode, *Steinernema carpocapsae* (Weiser) (Koehler *et. al.*, 1992). Nematodes placed on moist filter paper in petri dishes (50 nematodes per cm²) killed all adult cockroaches after 3 days of exposure at 25°C. During a 4-week long small chamber test (32 cm × 25 cm × 10 cm) in which 24 adult cockroaches (12 female, 12 male) were replaced each week, prototype bait stations containing 2 million nematodes caused a weekly mortality of about 80%. Separate food, water, and harborage were available throughout every test.

Groups of adult and nymphal cockroaches (6 female [carrying oothecae], 6 male, 12 younger instars [1-2], 12 older instars [4-5]) more like field populations were exposed for 4 weeks to stations containing 2 million nematodes to study age-related effects (cockroaches were *not* replaced). The overall cumulative weekly decrease was 50.3%, 60.5%, 75.8%, and 91.5% (corrected for control mortality)(Abbott, 1925). Virtually all females and older instars were killed the first week, significantly reducing the fecundity of the population. Most cockroaches surviving the first 2 weeks were young instar nymphs that are less susceptible to nematodes. These young nymphs were killed as they grew and became more susceptible.

In 2 apartment trials in 1991 and 1992, in Opelika, Alabama, German cockroach populations were reduced significantly by stations containing 2 million nematodes 1, 2, 4, 8, and 12 weeks post treatment. Reduction levels were comparable to decreases observed in apartments treated with Combat bait stations.

These studies demonstrate that entomopathogenic nematodes delivered in a control station represent a viable technology that can be used in a successful biological control program.

INTRODUCTION

Entomopathogenic Nematodes

Nematodes in the genus *Steinernema* parasitize a wide range of insect species (Poinar, 1975; 1979) including cockroaches (Koehler *et. al.*, 1992, Corpus and Sikorowski, 1992). Insect hosts are parasitized by a third stage infective juvenile (IJ) nematode that locates the insect, either by following CO₂ or temperature gradients or waiting for the host to pass by. *Steinernema* species enter the insect body through natural openings (i.e., spiracles, anus, mouth). Once inside the hosts haemocoel, the IJ releases a symbiotic bacterium (*Xenorhabdus* sp.) that multiplies, kills the host, and renders the host interior conducive to nematode reproduction. The nematodes feed upon the *Xenorhabdus*, develop into fourth stage juveniles and then into adults. *Steinernema* IJs develop into adult males or females that mate and give rise to a second generation of progeny. Several generations can develop in this manner, the number dependent upon the size of the insect host. When host resources are nearly depleted the nematodes simultaneously become IJs that are resistant to the environment outside of the host. Only this stage (IJ) can survive outside of an insect host in nature (Georgis, 1992).

Commercial Availability

Products containing Steinernematid nematodes have only recently become commercially available. A major reason has been the cost of production, a cost that has been reduced enough to compete with some chemicals only through the development of reliable in vitro rearing methods (Georgis, 1990). Most available products are labeled for use against agricultural or ornamental plant pests (Georgis, 1992).

Cockroach Control

In recent years the desire to reduce exposure to insecticides has supported the development of enclosed stations containing insecticidal baits for cockroach control. Several different insecticides

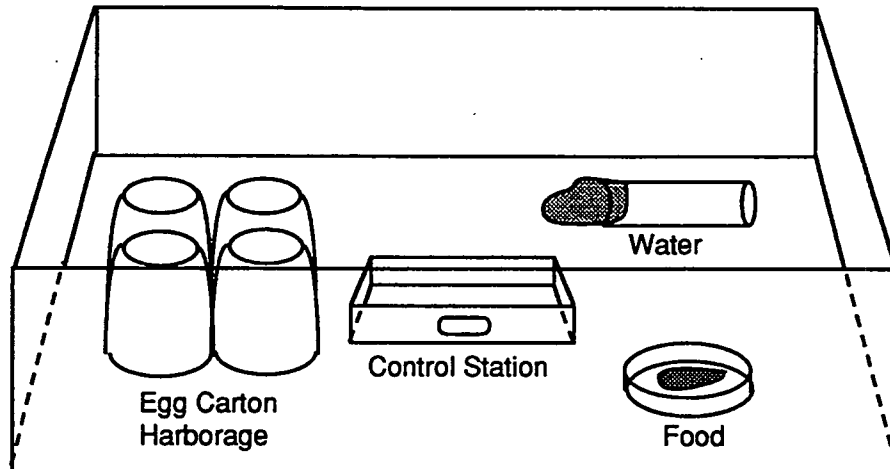


Figure 1. Small chamber unit including the nematode control station, food, water, and harborage.

have been employed; designs containing hydramethylnon have been among those that controlled German cockroaches (*Blattella germanica* (L.)) effectively (Appel, 1990).

Station Development Strategy

In order for a nematode-based product to effectively control a pest, an application strategy that ensures adequate exposure of the pest to nematodes must be employed (Begley, 1990; Klein, 1990; Georgis, 1990, 1992). Application of nematodes against soil-inhabiting pests is the most common situation. When moisture is sufficient a common reason for control failure is inadequate contact between the target and nematodes (Klein, 1990; Georgis and Gaugler, 1991). Employing nematodes against German cockroaches indoors involves bringing the nematodes and cockroaches together in an environment that preserves nematode viability and attracts, or at least does not repel the cockroaches. An enclosed control station is one way to achieve this. Our goal was to demonstrate that nematodes in an enclosed station could reduce German cockroach densities in apartments as well as the most efficacious insecticidal bait stations.

MATERIALS & METHODS

Cockroaches

German cockroaches used in all lab experiments were reared at biosys in aquaria containing food (Gainesburger), water (cotton wick inserted through the lid of a plastic jar) and cardboard harborage. The original cockroaches came from a colony kept at S. C. Johnson (Racine, WI) that is considered to not be resistant to any insecticides. All colonies were held at $25 \pm 2^\circ\text{C}$, $40 \pm 5\%$ relative humidity, and a 12:12 (light:dark) photoperiod. Cockroaches were anesthetized with CO_2 for easier transfer to experimental units.

Small Chamber Unit

Each group of cockroaches was housed in a clear plastic sweater box ($32\text{cm} \times 25\text{cm} \times 10\text{cm}$)(Fig. 1). A band of fluon (Northern Products, Inc.) extending 4–5cm down from the inner top edge prevented cockroach escape. Food (Gainesburger), water (cotton plugged glass tube) and harborage (10cm square piece of cardboard egg carton) were placed in each sweater box. All experiments were performed at $25 \pm 2^\circ\text{C}$, $40 \pm 5\%$ relative humidity, and a 12:12 (light:dark) photoperiod.

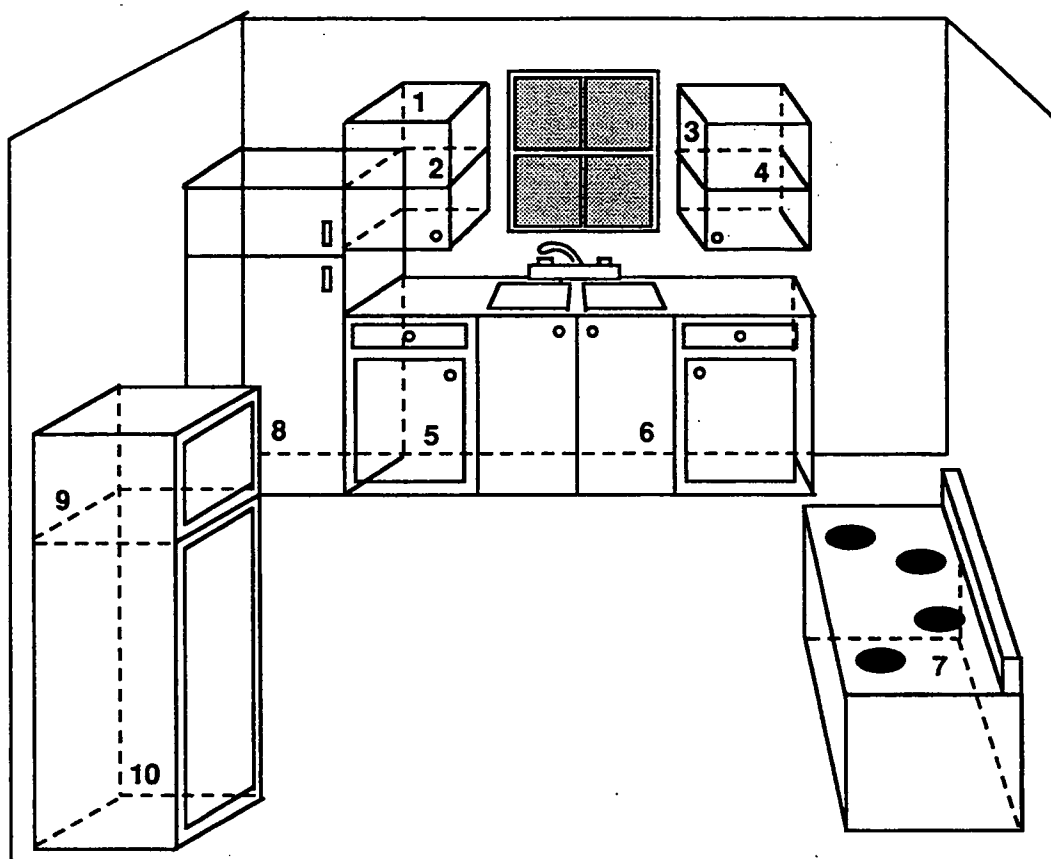


Figure 2. Placement of ten sticky traps in apartments.

Prototype Station

The nematode station consisted of a plastic shell (9cm × 9cm × 1.5cm) enclosing a water reservoir, a nematode source, and an interior configured to maximize surface area. Each station (Fig. 1) contained 2 million nematodes (*Steinernema carpocapsae* (Weiser) strain All) and about 110 grams of water initially.

Weekly Efficacy Test

The goals of this test were to demonstrate that the station remained as lethal to cockroaches the fourth week as it was the first week and to measure the water loss rate. Single prototype stations were placed in each of three sweater boxes. Three more did not receive stations (untreated control). Initially, 12 male and 12 female cockroaches were placed in each sweater box. The number of living and dead cockroaches was tallied every 7 days after which all cockroaches were replaced with a new set of 12 male and 12 female cockroaches. Food and water were replaced when cockroaches were replaced. This continued for 4 weeks.

All components of each bait station were weighed before assembly and again just after their reservoirs had been filled with water. Immediately thereafter these stations were placed into sweater boxes. When cockroaches were replaced, each bait station was weighed and placed back into the sweater box from which it came. All dead cockroaches were removed before the stations were weighed. Water reservoirs were not refilled.

Mixed-age Cockroach Tests

These tests were performed to see how the station would affect different cockroach age groups and population growth rates. Single prototype stations were placed in each of four sweater boxes. Four more did not receive stations (untreated control). Initially, 12 large nymphs (instar 4–6), 12 small nymphs (instar 1–3), six male and six female cockroaches were placed in each sweater box. All selected females were carrying oothecae. All cockroaches were anesthetized with CO₂ and tallied every 7 days. Developmental stage and sex (adults only) were recorded. Cockroaches were *NOT* replaced. Mortality was corrected for control mortality using Abbott's method (Abbott, 1925). Food and water were replaced when cockroaches are examined. This continued for seven weeks.

This experiment was repeated using a different batch (production run) of nematodes. The same protocol was followed except that this experiment was terminated after four weeks.

Apartment Trials

Thirty apartments were selected from 2- and 8-unit apartment buildings at the Opelika Housing Authority located in Opelika, Lee Co., Alabama to see if nematodes could significantly suppress cockroach infestations.

Cockroach populations were estimated using 10 sticky traps placed in the kitchen in locations that contacted a vertical surface such as a wall or part of an appliance (Fig. 2). Traps were collected 7 days after placement and evaluated in the lab. Cockroach populations were estimated before treatment and 1, 2, 4, 8, and 12 weeks after treatment.

Three groups of 6 apartments per group were chosen so that the initial distributions of cockroach infestations within each group were as similar between groups as possible. Twelve nematode stations were placed in approximately the same places (11 in the kitchen and 1 in the bathroom) in each of 6 apartments. Twelve Combat bait stations were placed in the same places as nematode stations in each of the second group of 6 apartments. No stations were placed in the remaining 6 apartments. Nematode stations were replaced 4 and 8 weeks after placement of the initial stations. Combat stations were not replaced because the label instructions did not indicate that replacement was necessary. The initial set of sticky traps was placed on 3 June 1991, treatments were placed on 18 June 1991, and 12-week sticky traps were collected on 17 September 1991.

This experiment was repeated between 7 July 1992 and 1 October 1992. The protocol of the 1991 trial was utilized except that Combat stations were replaced when nematode stations were replaced.

The mean percent change (precount compared to post treatment count) for the untreated control and nematode and Combat treatments were compared with the least significant means procedure on the log reduction ratio (SAS Institute, 1985).

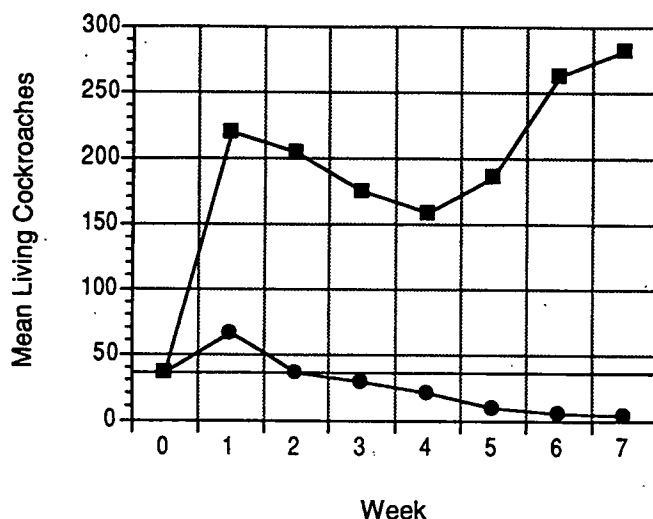


Figure 3. Mean number of living cockroaches (all stages combined) in chambers treated with nematode stations (●) or nothing (■).

RESULTS

Weekly Efficacy Test

Mean weekly cockroach mortality was 82%, 79%, 84%, and 79%. Cumulative percent water loss of the nematode station averaged 292%, 592%, 862%, and 964% weeks 1–4, respectively. Live IJs were observed nictating in 4-week old stations.

Mixed-age Cockroach Tests

During the first week, the number of cockroaches in both the nematode treatment and untreated control increased although the increase in the nematode treatment was only 30% as large as that observed in the untreated control. Between the second and seventh weeks cockroach populations exposed to prototype control stations decreased linearly each week. The untreated control experienced a weekly decrease through the fourth week followed by a weekly increase during weeks 5–7 (Fig. 3).

In the repeat test, the pattern of mortality of cockroaches exposed to nematodes was very similar to that observed in the first test; cockroach populations increased during the first week and declined each week thereafter. The population in the untreated control increased the first week, decreased the second and third week, and increased the fourth week (Fig. 4).

Both treatments (nematode and control) began with populations equally divided into three age groups (adults, instar 4–6 nymphs, instar 1–3 nymphs). First week increases consisted entirely of new first instar nymphs emerging from oothecae carried by females. Virtually all female and older instar (4–6) nymphs exposed to nematodes were killed the first week. In some cases oothecae were killed along with their mother. By the second week only a few males survived along with young nymphs. As the young nymphs developed they were killed by nematodes (Fig. 4).

In the untreated control, the number of older instar nymphs decreased each week through the third week as they became adults. This caused a gradual increase in the number of adults through the third week. Most mortality was experienced by young nymphs; they decreased both the second and third weeks. A large increase in older nymphs (developing from younger nymphs) and younger nymphs (from a new set of oothecae) occurred the fourth week. A slight decrease in adults occurred the fourth week (Fig. 4).

Apartment Trials

In the 1991 trial nematodes and Combat significantly reduced cockroach populations (mean percent reduction) compared with the untreated control; reductions caused by nematodes and Combat did not differ significantly (Table 1). A similar pattern was seen in the 1992 test although statistical significance occurred only the first and fourth week of the trial (Table 1).

In 1991 the mean number of cockroaches in all treatments decreased during the first two weeks. Thereafter, the numbers of cockroaches increased in the untreated control. Cockroaches in the Combat treatment increased after the fourth week while the nematode treatment saw a continuous decrease through the eighth week and a small increase by the twelfth week (Fig. 5).

In 1992 the mean number of cockroaches in the nematode and Combat treatments changed in virtually the same way, decreases occurring between every measurement. In general the number of cockroaches caught in the untreated control increased during the trial (Fig. 5).

DISCUSSION

The weekly efficacy measurements demonstrated that nematodes in the prototype remained viable and able to kill German cockroaches even though water declined to about 4% of the original supply by the fourth week.

Experiments involving mixed-age cockroach populations showed that the prototype has the ability to suppress rapidly growing cockroach populations in the lab. Adults and older (instar 4–6) nymphs were more readily killed than younger (instar 1–3) nymphs. The prototype reduced population growth in two major ways. First, it rapidly killed females and, thus, drastically reduced

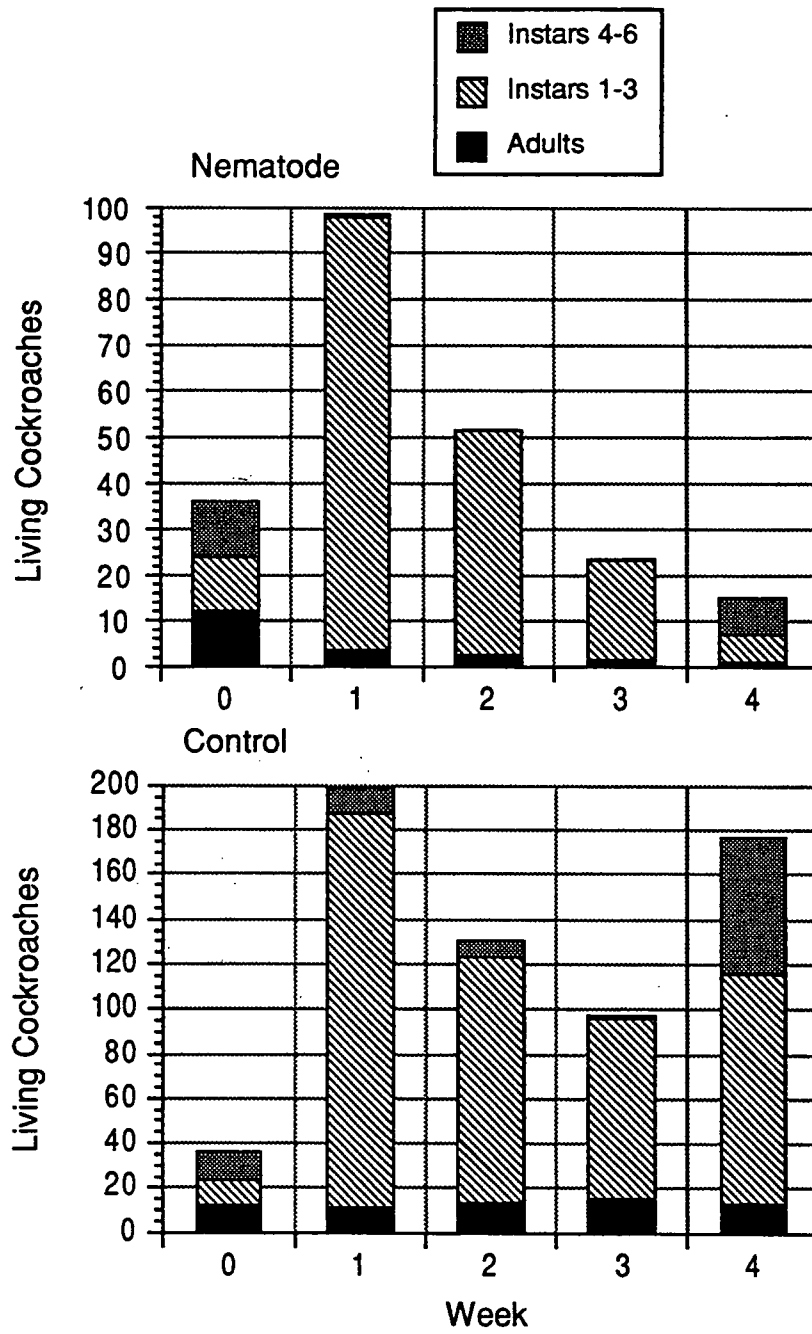


Figure 4. Mean number of living cockroaches (adults, 4-6 instar nymphs, instar nymphs) in chambers treated with nematode stations or nothing.

Table 1. Mean percent reductions of cockroach populations in apartments after treatment.

| Treatment | Precount | 1991 | | | | |
|-----------|----------|--------------------|--------------------|--------------------|---------------------|--------------------|
| | | Week 1 | Week 2 | Week 4 | Week 8 | Week 12 |
| Nematode | 326.67 | 33.8 ^a | 39.9 ^a | 52.0 ^a | 67.1 ^a | 57.5 ^a |
| Combat | 384.33 | 45.1 ^a | 42.3 ^a | 53.1 ^a | -19.6 ^{ab} | -3.9 ^{ab} |
| Control | 225.44 | -26.8 ^b | -23.7 ^b | -18.0 ^b | -35.5 ^b | -69.2 ^b |

| Treatment | Precount | 1992 | | | | |
|-----------|----------|-------------------|-------------------|--------------------|---------------------|---------------------|
| | | Week 1 | Week 2 | Week 4 | Week 8 | Week 12 |
| Nematode | 352.67 | 54.0 ^a | 55.5 ^a | 54.8 ^a | 52.3 ^a | 49.8 ^a |
| Combat | 337.83 | 48.8 ^b | 29.4 ^a | 45.7 ^b | 45.5 ^a | 33.4 ^a |
| Control | 355.67 | -6.8 ^b | 6.5 ^a | -55.2 ^b | -119.3 ^a | -223.3 ^a |

Mean within columns (within years) followed by the same letter are not significantly different (Least Significant Means, $p = 0.05$). Negative values indicate a mean percent increase instead of a reduction.

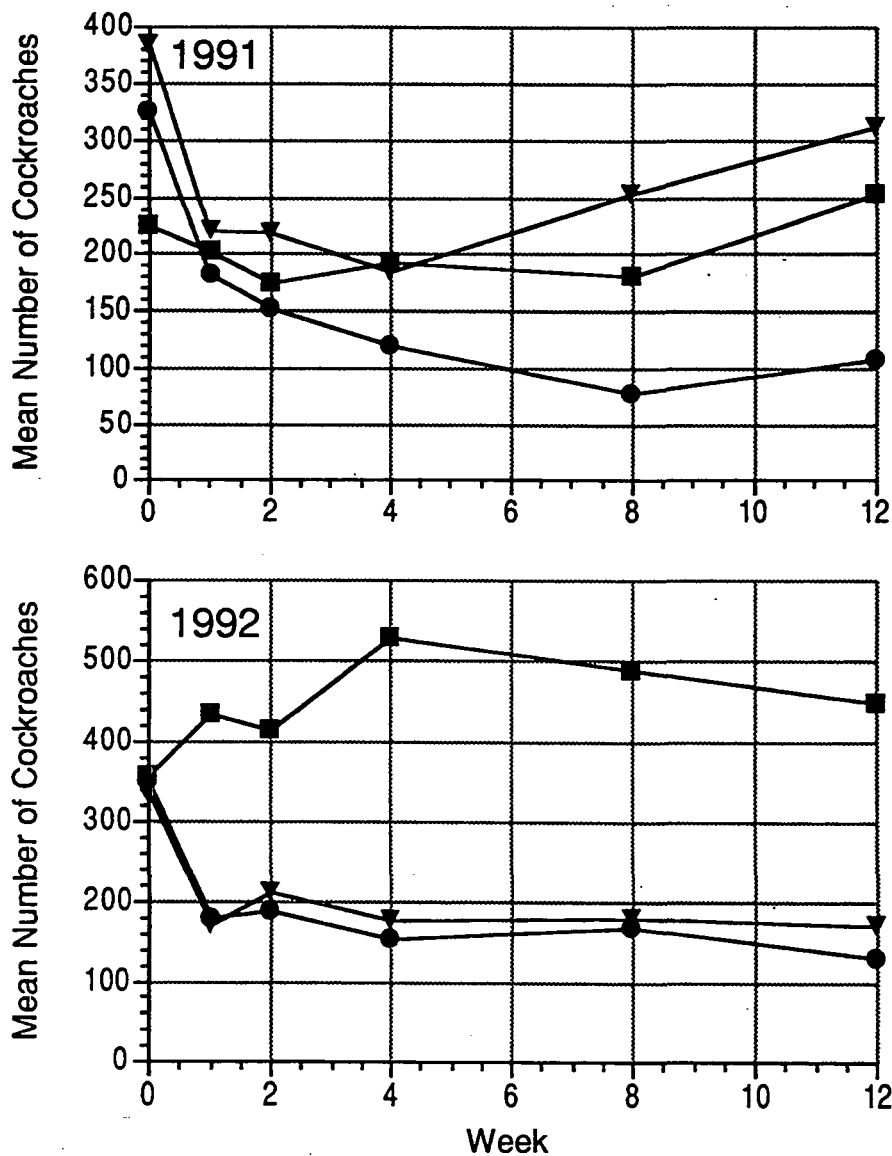


Figure 5. Mean cockroach catches in apartments treated with nematode stations (●), Combat (▼), or nothing (■).

the actively reproducing segment of the cockroach population. In some cases oothecae were killed along with the females that carried them. Second, the prototype killed older nymphs thereby preventing the development of new reproductives. Younger nymphs became more susceptible to nematode-induced mortality as they developed.

The lower-than-desired rate of mortality of younger instar nymphs deserves more study. Different developmental stages of many insects vary greatly in their susceptibility to nematodes. In some cases a physical barrier such as spiracular size or the presence of sieve plates prevents nematode entry into the host. Behavioral differences affecting the extent of contact between nematode and host also can play a major role in the extent of nematode induced mortality (Begley, 1990; Klein, 1990). These factors need to be addressed in further lab trials.

Both apartment trials demonstrated that stations containing nematodes can control German cockroaches as well as Combat bait stations. Because these apartment trials were meant to test the potential of a nematode-based cockroach control strategy, the Combat label rate was utilized. Future trials will examine the relationship of the number of stations per unit area, the cockroach population density, and the rate and degree of cockroach population suppression.

Improvements will be necessary to achieve more rapid and more complete control of German cockroach infestations. No attractants other than water emitted by the station were included in the prototype we tested. The presence of nematodes did not result in significantly increased repellency compared to a nematode station containing no nematodes (Appel *et al.*, 1993). Pheromones have been shown to decrease the repellency of some insecticidal baits (Rust and Reiersen, 1977) suggesting that the addition of an attractant might increase cockroach entry and infection rates.

We believe that our prototype represents a viable technology that, when more completely developed, will be an effective part of an integrated program for controlling German cockroaches.

REFERENCES

- Abbott, W. S. (1925). A Method for Computing the Effectiveness of an Insecticide. *J. Econ. Entomol.* 18: 265-267.
- Appel, A. G. (1990). Laboratory and Field Performance of Consumer Bait Products for German Cockroach (Dictyoptera: Blattellidae) Control. *J. Econ. Entomol.* 83: 153-159.
- Appel, A. G., Benson, E. P., Ellenberger, J. M., and Manweiler, S. A. (1993). Laboratory and Field Evaluations of an Entomogenous Nematode (Nematoda: Steinernematidae) for German Cockroach (Dictyoptera: Blattellidae) Control. *J. Econ. Entomol.* (in press)
- Begley, J. W. (1990). Efficacy Against Insects in Habitats Other Than Soil. In: *Entomopathogenic Nematodes in Biological Control*. pp. 215-232 (ed. by R. Gaugler and H. K. Kaya). CRC Press, Inc.
- Corpus, L. D. and Sikorowski, P. P. (1992). Susceptibility of *Periplaneta brunnea* (Dictyoptera: Blattellidae) to the nematode *Steinernema carpocapsae* (Rhabditida: Steinernematidae). *J. Med. Entomol.* 29: 707- 710.
- Georgis, R. (1990). Formulation and Application Technology. In: *Entomopathogenic Nematodes in Biological Control*, pp. 173-191 (ed. by R. Gaugler and H. K. Kaya). CRC Press. Boca Raton.
- Georgis, R. (1992). Present and Future Prospects for Entomopathogenic Nematode Products. *Biocontrol Science and Technology* 2: 83-99.
- Georgis, R. and Gaugler, R. (1991). Predictability in Biological Control Using Entomopathogenic Nematodes. *J. Econ. Entomol.* 84, 713-720.
- Klein, M. (1990). Efficacy Against Soil-inhabiting Insect Pests. In: *Entomopathogenic Nematodes in Biological Control*. pp. 195-214 (ed. by R. Gaugler and H. K. Kaya). CRC Press, Inc.
- Koehler, P. G., Patterson, R. S., and Martin, W. R. (1992). Susceptibility of Cockroaches (Dictyoptera: Blattellidae, Blattellidae) to Infection by *Steinernema carpocapsae*. *J. Econ. Entomol.* 85: 1184- 1187.
- Poinar, G. O., Jr. (1975). *Entomogenous Nematodes – A Manual and host list of insect-nematode associations*. Brill. Leiden.
- Poinar, G. O., Jr. (1979). *Nematodes for Biological Control of Insects*. CRC Press, Inc.
- Rust, M. K. and Reiersen, D. A. (1977). Using Pheromone Extract to Reduce Repellency of Blatticides. *J. Econ. Entomol.* 71: 704- 708.
- SAS Institute. (1985). *SAS Users Guide: Statistics. Version 5*. SAS Institute, Cary, N. C.