# Host plant resistance and entomogenous nematodes for controlling the northern corn rootworm, *Diabrotica barberi* (Coleoptera: Chrysomelidae).



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Biological control of *Diabrotica barberi* in Quebec.

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# Host plant resistance and entomogenous nematodes for controlling the northern corn rootworm, *Diabrotica barberi* (Coleoptera: Chrysomelidae).

## ABSTRACT

Resistance to northern corn rootworm (NCR), *Diabrotica barberi*, in the form of tolerance was found in inbred corn line Q179. Indications of antibiosis were also found in this line. Line CSJ-1 was susceptible to larval NCR attack. Evidence suggests that corn lines resistant to attack by European corn borer, *Ostrinia nubilalis*, are not also resistant to larval NCR attack.

Host-finding behaviour of larval NCR is directed, possibly in response to kairomones released by corn roots. The larvae are not attracted to the roots of a variety of grasses.

All NCR stages except the egg were susceptible to attack by the entomogenous nematodes *Heterorhabditis heliothidis*, *Steinernema feltiae*, and *S. bibionis*. *H. heliothidis* was found to be the most virulent in laboratory bioassays. In the field *S. feltiae* and *S. bibionis* both reduced larval NCR numbers but the insecticide fonofos performed better than either nematode in this respect. *H. heliothidis* and *S. bibionis* persisted longer in four soil types under field conditions than *S. feltiae*.

MSc Graham S. Thurston Dept. d'entomologie

# Contrôle de la chrysomèle des racines du maïs (*Diabrotica barberi*) par la résistance de la plante-hôte et l'utilisation de nématodes parasites.

## RESUME

La résistance à la chrysomèle des racines du maïs, (*Diabrotica barberi*), a été observée sous forme de tolérance dans la lignée de maïs Q179. Cette lignée a montré des effets antibiotiques sur les larves de chrysomèle. Des expériences indiquent également que des lignées de maïs résistantes à la pyrale du maïs, *Ostrinia nubilalis*, ne résistent cependant pas à l'attaque des larves de cette chrysomèle.

Le comportement des larves de chrysomèle des racines du maïs pour la recherche de l'hôte semble dicté par l'émission d'exudats provenant des racines de maïs. Les racines d'un grande nombre de graminées n'attirent pas les larves de cette chrysomèle.

Tous les stades de *D. barberi*, excepté l'oeuf, ont montré une susceptibilité aux nématodes. *Heterorhabditis heliothidis*, *Steinernema feltiae*, et *S. bibionis*. *H. heliothidis* s'est avéré le plus virulent dans les essais faits en laboratoire. Sur le terrain, *S. feltiae* et *S. bibionis* ont tous les deux réduit les nombres de larves de chysomèle des racines du maïs. Néanmoins l'insecticide fonofos a donné de meilleurs résultats que les deux nématodes. Sur le terrain, *H. heliothidis* et *S. bibionis* ont survécu plus longtemps que *S.feltiae* et de, dans quatre types de sol.

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## GENERAL INTRODUCTION

The northern corn rootworm (NCR), *Diabrotica barberi* Smith and Lawrence, is an insect belonging to the order Coleoptera, family Chrysomelidae. In common with the majority of members of this family the NCR is phytophagous. Corn rootworms (*Diabrotica* spp.) are serious pests of corn in the New World. The larvae feed on and in the roots of corn plants, resulting in weakened root systems and reduced nutrient and water uptake; this in turn leads to yield reductions. The adult beetles feed on corn silks, and yield losses have occurred in years when adult populations are high, due to incomplete pollination of the ears.

In the United States alone, corn rootworms are responsible for insecticides being applied to 12 to 16 million ha of corn-growing land and are costing American farmers an estimated $1 billion (US) each year in crop losses and insecticide application costs (Metcalf 1986). The northern corn rootworm is the only member of the genus *Diabrotica* that is found in Quebec. Since first becoming a problem in Quebec in the mid-1970's it has increased its range greatly in the corn-growing regions of the province and is now considered to be a serious pest of corn throughout this area (Martel *et al.* 1980).

In some cases the NCR can be controlled by normal crop rotation. However, recent evidence (Krysan *et al.* 1986) indicates that significant proportions of some NCR populations may diapause for two years and cause yield reductions in corn under a two year rotation scheme. A three-year rotation plan effectively controls the insect under these conditions. Rotation is unacceptable to many farmers, however, and many grow continuous monoculture corn (Olkowski 1986), compounding the problem by allowing populations of the pest to build up over a period of years. Even in corn grown in two-year rotation with a non-host plant, damage caused by NCR is being observed quite often (Krysan *et al.* 1986), necessitating the widespread use of soil insecticides to control the insect.

Insecticide applications are commonly made to corn crops as a preventive measure. Consequently, they are often made unnecessarily and this practice increases production costs and adds to environmental pollution. Sampling programmes that detect rootworm infestations before damage occurs have been developed (eg. Matin 1983). However, the techniques are somewhat laborious and therefore are not widely practiced. Predictive sampling programmes have the potential of reducing the frequency of application of insecticides. Without their implementation, the prophylactic use of insecticides results. Control of the NCR with soil insecticides can be acheived in many circumstances but the incidence of ineffective control is

increasing due to the development of insecticide resistance by the insect (Ball and Weekman 1962; Kantack 1983).

The problems inherent with the use of chemical insecticides (eg. insecticide resistance, environmental contamination, effects on non-target organisms), the lack of sampling programmes, and the impracticality in current production systems of following three-year crop rotation schemes, suggest that alternative techniques are required to control this insect pest.

Host plant resistance (HPR) has been considered as a control technique for NCR for many years and a few inbred lines of corn have been identified that are considered to be tolerant of NCR larval attack (Welch 1977; Ortman *et al.* 1974; Wilson and Peters 1973). However, the use of resistant plants for controlling the NCR is not common, possibly because of other disadvantageous traits (eg. poor growth characteristics). Host plant resistance to control another pest of corn, the European corn borer (ECB) (*Ostrinia nubilalis*), however, has been effective and inbred lines resistant to this pest have been identified and others developed (Hudon and Chiang 1985; Hudon *et al.* 1979); some of the most resistant lines are being used in experimental trials in North America and Europe (Hudon *et al.* 1984). NCR and ECB occur together on corn in Quebec; therefore if HPR is to be

used to control one of these insect pests, the candidate cultivar should also be resistant (or at least non-suseptible) to attack by the other pest. Lines of corn with known resistance to ECB have not been characterized in terms of their resistances to NCR.

Another control technique that shows promise is biological control with entomogenous nematodes. Recently, this technique has received increased attention from researchers and is appropriate for use against the NCR since the most important damaging stages of this insect dwell in the soil, the natural environment of the specific nematodes used. Laboratory tests have indicated that corn rootworm larvae are susceptible to attack by entomogenous nematodes but field trials have been inconclusive (Poinar 1979).

The experiments reported in this thesis were designed to:

1) Determine if a) inbred lines of corn known to be resistant to attack by ECB are also resistant to attack by NCR and b) those lines susceptible to ECB attack are also susceptible to NCR attack.

2) Determine if concurrent attack by NCR and ECB on a plant causes greater damage than the sum of the damages caused by each insect separately.

3) Determine if host-plant searching by newly-hatched NCR larvae is random or is directed in response to kairomones secreted by corn roots.

4) Determine the susceptibility of NCR to attack by several species and strains of entomogenous nematodes by conducting laboratory bioassays.

5) Determine if entomogenous nematodes are capable of penetrating corn roots to attack NCR larvae feeding within.

6) Examine the affects of four different soil types on nematode persistance.

7) Determine the practical efficacy of applying entomogenous nematodes to field soils to control larval NCR populations in a commercial grain corn crop.

# LITERATURE REVIEW

Corn rootworms (*Diabrotica* spp.) are serious pests of corn (*Zea mays* L.) throughout most of the corn growing regions of Canada and the United States of America. Together, the northern corn rootworm (NCR), *Diabrotica barberi* Smith and Lawrence, the southern corn rootworm (SCR), *Diabrotica undecimpunctata howardi* Barber, and the western corn rootworm (WCR), *Diabrotica virgifera virgifera* LeConte, are responsible for the use of insecticides on 12-16 million ha. of corn growing land in the USA and are costing American farmers in the range of $1 billion (US) per year in crop losses and insecticide treatment costs (Metcalf 1986).

In southwestern Quebec, the northern corn rootworm (NCR) is likely to develop as a more serious pest of corn as more corn is grown in continuous monoculture in this province (Martel *et al.* 1980).

## Taxonomy

The NCR is classified as follows (Krysan *et al.* 1983; Borror *et al.* 1976):

Order: Coleoptera

Superfamily: Chrysomeloidea

Family: Chrysomelidae

Tribe: Galeraucini

Genus: *Diabrotica*

Species: *barberi* Smith and Lawrence 1967

This beetle was first described as *Gallerauca longicornis* in 1824 from adult specimens collected from a wild cucurbit in Colorado (Say 1824). In 1837 this species was assigned to the genus *Diabrotica* (Smith and Lawrence 1967). The loss of Say's original specimens prompted Smith and Lawrence (1967) to designate a neotype for *D. longicornis*. In their revision of the genus the authors recognized two separate populations of *D. longicornis* which they gave subspecific status, designating the western population as *D. longicornis longicornis* and the eastern population as *D. longicornis barberi*. However, Krysan *et al.* (1983) found that the two subspecies described by Smith and Lawrence were reproductively isolated populations and hence they raised *D. l. barberi* to species status as *D. barberi*, the northern corn rootworm.

## Distribution

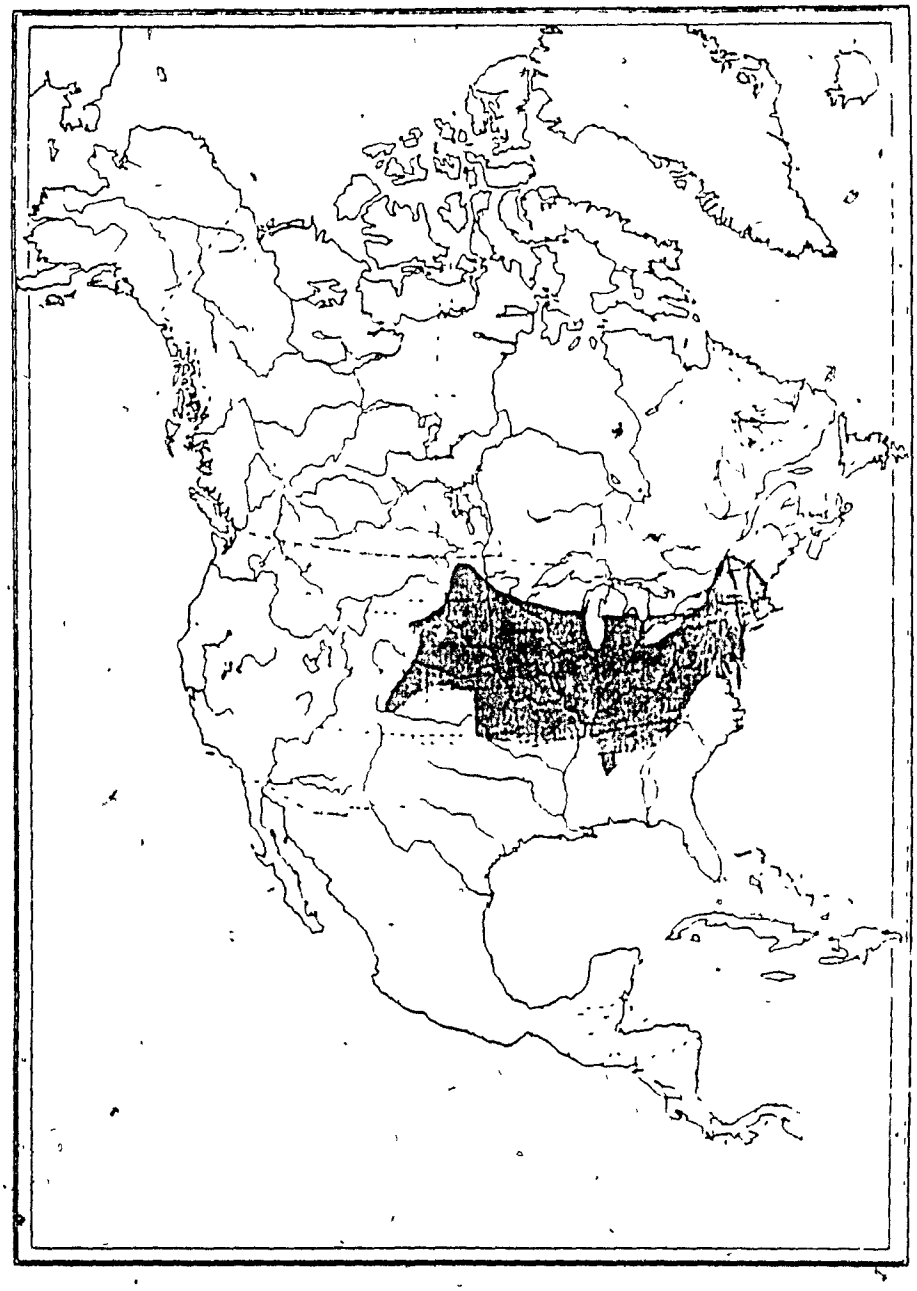
Although the genus *Diabrotica* is widely distributed in

the Americas, the distribution of *D. barberi* is restricted to the corn growing regions of North America east of the states of Montana, Wyoming, and Colorado and north of the states of Oklahoma, Arkansas, Mississippi, and Alabama, north to the Canadian border in the west, the Great Lakes in north central USA and into southern Ontario and Quebec in the east (Figure 1). The distribution of this insect in Quebec is shown in Figure 2.

The NCR was first observed in Canada in 1890 (Harrington 1894) as the adult stage feeding on flower heads of a common large thistle in New Brunswick. Harrington described the insect as probably living amongst the roots of some of the larger grasses but it is more probable that the adults he observed had been blown to that locality from corn growing regions to the west since the NCR has never again been found in that area.

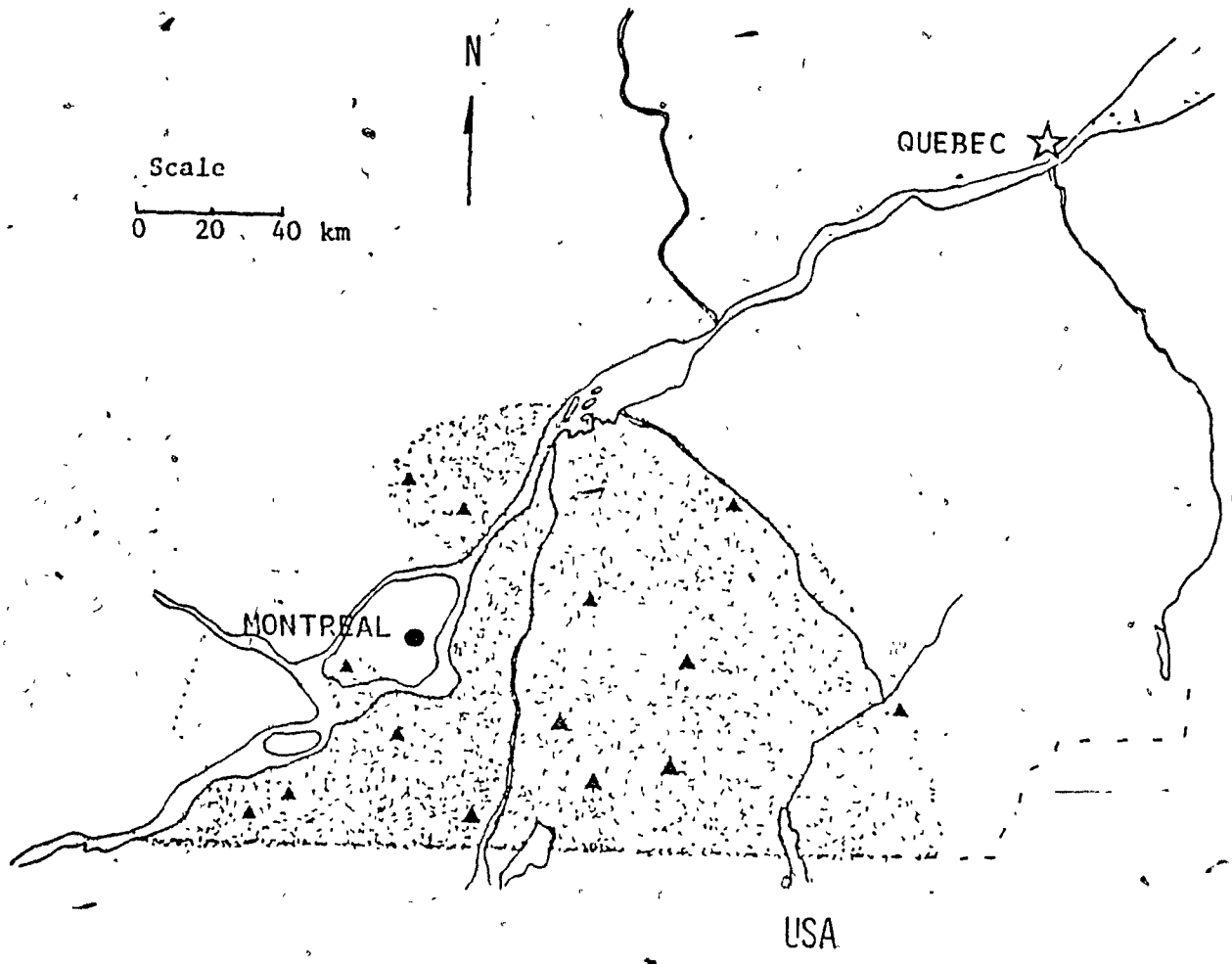
The NCR was first observed in the Province of Quebec in 1932 (Chagnon 1938) but was then apparently absent until 1975 when it was collected from cornfields near Napierville (Guibord 1976). Since then the NCR has expanded its range and is now found at moderately high population levels in all of the corn growing regions of southern Quebec (Martel *et al.* 1980).

Figure 1.



Distribution of *Diabrotica barberi*.

Figure 2.




▲ data from field observations and C. Ritchot (St.-Hyacinthe Research Station)

Distribution of *Diabrotica barberi* in Quebec.

## Biology

The NCR is a holometabolous insect that has one generation a year and goes through four stages in a generation: egg, larva, pupa, and adult. In general, eggs are laid in the soil of cornfields in the autumn, overwinter there and hatch the next spring. The young larvae disperse through the soil until they find the roots of a corn plant, upon which they begin feeding. Older larvae burrow into the roots of the plant and continue feeding. When they have completed feeding, the larvae chew exit holes in the root and emerge to pupate in the soil. The adults emerge a few days later and feed on the silk and pollen of corn plants. Later in the season, when the silks have dried out and the corn pollen is depleted, the adults migrate to the edges of the cornfield where they feed on the pollen of a variety of flowers. Mating occurs either on the corn plants or on other plant species at the edge of the field. After mating, the females return to the cornfields and deposit their eggs in the soil (Chiang 1973).

Egg-laying begins in late July or early August in southwestern Quebec (Dominique 1983) and terminates with first severe frost, usually around the second week of October (Tyler and Ellis 1975; Dominique 1983). The depth at which the eggs are deposited depends upon a number of factors affecting the substrate, including soil moisture and soil type (Kirk *et al.*

1968, Dominique *et al.* 1983). In general, the majority of eggs are found in the upper 10 centimetres of soil (Chiang 1965; Matin 1983).

The female beetles do not excavate an oviposition site, instead they use existing cracks in the soil surface to penetrate to a suitable depth for oviposition (Kirk 1979). Sisson and Chiang (1964) and Patel and Apple (1967) found that the majority of NCR oviposition occurred near the bases of corn plants, however Matin (1983) found the distribution of eggs to be random with respect to the corn plant.

The majority of the literature on diapause and overwintering of corn rootworm concerns the WCR. However, the WCR and the NCR are very similar in this respect so the information gained from one species can quite readily be applied to the other (Chiang 1973). The eggs of both of these corn rootworm species undergo diapause. Chilling does not necessarily break the diapause condition; it instead has the effect of synchronizing egg hatch (Branson 1976). Not all of the eggs of NCR necessarily undergo diapause; Patel and Apple (1967) found in a laboratory culture of NCR held at a constant 20-22° C that approximately 13% of the eggs hatched as early as nine days after oviposition. In addition, Krysan *et al.* (1986) determined that NCR eggs may diapause over two winters in some areas.

While overwintering the eggs are subjected to cold temperatures, therefore they must have some degree of cold tolerance. The eggs of NCR appear to be more cold hardy than those of WCR; after 42 days at -10° C, NCR egg hatch was 35% but after only 21 days at -10° C, WCR egg hatch was only 3.3% (Gustin 1983). Matin (1983) demonstrated the strong survival capacity of NCR eggs; after 336 days at 5°C, 64% of the eggs hatched.

During the winter, development of the embryo within the egg occurs only at temperatures above the development threshold which has variously been determined to be 9.5° C (Patel and Apple 1967), 9.7° C (Dominique and Yule 1983), and 11.5° C (Chiang and Sisson 1968). Dominique and Yule (1983) determined that the mean degree day (DD) accumulation above 9.7° C for eclosion was 340 in Quebec; Chiang and Sisson (1968) reported 400 DD above 11.5° C in Minnesota.

In Quebec, first egg hatch normally occurs during the first week of June, and hatching continues into July (Dominique 1983). Upon hatching, the young larvae migrate through the soil until they find the roots of a suitable host plant. Whether this migration is random or is directed in response to plant kairomones is at present unknown. Suttle *et al.* (1967) and Short and Luedtke (1970) determined that newly hatched WCR larvae could migrate at least one metre through the soil. NCR

larvae are assumed to behave in a similar fashion. Having found a suitable host plant, the larvae begin to feed on the young roots and continue feeding until pupation (Bryson *et al.* 1953). Young larvae feed on the outside of the roots but older ones burrow in and feed on the interior. The feeding activity of the larvae impairs the water and nutrient uptake by the plant (Gerloff 1976) and also weakens the general root structure, leaving the plant prone to lodging in strong winds and rainstorms. Larval feeding damage commences at egg hatch and continues until all larvae have pupated, this is usually around the beginning of August in Quebec (Dominique 1983). The mean time of development from egg hatch to the beginning of pupation in a laboratory colony held at 23° C was found to be 30 days (Dominique 1983).

The number of larvae that pupate is determined predominantly by soil conditions. Turpin and Peters (1971) found that larval survival was much better in clay soils than sandy soils. Lummus *et al.* (1983) agreed with these findings and also determined that soil moisture was another important factor in larval survival. Once the larvae have fully developed they emerge from the roots and pupate in the soil nearby. The duration of the pupal stage of the NCR at 23° C ranges from 14 to 20 days (Dominique 1983).

Adult emergence commences in mid-July and continues until

early September in Quebec (Dominique 1983). This agrees with Tyler and Ellis (1975), Bereza (1975), and Patel and Apple (1967) in northern USA and southern Canada. Adult beetles feed primarily on corn silk and pollen. When severe, this feeding can cause grain loss by preventing complete pollination of the corn (Chiang 1976; Capinera *et al.* 1986). After the corn silks have dried out, the adults migrate to areas outside of cornfields where pollen from other plants is available as food (Cinereski and Chiang 1968). The female beetles migrate back and forth between these food sites and the oviposition sites in cornfields (Cinereski and Chiang 1968) until they are killed by autumn frosts.

## Damage

The most damaging stage of the NCR is the larva. The adults also can cause some damage by ear feeding but this damage is rarely important. The larval feeding occurs on the corn plant at a time when it is developing rapidly and requires a constant influx of moisture and nutrients. Root damage at this stage can have serious effects on plant growth, especially in years with reduced rainfall (Gerloff 1976).

NCR larvae not only cause direct damage to roots by feeding but also provide avenues of entry for root rot organisms.

Howe and Britton (1970) and Palmer and Kommedahl (1969) presented observations on the dual damage to corn roots by NCR and *Fusarium* root rot and have shown that the presence of NCR feeding damage enhances the damage caused by the rot.

## Control Measures

Crop rotation is currently the only practical, non-chemical control technique for corn rootworms (Gerloff 1976; Mayo 1986). However, rotation is rarely used in intensive grain corn production practices because of financial considerations of the corn-producing system in North America (Olkowski 1986). Also, recent evidence indicates that the NCR can cause damage in corn in a two year rotation programme because a significant proportion of NCR eggs may overwinter for two years (Krysan *et al.* 1986).

Because rotation is seldom practiced, there has been heavy reliance on the use of chemical insecticides for the control of corn rootworms. Large scale soil treatments with insecticides to control *Diabrotica* have been made since the late 1940's in the United States and the incidence of ineffective control due to resistance to the insecticides has risen greatly since then (Ball and Weekman 1962; Kantack 1983).

In part, resistance to insecticides has become a problem

because insecticides are routinely applied to areas where they may not be needed. Soil insecticides are applied to 60% of the corn fields in the corn belt States to control corn rootworms, but studies have indicated that only about 11-19% of this area needs to be treated to reduce economic damage (Luckmann 1978). In one area in Nebraska where soil insecticides are routinely used on 90% of the corn fields, Stamm *et al.* (1985) demonstrated that the treated area could be reduced to 5-9% of the fields without serious rootworm damage.

Proper use of our current knowledge of the bionomics and ecology of the NCR and the other corn rootworms, in the application of integrated pest management technology could quite readily reduce the cost to farmers of treatment (Metcalf 1986). However, producers are not yet willing to change from prophylactic insecticide use to other approaches. In the meantime, there is a need for development of alternative control measures such as host plant resistance and other biological controls for integration into future corn rootworm management programmes. There is also a need to demonstrate that these alternative control measures are valid and practicable.

## Host Plant Resistance

Host plant resistance (HPR) of corn to the corn rootworm complex has been suspected since the late 1930's (Bigger *et al.* 1938). However, it was not until the late 1960's that research began on the possibilities of using HPR as a means of lessening the damage caused by rootworm larvae. Painter (1951) recognized three types of resistance: non-preference, antibiosis, and tolerance. Non-preference is the absence of attracting or feeding stimuli or the presence of feeding repellants or feeding deterrents in the plant. In this case the feeding insect prefers some other plant than the one showing non-preference. Antibiosis occurs when the plant adversely affects the pest through influence on its growth, survival, or fecundity. Tolerance is considered to be the ability of the plant to withstand insect attack without an appreciable loss in yield and without adversely affecting the insect population.

For maximum protection of a crop, non-preference is the most effective form of resistance, but so far germ plasm showing either non-preference or antibiosis to corn rootworms has been identified in only one study (Branson *et al.* 1983). Tolerance, in the form of large root systems, is the most common mechanism of larval corn rootworm resistance (Chiang and French 1980; Welch 1977; Wilson and Peters 1973).

Since larval NCR feed underground, evaluation of plant resistance to this insect is very difficult. Several evaluation techniques have been proposed. All are rough indicators of tolerance and the majority are destructive to the plant. Percent of plants lodged was the first indicator of tolerance to corn rootworm attack used (Bigger *et al.* 1941) and was used until fairly recently (eg. Owens *et al.* 1974). Plants with large root systems are less susceptible to lodging and will not be as affected by an NCR infestation as plants with smaller root systems. However, lodging is not caused solely by rootworm damage but is also influenced by soil type, moisture, weather conditions, protection from wind, and root toughness (Zuber *et al.* 1971). Because these other factors are involved, plant lodging is not a reliable indicator of plant tolerance to NCR. A more useful measure of root system size, root-pulling resistance, was proposed in 1968 (Ortman *et al.* 1968). In evaluating this as a measure of plant tolerance to rootworm attack Rogers *et al.* (1976) determined that root-pulling resistance is good for estimating corn lodging potential and therefore, indirectly, for rootworm tolerance. However, this technique relies on uniform soil type and moisture throughout the experimental plots and does not allow for comparisons between sites or between years. Nevertheless, this technique is still widely used (eg. Branson *et al.* 1983).

A root damage rating technique for evaluating the direct

effects of corn rootworm attack on the root systems of corn plants was proposed by Hills and Peters (1971). This method allows direct assessment of insect damage rather than potential tolerance. However, the root damage rating technique is only suitable for comparisons within a line of plants or between lines with the same sized root systems. Branson *et al.* (1981) found that a given level of rootworm infestation produces the same rating in root systems of all sizes. Therefore, since tolerance is considered to be a factor of root system size, the root damage rating system is not appropriate as a measure of tolerance. Nevertheless, the root-rating system is still in common use. A more appropriate measure of root damage for comparison between lines of corn is a system based on the proportion of roots damaged.

To date, studies have shown no indication that antibiosis to corn rootworms exists in corn (Chiang and French 1980). Considerable effort has been made to find and develop inbred lines of corn that are tolerant of rootworm attack (Wilson and Peters 1973; Rogers *et al.* 1977; Jenison *et al.* 1981). However, a danger exists because the widespread use of tolerant plants may be more conducive to pest population build-up (because of the ability of each plant to support a larger number of pests) than the use of more susceptible plants (Pathak 1970). Therefore, the use of tolerant plants should only be considered as a short-term or local management

tool (Chiang and French 1980).

**Entomogenous nematodes**

A control technique that shows promise is biological control with nematodes. In recent years the use of entomogenous nematodes for biological control of insect pests has received increased attention. In 1979, Poinar published a comprehensive work on entomogenous nematodes in which he recognized nine families of nematodes having members showing promise as biological control organisms for pest insects. This discussion will be limited to two of these families, Heterorhabditidae and Steinernematidae. Heterorhabditidae contains one genus, *Heterorhabditis*; within this genus only one species, *H. heliothidis*, will be considered. Steinernematidae contains several genera but only one, *Steinernema*, is of interest here. Two species, *S. feltiae* and *S. bibionis* will be considered. The selection of these particular nematodes for use in research for this thesis was based on earlier successes reported in the literature, the broad host range of these nematodes, and on availability of cultures (eg. Poinar 1979; Gaugler 1981).

At present there is some confusion in the literature as to the correct name of one of these genera and the correct specific epithet of one of the species in this genus. The

most common current usage in North America is *Steinernema feltiae;* however, several authors consider the correct name to be *Neoaplectana carpocapsae*. Poinar (1984) gives reasons for using *N. carpocapsae* but the nematode will be referred to here as *S. feltiae* because this is the name most commonly used in North America at the time of writing.

Both *Heterorhabditis* and *Steinernema* are obligate parasites of insects and are mutualistically associated with bacteria. Poinar (1979) describes these two genera along with their associated bacteria in considerable detail.

Mode of Action The modes of action of these two nematodes are similar. Third-stage juvenile (dauer) nematodes locate the host insect and initiate infection. The nematodes are attracted to the host by chemical cues such as $CO_2$ (Gaugler *et al.* 1980), and insect faecal components (Schmidt and All 1979). When a suitable host is located the nematodes invade the insect by the mouth, anus, or spiracles (Trigginani and Poinar 1976) and, in the case of *H. heliothidis*, by direct cuticular penetration (Bedding and Molyneux 1982). Once inside the insect's haemocoel, the alimentary tract of the nematode opens and the symbiotic bacteria (*Xenorhabdus nematophilus* in *Steinernema* spp. and *X. luminescens* in *H. heliothidis*), are released via the anus (Poinar 1966). The

bacteria multiply rapidly in the insect haemocoel and cause the death of the insect by septicemia (Poinar and Thomas 1967). Death usually results within 48 hours of the release of the bacteria into the haemocoel. The nematodes obtain nutrients as a result of the bacterial activity and reproduce within the cadaver of the insect (Poinar 1979).

The two genera have different modes of reproduction; *H. heliothidis* is partially parthenogenic. Nematodes in the genus *Steinernema* do not have a partially asexual reproductive strategy; otherwise the life history and mode of action is similar to that of *H. heliothidis*. The first-generation adults of *H. heliothidis* are all hermaphroditic females. These hermaphrodites deposit eggs in the insect cadaver which develop into a bisexual second generation. The young of the matings from this generation develop into either third generation hermaphroditic female adults or into dauer nematodes, depending on the availability of nutrients in the host (Khan *et al.* 1976). Dauer nematodes are produced as a response to crowding and lack of food. This stage is resistant to many environmental conditions unfavourable for the survival of adults and can survive for long periods of time in moist soil (Poinar and Leutenegger 1968). The newly-produced dauers exit the cadaver and begin searching for another host.

Relationship between bacterium and nematode The relationship between *Xenorhabdus* spp. and *Steinernema* and *Heterorhabditis* spp. is a mutualistic association. The bacteria have never been isolated from an environment other than the nematode intestine or a nematode-infested insect (Poinar 1979). The bacterial cells can not survive in other environments; they have a very transient existence in soil or water. When artificially introduced into the gut of an insect the bacteria have no effect, but even a few bacterial cells introduced into the insect haemocoel are sufficient to cause rapid death (Poinar and Thomas 1967). Axenic dauer nematodes are capable of penetrating the insect and entering the haemocoel, but are unable to reproduce, probably due to a lack of nutrients (Poinar and Thomas 1966). Thus, the nematode affords a protective environment for the bacterium and carries it into the haemocoel of a host insect, while the bacterium supplies nutrients, either directly or indirectly, that are necessary for nematode reproduction.

Use of entomogenous nematodes as biological control agents Entomogenous nematodes have been used for many years in attempts to control various insect pests (Poinar 1979; Benham and Poinar 1973). They have proven to be quite effective at reducing pest insect populations in some situations (eg. Thurston 1986; Georgis and Poinar 1984; Poinar *et al.* 1983;

Toba *et al.* 1983), but in many situations control has been poor. Solar radiation has been found to have a detrimental effect on the survival of these nematodes (Gaugler and Bousch 1978) and they are rather susceptible to desiccation unless they are in a protected environment (Webster 1973). Attempts are being made to encapsulate nematodes in calcium alginate gel to prevent or retard desiccation and protect them against UV radiation but as yet these attempts have not been successful (Kaya and Nelsen 1985; Shapiro *et al.* 1985). It is now generally accepted that use of entomogenous nematodes is most effective against insects found in cryptic habitats such as the soil (Akhurst 1986; Petersen 1982; Gaugler 1981; Poinar 1979).

Entomogenous nematodes are primarily used as insecticides in inundative releases. Little success has been obtained in establishing sufficient populations of the nematodes to maintain an insect pest infestation at low levels from year to year (Gaugler 1981).

*Diabrotica* control with entomogenous nematodes. *Diabrotica* spp. are susceptible to attack by steinernematids and heterorhabditids in laboratory situations. Since a portion of the life cycle of these beetles is in the soil, they are suitable candidates for control with these nematodes. Fronk (1950)

reported the presence of a *Neoaplectana* sp. (=*Steinernema* sp.?) in adult southern corn rootworm, *D. undecimpunctata howardi*, but discarded the nematode as a possible biological control agent because it was not very prevalent in nature. In 1968, Creighton *et al.* reported that the banded cucumber beetle, *D. balteata*, was highly susceptible to the DD-136 nematode (*Steinernema feltiae* DD-136 strain) in laboratory trials. However, when the nematode was used in small-scale field trials control was not adequate. This inadequate control was probably due to the fact that the nematodes were applied on the soil surface rather than below and were therefore exposed to deleterious environmental conditions (eg. ultraviolet radiation, desiccation). A larger-scale field experiment conducted in 1969 (Munson and Helms 1970) was unsuccessful at reducing *Diabrotica* spp. damage. In this case soil moisture conditions or nematode application procedures may have influenced the experimental outcome. Also, the strain of nematode used may not have been the most suitable for this insect and field conditions. Poinar *et al.* (1983) conducted a large-scale field experiment with the Breton strain of *S. feltiae* against corn rootworm larvae. They found that nematodes applied at the rate of 10,000 per linear metre in-furrow at the time of corn seeding significantly reduced larval corn rootworm populations compared to the control treatment. Also, they concluded that the nematodes were cheaper and significantly more effective than a soil insecticide, chlor-

pyrifos. However, these results should be viewed cautiously because of a poor experimental design (three separate fields were used, one for each treatment; different varieties of corn were used in the fields; soil type was not determined to be uniform in the fields; corn rootworm infestation was not determined to be uniform) and because only 30 plants were sampled from a 48 hectare area. Jackson (1985) compared four strains of *S. feltiae* for pathogenicity to WCR larvae in laboratory bioassays and found the Mexican strain to be the most virulent. Unfortunately, these laboratory results were not followed up with experiments in the field.

Thus, corn rootworms are susceptible to attack by entomogenous nematodes in the laboratory but the feasibility of using these nematodes in the field is still undetermined.

# HOST PLANT RESISTANCE EXPERIMENTS

## Materials and Methods

### Inbred Corn Response to NCR Attack (Greenhouse)

The responses of two inbred lines of corn with known resistance to European corn borer (ECB) to attack by larval NCR were assessed in greenhouse trials at Macdonald College. The lines of corn used (CSJ-1 - resistant to ECB, Q179 - susceptible to ECB) were selected in consultation with M. Hudon (Agriculture Canada Research Station, St. Jean-sur-Richelieu). The lines were selected for their resistance to ECB (either high or low), and for availability of seed stock. Treatments consisted of two infestation levels of NCR eggs (25 and 50 eggs/plant - each replicated five times) and an untreated control (replicated 10 times). The experiment was established in a randomized complete block design with five blocks. Corn was planted on 06 March 1985 three seeds to a pot and thinned to individual plants after emergence. Artificially lengthened daylight provided 14 hours of light per day. The plants were fertilized at each watering (twice a week) with a dilute solution of 20-20-20 Plant Prod$^{R}$ nutrient fertilizer. The treatment plants were inoculated with NCR eggs on 03 April 1985.

The eggs, obtained the previous autumn from field-collected adults, were overwintered in sterile soil at 5°C for at least 120 days (Dominique 1983) and incubated in sterile distilled water at 23°C for 10 days prior to inoculation of the plants. This was done to ensure fairly rapid hatch once the eggs were placed in the soil. Fifty eggs were reserved in sterile distilled water at 23°C so that an estimate of percent egg hatch could be obtained. All plants were harvested on 03 July after plant height (the distance from the top of the first node above ground to the base of the tassel) measurements were taken. Removal of part of a plant's root system lowers the growth rate of the shoot (Humphries 1958); therefore plant height was used as an indicator of plant success under different levels of NCR attack. The root mass was washed clean, root volume was determined using a water displacement technique (Figure 3), and the roots were searched for evidence of feeding damage. Root damage estimates were obtained by manually searching the roots and recording the number of roots eaten off within 3 cm of the stalk plus the number showing visible feeding scars. This number was then converted to a proportion of the total number of roots on the plant for comparative purposes.

The resulting data were analyzed using an analysis of variance procedure for unbalanced data (General Linear Models) available from Statistical Analysis Systems Institute, Inc.,

Figure 3.

Water displacement technique for determining root volume.

Cary, NC. (SAS 1982).

**Inbred Corn Response to NCR and ECB Attack**

The following hypotheses were tested in a propagation room at the Agriculture Canada Research Station at St. Jean-sur-Richelieu: 1) corn known to be resistant to attack by ECB is also resistant to attack by NCR; and 2) combined attack by NCR and ECB results in greater damage than attack by either of the insects alone.

Two inbred lines of corn were selected with known resisitance to ECB attack, CSJ-1 being resistant and Q179 being susceptible (Hudon, pers. com.; Hudon *et al.* 1979). Treatments consisted of plants infested with NCR, ECB, NCR+ECB, or left untreated as controls. The experiment was arranged in a completely randomized design and each treatment was replicated five times.

Corn individually planted in 12L pots on 26 February 1985 was grown under a light regime of L:D=14:10 with incandescent and full-spectrum fluorescent lighting. The plants were watered when necessary and 20-20-20 fertilizer (Plant Prod$^{R}$ nutrient fertilizer) was applied once per week. On 18 March 1985 the plants in the NCR and NCR+ECB treatments were inocu-

lated with 50 NCR eggs. The eggs were pipetted into 5cm holes in the soil around the base of the plants and covered with soil. NCR eggs were obtained from field-collected adults, overwintered in soil at 5° C for more than 120 days (Dominique 1983), and incubated in distilled water at 23°C for 10 days prior to the inoculation of the plants. Percent egg hatch was determined by conserving 50 eggs in distilled water at 23°C until they hatched. On 16 April 1985, the plants in the ECB and NCR+ECB treatments were inoculated with one ECB egg mass of approximately 20 eggs (obtained from the St. Jean culture of M. Hudon) pinned to a leaf near the stalk. All plants were harvested on 29 May 1985 after plant heights were measured. Plants were cut off at ground level and root volume and root damage measurements were made.

The resulting data were analyzed using an analysis of variance procedure for balanced data (ANOVA) of SAS (SAS 1982).

**Inbred Corn Response to NCR Attack (Field Microplots)**

An experiment was conducted in 1x2 m microplots at the seed farm of Macdonald College to assess the tolerance of several inbred lines of corn to attack by larval NCR. Four

inbred lines of corn were selected; two lines known to be resistant to ECB attack (CSJ-1 and A619) and two known to be susceptible to ECB attack (Q179 and Bc23) (Hudon *et al.* 1979; Hudon and Chiang 1985).

Since Matin (1983) experienced problems with artificial infestation of these microplots with NCR eggs, possibly due to predation by ants and mites, the plants in this experiment were inoculated with NCR eggs in pots in the greenhouse and later transplanted to the microplots after the eggs had hatched and the larvae had become established in the roots.

The corn was planted in 10x18 cm pressed peat Fertil$^{R}$ pots in the greenhouse on 14 June 1985. NCR eggs were incubated in distilled water for 10 days and on 27 June half of the plants were inoculated with 50 eggs each. Percent egg hatch was estimated by incubating 50 eggs in sterile distilled water at 23$^{o}$C until hatch was complete. Three weeks later, on 19 July, all plants were transplanted in a randomized complete block design into the microplots, with each microplot constituting a block. Two plants of each line were planted in each plot, one control (without NCR) and one treatment (with NCR), totalling eight plants per plot. Each treatment was replicated six times (six blocks). The plants were grown under natural conditions except for supplementary irrigation when required. The plants were harvested at maturity (23

Sept.) and plant heights were measured. The root systems were dug up and assessed for NCR damage.

The resulting data were analysed using an analysis of variance procedure for unbalanced data (GLM; SAS 1982).

**Host Preference Trials**

In order to determine whether searching by larval NCR for host plant roots is random or is directed in response to a chemical gradient from corn roots, a simple laboratory choice-chamber test for host attractiveness was conducted.

A three-chambered choice-chamber was constructed from 5.5 cm diameter plastic petri dishes and 0.7 cm inside diameter glass tubing following the directions of Branson and Ortman (1969). The substances that were being tested for attractiveness were placed on moist filter paper in either of the end dishes and 10 newly-hatched NCR larvae were released onto moist filter paper in the centre of the middle dish. The petri dishes were tightly sealed and the entire apparatus was placed in a dark box in a 23° C incubator. The position of each larva was recorded at hourly intervals. The trials ran for eight hours or until all the larvae reached the end

dishes. The choices available to the larvae were: distilled water -- distilled water, field corn roots (Pioneer hybrid 3994) -- field corn roots, field corn roots -- grass roots (Tendergreen$^{R}$ lawn seed mixture [creeping red fescue, Kentucky bluegrass, annual rye grass]), field corn roots -- distilled water, line Q179 roots -- line CSJ-1 roots. In each case the same mass (0.5 g) of fresh roots was put in the end petri dishes so that any response recorded was due to the quality of the material rather than the quantity. The resulting data were analyzed by the contingency-table chi-square technique after determining the homogeneity of the variances (Steel and Torrie 1980).

## Results and Discussion

### Inbred Corn Response to NCR Attack (Greenhouse)

Egg hatch was determined to be 72%. Neither line CSJ-1 nor line Q179 showed significant difference from the controls in plant height or root volume at either of the two egg densities used (Table 1). Root damage was significantly greater than in the control treatments for both lines in those plants infested at the rate of 50 eggs/plant, with CSJ-1 showing more

## Table 1.

Mean plant height, root volume, and proportion of roots damaged of two *Zea mays* inbred lines subjected to different rates of infestation with *Diabrotica barberi* (NCR) eggs.

| Treatment (# NCR eggs) | Height (cm) | | Volume (mL) | | Root damage | |
|---|---|---|---|---|---|---|
| | Q179 | CSJ-1 | Q179 | CSJ-1 | Q179 | CSJ-1 |
| CONTROL | 150 a | 148 a | 384 a | 364 a | 0 a | 0 a |
| 25 | 153 a | 156 a | 345 a | 315 a | .036 ab | .048 a |
| 50 | 141 a | 122 a | 320 a | 258 a | .079 b | .314 b |

Means in the same column followed by the same letter are not significantly different. Duncan's multiple range test. (p=0.05).

damage than Q179, indicating its greater susceptibility to larval NCR attack (Table 1). Line CSJ-1 was more heavily damaged at 50 than at 25 eggs/plant whereas line Q179 was not. These results could be due to Q179 being less attractive to the rootworms or to this line exhibiting a degree of antibiosis to NCR larvae.

Even though significant root damage was noted at the highest egg density in both lines, the absence of significant decreases in plant height height and root volume suggests the presence of a degree of tolerance to NCR larval attack. The amount of damage that can be tolerated appears to be high in line CSJ-1, with a mean proportion of roots damaged of 0.314. The highest tolerable amount of damage in both lines remains to be determined.

The mean plant height of both lines unexpectedly increased over that of the control at the moderate infestation level and decreased at the high infestation level. These trends of increase in plant height could be due to the phenomenon of 'overcompensation'. Overcompensation, a process whereby plant growth is higher in the presence of moderate levels of herbivory has been found to occur in corn (Dyer 1975; White and Scott 1983) and in other plants (McNaughton 1983).

**Inbred Corn Response to NCR and ECB Attack**

Plant yields could not be measured due to poor pollination, consequently damage assessments were based on plant height, root volume, and root damage. Egg hatch was determined to be 74%. The results of this experiment (Table 2) were comparable to the previous experiment in that CSJ-1 was more susceptible to NCR attack than Q179. In addition, line Q179 was found to be susceptible to ECB attack while CSJ-1 was resistant, in agreement with Hudon *et al.* (1979).

Line CSJ-1 showed no significant response to infestation by either insect in terms of plant height and root volume (Table 2); the trends, however, indicate that this line was damaged to a greater extent by NCR than ECB. With a higher NCR egg density these trends would possibly become significant.

Plant height and root volume of line Q179 were significantly affected by ECB treatments but not by NCR treatments, suggesting that this line is susceptible to ECB attack but tolerant of NCR attack.

In no case was the damage caused by the combined attack of the two insects greater than the damage caused by one of the insects alone, disproving the original hypothesis

Table 2.

Mean plant height, root volume, and proportion of roots damaged of two *Zea mays* inbred lines subjected to infestation with *Diabrotica barberi* (NCR) and *Ostrinia nubilalis* (ECB) eggs.

| Treatment | Height (cm) | | Volume (mL) | | root damage | |
|---|---|---|---|---|---|---|
| | Q179 | CSJ-1 | Q179 | CSJ-1 | Q179 | CSJ-1 |
| CONTROL | 123 a | 122 a | 323 a | 215 a | 0 a | 0 a |
| NCR | 127 a | 90 a | 266 a | 174 a | .374 b | .253 bc |
| ECB | 106 b | 123 a | 138 b | 198 a | .030 a | .125 ab |
| NCR+ECB | 106 b | 94 a | 142 b | 140 a | .308 b | .387 c |

Means in the same column followed by the same letter are not significantly different. Duncan's multiple range test. (p=0.05)

(combined attack by NCR and ECB results in greater damage than attack by a single insect species). In line Q179 plant height and root volume were the same for the ECB and the NCR+ECB treatments. In line CSJ-1 plant height and root volume were similar in the NCR and NCR+ECB treatments. Cumulative damage was not observed in this experiment probably because one line of corn (CSJ-1) was resistant to ECB while the other (Q179) appears to be resistant to attack by NCR. Given a line of corn that is susceptible to both of these insects, cumulative damage would be expected. To further test hypothesis stated above the experiment should be repeated with a line of corn known to be susceptible to attack by both insect species. Also, to determine the degree of resistance of Q179 to larval NCR attack this experiment should be repeated using a series of higher NCR egg densities.

The results of this experiment indicate that the factor conferring resistance to ECB attack is not the same one conferring resistance to NCR attack. The most common mechanism for ECB resistance in corn is the presence of the cyclic hydroxamate 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) in plant tissues (Klun *et al.* 1967). No chemical resistance factor for NCR has been found (Chiang and French 1980). Tolerance, in the form of large root systems, appears to be the most common resistance mechanism of corn to larval NCR.

**Inbred Corn Response to NCR Attack (Field Microplots)**

Yield measurements were unobtainable for this experiment. Because the experiment was initiated late in the season, the corn silked later in the year than field corn in the area. Since corn silks are a preferred food of adult NCR (Dominique 1983), these relatively few plants attracted large numbers of beetles, resulting in the silks being eaten back to the ear and pollination being inhibited.

When the treatments were compared for each line of corn only CSJ-1 showed a significant reduction in plant height while all lines except Q179 had a significant increase in root damage (Table 3).

Lines A619, Bc23, and CSJ-1 suffered significant root damage but this damage only affected the height of one line, CSJ-1. Q179 is the only line that showed non-significant root damage, reinforcing the conclusions from the previous experiment -- that there is either a lesser degree of attraction to this line or there exists some antibiosis effect that causes a reduction in root damage. CSJ-1 was the only line that suffered reduced height growth as a result of larval NCR feeding damage; this finding supports the results of the two previous experiments which indicated that this line is susceptible to NCR attack.

Table 3.

Mean reduction in plant height compared to control and proportion of roots damaged of four *Zea mays* inbred lines subjected to *Diabrotica barberi* infestation in field microplots.

| Inbred Line | Height reduction (cm) | Root damage |
|---|---|---|
| A619 | 6.1 | 0.133 + |
| Bc23 | 6.0 | 0.181 + |
| CSJ-1 | 15.6 ** | 0.154 + |
| Q179 | 5.3 | 0.076 |

** significant height reduction (p=0.05)
+ significantly different from zero (p=0.05)

In this experiment CSJ-1 suffered 15.4% root damage, causing a reduction in height growth. In the first experiment (in the greenhouse) this line suffered over 30% root damage but no reduction in height growth resulted. In the microplot experiment the growing conditions were less than ideal (hot and dry after transplant) while in the greenhouse the growth conditions were close to optimal, showing that plants grown under optimum conditions are able to tolerate greater amounts of herbivory than those plants grown under more marginal conditions.

**Host Preference Trials**

In all cases within a paired match the variances for the replicates were homogeneous so the data were lumped and chi-square ($chi^2$) analyses were conducted on the resulting values.

When the choice offered to the NCR larvae was distilled water in either end dish they did not migrate out of the central dish. When field corn roots and distilled water were offered as choices, the larvae all consistently migrated towards the roots, indicating that larval searching behaviour is not random ($chi^2=56.5$, $2df, p<0.01$). When the same variety of field corn roots was placed in both end chambers the larvae

were equally attracted and they were distributed evenly between the two chambers ($chi^2$=0.4, 1df, ns).

To determine whether NCR larvae were differentially attracted to different lines of corn, a choice between the same mass of Q179 and CSJ-1 roots was offered. Most of the larvae migrated to an end chamber but the distribution between the two chambers was equal ($chi^2$=0.5, 1df, ns), indicating that the larvae had no preference for one line of corn over the other.

When corn roots were offered in choice with grass roots, the larvae preferentially selected corn roots. Larvae were attracted to both types of roots but the distribution of larvae in the choice-chamber was not random ($chi^2$=19.54, 2df, $p<0.01$), indicating that corn roots are more attractive to NCR larvae than the roots of grass plants.

There are two ways in which insects could aggregate around an odour source (Sutherland 1972). The insects could wander randomly until they come into contact with an arrestant or they could be attracted (make oriented movements) to the source. The results of this experiment indicate that migration of NCR larvae is directed towards corn roots, possibly in response to kairomonal secretions. Branson (1982), in a similar chioce-chamber experiment reached the same conclusion

for *Diabrotica virgifera virgifera*, and it is known that several other subterranean phytophagous insects locate host plants by directed movements (Calkins *et al.* 1967; Sutherland 1972; Doane *et al.* 1975; Jones and Coaker 1977). This host-finding mechanism has also been found in plant parasitic nematodes (Riddle and Bird 1985).

In tests with western corn rootworms, Branson (1982) determined that the larvae were equally attracted to corn and several other plants including Kentucky bluegrass, one of the components of the grass seed mix used in the present experiment. The results of this experiment do not agree with those of Branson. The different results are possibly due to the fact that *D. virgifera* larvae are oligophagous (Branson and Ortman 1967a; 1970) whereas *D. barberi* larvae are primarily monophagous with minimal feeding on other plants (Branson and Ortman 1967b; 1971). Since *D. virgifera* has a wider host range, it would be expected to be attracted to a greater variety of plants and respond to a wider range of attractant chemicals.

It was proposed in an earlier section that inbred line Q179 showed an antibiosis effect towards larval NCR, or was less attractive than line CSJ-1. The results of this experiment show that NCR larvae had no preference for one of these two lines. Therefore Q179 must have some degree of antibiotic

activity to larval NCR.

## NEMATODE EXPERIMENTS

### Materials and Methods

#### Infectivity Tests

Infectivity tests were conducted to determine whether the nematode *Steinernema feltiae*, All and Mexican strains, (obtained from BIOSIS, Palo Alto, CA) can infect all life stages of NCR.

Field-collected eggs, larvae, pupae, and adults of NCR were tested for susceptibility to attack by two strains of *S. feltiae*. Ten NCR individuals were placed on damp filter paper in 9 cm petri dishes and a suspension of 100 nematodes per NCR individual was added. The dishes were sealed and placed in a 23° C incubator in the dark. After seven days the NCR were

removed, dissected, and observed by microscope to detect the presence of nematodes. Each trial was replicated at least two times. The stadia tested were egg, first, second, and third instar larvae, pupa, and adult.

**Adult NCR Bioassay**

Laboratory bioassays were conducted to determine the susceptibility of adult NCR to attack by two strains of the entomogenous nematode *S. feltiae* (All and Mexican strains). These bioassays were only meant to be approximate in order to determine whether one strain was more virulent to NCR and roughly what doses are effective against NCR for use in further experiments.

Field-collected adult beetles were subjected to different densities of the two strains of nematode on moist filter paper in 9 cm petri dishes. A known number of infective nematodes was added to the filter paper in 2 mL volumes of sterile distilled water. Ten adult NCR were then added. The densities of nematodes used were 200, 100, 50, 25, 10, and 0 per individual beetle. The dishes were sealed tightly with Parafilm$^{R}$ and incubated at 23$^{o}$C under a 14:10 light-dark regime. The dishes were examined 72 hours after inoculation with the nematodes and any beetles that appeared to be dead

(not responding to prodding with a dissecting probe) were dissected and the number of beetles infected with nematodes was recorded. The data were transformed to probit mortality and log dose (Finney 1971) and LD50 values were calculated.

**Accessibility Trials**

Since it was not known whether entomogenous nematodes could penetrate the roots of corn plants to attack NCR larvae feeding within the roots, accessibility trials were conducted.

Grain corn was planted in pots in an incubator on 26 May 1986. The light regime of the incubator was 14:10 light/dark and the temperature was 25° C by day and 18° C by night. On 06 June 50 NCR eggs that had been incubated in distilled water at 23° C for 10 days, so they were ready to hatch within a few days, were added to each pot. On 17 July, 50,000 infective juvenile nematodes were added to each pot. The nematodes used were *Steinernema feltiae* All and Mexican strains, *S. bibionis* Sn strain, and *Heterorhabditis heliothidis* NC strain. The two additional nematode species were added because a review of the literature revealed that large differences exist in the virulences of different nematode species and strains to a variety of insect species. Treatments and controls consisted of four pots each with and without nematodes added, respec-

tively. The plants were all harvested on 01 July when the NCR larvae were be in the second instar. The roots were washed and searched for evidence of feeding damage and dissected to extract NCR larvae. NCR larvae found were dissected to determine the presence of nematodes.

To determine whether nematodes were present in the soil at the end of the experiment two late instar greater wax moth (*Galleria mellonella*) larvae were placed in the soil of each pot. Nematodes present in the soil would migrate toward these insects and attack them. After five days the larvae were removed from the soil, dissected and the presence of nematodes was recorded.

**Larval NCR Bioassays**

Laboratory bioassays were conducted against newly-hatched first instar NCR larvae. The nematodes used were *S. feltiae* All and Mexican strains, *S. bibionis* Sn strain, and *H. heliothidis* NC strain (obtained from BIOSIS, Palo Alto, CA). NCR larvae were placed 10 to a dish on moist filter paper in 9 cm plastic petri dishes. An aliquot of a nematode suspension was added to the filter paper so that either 200, 100, 50, 25, 10, or 0 nematodes/larva were found in the dishes. The amount of fluid added to the dishes was made up to 2 mL with dis-

tilled water and small diameter corn roots were added as food for the larvae. The dishes were sealed tightly and placed in a 23° C incubator in complete darkness. The dishes were checked each day and the numbers of dead larvae were recorded. Fresh corn roots were added as required. The trials ran as long as larvae remained alive in the dishes. Each treatment was replicated a minimum of four times. Probit analysis was conducted on the data and LD50 values were calculated.

**Nematode Persistence in Soil**

A field microplot experiment was conducted to determine the longevity of the nematodes in four soil types under field conditions.

The same four nematode species/strains used in earlier experiments were used in these trials. The four soil types were: Ste. Rosalie clay, St. Bernard loam, muck, and sandy soils. The physical characteristics of each soil type are given in Table 4. One by two metre microplots located at the seed farm of Macdonald College were subdivided with 4 mil polythene sheeting to a depth of 35 cm into four equal subplots. A different nematode species/strain was added to each of these subplots; aqueous suspensions of 100,000 infective juveniles were injected into the soil to a depth of ap-

Table 4.

Type, composition, and pH of soils in field microplots.

| Soil type 1) | Soil Composition (%) | | | | Soil pH |
|---|---|---|---|---|---|
| | Clay | Silt | Sand | Organic matter | |
| Ste. Rosalie Clay | 58 | 20 | 18 | 4 | 6.1 |
| St. Bernard Loam | 20 | 19 | 59 | 2 | 5.8 |
| Muck | 8 | 21 | 10 | 61 | 6.5 |
| Uplands Sand | 6 | 5 | 88 | 1 | 6.4 |

1) After Lajoie (1960).

proximately 5 cm. The soil type was randomized between plots and the nematode type was randomized within plots. Each treatment was replicated twice in each soil type. Soil samples of 500 mL were taken approximately weekly from each subplot, placed in 1 L plastic ice cream containers, brought back to the lab and baited with two or three late instar *Galleria melonella* larvae placed in the centre of the mass of soil. The containers were placed in a 23° C incubator for six days after which the larvae were removed from the soil and dissected. The numbers of adult nematodes within the bodies of the larvae were recorded in an effort to quantify the numbers of nematodes present in the microplots. Nematodes were applied to the plots on 05 June 1986 and sampling continued until 16 September 1986.

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**Field Control of NCR Larvae**

A field experiment was conducted on the farm of Monsieur P. Bellefroid at Pike River, P.Q. to determine the efficacy of using entomogenous nematodes to control larval NCR in a grain corn crop.

The nematodes used in this experiment were *S. bibionis* Sn strain and *S. feltiae* Mexican strain. *S. feltiae* was selected

for use in this experiment because it had been used in earlier field experiments against *Diabrotica* spp. (Rhorbach 1969; Poinar *et al.* 1983) and because it had performed well in laboratory bioassays. The Mexican strain of *S. feltiae* was selected over the All strain because it performed better than the All strain in one laboratory bioassay against *Diabrotica* (Jackson 1985) and because sufficient numbers were available, whereas insufficient numbers of the All strain were on hand.

*S. bibionis* was selected because it was the least virulent to NCR in laboratory bioassays (this report) and because it is consistently less virulent than *S. feltiae* (Molyneux *et al.* 1983; Belair and Boivin 1985; Kondo and Ishibashi 1986). It was selected as a contrast to the more highly virulent *S. feltiae*.

The experiment was established in a randomized complete block design with 10 blocks and eight treatments. The experiment was originally set up with only four treatments but four days later another four treatments were added after more information on rate of application of the nematodes was obtained from the nematode supply company. The original four treatments, established 05 May 1986, consisted of two nematode treatments, an insecticide treatment, and an untreated control. The nematodes were applied in-furrow on top of the seeds at seeding time, using a modified plastic wash bottle,

at the rate of 10,000 infective juveniles per metre of corn row. The seeds were then covered with soil to a depth of 5 cm. The insecticide used was fonofos (Dyfonate$^{R}$ 20G) in granular form and was applied in-furrow at a rate equivalent to the manufacturer's recommended rate of application (0.85 kg/ha AI). With the material being used in this experiment this amounted to 0.56 grams per 10 metres of corn row. The granular insecticide was applied using a hand-held dispenser constructed and described by El Hag (1987). After application of the insecticide, the seeds were covered with soil to a depth of 5 cm. The second four treatments were established in the rows of corn immediately adjacent to the first part of the experiment on 09 May 1986. The insecticide and control treatments were repeated and both species of nematodes were applied at the rate of 100,000 infective juveniles per linear metre of corn row.

Each subplot (treatment within a block) consisted of two 10 m rows. Between each subplot unit were two untreated (buffer) rows planted to corn. Four buffer rows were left between the two sets of treatments.

The subplots were sampled for NCR larvae on 07 and 08 June. No samples were taken from the end metre of any subplot, to reduce edge effect. Three corn plants per subplot were cut off at ground level and the roots were dug out in a

20x20x15 cm block of soil. These samples were brought back to Macdonald College and the soil and roots were searched by hand for the presence of NCR larvae. On 21 and 27 September, 25 plants per subplot were selected at random, their heights (from the bottom of the tassel to the first node above ground) were measured, and the ears were removed and brought back to Macdonald College where they were dried in a commercial corn drier and weighed for yield estimates.

All data were analyzed using an analysis of variance procedure for balanced data (ANOVA) of SAS (SAS 1982). The data were analyzed to determine if location (*i.e.* first four treatments *vs*, second four treatments) had any affect. If no affect was found the data were pooled for further analysis.

## Results and Discussion

### Infectivity Tests

All stages of NCR except the egg stage were susceptible to attack by both nematode strains (Table 5). Thus it was concluded that entomopathogenic nematodes might be effective as biological control agents against all life stages of NCR

Table 5.

Percent infection of *Diabrotica barberi* (NCR) stages with *Steinernema feltiae*, All and Mexican strains.

| NCR stage | # NCR | % NCR with nematodes | |
|---|---|---|---|
| | | All strain | Mexican strain |
| egg | 40 | 0 | 0 |
| L1 | 20 | 95 | 95 |
| L2 | 20 | 100 | 95 |
| L3 | 20 | 90 | 95 |
| pupa | 20 | 90 | 85 |
| adult | 40 | 95 | 98 |

except the egg. However, it is not known whether all of these life stages are susceptible to nematode attack in the natural environment. First instar larvae, pupae, and teneral adults are found in the soil and may therefore be susceptible. Second and third instar larvae, however, are found within the roots of corn plants and consequently may not be accessible to nematode attack.

**Adult NCR Bioassay**

Both nematode strains were pathogenic to adult NCR and the responses of the insect to the nematodes were almost identical (Figure 4, Table 6). The slopes of the two regression lines were the same judged by a Student's t-test for slope comparison (Zar 1984) ($t=0.315$, $p=0.05$) and the regression lines fit the data, as determined by a $chi^2$ test of goodness of fit (Table 6). Calculated LD50 values were not significantly different, using the criterion of non-overlapping 95% fiducial limits.

The low LD50 values and the relatively steep slopes of the regression lines suggest that these nematodes are quite virulent to NCR and may be good candidates for biological control of NCR. The results of this experiment are not directly applicable to a field situation, however, because of the dif-

Figure 4.

Probit mortality *vs.* log dose (# nematodes per *Diabrotica barberi* adult) from Petri dish bioassays with *Steinernema feltiae*, All and Mexican strains.

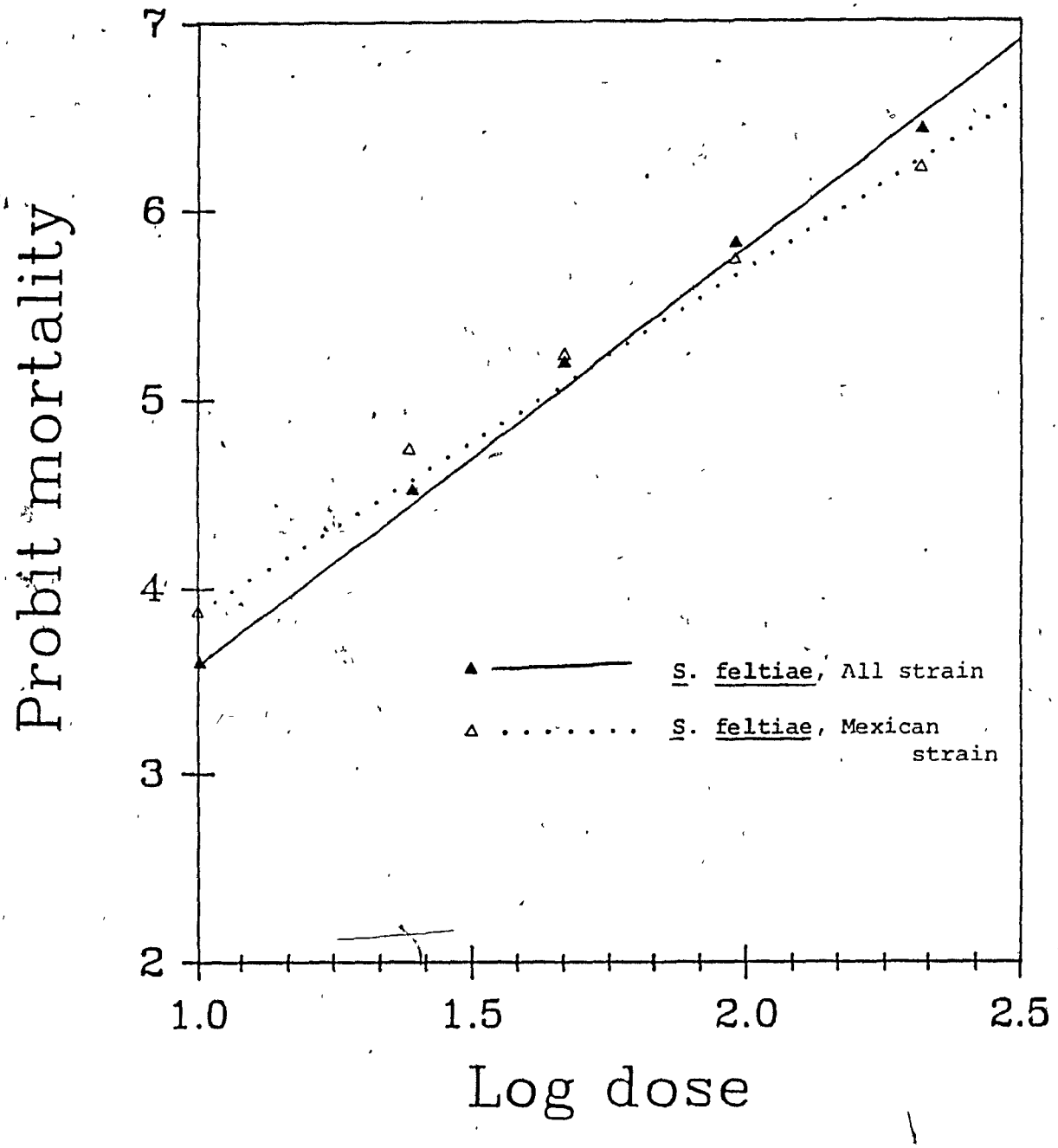


Table 6.

Toxicity of *Steinernema feltiae* All and Mexican strains to *Diabrotica barberi* adults based on Petri dish bioassays.

| Nematode | LD50 \a | 95% fiducial limits \a | $Chi^2$ | Slope |
|---|---|---|---|---|
| *S. feltiae* (Mexican) | 42 | 32-55 | 0.67 | 1.82 |
| *S. feltiae* (All) | 44 | 35-54 | 1.32 | 2.21 |

\a nematodes per adult NCR

ferent environmental regimes. Many laboratory investigations have acheived good control of insect pests, but when experiments were conducted in the field unsatisfactory control resulted (eg. Lewis and Raun 1978; Zelazny 1985). Many of these trials failed because of a poor understanding of the environmental tolerances of the nematode used. Nevertheless, positive laboratory results indicate that the nematode may be a suitable candidate for field experiments.

**Accessibility Trials**

None of the nematodes were capable of infecting NCR larvae within corn roots. When the corn roots were searched, an average of 9.0 NCR larvae per treatment was found and the number recovered was consistent for all treatments (Table 7). All of the larvae found were alive and feeding inside the roots and when they were dissected no nematodes were found.

When the soil was baited with *Galleria* larvae after the corn plants were removed, nematodes were recovered from zero of the four control pots and 15 of the 16 treatment pots (Table 7), indicating that the lack of nematodes in the NCR larvae was not due to a lack of nematodes in the soil but is possibly due to the inability of the nematodes to penetrate the roots or follow the very narrow feeding tunnels of first

Table 7.

Numbers of *Diabrotica barberi* (NCR) larvae recovered from *Zea mays* plants treated with *Steinernema feltiae*, *S. bibionis*, and *Heterorhabditis heliothidis* and recovery of nematodes from the larvae and the soil.

| Nematode (strain) | # NCR larvae recovered (4 plants) | # NCR larvae with nematodes | # pots with nematodes |
|---|---|---|---|
| Control | 9 | 0 | 0 |
| *S. feltiae* (Mexican) | 10 | 0 | 4 |
| *S. feltiae* (All) | 9 | 0 | 3 |
| *S. bibionis* (Sn) | 9 | 0 | 4 |
| *H. heliothidis* (NC) | 8 | 0 | 4 |

instar NCR.

Since entomogenous nematodes appear to be incapable of infecting NCR larvae feeding inside corn roots, control programmes using these nematodes should be directed against other life stages of the NCR. The logical stages to use nematodes against are the first instar larvae, pupae and teneral adults which are all found in the soil. First instar larvae were selected for use in larval bioassays based on the results of this experiment.

**Larval NCR Bioassay**

The four nematode species/strains tested were pathogenic to newly-hatched first instar larval NCR. An analysis of covariance on the probit-log dose regression lines (Zar 1984) indicated no significant differences between slopes ($F=0.969$, $p=0.05$). A $chi^2$ goodness of fit analysis (Hubert 1984) indicated that all of the regression lines fit the data (Table 8).

Of the nematodes tested, *H. heliothidis* was the most virulent to larval NCR at the LD50 level, followed by *S. feltiae* Mexican strain, *S. feltiae* All strain, and *S. bibionis*, in that order (Table 8). When the calculated LD50 values are compared, using the criterion of non-overlapping 95% fiducial

Table 8.

Toxicity of *Steinernema feltiae*, *S. bibionis*, and *Heterorhabditis heliothidis* to first instar larval *Diabrotica barberi*, based on Petri dish bioassays.

| Nematode (strain) | LD50 1) | 95% fiducial limits 1) | $Chi^2$ | Slope |
|---|---|---|---|---|
| *H. heliothidis* (NC) | 37 a* | 27-51 | 6.91 | 1.37 |
| *S. feltiae* (Mexican) | 49 ab | 33-72 | 0.17 | 0.91 |
| *S. feltiae* (All) | 67 ab | 46-97 | 5.59 | 1.11 |
| *S. bibionis* (Sn) | 100 b | 56-180 | 4.32 | 0.81 |

1) nematodes/insect

* values followed by the same letter are not significantly different. Duncan's multiple range test (p 0.05).

limits, *H. heliothidis* is found to be significantly more virulent than *S. bibionis* but is not different from any of the other nematodes.

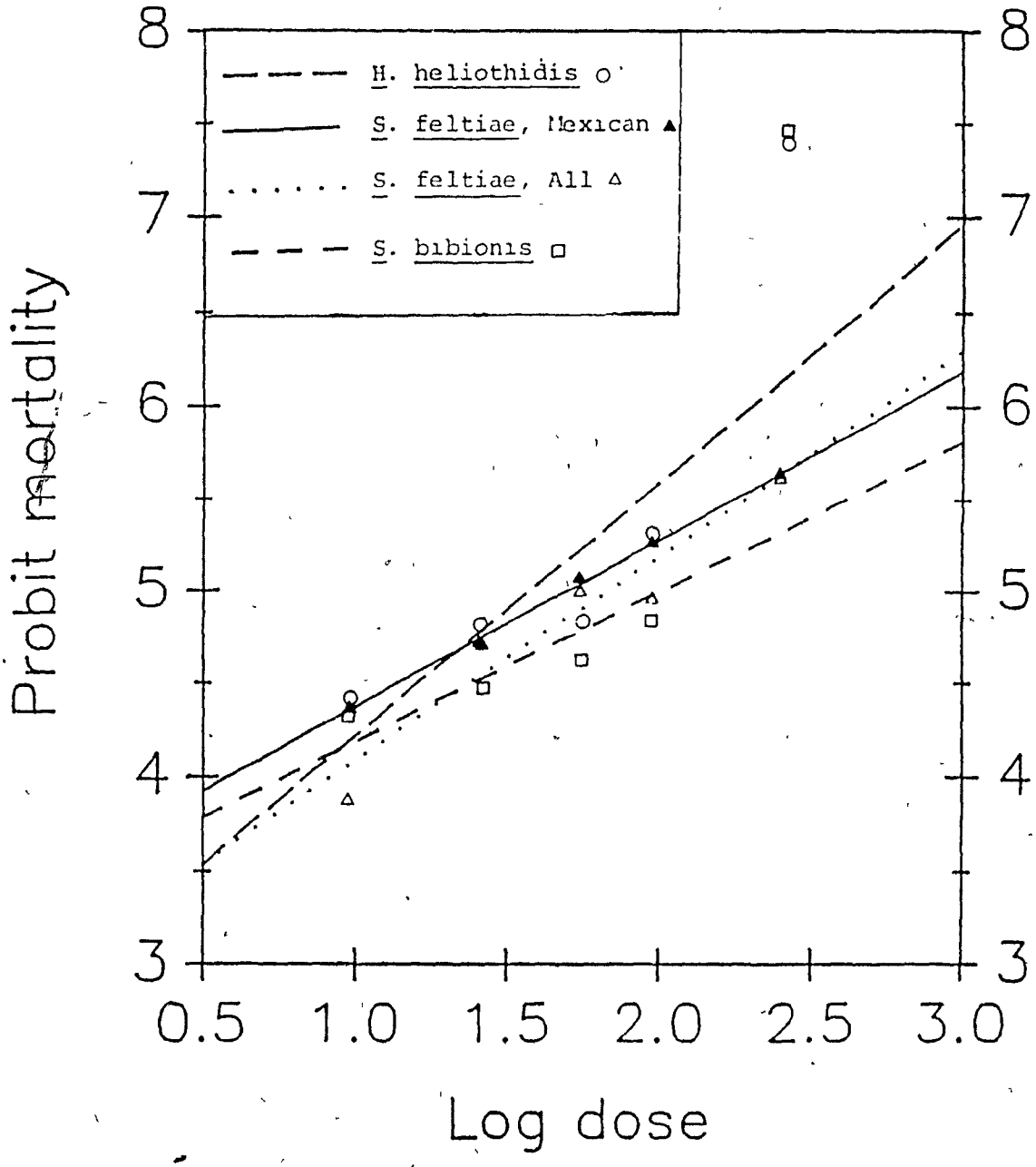
Inspection of the probit-log dose regressions (Figure 5) indicates that, although the slopes of the regression lines were found not to be different by an analysis of covariance, the differences in the virulences of the nematodes become greater at higher dose levels. A repetition of the experiment using more doses at higher levels would clarify the relationships between the lines.

Within a species, differences in virulences of nematode strains may exist (Jackson 1985; Molyneux *et al.* 1983). In this bioassay the Mexican strain of *S. feltiae* was 1.4 times more virulent than the All strain. Although this difference was not significant under the bioassay conditions it may nevertheless be important in more natural conditions. Jackson (1985) in soil bioassays with four strains of *S. feltiae* against western corn rootworm larvae found that the Mexican strain was significantly more virulent than the All strain.

A possible explanation for *H. heliothidis* being the most virulent nematode in these trials is that in addition to entering the host insect through its mouth, anus and spiracles, this nematode is capable of cuticiular penetration (Bedding and Molyneux 1982). This additional mode of infection prob-

Figure 5.

Probit mortality *vs.* log dose (# nematodes per *Diabrotica barberi* first instar larva) from Petri dish bioassays with *Heterorhabditis heliothidis*, *Steinernema feltiae*, All and Mexican strains, and *S. bibionis*.



ably increases the virulence of *H. heliothidis* to many insects, especially smaller ones such as NCR that have relatively small body openings that may restrict the passage of some nematodes.

Large body size is probably important in *S. bibionis* having a relatively low virulence to NCR larvae. Infective stage *S. bibionis* have a mean greatest body width of 29 µm, while *S. feltiae* is 23 µm wide and *H. heliothidis* is 24 µm wide (Poinar 1979). The larger size of *S. bibionis* may inhibit its movement through the small body openings of NCR larvae while the other, smaller, nematodes are not hindered in this manner.

This study has determined that differences exist in the virulence of several nematodes to first instar NCR larvae. The differences noted are relative and are based entirely on the conditions of this particular experiment. Therefore, the results from this experiment can not be quantitatively compared to results from other experiments. However, some qualitative comparisons can be made. The results of this experiment compare favourably with those of several authors but, contradict the findings of others. Zelazny (1985) found that the All and Mexican strains of *S. feltiae* were equal in virulence to *Oryctes rhinoceros*, an insect pest of coconut, whereas Sosa and Beavers (1985) found that the Mexican strain

of *S. feltiae* was significantly more virulent to *Ligyrus subtropicus* than the All strain. *S. feltiae* appears to be consistently more virulent than *S. bibionis* (Molyneux *et al.* 1983; Belair and Boivin 1985; Kondo and Ishibashi 1986). *H. heliothidis* was less virulent than *S. feltiae* and *S. bibionis* to the carrot weevil, *Listronotus oregonensis*, (Belair and Boivin 1985) but was more virulent than *S. feltiae* and *S. bibionis* to the sheep blowfly, *Lucilia cuprina* (Molyneux *et al.* 1983).

These varied results demonstrate that a nematode that is suitable for use against one insect pest species may not necessarily be the most suitable nematode to use against another insect. Therefore, several different nematode species or strains should be screened for virulence before being used in field applications.

Additional studies are required to determine the causes of the different virulences seen between the nematode species and still other studies are required to determine how the nematodes respond in a more natural environment. It is possible that the minor differences in virulence seen under these laboratory conditions could become more important under more natural conditions. The conditions in a Petri dish bioassay and in the natural environment are very different and therefore the factors that allow a nematode to perform well in a

lab bioassay may have no relation to the suitability of that nematode in the field. For example, the ability to find the host insect is important in a natural situation but is unimportant in the closed system of a Petri dish bioassay. Therefore a nematode with an inefficient searching ability would not be eliminated from consideration for use in field trials based on the results of a laboratory bioassay. In addition, since laboratory bioassays tend to be conducted under conditions optimum for the nematode, those nematode strains or species that are poorly adapted to the particular field environment of the target insect will not be identified and eliminated from field trials.

**Nematode Persistence in Soil**

*S. bibionis* and *H. heliothidis* both persisted longer in all soil types than either strain of *S. feltiae* (Table 9). Nematode persistence was always less in the sand and muck soils than the clay or loam soils.

Sand has a very low water-retention capacity because of its large pore spaces (Brady 1984). Therefore, sandy soils dry out more rapidly than other soil types with smaller pore spaces (eg. clay or loam soils) and reduce the viability of

Table 9.

Persistence of *Steinernema feltiae*, *S. bibionis*, and *Heterorhabditis heliothidis* in four soil types under field conditions.

| Nematode (strain) | Soil Type | SAMPLING DATE (1986) June 20 | June 30 | July 08 | July 15 | July 25 | Aug 05 | Aug 12 | Aug 19 | Aug 26 | Sept 16 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *S. feltiae* (Mexican) | Sand | + | + | + | - | - | - | - | - | - | - |
| | Loam | - | - | + | + | + | - | - | - | - | - |
| | Clay | + | + | + | + | + | - | - | - | - | - |
| | Muck | + | - | + | - | - | - | - | - | - | - |
| *S. feltiae* (All) | Sand | - | + | + | - | - | - | - | - | - | - |
| | Loam | - | - | + | - | + | - | + | - | - | - |
| | Clay | + | + | - | + | + | + | - | - | - | - |
| | Muck | + | + | + | + | + | - | - | - | - | - |
| *S. bibionis* (Sn) | Sand | + | + | + | + | + | + | + | - | - | - |
| | Loam | + | + | + | + | + | - | + | - | + | + |
| | Clay | + | + | + | + | + | + | - | + | + | - |
| | Muck | - | - | + | + | - | + | + | - | - | - |
| *H. heliothidis* (NC) | Sand | + | + | + | + | + | + | - | - | - | - |
| | Loam | - | - | + | - | + | + | + | + | + | + |
| | Clay | + | - | + | - | + | - | + | - | + | + |
| | Muck | + | + | - | + | + | + | + | - | + | - |

\+ nematodes present
\- nematode presence not detected

nematodes in that environment. The poor persistence of the nematodes in the sandy soil in this experiment was probably due to the periodic dry conditions during the season. This agrees with Molyneux and Bedding (1984), who found that nematode parasitism was reduced at low moisture potentials, especially in sandy soils.

Muck soils have very high water-holding capacities and are often poorly drained (Brady 1984). Therefore they often become waterlogged earlier and stay waterlogged longer than other soils. Molyneux and Bedding (1984) found that nematode parasitism at high soil moisture potentials was reduced. Since diffusion of oxygen from the atmosphere into the soil varies directly with the amount of air in the soil (Wallace 1968) and nematodes respire aerobically (Burman and Pye 1980) reduction of parasitism at high moisture levels could be due to a lack of available oxygen for respiration. The poor nematode persistence in muck soil observed in this experiment is thus possibly due to the adverse affects of saturated soil on these nematodes.

Nematode persistence in the clay and loam soils was good probably because of their moderate water-holding capacities and good drainage. These soils probably did not dry out as rapidly or as often as the sandy soil, nor did they stay saturated for as long after a rainfall as the muck soil.

It appears (Table 9) that *S. feltiae* is not as well-adapted as *S. bibionis* or *H. heliothidis* for long-term (several months) survival in any of the soil types tested. This should be taken into account when selecting a nematode for use in field trials, especially if establishment of a nematode population in the field is desired. Nevertheless, *S. feltiae* may be suitable for field applications, where it is used as an insecticide in inundative release and long-term survival is not important.

**Field Control of NCR Larvae**

In no case did location (*i.e.* the two parts of the experiment) have a significant affect on the results (ANOVA, $p<0.01$), therefore the control and insecticide treatments were pooled and the experiment was analyzed as a single unit. Analysis of the larval data revealed a significant treatment effect so a Duncan's multiple range test was conducted on the mean number of larvae per plant in each treatment (Table 10).

The number of larvae per plant was very low in all treatments. The field populations of the northern corn rootworm in the summer of 1986 were unusually low, probably because of a very early but wet spring that allowed the larvae to hatch

Table 10.

Effect of field applications of *Steinernema feltiae*, *S. bibionis*, and the chemical insecticide fonofos on *Diabrotica barberi* larval number and *Zea mays* plant height and grain yield.

| Treatment | Mean number of larvae per plant | Mean plant height (cm) | Mean grain yield (g/25 plants) |
|---|---|---|---|
| Control | 2.17 a | 217 ab | 2874 ab |
| *S. bibionis* (10,000/m) | 1.50 ab | 214 c | 2785 b |
| *S. feltiae* (10,000/m) | 1.43 b | 214 c | 2790 b |
| *S. bibionis* (100,000/m) | 1.17 b | 219 a | 2987 a |
| *S. feltiae* (100,000/m) | 1.17 b | 220 a | 2925 ab |
| fonofos (0.85g/ha) | 0.45 c | 216 bc | 2945 a |

Means in the same column followed by the same letter are not significantly different. Duncan's multiple range test (p=0.05)

early but prevented the farmers from planting their corn for several weeks. This could have resulted in an unavailability of food for the newly-hatched larvae so they starved to death. Possibly, the only rootworms that survived to attack the corn were the few that hatched later in the spring.

Even though larval numbers were very low, several of the treatments significantly reduced the number of larvae per plant compared to the number found in the control treatment (Table 10). The insecticide, fonofos, caused the greatest reduction in larval numbers, reducing them significantly from the control and also from all nematode treatments. Both nematode species applied at the rate of 100,000 nematodes per linear metre gave significant larval reductions from the control whereas only *S. feltiae* gave a significant reduction when applied at the rate of 10,000 per linear metre. These results do not support the results of Poinar *et al.* (1983), who found that *S. feltiae* applied at the rate of 10,000/m reduced larval populations of mixed corn rootworm species significantly more than did an insecticide, chlorpyrifos.

Significant treatment effects were also observed when plant height and grain yield data were analyzed. Duncan's multiple range tests were conducted on these data, the results are reported in Table 10. The differences between the treatments are all very small, probably because of the unusually

low NCR population in the field. The difference in plant height between the treatments with the maximum and minimum effects is only 2.7%, yet this difference was determined to be significant probably because of a low variability of plant height within plots. The difference between the lowest and highest grain yield is also very low, 6.8%. These differences are probably not biologically relevant.

The only treatments that were significantly different from control for plant height were *S. feltiae* and *S. bibionis* applied at the rate of 10,000/m. Plant heights in these treatments were significantly less than in the control treatment. However, the actual differences are so small that they are probably not biologically relevant. When yield data were analyzed no treatment was found to be significantly different from control although the highest yields were obtained in the insecticide treatment and in the 100,000/m treatment of both nematodes.

The results of this experiment suggest that either nematode applied at a rate of 100,000 infective juveniles per linear metre of corn row can effectively reduce larval NCR populations and may prevent yield reductions in corn in years with high NCR populations. The results also indicate that the control achieved by the nematodes is not as great as control by fonofos. Nevertheless the nematodes are probably suitable

alternatives to chemical insecticides because the NCR larval population reduction observed is possibly sufficient to reduce the pest to below the economic threshold. To clarify the results of this experiment it must be repeated in fields with higher NCR populations. This could be accomplished by repeating the experiment for several years or by artificially infesting the field with NCR eggs (Palmer *et al*. 1977).

## SUMMARY AND RECOMMENDATIONS

Laboratory and field studies indicate that host plant resistance and the use of entomogenous nematodes are potentially effective biological control techniques for northern corn rootworm.

Resistance to larval NCR attack exists in inbred lines of corn primarily in the form of tolerance but indications of an antibiosis effect were obtained in line Q179. Both inbred lines were capable of tolerating some NCR feeding. Line CSJ-1 was more strongly affected by larval NCR feeding than Q179, suggesting that CSJ-1 is susceptible while Q179 is resistant. The identification of inbred lines with high tolerance levels and the development of hybrid varieties from these inbreds, as has been done successfully in Quebec against European corn borer, are research directions that may prove to be fruitful. In addition, further investigations into the possible antibiosis of line Q179 should be conducted. Because the European corn borer and the northern corn rootworm often occur together in Quebec cornfields, a search for lines of corn resistant or tolerant to both of these insects should be initiated, beginning with a characterization of the inbred lines known to be resistant to ECB.

The northern corn rootworm is susceptible to attack by

entomogenous nematodes and was determined to be a good candidate for biological control using these organisms. Based on the results of laboratory bioassays and field persistence trials *Heterorhabditis heliothidis* was determined to be the most suitable nematode to use in control programmes against NCR. However, the virulence of this nematode to NCR under field conditions remains to be determined. *Steinernema feltiae* and *S. bibionis* both significantly reduced numbers of NCR larvae in the field but no increase in corn yield resulted, probably because of the low populations of NCR involved. Better control of NCR larvae in the field was achieved with a chemical insecticide, fonofos, than with nematodes applied at the rate of 100,000 per linear metre of corn row. Further experiments need to be conducted to determine whether better control in the field can be achieved with another nematode species or strain.

With further development, host plant resistance and entomogenous nematodes hold promise for successful biological control of NCR. These two control techniques are compatible with one another and are suitable for inclusion in an integrated pest management programme. The development of such a programme would eliminate some of the problems associated with chemical insecticides and, over the long term, possibly provide more effective control than that provided by using chemical insecticides alone.

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