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UNIVERSITÉ D'OTTAWA
UNIVERSITY OF OTTAWA

To my parents

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LIST OF ABBREVIATIONS

ai: active ingredient

AZA: azadirachtin

EC: emulsifiable concentrate

H_2-AZA: dihydro-azadirachtin

$^3H_2$-AZA: tritiated-dihydro-azadirachtin

HPLC: high pressure liquid chromatography

IGR: insect growth regulator

LSC: liquid scintillation counting

NSKE: neem seed kernel extract

PBO: piperonyl butoxide

TLC: thin layer chromatography

ABSTRACT

The use of neem products to control the European corn borer, Ostrinia nubilalis (Hübner), in sweet corn was investigated. Three years of field studies were conducted comparing various foliar-spray applications of a neem seed kernel extract (NSKE), azadirachtin (AZA, the active ingredient of neem extracts), and Ambush (a synthetic pyrethroid).

The results included: (1) NSKE sprayed prior to artificial infestation of the corn plants provided excellent protection (comparable to Ambush) against borer damage and greatly reduced larval populations. (2) Neem extract formulations from Safer Ltd. were the most effective. (3) The rate of application or number of applications were not determining factors in altering the efficacy of neem products. (4) Exceptional weather conditions (high temperatures, dry conditions) may be detrimental to the efficacy of foliar applied neem products. (5) Some neem treatments were found to increase corn stalk breakage possibly by altering the behavior of the larvae.

Laboratory evaluations using no-choice leaf-disk bioassays showed the antifeedant action of AZA and the negligible effect of PBO (piperonyl butoxide) and Citowett (used in field formulations) on the feeding behavior of 3rd instar larvae. Growth and development studies of O. nubilalis fed a diet containing 0, 1, 3, 10, 30, 100 and 1000 μg NSKE g^{-1} diet were conducted. 10 μg NSKE g^{-1} diet (50 ng AZA g^{-1} diet) fed continuously to the larvae were larvicidal and concentrations as low as 3 μg NSKE g^{-1} diet (16.6 ng AZA g^{-1} diet) significantly reduced larval, pupal and adult weights, increased the larval and pupal periods, decreased adult emergence and caused wing deformities. AZA content of the extracts was found to be a critical factor in the IGR (insect growth regulator) effects of NSKE.

The systemic action of H_2-AZA was studied using the tritiated form of the compound.

Foliar uptake of $^{3}H_{2}$.AZA by sweet corn seedlings was minimal (<15%) 24, 48 and 72 hours following application. The absorbed ^{3}H-material was found to translocate mostly to the roots and root medium. Translocation of the label to other leaves was also detected.

RESUME

Une étude a été menée sur l'utilisation des produits à base du margousier (ou neem) pour contrôler la pyrale du maïs, Ostrinia nubilalis (Hübner), envahissant le maïs sucré. Trois saisons d'étude sur le terrain ont été nécessaires afin de comparer différents arrosages foliaires à base d'extraits de neem (NSKE), d'azadirachtine (AZA, l'ingrédient actif des extraits de neem), et d'Ambush (un pyréthrinoïde synthétique).

Les résultats de ces études démontrent que: (1) Un arrosage à base de NSKE précédent l'infestation artificielle de plants de maïs avec les oeufs de la pyrale est aussi efficace que Ambush (application conventionnelle) à protéger le maïs sucré des dommages causés par la pyrale, et réduit considérablement la population larvaire. (2) Les extraits de neem préparés par la compagnie Safer Ltd. ont été les plus efficaces. (3) Les taux ou le nombre d'arrosages avec les NSKE ne sont pas des facteurs qui influencent leur efficacité. (4) Certaines conditions climatiques (températures élevés, sécheresse) peuvent agir négativement sur l'efficacité des produits à base de neem. (5) Certains traitements à base de neem ont augmenté le minage de la tige, possiblement en modifiant le comportement larvaire.

Des études en laboratoire ont démontré l'action anti-appétante de l'AZA ainsi que l'effet négligeable du PBO et Citowett (produits compris dans la fabrication des solutions utilisées sur le terrain) sur O. nubilalis du troisième stade larvaire. Des études de croissance et de développement où des larves d'O. nubilalis ont consommé de la nourriture contenant 0, 1, 3, 10, 30, 100 et 1000 μg NSKE g^{-1} ont été menées. Une concentration de 10 μg NSKE g^{-1} (50 ng AZA g^{-1}) est létale tandis que des effets néfastes tels qu'une baisse importante du poids larvaire, des pupes et des adultes, une augmentation de la période larvaire, une baisse du taux d'émergence chez les adultes et des malformations au niveau des ailes, ont été notés aux concentrations plus basses. Ces

études ont aussi démontré que la concentration d'AZA est un facteur important régissant les effets de régulateurs de croissance des NSKE.

Une étude sur le mouvement systémique de l'AZA dihydrogénée (H_2-AZA), à l'aide d'H_2-AZA tritiée a démontré que l'assimilation de l'H_2-AZA tritiée par les feuilles de jeunes plants de maïs sucré, 24, 48 et 72 heures après son application, est faible (<15%). Le matériel radioactif assimilé a été retrouvé principalement au niveau des racines et du milieu aqueux dans lequel elles baignaient. Les feuilles non-traitées contenaient aussi des traces de radioactivité.

A - INTRODUCTION

1.0 In Search of Novel Approaches to Pest Control

Modern agricultural practices have brought about environmental and health related concerns. The nature of these problems is complex and encompasses, amongst many others, large scale infestations by insect pests. Modern agriculture's problems arose from the development and breeding of crops with high yields and superior agronomic traits, and cultivating these genetically uniform hybrids over large areas.

It is well known that most phytophagous insects will find their hosts more efficiently when no other plant species are present to interfere (Strong et al, 1984). Monocultures thus offer a perfect environment with an unlimited food source for an insect to colonize. The treatment against the symptoms caused by insect pests i.e. loss of product and thus profit has been synonymous with the use of synthetic pesticides. This effort to rid monocultures of damaging insects was designed to be efficient and convenient with a quick and noticeable increase in profits (Pedigo, 1989).

Unfortunately the disadvantages of synthetic pesticides have become overwhelming: their non-specificity, the persistence of by-products, the high incidence of misuse, and the development of resistance in pest populations (Pedigo, 1989). From the negative impacts that synthetic pesticides have had on agriculture itself, the environment and human health, alternative pest control methods have stemmed and are evolving towards an agricultural system associated with balance and decentralization. The present effort to reduce synthetic pesticide application, or integrated pest management (IPM), combines techniques that aim for efficiency, a more conscientious use of conventional pesticides and substitution of these non-specific chemicals. IPM's first essential step is monitoring the pest and having a thorough knowledge of its life cycle (Pedigo, 1989). Monitoring includes techniques such as regular inspection of the crop and its environment, traps with

attractants such as pheromones and interception traps. Once the status of the pest population and its economic threshold are established, control measures can then be considered. These pest control methods embrace a wide range of strategies such as;

1) biological controls which include parasites (parasitic wasps, flies, nematodes), predators (ladybird beetles, wasps, spiders, birds), pathogens (bacteria and/or their metabolic products, fungi, viruses, protozoa, mycoplasma),

2) pheromones (sex attractants) that disrupt the normal mating behavior of certain pests,

3) introduction of sterile or genetically incompatible males in certain pest populations,

4) the development of resistant crop varieties,

5) pesticides that are better formulated and are applied using improved techniques and

6) safer pesticides such as botanical insecticides that have low mammalian toxicity and are non-persistent in the environment, antifeedants, insect growth regulators (IGR) that specifically affect the insects' development, hormones, sterilants, abrasive dusts (Pedigo, 1989).

The alternatives listed above have proven to be effective and will certainly occupy a more prominent role in pest control, but they are still merely treatments to cure some of the symptoms of our ailing agricultural system. A holistic approach where the agrosystem is based on cultural methods rather than curative ones seems to be the key to a "sustainable" form of agriculture. Within such a system factors to be considered would include;

1) site selection (soil type, fertility and structure, climate, site history),

2) crop selection (resistant varieties, varieties suited for soil and climate, varieties able to compete with weeds),

3) planting methods (crop rotation, intercropping, appropriate planting time to avoid pest, management of field borders),

4) maintenance of site (tillage, irrigation, drainage, organic fertilizers, introduction of

beneficial organisms, pest monitoring),

5) in the advent of pest outbreak: mechanical removal of pest if feasible, introduction of pheromone traps, repellents, parasites, pathogens or predators, biological control, application of safe insecticides, IGRs or behavior modifiers that are effective, specific and have low persistency and

6) harvesting and storage (times harvest to avoid late pests, destruction of crop residues that are overwintering sites, storage of pest free produce).

Profound changes in society's demands, economy and politics must occur for such cropping systems to become universal. Hopefully, we are heading in that direction. Presently, we are still seeking substitutions to the use of conventional pesticides to protect our crops. As previously mentioned, botanical insecticides are an important pest control strategy since they often include compounds that strictly affect insect metabolism, such as IGRs, or their behavior such as antifeedants. The neem tree (Azadirachta indica A. Juss) offers one of the most promising botanical insecticides.

2.0 Neem: Its Properties and Uses

2.1 Botanical and Cultural Aspects

Azadirachta indica A. Juss. (Meliaceae); syn. Melia azadirachta L., Melia indica Brandis, Neem tree, Indian lilac, or Margosa tree. Also referred to as "neeb" in Arabic, "neem" in Hindi and Urdu, "azaddirakht" in Persian, and "nimb" in Sanskrit. (Ahmed and Grange, 1986).

The neem tree is a fast-growing evergreen tree that attains heights of 7 to 20 m at maturity and may live to be 200 years old (Radwanski, 1977). Trees produce fruit in 4 to 5 years and are fully productive in 10. Fruit consist of a fleshy pericarp which surrounds the oil-rich kernel. The mature tree produces from 37 to 50 kg of fresh fruit annually yielding an average of 5.5 kg of kernel from which approximately 2.5 kg of

neem oil can be extracted (Koul et al, 1990).

From its native arid Indian sub-continent, neem is now widely distributed in South Asia, Africa, Central and South America, Australia and tropical areas of North America (Schmutterer, 1985; Ahmed et al, 1986). In many countries, the introduction of neem has served reforestation purposes and production of fuel wood. Azadirachta indica is also used as a boulevard or shade tree and most importantly as a supply of botanical pesticide (Schmutterer, 1990a). Neem plantings such as the ones in the Philippines (introduction of the neem tree over 40,000 ha of forest land) require virtually no attention; management needs such as fertilizers and irrigation are low (Radwanski, 1977). Subhumid to semiarid conditions constitute ideal environments in which neem trees can thrive. They can be established in warm regions where precipitation is less than 500 mm $year^{-1}$, without irrigation. Neem trees will grow from seedlings, saplings or ripe seed (Radwanski and Wickens, 1981).

2.2 Historical Background

The uses of neem for medicinal and pest control purposes have been known to Indians since antiquity and are mentioned in classical texts of Ayurveda (van der Nat et al, 1991). The first study of the repellent properties of neem was reported by Pradhan et al (1962) who found that a 0.001% aqueous suspension of ground neem kernels was sufficient to deter adult locusts from feeding. Subsequently, field tests using 0.1% aqueous ground neem kernel extract were conducted. This aqueous neem suspension was sprayed on different crops at 300 to 600 L ha^{-1}; feeding by desert locusts was completely halted, the effect lasting 2 to 3 weeks (Pradhan and Jotwani, 1968). Since then, the number of publications on the uses and properties of neem as a crop protectant has been overwhelming.

2.3 A Panoply of Uses

All parts of the neem tree have at one time or another been utilized to serve man. A simple listing of its properties and uses will enable the reader to appreciate the versatility and complexity of neem's products (Table 1). The pesticidal properties of neem will be reviewed in greater detail. First, a look into the chemistry of neem will reveal the ingredients that are the source of its pest control capabilities.

2.4 Active Ingredients of Neem

The chemical composition of most parts of the neem tree has been the source of many studies and reviews (Kraus, 1984; Schmutterer et al, 1981; Schmutterer and Ascher, 1984; Siddiqui et al, 1988; Jones et al, 1989; Mitra and Misra, 1967; Keher and Nagi, 1949; Tirimanna, 1984; Fujiwara et al, 1982). More than 100 compounds have been isolated from the leaves, bark or neem seed kernels and chemically characterized. Neem constituents comprise protolimonoids, limonoids also referred to as tetranortriterpenoids, pentanortriterpenoids, hexanortriterpenoids, and nontriterpenoidal compounds (Koul et al, 1990; van der Nat et al, 1991) (Figure 1, Table 2).

2.4.1 Azadirachtin

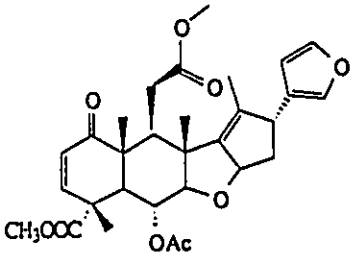
Azadirachtin (AZA), a tetranortriterpenoid is regarded as the most active principle in neem seed kernels because of its numerous effects on insects (Rembold, 1989; Isman et al, 1990; Schmutterer, 1990a) (Figure 2a). AZA has been found to be a deterrent (Butterworth and Morgan, 1971), an antifeedant (Arnason et al, 1985; Kubo and Klocke, 1982), a growth regulator (Rembold, 1987), a fecundity and fitness reducing compound (Rembold et al, 1987; Rembold et al, 1984; Dorn et al, 1987), and an antiovipositional compound (Rice et al, 1985).

TABLE 1: Properties and uses of Neem Products (Koul et al, 1990)

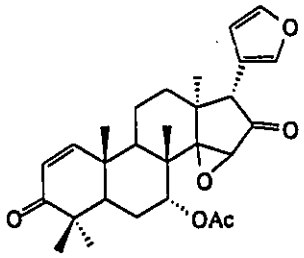
PROPERTIES AND USES	NEEM PRODUCT
MEDICINAL PROPERTIES	
antimalarial	seed oil
febrifuge	
vermifuge	
antihelminthic	
antiseptic	
antimicrobial (mycobacteria, pathogens: Staphylococcus typhosa, Salmonella paratyphi, Vibrio cholerae iriba, Klebsiella pneumoniae)	
control of bronchitis	
dermatological agent (heals skin disorders: ringworm, scabies	extract of dried neem leaves
fungal growth on animals and humans	neem oil extract
viral skin infections in rabbits and monkeys, exzema, scabies)	aqueous leaf extract
antileprotic	aqueous leaf extracts
antimalarial	
analgesic	neem oil extract
antipyretic in guinea pigs	neem oil
antiinflammatory	
antihistaminic	
diuretic (dogs and humans)	nimbidinic acid from neem seeds
antitumour agent (epidermoid carcinoma, parotid tumours)	neem seed oil
antifertility properties in mice	aqueous neem leaf extract, neem kernel extract
retards spermatogenesis in male rats	neem cake
prevents pregnancy in female albino rats	neem oil
INDUSTRIAL AND PHARMACEUTICAL USES	
soap (Margo soap)	neem oil
dog shampoo and soap in the control of ticks and fleas	
dentifrice, heals gum inflammations and paradontosia (Nimodent)	extract of neem bark
toothpaste and mouthwash (Silvose T, Silvose TRS,	
Dr. Grandel's Neem toothpaste)	
contraceptive (Sensal for intravaginal use)	neem oil
local sedative (Olosyn for external application)	neem leaf preparation
oral tablets (JK22 recommended for diabetes mellitus of adults,	neem leaf decoction
nonketonic diabetes, insulin fast or sensitivity)	
wound dressing	neem oil
neem cream (Marguentum Forte ointment for dermatological use)	neem oil
edible oil	debittered neem oil
cattle feed supplement (Pasutone to kill intestinal worms)	neem leaf powder
fly and mosquito repellent (Nemlet)	neem oil
AGRONOMIC USES	
pesticide formulation (Margosan-O concentrate recommended for use	neem seed extract
against insect pests on nonfood crops	
organic manure	neem cake, neem oil
USE AS FEED FOR FARM ANIMALS	
poultry feed	extracted seed materials mixed with feed seed
buffalo feed	neem seed meal rich in protein and free from fat and lipid
feed for milking cows	neem seed cake
sheep feed	deoiled neem cake mixed with maize

FIGURE 1

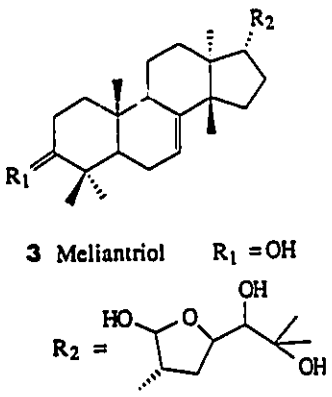
Some important tetracyclic triterpenoids and their derivatives from A. indica. (Koul et al, 1990



1 Nimbin

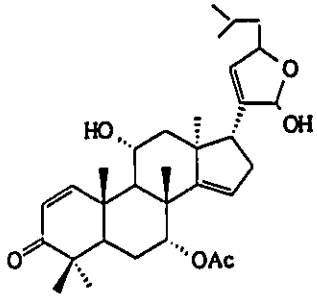


2 Nimbinin

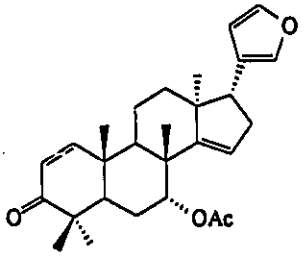


3 Meliantriol R_1 = OH

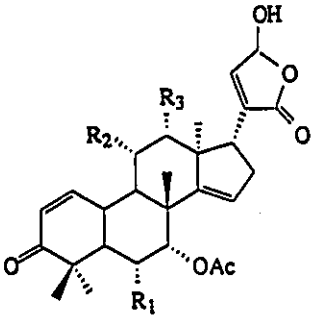
R_2 =



4 Azadirachtol



5 Azadirone



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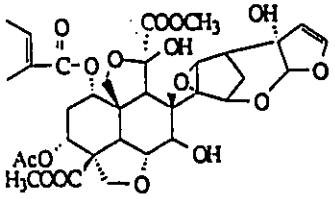
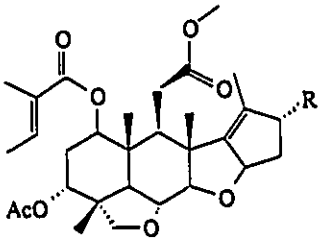
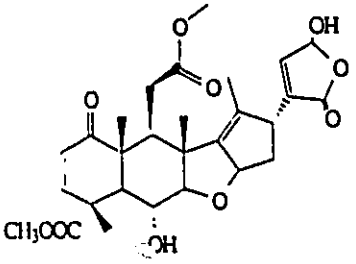


7 Azadirachtin

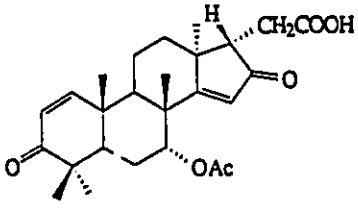


8*a* Salannin R =

8*b* Salannolide R =



9 Desacetylnimbinolide



10 Nimolicinoic acid

TABLE 2: Chemical Profile of the Neem Tree (van der Nat et al, 1991)

COMPOUND	PART OF TREE FROM WHICH ISOLATED
Group 1 Tetracyclic triterpenes, Protolimonoids (=protomeliacins=melianes)	
Meliantriol	Seed oil
Nimbocinone	Fresh, undried winter leaves
Group 2 Apo-tetracyclic triterpenes, apo-protolimonoids, derived from group 1 by an apo-rearrangement	
Azadirachtol	Fresh, ripe fruits
Group 3A Limonoids derived from Group 2 by a loss of 4 C-atoms from the side-chain followed by a rearrangement of the rest to a Y-hydroxybutenolide ring. Pentacyclic nortriterpenoids	
Isonimbocinolide	Fresh, undried winter leaves
Nimocinolide	Fresh, undried winter leaves
Isonimocinolide	Fresh, undried winter leaves
Group 3B Limonoids derived from group 2 by a loss of 4 C-atoms from the side-chain followed by a rearrangement of the rest to a furan ring. Pentacyclicnortriterpenoids	
Azadirone	Oil from dried fruits, fresh fruits
Azadiradione	Oil from dried fruits, fresh fruits
17-Epi-azadiradione	Dried fruits, dried seeds
17-B-Hydroxyazadiradione	Dried fruits, dried seeds
17-a-Hydroxyazadiradione	Fresh fruit pulp
7-Desacetyl-7-benzoylazadiradion	Dried seeds, fresh fruits
Nimocinol	Undried winter leaves
Nimocin	Fresh fruits
Nimbocinol	Fresh, ripe fruits
Epoxyazadiradione	Oil from dried fruits, fresh fruits
= Nimbinin	Seed oil
1a-Methoxy-1,2-dihydroepoxyazadiradiaone	Dried seeds
1B,2B-Diepoxyazadiradione	Dried seeds
7-Desacetyl-7-benzoylepoxyazadiradione	Dried seeds
Meldenin diol	Green leaves
Meldenin	Seed oil
Isomeldenin	Fallen yellow leaves
7-Actylneotrichilenone	Dried seeds
Vepinin	Seed oil
Nimbolin A	Trunk wood
Nimbidinin	Seed oil
Vilasinin	Green leaves
1,3-Diacetylvilasinin	Seed oil
4a,6a-Dihydroxy-A-homoazadiron	Dried leaves
Azadirachtanin	Fresh leaves

COMPOUND	PART OF TREE FROM WHICH ISOLATED
Group 4A Limonoids derived from Group 3A by oxidative fission of ring C. Ring C-seco limonoids with a Y-hydroxybutenolide ring	
Salannolide	Seed oil
Isonzadirolide	Fresh winter leaves
Margosinolide	Fresh, green twigs
Isomargosinolide	Fresh, green twigs
Group 4B Limonoids derived from Group 3B by oxidative fission of ring C. Ring C-seco limonoids with a furan ring. This furan group may be further oxidized, as in the azadirachtin C-seco limonoids	
Azadirachtin	Seed
22,23-Dihydro-23-B-methoxyazadirachtin	Seed
3-Tigloylazadirachtol	Seed
Nimbin	Seed oil, flowers, root bark, trunk bark
6-Desacetylnimbin	Seed, bark
Nimbandiol	Seed oil, dried leaves
6-O-Acetylnimbandiol	Seed oil
4-Epinimbin	Seed oil
Nimbinen	Seed oil, dried leaves, bark
6-Desacetylnimbinen	Seed oil, dried leaves, bark
Desacetyldihydronimbic acid	Fresh leaves
Nimbolide	Fresh leaves
Nimbolin B	Trunk wood
Nimbidic acid	Seed oil
Salannin	Seed oil
3-Desacetylsalannin	Seed oil, dried leaves
Salannol	Seed oil
2,3-Dehydrosalannol	Dried leaves
Group 5 Limonoids derived from Group 3B by oxidation of ring D to a lactone	
Gedunin	Oil from dried fruits, fresh fruits
7-Desacetyl-7-benzoylgedunin	Dried seeds
Nimolicinol	Fresh, ripe fruits
Other Compounds	
Nonlimonoidal compounds	
Hydrocarbones	Fruit, seed oil
Fatty acids	Fruit, seed oil
Sterols	Fruit, seed oil
Phenols	Fruit, seed oil
Glycosides	Fruit, seed oil
Flavanoids:	Leaves
Nimbaflavone	
Nimbochalcin-a	
Nimbocetin-a	
Dihydrochalcone	
Quercetin	

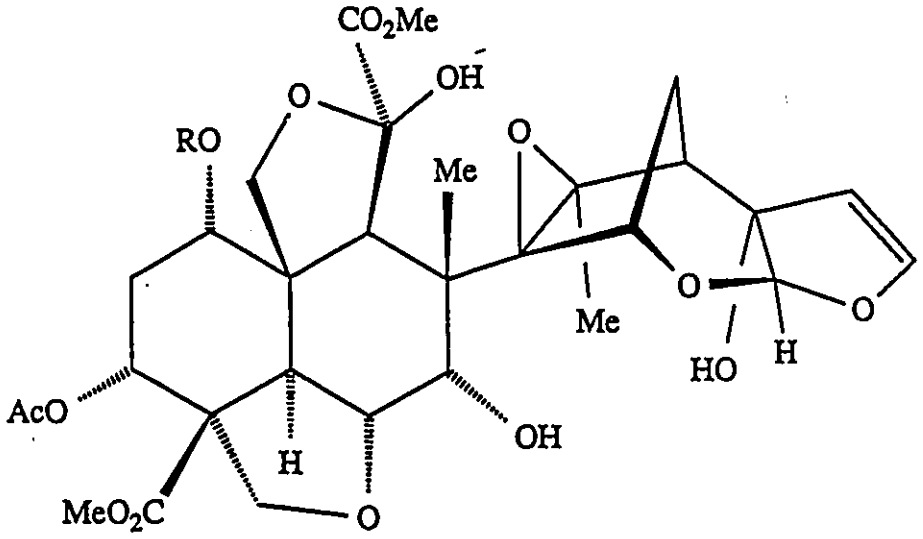
COMPOUND	PART OF TREE FROM WHICH ISOLATED
Other Compounds ...cont	
Coumarins: Scopoletin	Leaves
Alkanes	Leaves
Sulfur compounds: Trisulfides Tetrasulfides A series of 25 volatile sulfides	Leaves, seed oil

The amount of AZA extractable from neem seed kernels varies depending on the source of the kernels; there are geographical as well as genetic reasons for these variations and the length of storage of the kernels can also decrease the AZA content (Ermel et al, 1987; Singh, 1987). Schmutterer (1990a) reports the highest AZA yield to be 10 g kg^{-1} neem seed kernels. The amount of AZA in different neem oils extracted from the kernels can vary from less than 50 ppm to over 4000 ppm (Isman et al, 1990).

Various isomers of AZA have been identified qualitatively and quantitatively by HPLC procedure (Rembold et al, 1984). Chemical alterations of the AZA molecule are also possible. Hydrogenation of the 22,23- double bond or its tritiation are achieved successfully (Rembold et al, 1984) (Figure 2b); hydrogenated analogues are very stable and also highly biologically active, with the same potency as their natural counterparts (Morgan, 1982; Rembold et al, 1984; Yamasaki and Klocke, 1987; Barnby et al, 1989). Synthesis of AZA or bioactive analogues has been attempted (Ley et al, 1987) but the highly complex nature of the molecule presents a barrier to the success of the procedure.

FIGURE 2a

Structural formula of azadirachtin (Bilton et al, 1987)



AZADIRACHTIN

R = tigloyl,

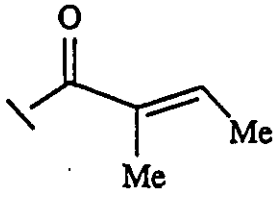
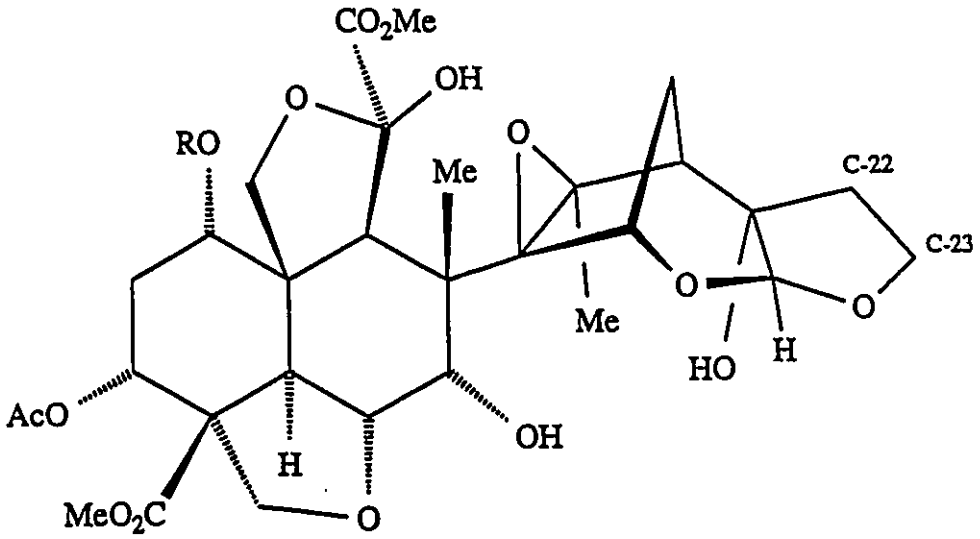


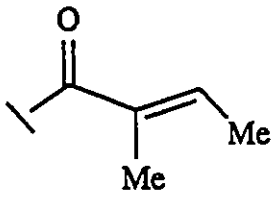
FIGURE 2b

Structural formula of H_2-azadirachtin (Bilton et al, 1987)



DIHYDROAZADIRACHTIN

R = tigloyl,



Structure-bioactivity relationships of AZA have been carried out. Results from Yamasaki and Klocke's (Yamasaki and Klocke, 1987) experiments have shown that the hydroxyl groups are essential for activity, while maximum activity requires a lipophilic region on the molecule. They propose that the lipophilic side of the molecule is necessary for transport phenomena while the hydrophilic side is involved in binding to specific proteins.

2.5 Influence of Neem Products on Insects

Neem compounds reportedly affect more than 100 species of insects, mites and nematodes, including economically important pests such as the desert and migratory locusts, rice and maize borers, pulse beetle, rice weevil, rootknot, nematodes, and mites (Warthen, 1985; Schmutterer, 1985; Schmutterer, 1990a). AZA, the main bioactive ingredient of neem has been shown to influence over 200 species of insect pests in seven orders, which represents more than 90% of the tested species (Warthen, 1989; Saxena, 1989). Neem products can thus be referred to as medium-to-broad spectrum pesticides (Schmutterer, 1990a). Modes of control by neem products include antifeedant, growth regulation and inhibition, oviposition deterrent, hormonal and pesticidal action in larval and/or adult stages, and antifertility action (Warthen, 1985). The major interest in neem products as pest control products lies in their antifeedant and growth regulatory properties.

2.5.1 Antifeedant Properties

An antifeedant compound or a chemical inhibiting feeding behavior can act in several ways;

1) the insect may be actively repelled by the compound. Perhaps without any contact, the insect moves away from it, i.e. a repellent,

2) further feeding action such as biting may be suppressed following a first contact, i.e. a suppressant, or

3) the insect is deterred from further feeding following biting of the leaf, i.e. a deterrent (Chapman, 1974).

These antifeedant properties of neem derivatives have been shown in several orders of insects (Jacobson, 1986). The desert locust, Schistocerca gregaria, is especially sensitive to the phagodeterrent properties of neem. AZA completely inhibits feeding in this species of locust at 1.5-6.0 X 10^{-8} M solution (10-40 $\mu g\ L^{-1}$) (Butterworth et al, 1971). Homopterous insects such as Nilaparvata lugens, Sogatella furcifera and Nephotettix virescens fed significantly less than control insects when rice plants were sprayed with 1 to 50% neem oil emulsion (Heyde et al, 1984).

The bioactivity of the antifeedant compound also depends on the treated plant. A 1% aqueous emulsion of an ethanolic neem seed kernel extract (NSKE) deterred the Japanese beetle, Popillia japonica, from feeding on soybean leaves whereas leaves of roses and grapes were left unprotected with the same treatment (Ladd, 1981).

Antifeedant chemicals other than AZA have also been identified in neem and these include meliantriol, salannin, nimbin, nimbidin and nimbolin (Warthen, 1989).

The feeding behavior of many lepidopterous insects such as Spodoptera littoralis, S. frugiperda, Heliothis virescens, Helicoverpa zea, H. armigera, Manduca sexta, Ostrinia nubilalis, has been shown to be altered by AZA, the oligophagous insects being more sensitive than polyphagous ones (Simmonds and Blaney, 1984). Laboratory and field studies on the antifeedant action of neem compounds on lepidopterans are numerous.

For example, Arnason et al (1985) found that the 50% protective concentration for Ostrinia nubilalis neonate larvae feeding on corn disks treated with AZA was 3.5 ppm, and 24 ppm for third instar larvae. The antifeedant activity of AZA on O. nubilalis may involve all three components of an antifeedant i.e. a repellent, suppressant and deterrent

action, as well as a toxic effect where the insect, having ingested the treated diet, becomes intoxicated and no longer feeds. In another lepidopteran, neem oil and an aqueous NSKE at 0.6% proved to be effective antifeedants for S. littoralis in field trials and in laboratory experiments (Meisner et al, 1981).

The use of antifeedants in crop protection requires that the compound possess certain qualities to be economically and practically useful. For instance, the antifeedant should be translocated to untreated parts of the plant, otherwise the insect will feed on untreated new growth (Chapman, 1974). Systemic movement is especially important in the use of antifeedants to control stalk boring insects. The systemic action of antifeedant ingredients in neem was first reported by Gill and Lewis (1971). Many studies have since shown that bioactive ingredients in neem translocate from a treated plant part to untreated parts (Meisner et al, 1986; Larew, 1988; Larew et al, 1987). However, the evidence has so far been indirect and conflicting (Ladd et al, 1978; Larew, 1988). From the laboratory studies on the systemic movement of neem's active compounds, factors affecting the bioactivity of these compounds following translocation include the species of insect tested, the plant species used in the bioassay, and the formulation of the neem material. It is thus of great importance that direct evidence for the uptake and translocation of neem products be pursued. The use of a radioactive tracer would incontrovertably determine if there is movement of neem derivatives throughout a plant.

2.5.2 Growth Regulation Activity

Neem derivatives such as a methanolic leaf extract and AZA were first found to be effective IGRs in 1972 by Leuschner and Ruscoe. Many neem derivatives have since been shown to be effective IGRs on many species. These extracts or derived products include whole fruit extracts (Naqvi, 1987), acetone extracts (Islam, 1987; Meisner et al, 1986), simple crushed neem seed kernels (Parmar et al, 1985), water extracts (Dreyer,

1987; Meisner et al, 1986; Kirsch, 1987), alcohol extracts of neem seed kernels (Schluter, 1981; Sombatsiri et al, 1987), alcohol extracts of leaves (Leuschner, 1972), neem oil (Heyde et al, 1984; Isman et al, 1990), extracts from neem oil (Isman et al, 1990; Parmar et al, 1987; Dreyer, 1987), neem cake (Saxena et al, 1984; Joshi, 1987), enriched NSKE (Heyde et al, 1984), AZA and related compounds (Arnason et al, 1985; Koul, 1984, 1985; Koul et al, 1987; Rembold, 1989), and the commercial product Margosan-O (Koul, 1988a, 1988b; Larson, 1987).

The effects of neem derivatives as IGR that have been most observed include delay and/or inhibition of molt into successive instars, disturbance of the molting process and delay, disturbance or inhibition of ovarian development.

Lepidopterous insects are amongst the most highly sensitive to the growth regulating activity of neem derivatives (Schmutterer, 1990a). For instance a diet containing 5-50 ppm of methanolic neem kernel extract was insecticidal to Manduca sexta fifth instar larvae (Haasler, 1984). The same methanolic extract at 2 ppm prevented the formation of viable pupae, or larvae molted into malformed non-viable pupae. When normal looking pupae developed to the adult stage, many had malformed wings and proboscis. The development of abnormal wings is frequently seen in larvae fed with neem treated diets (Schmutterer, 1990a).

In a growth study of Heliothis zea, the corn earworm, the ED_{50} (a 50% effective dose when compared to untreated larvae) for growth inhibition was 0.7 ppm AZA. Molting was strongly inhibited with an EI_{95} (effective concentration for 95% inhibition of ecdysis) of 2 ppm (Kubo and Klocke, 1982). As little as 0.35 μg AZA insect^{-1} was enough to completely inhibit ecdysis in Oncopeltus fasciatus (Redfern, 1979). The effects of AZA on the European corn borer (ECB) Ostrinia nubilalis, are very similar to the ones observed for other lepidopterous species (Arnason et al, 1985). However, ecdysis inhibition was observed in only 2 out of 30 insects at 1.0 and 10.0 ppm AZA incorporated

in the diet of the larvae. As suggested by Arnason's group (Arnason et al, 1985), "failure to grow was apparently due to the toxic effects of AZA rather than an inability to moult". Another study using the ECB showed that an aqueous NSKE sprayed on corn seedlings was an effective IGR at 0.25% to 1.0% concentrations; at 1.0% NSKE no larvae pupated (Meisner et al, 1986).

2.5.3 Mode of Action

Several studies have been carried out during recent years to elucidate the physiological effects of extracts from A. indica and pure AZA on the growth and development of insects. The morphological effects of AZA on insect development include growth retardation, molting inhibition, inhibition of egg maturation in the adult stages, and in several insect species, induction of sterilization (Dorn et al, 1987; Redfern et al, 1981; Rembold, 1989; Rembold et al, 1984; Arnason et al, 1985; Sieber and Rembold, 1983; Subrahmanyam and Rao, 1986).

Most studies have used AZA as a pure material to research the modifications in the endocrine control mechanisms induced by this compound. Because of its structural similarity to ecdysteroids (moulting hormones), AZA was first believed to act on the hormonal balance of insects by substituting for the existing ecdysteroids or adding to the pool of hormones (Ruscoe, 1972; Leuschner, 1972). The main action of AZA on the hormonal control of insects has since been clarified from studies with Locusta migratoria (Sieber and Rembold, 1983). A reduction or delay in the ecdysteroids (molting hormones) titer have been shown to occur in AZA-treated locusts. Test insects were injected with 2.5 μg AZA g body weight^{-1}. The treated nymphs showed a much slower and gradual increase/decrease of hemolymph ecdysteroid titer concentrations compared to untreated insects. Higher doses of AZA completely inhibited molting and these insects had much smaller bursts of ecdysteroid concentrations compared to controls.

The mechanism by which the release of the hormones from the neuroendocrine system is blocked is not yet clearly understood. However, a study of the effects of AZA on the endocrine events of Bombyx mori excluded the possibility of AZA interfering directly with prothoracic hormone function or prothoracic gland (which secretes ecdysones) excretion (Koul et al, 1987).

Toxicokinetic and autoradiographic studies with labelled H_2-AZA have shown high accumulation of the tritiated, chemically unchanged compound, in the basal and inner regions of the Malpighian tubules of L. migratoria, even after a long period of time (Rembold et al, 1988). The labelled compound was also shown to accumulate at the periphery of the blood brain-barrier as well as along the axons terminating in the storage lobe of the corpus cardiacum (receives prothoracictropic hormones secreted by the protocerebrum)(Subrahmanyam et al, 1989). The AZA laden axons might possibly be blocking the release of hormones from the corpus cardiacum (hormones having the prothoracic gland as target organ) into the hemolymph, thus causing the delay in ecdysteroid titer concentrations, resulting in molting inhibition and other developmental abnormalities.

3.0 Potential of Neem Products as Insecticides

The properties of neem-based products that have so far been described are more than highly sufficient to warrant their use as commercial pesticides. However, in 1990 no commercial neem insecticides were available outside of India other than Margosan-O, which is recommended for use against insect pests of non-food crops. There are several reasons why neem-based insecticides have not yet been registered and commercialized on a wide basis (Walter and Knauss, 1990). These include;

1) the instability of AZA. Several studies have shown that AZA is an easily degradable molecule. For instance, Stokes and Redfern (1982) found that AZA is sensitive to UV

light. Larson (1989) found that pH of the solution and the AZA concentration affects the stability of the molecule. The sensitivity of the AZA molecule has also been found to vary with temperature, UV light, pH, rainfall or other environmental factors when it is applied to plant material (Schmutterer, 1990a)

2) the variability of a natural raw material. AZA content of neem kernels has been found to vary with the geographical location of the tree. AZA content varied from 4.8% in samples from Nicaragua and Indonesia, to much lower yields of 1.9% and 1.5% in samples from Sudan and Niger (Ermel et al, 1987; Singh, 1987)

3) contents of the final product is often inconsistent. Isman et al (1990) found a wide range of AZA concentrations in 12 neem oil samples obtained from India. Two of the oils had no detectable amounts of AZA while the concentration of the others ranged between 200-4000 ppm AZA

4) potential phytotoxicity. Neem oil tested on tomato, in field trials in the Dominican Republic against Aculop lycopersici, was phytotoxic to the plants. Deleterious effects on other crop plants was noted in onion, potato and cabbage (Kirsch, 1987; Zehnder and Warthen, 1988). In the cabbage crop, the neem oil-treated heads of white cabbage were smaller than untreated ones. The outer leaves of cabbage and onion were destroyed by neem oil applications. Neem seed extract and a commercial preparation of AZA were used as basal soaks to protect chrysanthemum cuttings from Liriomyza trifolii (Oetting et al, 1990). The treatment affected the growth of the plants, the reduction in growth being directly proportional to the concentration of the solution and the length of the soak. However, most of these problems can be overcome by using ethanolic extracts (personal communication, Arnason).

Although there are technical problems with the use of neem products as crop protectant, there are numerous factors that render this botanical pesticide a good candidate. These include:

1) low toxicity to warm-blooded animals; The historical background of neem uses by many people as protectant of stored grains or clothes, and its every day use in oral hygiene (twigs are used as toothpicks) and medicine (van der Nat et al, 1991) strongly suggest that neem products have low mammalian toxicity. Results from a series of toxicological tests required by the EPA (Environmental Protection Agency) for the registration of Margosan-O in the US, revealed the low toxicity of the compound (Larson, 1990). For instance, the acute oral toxicity (LD_{50}) to rats was in excess of 5 ml kg^{-1} body weight (the limit of the required test). The traditional Ames mutagenicity test using five strains of Salmonella typhimurium showed that Margosan-O concentrate was non-mutagenic. An AZA-enriched neem extract given in a dose of 5000 mg kg^{-1} body weight to rats showed no acute oral toxicity (Schmutterer, 1984). In doses of 5000 to 8750 mg kg^{-1} body weight, a methanolic NSKE also showed no acute oral toxicity in rats. However, some studies of pure compounds extracted from neem, neem oil extracts or neem seed oil have demonstrated toxic effects. For example, aqueous extracts of fresh or dried neem leaves given orally at doses of 50–200 mg kg^{-1} body weight to guinea pigs caused weight loss and bradycardia, and diarrhea in animals eating green leaves (Ali, 1987). Lethal effects in children were reported following the application of neem oil to cure minor ailments (Sundaravalli et al, 1952). It is thought that the oil might have been contaminated by aflatoxin-producing strains of Aspergillus flavus.

2) low or no toxicity to non-targeted insects and natural enemies of pests; Coccinellids predatory on the aphid Melanaphis sacchari, a sorghum pest, were unaffected by the application of neem oil whereas the target pest was successfully controlled (Srivastava and Parmar, 1985). A study using the parasitoid Telenomus remus revealed that a 2% aqueous NSKE treatment of the egg masses of Spodoptera litura had no influence on oviposition by the parasitoid (Joshi et al, 1982). Emergence of the parasitoid was normal but their longevity was reduced if the treatment was done before oviposition.

Longevity of the parasitoids was increased if the treatment was carried out after oviposition. However, negative effects of neem products on beneficial parasites have also been reported. Arnason et al (1987) conducted a study on the effects of AZA on Diadegma terebrans, a parasitoid of Ostrinia nubilalis. These preliminary results suggested a significant reduction in the pupal weight and an increase in time of pupation in parasitoids of ECB larvae reared on diets containing 0.1 to 0.3 ppm AZA, compared to parasitoids of larvae reared on control diets.

Honeybee workers were unaffected after application as a direct contact product in concentrations up to 4418 ppm AZA ha^{-1} (Larson, 1990). Although earthworms were first deterred from soil treated with ground neem leaves and neem seed kernels, their weight significantly increased following their penetration in the treated soil (Rossner and Zebitz, 1987). Earthworm progeny also increased by 25% in soil treated with 5% v/v ground neem kernels.

3) Short residual effect of neem products; Most botanical pesticides are not persistent under field conditions (Schmutterer, 1990a). Environmental factors such as UV light, rainfall, pH, temperature contribute to a high rate of degradation of natural compounds. The persistence of neem-based products has been found to be approximately 5 to 7 days (Barnby et al, 1989; Schmutterer, 1990a), which compares to presently used synthetic pesticides such as the organophosphates. Organophosphates are unstable in the presence of light and quickly break down into nontoxic compounds; breakdown can occur within a few hours or days (Pedigo, 1989).

Antifeedant effects of an aqueous NSKE, a neem extractive and neem oil were tested on S. littoralis in the field (Meisner et al, 1981). All three products at 0.6% on alfalfa were found to have strong residual antifeedant effects after 24 hours. The aqueous NSKE had the strongest residual antifeedant effect when applied to sugar beet; it lasted approximately 3-4 days. A study with O. nubilalis revealed that larval survival on 2 and

6 day old residues of 1% aqueous NSKE sprayed on corn seedlings was significantly lower than on control plants (Meisner et al, 1985). Average weight of larvae 13 days after the beginning of the experiment were 1/7, 1/8 and 1/8 of the control, on 2, 6 and 8 day old residues, respectively. Pupation was completely inhibited in all treatments, even on 8 day old residues. The residual effect of 0.025% AZA was also studied 2, 4 and 6 days post-treatment. Ten days after the start of the experiment, weights of larvae exposed to treated seedlings were significantly lower than those of control insects. Pupae of larvae feeding on seedlings with 2 day old residues did not develop and only 11% and 16% of those fed on seedlings with 4 and 6 day old residues pupated, respectively. Shorter residual effects were noted in a study using fall armyworm larvae (Wood, 1990). Corn plants in the field were sprayed with 600 mg AZA L^{-1} and leaves were collected 24 and 72 hours following the treatment. The antifeedant effect of AZA was observed only in 24 hour old residues.

The low persistence of neem-based products represents both an advantage and a disadvantage in crop protection. The short lived natural pesticides are invariably safer to use for the environment, as well as for the producer and the consumer, compared to synthetic pesticides. However, because of the low residual effect of neem products, repeated applications during a growing season at intervals of 7 to 10 days may be necessary (Schmutterer, 1990a). The use of these antifeedants and growth regulators in an IPM program would be ideal. Neem products could be utilized in conjunction with other means of pest control such as biological products (microbials), parasites and natural predators, resistant crop varieties, sterilants, or sound and moderate use of synthetic pesticides.

The use of neem products in developing countries by resource-poor farmers is strongly encouraged since many can grow their own neem trees, produce non-toxic insecticides that are not costly for their crops and stored products (Schmutterer, 1985;

Schmutterer, 1988). In North America, these products could be well integrated in pest control programs. Major economic crops, such as sweet corn, are devastated by pests such as the European corn borer. The use of neem-based products in the control of O. nubilalis in sweet corn is the basis for the present study.

4.0 The Host Plant and its Insect Pest

4.1 The Crop value of Sweet Corn

Sweet corn represents one of the most economically important crops in North America. In 1983, a total of 8.5 tons ha^{-1} was produced in Canada both for fresh market and for the processing industry, while 11.3 tons ha^{-1} were produced in the USA (Hudon and Leroux 1986a). In Quebec alone, sweet corn was cultivated over 10,400 hectares in 1986, the second largest area for vegetable production following that of potatoes (Hudon and Leroux, 1986a). The value of the crop exceeded 10 million dollars. In 1990, Quebec saw its production of sweet corn increase both for the fresh and processing market (Belais, 1990). Over 12,000 hectares are now being used for sweet corn production, an area representing the largest one destined to vegetable production.

4.2 Sweet Corn Cultivars

There exists a vast array of sweet corn cultivars and they are rapidly changing because of the new high-sugar components introduced by the seed companies over the last few years (Ontario Ministry of Agriculture and Food, 1990). The major types of sweet corn cultivars are grouped according to the gene responsible for their degree of sweetness. For example, regular sweet corn represents those cultivars containing the "su" gene that have been grown traditionally . These contain an average amount of sugar which rapidly changes to starch following harvest if the cob is not quickly stored at cool temperatures. Sugar enhanced sweet corn contains the "se" gene and produces much higher levels of

sugar than the regular cultivars. The kernels will retain a sweet flavor longer after harvest simply because of the higher sugar content- eventually, the sugar is transformed to starch. The kernels of these cultivars have a very thin pericarp and do not withstand very well a mechanical harvester. Supersweet sweet corn contain the "SH_2" gene. Unlike the two previous cultivars, supersweet corn does not readily convert sugar to starch and will stay sweet for a longer period of time after having reached harvest maturity. SH_2 cultivars can therefore be harvested later and will retain their sweetness longer in the consumer's home. However, as the corn becomes more mature, the pericarp becomes thicker. Rapid cooling helps to retard this change.

4.3 Cultivation Practices

Cultivars containing the "se" gene and the "SH_2" gene have lower seed vigor and poorer germination; special planting practices are recommended (Ontario Ministry of Agriculture and Food, 1990). Planting should be done when soil temperature is above 18°C, seeding rates should be 20% higher than normal, seeds should be planted at a depth of only 1.5 cm, and soil should be moist when planting or irrigated after planting. Supersweet sweet corn kernels should be handled and planted gently since the kernels can easily crack.

When grown in isolation, "se" and "SH_2" cultivars will best express their high sugar content. They should be grown away from field corn or other types of corn (approximately 75 meters away or if grown within 75 meters, the 2 types of corn should have a 2 week difference in tasselling time).

Days to maturity of sweet corn varies according to the temperature. A particular cultivar may reach maturity in 85 days if planted in early May but require only 65 days to reach maturity if planted in mid-June. In cooler regions, it will mature later.

4.4 Pests of Sweet Corn

A variety of pests and diseases plague sweet corn crops. These include vertebrates such as the red-winged black bird, crows, racoons and skunks; diseases such as leaf blights, stalk rots and ear rots, common smut and head smut; and insects such as seed maggots and wireworms, the fall armyworm, aphids, corn earworm, corn rootworm and the European corn borer (ECB).

4.5 The European Corn Borer

4.5.1 Host Plant and the Insect's Life Cycle

The European corn borer, Ostrinia nubilalis (Hubner) (Lepidoptera: Pyralidae), is a pyralid moth of the subfamily Pyraustinae. Its most important host plant in North America is Zea mays. The corn borer is a highly polyphagous insect and will establish itself in almost every herbaceous wild or cultivated plant with large stems (Hudon and Leroux, 1986a; Beck 1987). Over 223 species of plants have been reported as host plants of the ECB (Lewis, 1975). The wide range of cultivated plants reported to be infested by Ostrinia nubilalis include hemp, hop, sorghum, millet, field pea, soybean, wheat, cotton, gladiolus, dahlia, apple, peach, raspberry, eggplant, sweet pepper, hot pepper, paprika, snap bean and potato (Hudon and Leroux, 1986a).

The ECB can have from one to many generations (multivoltinism) or broods per season. However, in most corn producing regions, the insect is bivoltine, i.e. there are two generations per season (Hudon and Leroux, 1986a). The corn borer overwinters as a mature larva in diapause, in the remaining stalk, stubble or ear. It pupates in spring and emerges as a moth. The female of the first generation mates soon after emergence and lays its eggs on the underside of leaves of the host plant, close to the midrib. When the host plant is corn, egg laying coincides with the whorl stage of the plant's development.

Eggs are white and in masses of 15-35. One adult female can lay over 500 eggs

during its lifespan. Eggs hatch in about seven days. First and second instar larvae feed initially on leaf tissues within the whorl of the plant. Third and fourth instar larvae will feed on sheath and collar tissues of the developing plant. At tasselling time, fifth instar borer move downward to the ear zone and all parts of the stalk, feeding and tunnelling in the stalk, tassels, ear shoots and leaf sheaths. Late instars will feed on the pith of stalks and slowly migrate downwards as the season advances.

In areas where the ECB is bivoltine, the mature larva of the first generation will bore a hole in the stalk for later emergence as an adult, and pupate in the tunnel. Second-generation larvae feed on shedded pollen accumulated in the leaf axils, sheath and collar tissues. These second brood larvae will later feed on the ears and shanks before tunnelling into the stem where they will overwinter.

4.5.2 Past and Present Status

The ECB, an insect of economic importance on the European continent, was accidentally introduced to North America. It was first reported near Boston and in Ontario (Vinal, 1917). However, the larvae were known to growers as early as ten years before the first official reports, in St-Thomas-Port-Stanley, Ontario (Keenan, 1927). The borers were probably introduced in more than one area in eastern USA and Canada in shipments of broomcorn from Austria, Hungary and Italy (Hudon and Leroux, 1986a). The first infestations in Massachusetts, New York and Ontario were in areas surrounding broom factories. The wastes from these factories were probably the means by which the species spread (Hudon et al, 1989). In the USA, the ECB had invaded a total of 18 states, spreading westward (Montana, Wyoming, Colorado, Oklahoma) and southward (Texas, Louisiana, Mississippi, Alabama, Georgia, Florida). It is prevalent today in 40 states and eight Canadian provinces, from the Maritimes to the Prairie provinces, Lethbridge, Alberta being its northwestern limit (Palmer et al, 1985; Hudon et al, 1989).

North American borer populations belong to three ecotypes (Hudon et al, 1989). These are based on geographical variation and biological characteristics of the borers (photoperiod, thermoperiod, rates of growth, feeding habits and diapause). A northern ecotype (populations in northern Minnesota, Quebec, New York and Ontario) is univoltine, a central ecotype (populations in the central corn belt of USA and the south west of Ontario) is bivoltine and a southern ecotype (populations where growing seasons are long, in Alabama, Georgia, Missouri, North Carolina) is multivoltine.

Mortality in the early larval instars is high and can reach 90% (Reid, 1988), while over-wintering larval mortality represents only 1.6% of total 5th instar larvae (Hudon and Leroux, 1986a). However, Hudon and Leroux (1986c) report that in Southwestern Quebec, only 1.3% borer survival is necessary to maintain economic levels of infestation.

Agricultural, biological and ecological factors have contributed in maintaining O. nubilalis populations (Hudon and Leroux 1986c): variable life cycles, length of egg laying period, low level of mortality in overwintering larvae, low levels of parasitic activity, mismanaged cultural practices and the increase in cultivated area of grain and forage corn.

4.5.3 Economic Importance

The bivoltine strain of ECB is more injurious to sweet corn than the univoltine since second brood larvae feed at the ear zone and bore into the husks, kernels and shanks (Hudon et al, 1989). Sweet corn for the processing market will also be contaminated by second generation larvae. The univoltine strain is more injurious to grain corn because it tunnels in the stalks causing lodging problems at harvest.

Total annual losses due to ECB damage have been reported to exceed 200 million dollars (Hudon and Leroux, 1986a). In Canada, economic thresholds accepted in fresh sweet corn production are 5% infested ears in the case of fresh market corn and 10% infested ears for processing corn. Control of ECB infestations thus represents high

monetary and time investments for the sweet corn producer.

4.6 Controlling the European Corn Borer

4.6.1 Historical Perspective

In order to prevent the spread of the borer, the Canadian Federal government first implemented quarantine measures in 1925 (Lagloire, 1943). These included the banning of export of corn from 16 counties of Quebec, and later on this ban applied to the whole province. Provincial government imposed in 1933 a Plant Protection law. The law was concerned with handling, inspection, classification of corn produce and was strictly enforced. Enforced measures to control the borer included the burning of old stalks, or plowing them down; the corn crop residues from the previous seasons had to be destroyed before June 1 (it was then believed that 80% of the overwintered larvae were still present until June 1). The most successful measures to control borer populations were eventually found to be efficient clean-up measures, production of silage corn, crop rotation, and cultural practices such as deep plowing of the remaining stalks (Gauthier, 1941).

The introduction of hybrid corn plants which were vigorous and tolerant to corn borer attack was a major factor in attempts to control borer populations (Wressell, 1958). The use of insecticides in the 1930's was either too expensive or often ineffective in controlling borer infestations (Hudon and Leroux, 1986a). As new synthetic insecticides were discovered and became more accessible to the corn producer, their use became more popular as a practical way to protect corn crops from the ravages of the ECB.

4.6.2 The Era of Chemical Insecticides

In 1937, Batchelder devised an insecticide treatment schedule against the ECB: insecticides should be applied 4 or 5 times at 5 days intervals beginning at the time eggs started to hatch (Hudon, 1969). At the time, the number of applications was more

important than proper timing of applications. It was reported in 1950 that satisfactory commercial control could be obtained on extra-early-maturing corn with 4 applications at intervals of 5 days, and with 3 applications at the same interval when tassels developed after June 15 (Hudon, 1969). Under low borer infestation, it was found that multiple applications of DDT at weekly intervals had no advantage over 2 applications. In 1965, an excellent commercial control of the corn borer was developed by combining 1 application of DDT at 1 lb acre^{-1} applied before tasselling, and 2 weekly applications of carbaryl at 1 1/2 lb acre^{-1} during ear formation (Hudon, 1969). Use of DDT on corn was later banned in 1970 (Hudon and Martel, 1977).

In the 1970's, organophosphates such as trichlorfon, stirofos, leptophos, and carbamates such as methomyl, carbofuran and carbaryl replaced the highly persistent and contaminating organochlorines such as DDT (Hudon and Martel, 1977). Organophosphates and carbamates became the common insecticides to control the ECB; they gave good commercial control with less than 5% infested ears. Timing of application also became an important factor; the early larval instars were found to be much more susceptible to the insecticides than later instars.

The first pyrethroid, allethrin, was developed in 1949 (Pedigo, 1989). Because of their great effectiveness and safety of application, pyrethroids quickly became the fastest growing group of modern insecticides. Third-generation pyrethroids, fenvalerate and permethrin, were introduced in 1972 and 1973, respectively (Pedigo, 1989). Ambush and Pounce (two permethrins) are now recommended insecticides against the ECB (Ontario Ministry of Agriculture and Food, 1990). Fourth generation pyrethroids, such as cypermethrin, deltamethrin, fluvalimate, flucythrinate, are even more potent insect poisons than are the third-generation chemicals (Pedigo, 1989). Cymbush and Ripcord, two cypermethrins, are also recommended by the Ontario Ministry of Agriculture and Food in the control of the ECB. Other recommended insecticides include two carbamates,

carbaryl and carbofuran and an organophosphate, methomyl. Economically, the pyrethroids compare favorably in price with the organophosphates and carbamates because of their low application rates.

Spraying programs are based on the presence of pinhole feeding detected through field inspections and are done prior to tasselling (Ontario Ministry of Agriculture and Food, 1990). A single application to several applications (up to 7) may be necessary. Applications at 5-day intervals usually give satisfactory control but the interval may vary with temperature and the material used. Early maturing sweet corn in areas where the bivoltine strain of ECB is present may require only 2 applications.

Through the use of pheromone traps, Quebec sweet corn producers are now able to detect on their own land the arrival of adult moths, and thereby decrease the insecticide application and improve the timing of application. Regional detection services were offered to the producers but since many producers plant at different times to assure a continuous harvest throughout the growing season, these data were not accurate enough. These prepared pheromone trap kits can be purchased at cooperatives (Paradis, 1991).

4.6.3 Borer Management: An Integrated Approach

In the quest of alternative methods to the use of synthetic pesticides in the control of the ECB, integrated pest management programs are now being intensely studied in field trials. Natural enemies such as parasitoids, predators (birds, insects), bacteria, fungi, protozoa and viruses have been tested alone or in combination with pesticides (Hudon et al, 1989). However, combined IPM i.e. pesticides + alternative control agent has been shown to be indispensable, especially for sweet corn, popcorn and seed corn since economic thresholds are very low.

4.6.3.1 Parasitoids

Among the many parasitoids of the ECB (Lydella thompsoni, Eriborus terebrans, Macrocentrus grandii, Sympiesis viridula, Trichogramma spp), Trichogramma nubilale seems to be a species most capable of controlling borer populations (Hudon et al, 1989). Mass production of the parasitoid remains a major problem. High levels of parasitoids are required to give effective levels of parasitism. Burbutis and Goldstein (1983) have been able to successfully produce T. nubilale on O. nubilalis and estimate that a release of 59,000 parasitoids ha^{-1} would parasitize 80% of the borer population.

4.6.3.2 Predators

Insect predators such as coccinellids, chrysopids and nabids, are predators of the ECB's egg stage (Hudon et al, 1989). Hudon and Leroux (1986c) reported an annual egg mortality of 6.1% due to predation by the coccinellid Coleomegilla maculata. However, the efficacy value of these predators is greatly reduced by a parasite of the coccinellid, Perilitus coccinellae. Birds have also been reported to be an important predator of ECB, especially of fall and spring larvae (Hudon and Leroux, 1986c). In New York, the downy woodpecker has been reported to reduce by 20-30% the overwintering larval population.

4.6.3.3 Diseases

There are few bacteria that are pathogens of larvae of O. nubilalis (Hudon et al, 1989). Bacillus thuringiensis has been used in field studies. For instance, Lynch et al (1977) found that granular formulations gave significantly more control than spray formulations. They also discovered that a high concentration of the granular formulation was the important factor in better ECB control as compared to greater volume.

Nosema pyrausta, an obligatory intracellular protozoan parasite of the ECB, has been reported to be the most effective of naturally occurring pathogens (Lewis and Lynch,

1978). N. pyrausta reduces egg hatch and larval developmental rate, fecundity, and adult longevity (Windels et al, 1976). Field studies have been conducted using the protozoan alone or in combination with insecticides (Lublinkhof et al, 1979). The authors reported that if N. pyrausta could be established in a population, its effect in combination with insecticides such as carbaryl and carbofuran would provide a very effective pest-management strategy for suppressing ECB populations.

Beauvaria bassiana, a fungus infecting early instars of ECB larvae, showed some promise in controlling borer populations (Feng et al, 1985). For instance, in Minnesota, high levels of larval mortality from B. bassiana were found in fall surveys (Winnie et al, 1981).

Although naturally occurring pathogenic viruses have never been reported, a nuclear polyhedrosis virus has been shown to be pathogenic to ECB larvae (Lewis et al, 1977). Field trials where such viruses were applied demonstrated significant reductions in number of larvae per plant and plant damage (Lewis et al, 1982).

4.6.3.4 Cultural Practices

Cultural practices recommended at the beginning of the century, such as deep plowing (at least 20 cm so that the infested stalks do not become exposed), crop rotation and intercropping are all still of great value (Hudon et al, 1989). For instance, intercropping corn with red clover was found to reduce significantly borer damage (Lambert et al, 1987).

4.6.3.5 Plant Resistance

Corn breeding to develop resistance to feeding by ECB larvae has been a major step in the control of borer populations and plant damage. Plant breeding has been the source of new corn genotypes which give a satisfactory degree of resistance to leaf feeding by

first-generation borers (Hudon et al, 1989). However, resistance of these varieties is very low to sheath and collar feeding by second-generation borers.

Sweet corn cultivars are highly susceptible to the corn borers. Although many resistant cultivars do not show activity against second-generation borers, one cultivar, B52, offers a degree of protection (Pedigo, 1989). This character has been successfully transferred to sweet corn cultivars where second generation damage is most important. Two other cultivars, B86 and BS9C5, are resistant to both generations of borers.

The biochemical factor associated with leaf feeding resistance to the ECB has been identified as 2,4-dihydroxy-7-methoxy–1,4-benzoxazin-3-one, or DIMBOA (Klun et al, 1967). DIMBOA acts as an antifeedant, suppresses larval development and increases mortality (Hudon et al, 1989). Reid (1988) conducted both laboratory and field studies on the role of DIMBOA in leaf-feeding resistance in 37 inbred lines of corn, indigenous landraces of Mexico, highly resistant lines, 3 CIMMYT maize pools, 3 Canadian synthetic lines, a multi-borer resistant line and 10 Argentine landraces. The laboratory leaf-feeding resistance evaluation correlated with both seedling DIMBOA concentrations and field evaluations of leaf-feeding resistance. However, other factors such as silica and lignin content also appear to be involved in conferring resistance to corn plants (Guthrie et al, 1986).

4.6.3.6 Botanicals

As previously mentioned, botanicals such as the pyrethroids with their quick knockdown effect are highly effective in controlling the ECB. However, "softer" compounds such as neem based products are apt to play an important future role in control of the borer in sweet corn. The efficacy of neem products against various insect pests has often been compared to pyrethroids; field studies have even demonstrated the superior control obtained with neem extracts (Isman et al, 1990; Schmutterer ,1990b).

Neem has even been shown to be effective in controlling insects resistant to pyrethroids (Radcliffe and Lagnaoui, 1990).

These new generation pesticides may become alternative insecticides or may be used in combination with presently used pesticides to achieve higher degrees of protection.

B - PROJECT OBJECTIVES

Based on a previous laboratory study (Arnason et al, 1985) of the antifeedant and insecticidal properties of azadirachtin towards the European corn borer and a preliminary field evaluation of a neem seed kernel extract (NSKE) and azadirachtin (AZA) against the pest in sweet corn, further field evaluations and laboratory studies were proposed. The main hypothesis of the research project is that neem-based products are effective under field conditions as a means of protecting sweet corn against the ECB.

The research had two objectives:

1. To examine the effect of neem-based products on the ECB:

   1.1 To evaluate, in field studies, the efficacy of AZA and neem extract formulations on the ECB infesting sweet corn by determining the number of damaged kernels, the number of ECB larvae present on the cob and the damage done to the stalks of the mature corn plants. Factors such as rate, time and number of applications, and various neem formulations were considered.

   1.2 To determine, in leaf disk feeding trials, the antifeedant properties of pure AZA and the efficacy of the formulation used in the field against the ECB larvae.

   1.3 To study, in laboratory evaluations, the insecticidal and/or growth disrupting effects of NSKE incorporated in artificial diet on the ECB.

2. To examine the absorption and translocation of H_2-AZA and AZA in corn seedlings:

   2.1 To determine, by means of a tritiated tracer, if H_2-AZA is absorbed by the leaf surface of corn seedlings and if so, if it is translocated to areas of new growth or to other parts of the seedling.

   2.2 To study the absorption and translocation of the antifeedant compound following

foliar application of AZA on corn seedlings, by means of feeding trials using corn borer larvae.

C - MATERIALS AND METHODS

1.0 The Effects of Neem and Azadirachtin on the European Corn Borer.

1.1 Chemistry

1.1.1 Preparation of Neem Products: NSKE, Azadirachtin, and H_2-Azadirachtin

Dried neem kernels were collected in May by Dr. Krishnamurty in New Delhi, India. The neem seed kernel extract (NSKE) was prepared at the chemistry department of the University of Ottawa by J. Kaminski following Nakanishi's procedure (Nakanishi, 1975). The NSKE yield for 1987 was 1 g extract 10 g seed^{-1}. In 1988 and 1989 the yield was 0.4 g extract 10 g seed^{-1}.

AZA, was further purified from the neem extract by J. Kaminski following the procedure established by Nakanishi. A white crystalline powder was obtained. A portion of the pure AZA was hydrogenated by J. Kaminski following the reduction procedure of Butterworth and Morgan (Butterworth et al, 1972) to obtain 22,23 H_2-AZA.

1.1.2 Identification and Purity of the Azadirachtins

The identities of AZA and H_2-AZA were confirmed by several methods. J. Kaminski confirmed the identity of the compounds by spectroscopy (FAB, NMR).

The pure compounds were further identified and characterized by thin layer chromatography (TLC) and high pressure liquid chromatography.

TLC was performed on Kodak chromatogram plastic sheets (#13181, silica gel with fluorescent indicator) 100 micron thick or TLC aluminum sheets pre-coated with silica gel 60 F_{254}, 200 micro thick. The AZA crystals were dissolved in 100% acetone (B.D.H.) and delivered to the chromatogram sheet with a 10 μl pipette as spots 4 mm in diameter. The 2.5 X 8.5 cm plates were placed in developing chambers that had previously been saturated with the chosen developing solvents. TLC developing solvent systems

consisted of 100% ethylacetate (B.D.H.) and chloroform:ethylacetate:methanol (B.D.H.) (4:4:1 v:v:v). On completion of the run, the plates were dried and the spots were then visualized by atomizing a 5% sulphuric acid solution in methanol on the plates.

To identify and confirm the purity of AZA and H_2-AZA by HPLC, a Beckman HPLC equipped with a Model 165 Variable Wavelength detector, a Model 110A Solvent Metering Pump System, and a Model 420 System Controller Programmer was used.

The crystalline compounds were dissolved in MeOH:H_2O (80:20 v:v) and filtered through 0.5 μm nylon millipore filters with a 1.5 ml Luer lock syringe. Twenty microliters of the filtered samples were injected into the system under the following conditions: Ultrasphere ODS reverse-phase C18 column; isocratic elution with 40% acetonitrile (HPLC grade, B.D.H.)/60% water as a first solvent system, and 70% MeOH (HPLC grade, B.D.H.)/30% water as a second solvent system (isocratic elution); flow rate of the solvent system=1 ml min^{-1}; detection wavelength=210 nm (Schneider et al, 1987).

1.1.3 Quantification of Azadirachtin in the NSKE

AZA was quantified in the NSKE by J. Kaminski. The extract was chromatographed on silica gel prepared in chloroform, using chloroform and 1:1 mixture of chloroform and ethyl acetate as elutents. The AZA fractions were collected by scraping the silica gel at the area corresponding to the R_f value of azadirachtin. The powder was filtered to remove the silica gel. The compound was then prepared for analysis by HPLC.

1.2 Field Studies

1.2.1 Source and Variety of Sweet Corn

Illini Gold hybrid sweet corn was chosen as the experimental plant for its high susceptibility to the ECB. The seed was grown in Idaho (lot no. 77530-33151-T.O.B.) and purchased from Harris Moran Seeds. Seeds had been treated with captan, captafoldiflotan, carboxinvitavax and thiram-arasan.

1.2.2 Experimental Sites

The Ottawa Experimental Farm was the site of the experimental fields. A field had a surface of 31 m X 28 m which represented a total surface area of 868 m^2 or 0.0868 ha. In 1988, the two experimental fields were separated by a gravel road whereas in 1989, the two fields were situated on the same side of the gravel road but separated by a 2 m walk (Figures 3-5).

1.2.3 Preparation of Soil and Sowing of Corn

The fields had been ploughed prior to the sowing date. The seeder was set at 90 cm which corresponded to the width between rows. The planter plate was chosen to sow the seeds at a 15 cm interval (planter plate no. IHC–16C–17C); depth of the sown seeds was approximately 2 in. In 1988, a fertilizer 46-0-0 (N-P-K) was applied to the soil simultaneous to sowing, at a rate of 115–129 kg ha^{-1}. In 1989, 36-0-0 fertilizer was applied to the soil 1 month following the sowing date. A trough 2 cm deep was dug 20 cm from the corn plants, along the rows. The fertilizer was slowly dribbled from a container into the troughs along the rows.

Reseeding was required in both 1988 and 1989 seasons to fill in gaps where sowed seeds had not germinated. In 1988, 13 days following the sowing date, seeds that had been soaked overnight in water were planted in wide gaps between corn seedlings. In 1989, 14 days following the sowing date, corn seedlings at the V-3 stage (Ritchie and Hanway, 1984) were transplanted in the gaps between the emerged seedlings.

FIGURE 3

Experimental design of 1987 field study - Ottawa Central Experimental Farm.

* Each plot is represented by a treatment number (Tables 3 to 7). Treatments were randomly assigned to the 10 plots in each block.

** 3 border rows

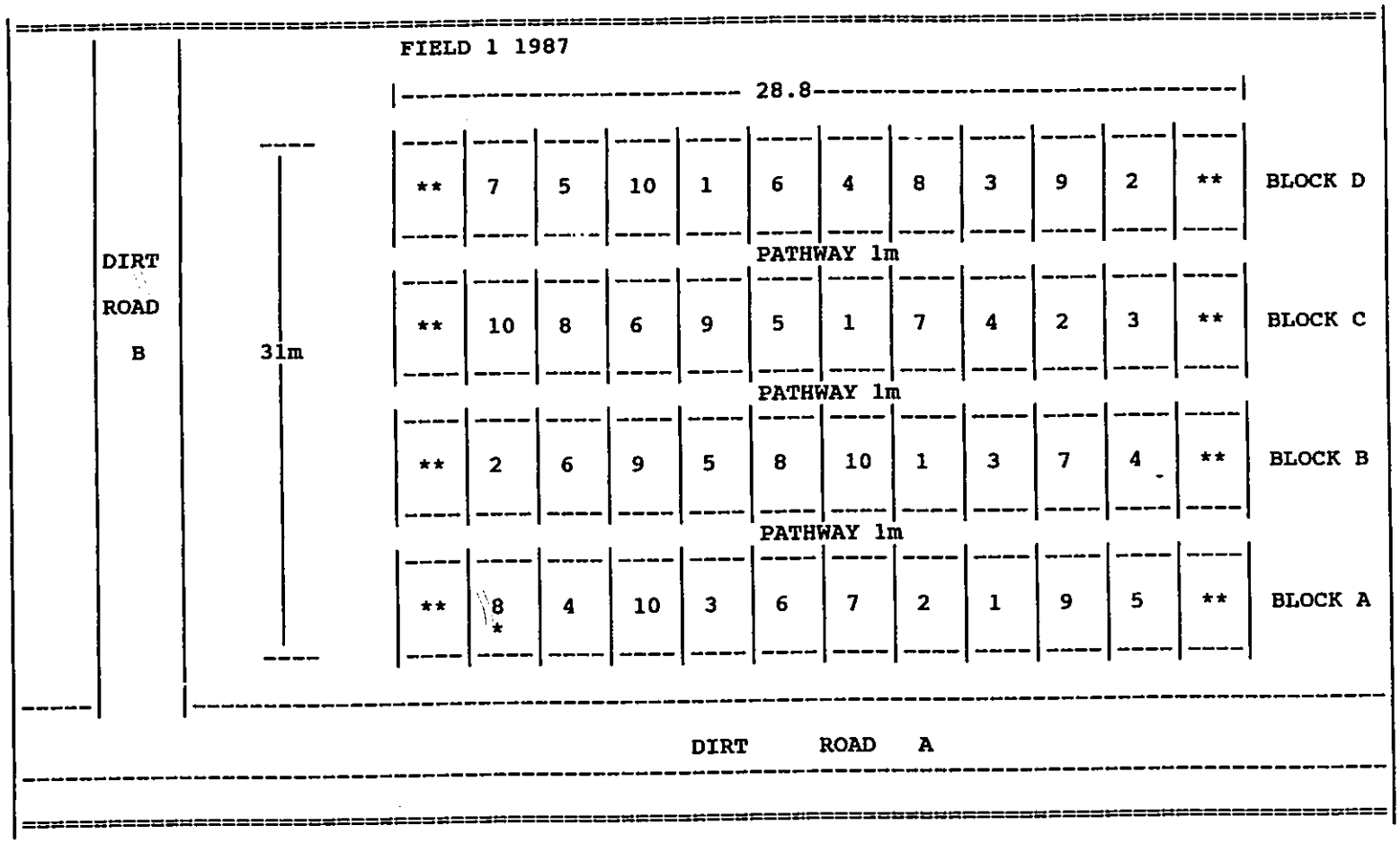

FIELD 1 1987
28.8
DIRT ROAD B
31m
** 7 5 10 1 6 4 8 3 9 2 ** BLOCK D
PATHWAY 1m
** 10 8 6 9 5 1 7 4 2 3 ** BLOCK C
PATHWAY 1m
** 2 6 9 5 8 10 1 3 7 4 ** BLOCK B
PATHWAY 1m
** 8 4 10 3 6 7 2 1 9 5 ** BLOCK A
DIRT ROAD A

FIGURE 4

Experimental design of 1988 field studies - Ottawa Central Experimental Farm.

Respective areas occupied by the two fields are shown as well as the random distribution of the treatment plots in each block. Dimensions are the same as the 1987 field study.

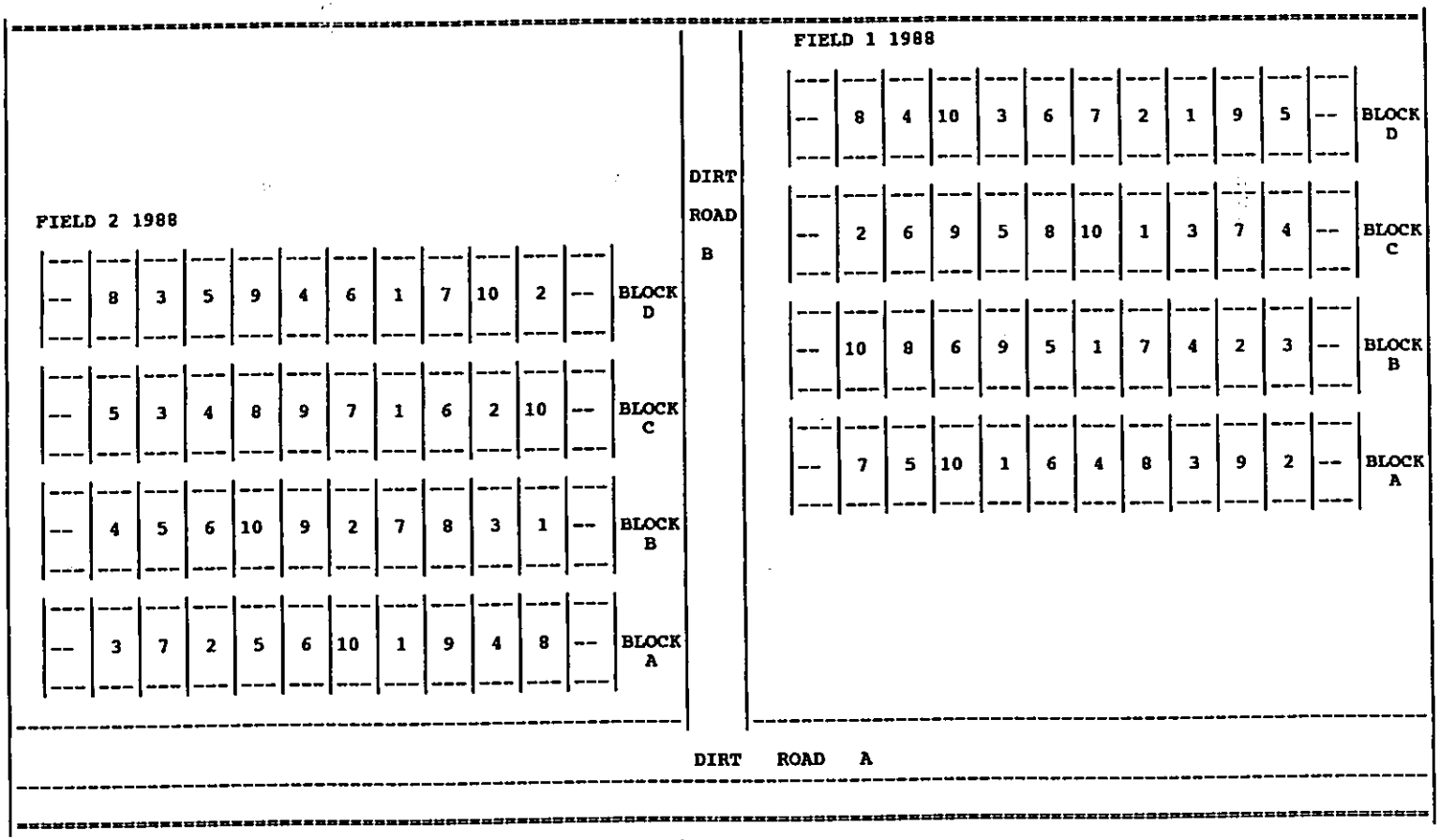

FIELD 2 1988
8 3 5 9 4 6 1 7 10 2
BLOCK D
5 3 4 8 9 7 1 6 2 10
BLOCK C
4 5 6 10 9 2 7 8 3 1
BLOCK B
3 7 2 5 6 10 1 9 4 8
BLOCK A
DIRT ROAD B
FIELD 1 1988
8 4 10 3 6 7 2 1 9 5
BLOCK D
2 6 9 5 8 10 1 3 7 4
BLOCK C
10 8 6 9 5 1 7 4 2 3
BLOCK B
7 5 10 1 6 4 8 3 9 2
BLOCK A
DIRT ROAD A

FIGURE 5

Experimental design of 1989 field studies - Ottawa Central Experimental Farm.

Respective areas occupied by the two fields are shown as well as the random distribution of the treatment plots in each block. Dimensions are the same as the 1987 field study.

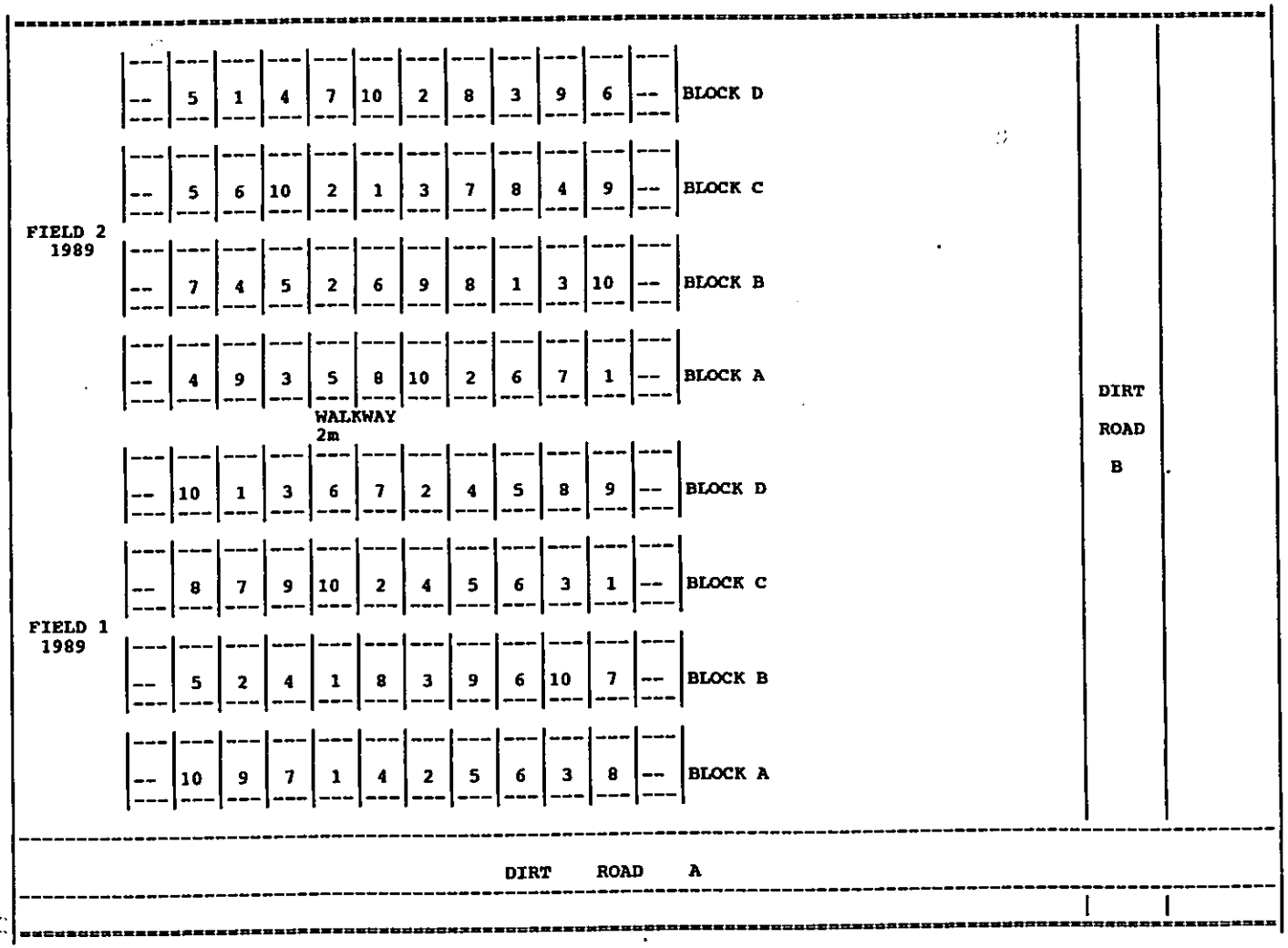
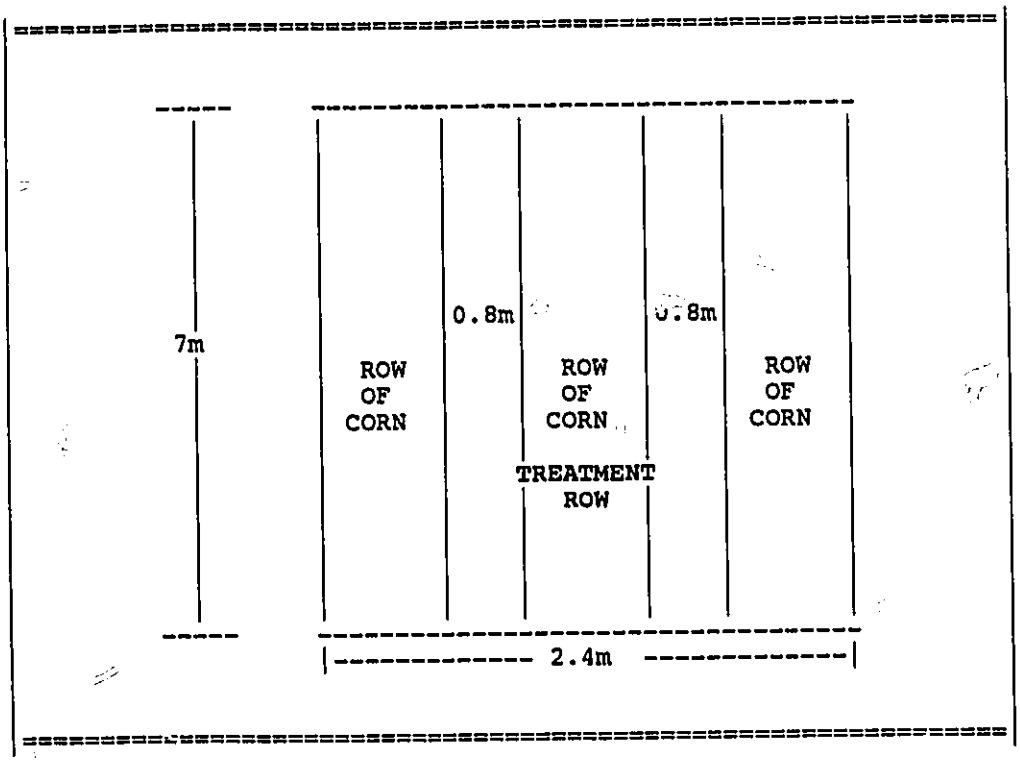

FIELD 2
1989
-- 5 1 4 7 10 2 8 3 9 6 -- BLOCK D
-- 5 6 10 2 1 3 7 8 4 9 -- BLOCK C
-- 7 4 5 2 6 9 8 1 3 10 -- BLOCK B
-- 4 9 3 5 8 10 2 6 7 1 -- BLOCK A
WALKWAY
2m
-- 10 1 3 6 7 2 4 5 8 9 -- BLOCK D
-- 8 7 9 10 2 4 5 6 3 1 -- BLOCK C
FIELD 1
1989
-- 5 2 4 1 8 3 9 6 10 7 -- BLOCK B
-- 10 9 7 1 4 2 5 6 3 8 -- BLOCK A
DIRT
ROAD
B
DIRT ROAD A

FIGURE 6

Typical treatment plot showing plot dimensions.

The design applies to treatment plots in all field studies.


7m
0.8m
0.8m
ROW OF CORN
ROW OF CORN
ROW OF CORN
TREATMENT ROW
2.4m

1.2.4 Maintenance of Fields

Fields were first weeded by hand and hoe at the early V-4 to V-5 stage of growth. Weeding was then done on a regular basis to maintain the density of weeds at a minimal level. In 1989, a motor-powered tiller was used to cultivate the soil between the rows of corn plants. The rows of corn plants were thinned to 35-40 plants per treatment row leaving approximately 32 cm between each corn plant. A final plant stand count was estimated at 62,500 plants ha^{-1}.

1.2.5 Experimental Design

The fields were designed to have 4 replicates consisting of 10 treatments each in a complete randomized fashion (Figures 3-5). Each treatment corresponded to a plot which was composed of 3 rows, 7 m long and approximately 0.8 m apart (Figure 6). The middle row of each plot was designated as the treatment row and marked with a white stake at the beginning of the row. Each plot had a surface of 16.8 m^2. Each treatment had a total surface area of 16.8 m^2 X 4 replicates or 67.2 m^2. Each replicate had 3 border rows on each side. The first and 3 to 5 last plants of each row also served as border plants. All treatment rows had 25 to 30 treatment plants corresponding to a 5 m treatment row. Each field had an average of 920 treatment plants.

1.2.6 Corn Borer Artificial Infestation

Selected treatment plots were artificially infested (first infestation) with ECB egg masses when the plants were approximately at the V–12 stage of growth. In 1988, the egg masses were supplied by Dr. M. Hudon of Agriculture Canada, St. Jean-sur-le-Richelieu, Quebec. In 1989, the egg masses were supplied by Dr. Hamilton of Agriculture Canada, at the Ottawa central experimental farm. The egg masses had been laid by the adult moths on sheets of waxed paper which were cut into uniform 1 cm diameter discs, each disc

having 1 to 2 egg masses. The number of eggs per average egg mass was estimated at 24. A large egg mass would often contain more than 25 eggs, a medium one from 11 to 25 eggs and a small egg mass could contain less than 11 eggs.

The eggs were received in a plastic bag with a wet sponge to maintain a high humidity. The egg masses in the black head stage were separated from the others and used immediately or placed in a cold room at 12°C to be used the next day. Egg masses which had not attained the black-head stage were used the next day; these eggs were placed in an incubator at 85% R.H. and 27°C or were placed in the cold room at 12°C, to be used for the second infestation in 5 days.

TABLE 3: Field season 1987 - Neem product treatments, their rates and timing of applications, and timing of ECB infestation.

TREATMENT	DATES OF ARTIFICIAL INFESTATION	RATE OF APPLICATION	NUMBER OF APPLICATIONS	DATES OF APPLICATION
1-CONTROL	----	----	----	----
2-CONTROL	July 23-30	----	----	----
3-AZADIRACHTIN	----	10 g ai ha^{-1}	2	August 5-11
4-AZADIRACHTIN	July 23-30	10 g ai ha^{-1}	2	"
5-AZADIRACHTIN	----	100 g ai ha^{-1}	2	"
6-AZADIRACHTIN	July 23-30	100 g ai ha^{-1}	2	"
7-NSKE	----	0.31 kg ex ha^{-1}	2	"
8-NSKE	July 23-30	0.31 kg ex ha^{-1}	2	"
9-NSKE	----	1.56 kg ex ha^{-1}	2	"
10-NSKE	July 23-30	1.56 kg ex ha^{-1}	2	"

--- Indicates no artificial infestation with ECB egg masses or no neem product treatments.
ai active ingredient
ex extract

TABLE 4: Field season 1988 - Field 1 - Neem product treatments, their rates and timing of applications, and timing of ECB infestation.

TREATMENT	DATES OF ARTIFICIAL INFESTATION	RATE OF APPLICATION	NUMBER OF APPLICATIONS	DATES OF APPLICATION
1-CONTROL	----	----	----	----
2-CONTROL	July 27-29	----	----	----
3-AZADIRACHTIN	----	10 g ai ha^{-1}	2	August 10-16
4-AZADIRACHTIN	July 27-29	10 g ai ha^{-1}	2	"
5-AZADIRACHTIN	----	100 g ai ha^{-1}	2	"
6-AZADIRACHTIN	July 27-29	100 g ai ha^{-1}	2	"
7-NSKE	----	0.31 kg ex ha^{-1}	2	"
8-NSKE	July 27-29	0.31 kg ex ha^{-1}	2	"
9-NSKE	----	1.56 kg ex ha^{-1}	2	"
10-NSKE	July 27-29	1.56 kg ex ha^{-1}	2	"

--- Indicates no artificial infestation with ECB egg masses or no neem product treatments.
ai active ingredient
ex extract

TABLE 5: Field season 1988 - Field 2 - Neem product treatments, their rates and timing of applications, and timing of ECB infestation.

TREATMENT	DATES OF ARTIFICIAL INFESTATION	RATE OF APPLICATION	NUMBER OF APPLICATIONS	DATES OF APPLICATION
1-CONTROL	----	----	----	----
2-CONTROL	July 27-29	----	----	----
3-NSKE	"	0.79 kg ex ha^{-1}	2	August 12-18
4-NSKE	"	3.12 kg ex ha^{-1}	2	"
5-SAFER'S EC	"	1.56 kg ai ha^{-1}	2	"
6-AMBUSH	----	15.6 g ai ha^{-1}	2	August 11-19
7-AMBUSH	July 27-29	15.6 g ai ha^{-1}	2	"
8-NSKE	"	1.56 kg ex ha^{-1}	4	August 12-18 23-26
9-NSKE	July 28-30	1.56 kg ex ha^{-1}	2	Pre-infest. July 28-30
10-NSKE	"	1.56 kg ex ha^{-1}	4	Pre-infest. July 28-30 Post-infest. August 12-18

--- Indicates no artificial infestation with ECB egg masses or no neem product treatments.
ai active ingredient
ex extract

TABLE 6: Field season 1989 - Field 1 - Neem product treatments, their rates and timing of applications, and timing of ECB infestation.

TREATMENT	DATES OF ARTIFICIAL INFESTATION	RATE OF APPLICATION	NUMBER OF APPLICATIONS	DATES OF APPLICATION
1-CONTROL	----	----	----	----
2-CONTROL	July 23 August 4	----	----	----
3-SAFER"S NEEM1	"	1.34 kg ex ha^{-1}	7	August 10-14-17-21-24-28-31
4-AZADIRACHTIN	"	10 g ai ha^{-1}	2	August 10-17
5-SAFER'S NEEM2	"	1.47 kg ex ha^{-1}	7	August 10-14-17-21-24-28-31
6-AZADIRACHTIN	"	100 g ai ha^{-1}	2	August 10-17
7-NSKE	----	0.31 kg ex ha^{-1}	2	"
8-NSKE	July 28 August 4	0.31 kg ex ha^{-1}	2	"
9-NSKE	----	1.56 kg ex ha^{-1}	2	"
10-NSKE	July 28 August 4	1.56 kg ex ha^{-1}	2	"

--- Indicates no artificial infestation with ECB egg masses or no neem product treatments.
ai active ingredient
ex extract

Infestation dates were similar for the three consecutive field seasons (Tables 3-7). For the first infestation, 2 egg masses were placed in the whorl of the treatment plants which were approximately at the V–12 stage of growth. The second infestation was done approximately 5 days following the first infestation by placing 2 egg masses between the stem and the young ear of the plant. Since the Illini Gold hybrid has an average of 2 cobs per plant, a total of 4 egg masses per plant was deposited in the second infestation (Grier and Davis, 1980).

TABLE 7: Field season 1989 - Field 2 - Neem product treatments, their rates and timing of applications, and timing of ECB infestation.

TREATMENT	DATES OF ARTIFICIAL INFESTATION	RATE OF APPLICATION	NUMBER OF APPLICATIONS	DATES OF APPLICATION
1-CONTROL	----	----	----	----
2-CONTROL	July 23 August 4	----	----	----
3-AMBUSH	----	15.6 g ai ha^{-1}	2	August 11-18
4-AMBUSH	July 28 August 4	15.6 g ai ha^{-1}	2	"
5-NSKE	"	1.56 kg ex ha^{-1}	7	August 10-14-17-21-24-28-31
6-NSKE	July 29 August 8	1.56 kg ex ha^{-1}	2	Pre-infest. Jul. 29-Aug. 8
7-NSKE	"	1.56 kg ex ha^{-1}	5	Pre-infest. Jul. 29-Aug.8 Post-infest. Aug.11-18-25
8-NSKE	July 28 August 4	0.79 kg ex ha^{-1}	2	August 11-18
9-NSKE	"	3.12 kg ex ha^{-1}	2	"
10-NSKE	"	1.56 kg ex ha^{-1}	7	Dusk Aug.10-14 17-21-24-28-31

--- Indicates no artificial infestation with ECB egg masses or no neem product treatments.
ai active ingredient
ex extract

1.2.7 Preparation of Neem-Product Formulations for Spray Application

The neem product treatments to be tested in 1988 were chosen based on the 1987 field trial conducted by J. Apablaza , J.T. Arnason and J. Kaminski (unpublished). A second field was added in 1988 and 1989 to test different neem product-treatments and spraying regimes (Tables 3-7).

1.2.7.1 NSKE Formulations and Safer's Neem Emulsifiable Concentrates & Formulations

All solutions were prepared on the eve of the spraying day and stored in the refrigerator. To insure proper emulsion of the neem extract with the solvents, the required amount of NSKE was first diluted in a small volume of 100% methanol, and piperonylbutoxide (4,5-Methylendioxy–2-propyl-benzyl-diethylenglykol-butylether) (PBO), Fluka) was added to the mixture as well as 0.1 % Citowett Plus (Spreader Sticker Adjuvant 50% guar, triton XR, octylphenoxy-polyethoxyethanol, B.A.S.F.). This slurry was then completely dissolved in the remaining methanol. Distilled water was added to the mixture to yield a NSKE:PBO (1:1)-0.1% Citowett–25% MeOH/75% H_2O solution. The final formulation was thoroughly mixed on a magnetic stirrer. The NSKE formulations were prepared in volumes of 500 ml or 250 ml (Table 8).

In 1988, an emulsifiable concentrate was prepared with Safer Ltd.'s products. The blend consisted of 6.25 g NSKE, 6.25 g PBO, 1 g of an emulsifier blend (0.85 g NPPOE 6 +0.15 g NPPOE 8) and 16.5 g cottonseed oil which produced 30 g of emulsifiable concentrate. By diluting 15 g of the concentrate in 235 ml distilled water, 250 ml of a 6% solution were available for spraying.

Safer's neem formulations of 1989 were prepared by adding the emulsifiable concentrate to a volume of distilled water. Safer's Neem 1 and Neem 2 were diluted to yield a 1:90, and a 1:85 solution, respectively. Once prepared, Neem 1 contained 80% Neem oil and 14.4% emulsifier blend while Neem 2 contained the same ingredients plus 5.6% pyrethrin.

1.2.7.2 Preparation of the Azadirachtin Formulations

To prepare the AZA formulations, AZA crystals were dissolved in a small volume of 100% methanol. PBO was added to the clear solution in a 5:1 ratio (PBO:AZA).

TABLE 8: Spray formulations' constituents for 1987, 1988, 1989 field seasons

TREATMENT	RATE	INGREDIENTS OF FORMULATION
AZADIRACHTIN	10 g ha^{-1}	azadirachtin.....0.08 g ai L^{-1} piperonyl butoxide.....0.4 g L^{-1} citowett plus.....1.0 ml L^{-1} methanol.....250 ml L^{-1} distilled water.....750 ml L^{-1}
AZADIRACHTIN	100 g ha^{-1}	azadirachtin.....0.8 g ai L^{-1} piperonyl butoxide.....4.0 g L^{-1} citowett plus.....1.0 ml L^{-1} methanol.....250 ml L^{-1} distilled water.....750 ml L^{-1}
NSKE	0.31 kg ha^{-1}	neem kernel extract.....2.5 g ex L^{-1} azadirachtin.....6.25 mg ai L^{-1} piperonyl butoxide.....2.5 g L^{-1} citowett plus.....1.0 ml L^{-1} methanol.....250 ml L^{-1} distilled water.....750 ml L^{-1}
NSKE	0.79 kg ha^{-1}	neem kernel extract.....6.25 g ex L^{-1} azadirachtin.....15.6 mg ai L^{-1} piperonyl butoxide.....6.3 g L^{-1} citowett plus.....1.0 ml L^{-1} methanol.....250 ml L^{-1} distilled water.....750 ml L^{-1}
NSKE	1.56 kg ha^{-1}	neem kernel extract.....12.5 g ex L^{-1} azadirachtin.....31.3 mg ai L^{-1} piperonyl butoxide.....12.5 g L^{-1} citowett plus.....1.0 ml L^{-1} methanol.....250 ml L^{-1} distilled water..... .....750 ml L^{-1}
NSKE	3.12 kg ha^{-1}	neem kernel extract.....25 g ex L^{-1} azadirachtin.....62.5 mg ai L^{-1} piperonyl butoxide.....25 g L^{-1} citowett plus.....1.0 ml L^{-1} methanol.....250 ml L^{-1} distilled water.....750 ml L^{-1}
SAFER'S EC	1.56 kg ha^{-1}	neem kernel extract.....12.5 g ex L^{-1} azadirachtin.....19 mg ai L^{-1} piperonyl butoxide.....12.5 g L^{-1} NPPOE 6.....1.7 g L^{-1} NPPOE 8.....0.3 g L^{-1} cottonseed oil.....33 g L^{-1}
AMBUSH	15.6 g ha^{-1}	ambush EC.....10 ml L^{-1} permethrin.....0.125 g ai L^{-1} distilled water.....990 ml L^{-1}
SAFER'S NEEM1	1.34 kg ha^{-1}	neem extract.....11.12 g ex L^{-1} azadirachtin.....19 mg ai L^{-1} distilled water.....988.88 ml L^{-1}
SAFER'S NEEM2	1.47 kg ha^{-1}	neem extract.....11.8 g ex L^{-1} azadirachtin.....19 mg ai L^{-1} pyrethrum.....0.65 g ai L^{-1} distilled water.....988.2 ml L^{-1} emulsifier.....14.4%

0.1% Citowett was mixed into the above solution. The remaining methanol and a volume of distilled water further diluted the mixture to yield a 1:5 AZA:PBO-0.1% Citowett- 25% methanol/75% water solution.

1.2.7.3 Preparation of AMBUSH Formulation

The AMBUSH (C.I.L) (12.5 g permethrin per litre) formulation was prepared according to the recommendations of C.I.L. for control of ECB on sweet corn. 5.0 ml of AMBUSH were mixed with 500 ml distilled water (1% solution).

1.2.8 Spraying Technique

In 1987, a Chapin compressed air sprayer, model no. 152, 7.6 litre capacity was used by J. Apablaza to spray the test plants with azadirachtin and the NSKE. The tank was filled with 130 ml of the appropriate solution and applied on the test rows to deposit 50 ml. After each row application, the residue in the tank was measured and refilled to 130 ml. The average volume applied per plot was 54.5 ml. The spray was directed toward the top of the corn plant but did not necessarily cover the tassels.

In 1988 and 1989, application of the neem products was done with a hand-held pressurized sprayer Model 59-3851-8 of a 1 litre capacity. 100 ml of the prepared solutions were poured into the sprayer. To insure an equal pressure and a uniform spray throughout all of the applications, the pressure pump was activated 20 times immediately before spraying the plants. The treatment plants were sprayed at the level of the whorl for the first pre-infestation sprays or at the level of the developing ears for all subsequent treatments. When a row had been sprayed, the remaining volume of solution was measured in a 100 ml graduated cylinder. This volume was recorded after each application; volumes ranging from 50 to 60 ml of solutions were applied to each treatment row.

1.2.9 Spraying Regimes

In all three field seasons, the first post-infestation sprayings were done approximately 14 days following the first infestation when small lesions <1 cm long (identified as burrowing tunnels of young corn borer larvae) could be observed on the top leaves of the corn plants.

NSKE formulations for the 1988 and 1989 pre-infestation treatments were applied at the level of the whorl prior to the first infestation and at the level of the developing ear prior to the second infestation. Before infesting the plants with ECB egg masses the sprayed plants were allowed to dry for 1 to 2 hours until no visible wetness could be observed.

1.2.10 Harvest

The cobs were harvested at maturity (R-3 and R-4 stage of growth). The harvest dates for the three field seasons were as follows: 1987-August 25; 1988-August 31 (field 1), September 5 (field 2); 1989-September 5 (fields 1 and 2). The cobs were chosen at random in each treatment row but "marketable-size" cobs (i.e. full-sized cobs) were preferably picked. In 1987, 10 ears per treatment row were harvested for a total of 400 ears. In 1988, the same number of ears was picked from field 1 but in field 2, the harvest was increased to 15 ears per plot (total of 600). To increase further the sample size of the populations, 20 cobs per treatment row (total of 800 ears per field) were harvested from fields 1 and 2 in 1989.

1.2.11 Evaluation of Damage to Crop

Each cob was evaluated for corn borer damage by counting the number of kernels that had been totally or partially eaten by the larvae. Fully developed kernels as well as partially developed kernels were considered.

The number of larvae found on the cob and in the husk was determined for each cob. In 1987 and 1988, the damage evaluation of field 1 took place on the harvest day. The cobs from field 2 of 1988 and from field 1 and 2 of 1989 were stored in an freezer at $-10^{o}C$ to cease further feeding by the larvae; evaluation of damage could thus be conducted at a later date.

Following the harvest of field 1 in 1987, the damage done to the stalks of the plants by burrowing larvae was measured. The stalk damage parameters were taken during the second and third week of September. The corn borer larval tunnelling was determined by first measuring the height of the plant, removing its leaves and splitting the stalk down its center from top to bottom. One point was awarded for each penetration site and one additional point for each 2.5 cm of tunnelling beyond the 2.5 cm from the penetration site (Grier and Davis, 1980). A ratio of tunnel length to plant height was then calculated.

A similar type of stalk damage evaluation was conducted in 1989 for field 1 and 2. A method developed by Guthrie et al (1960) was used to evaluate the degree of plant breakage (due to borer tunnelling) prior to the harvest. This evaluation consisted of rating each plant on a scale of 1 to 10. A rating of 1 described an unbroken plant, 2 or 3-plants with broken tassels, 4 or 5-stalks were broken above the ear, and 6 to 10-the stalk was broken beneath the ear.

1.2.12 Statistical Analysis of Field Data

Analysis of the field data was kept separate for each year because of varying climatic conditions from one season to the other and also because of the changing locations of the fields.

All data was analyzed with SAS General Linear Models Procedures. The means of the number of damaged kernels per cob, the number of larvae, the tunnelling ratios

(1987) and stalk damage ratings (1988) were computed and tested against a normal distribution (Shapiro-Wilk statistic W when the sample size was less than fifty-one and Kolomogorov D statistic when the sample size was greater than fifty). Since more than 50% of the treatments in each year did not follow a normal distribution, nonparametric statistics (NPAR1WAY procedure) were used to analyze the data.

A block analysis was first done to see if the means of the replicates of each treatment could be regrouped. The means for all treatments in a field were then submitted to an ANOVA (analysis of variance). The means of each treatment were then compared to their respective control with the Wilcoxon rank-sum nonparametric test and reported as being significantly different from their control at the p=0.05 level of significance for the test statistic Z (Zar, H. 1974). Treatments were also compared among themselves.

A "calibration curve" (Blau et al, 1978) was generated using the mean number of damaged kernels/cob and the mean stalk damage (stalk tunnelling or stalk breakage rating) of each control plot (8 control plots including naturally and artificially infested plots) plotted against the mean number of larvae cob^{-1} of these plots. The control data were submitted to Spearman's rank correlation procedure and were tested for simple linear dependency (Zar, H. 1974). The data obtained from the neem-product treated plants (the 4 plots for each treatment were represented individually) were plotted along this calibration curve. The number of treated plots falling above or below the calibration curve was then tabulated; the neem products could then be assessed as to their overall efficacy at decreasing the damage done by the ECB. Linear regression was carried out for the correlated variables using Sigmaplot version 1.10 on an IBM personal computer.

1.3 Laboratory Studies

1.3.1 Azadirachtin Antifeedant No-Choice Bioassays

In order to test the antifeedant activity of AZA and the combination of AZA with the compounds used in the field formulations, leaf-feeding trials with corn borer larvae were conducted using a method established by Ascher and Glotter(1981) and adapted by Reid (1988).

1.3.1.1 Apparatus

The apparatus was borrowed from Dr. J. Lambert's laboratory at Carleton University. It consisted of a series of petri plates arranged in such a fashion as to enclose a feeding arena from which corn borer larvae could not escape. A ball of absorbent cotton was placed in the inverted top of a 5 cm diam. petri plate and covered with a filter paper (Whatman #1) which was moistened with distilled water to maintain humidity and leaf turgescence. A leaf section (approximately 1.5 cm to 2.0 wide and 3.5 cm long) was placed on the filter paper on top of the cotton. The bottom of a 5 cm diam. petri plate with a 1.3 cm diam. hole cut out from its center was placed over the leaf section (upper surface exposed) and tightly sealed. At this stage of the set-up, the compounds to be tested were applied to the exposed surface of the leaf (138 mm^2). Three early third instar larvae were then placed in the feeding arena. The exposed leaf area was covered with a rigid opaque strip which was bent so as to form a "bridge" over the leaf surface; this provided the larvae with a dark area to which they might be attracted. The feeding arena was then covered with an inverted top of a petri plate and tightly sealed with masking tape. The containers were placed in an environmental chamber for 24 hours. This time period was sufficient to ensure appreciable feeding damage in the controls and did not allow enough time for the leaves to dry up or larvae to die. Since AZA easily degrades under UV light (Stokes and Redfern, 1982; Arnason, personal communication),

a limited amount of time was available for the testing period. Ten replicates were made for each compound tested. The entire experiment was repeated three times.

1.3.1.2 Corn

Illini gold corn was grown at the Environmental Laboratory Biology Annex, Carleton University during the winter months (day:night temperature of 25:20°C, photoperiod of 18 hours light:6 hours dark) and in the greenhouse of the University of Ottawa during the summer months. Four to five seeds were planted per 6 inch pot in commercial potting soil and watered regularly. The third leaf of seedlings at the V2 stage was used in the feeding bioassays.

1.3.1.3 Corn Borer Larvae Rearing and Selection

European corn borer larvae for this study and the growth and development studies were reared and maintained on a meridic diet according to the method of Guthrie et al, 1971 in a controlled environmental chamber (Conviron Model E7), at 25°C during the 16h light period and 19°C for the 8h dark period. Relative humidity was maintained at 85%. The eggs for the initial colony had been supplied by M. Hudon (Agriculture Canada, St.Jean, P.Q.). Early third instar larvae were selected from the culture on the basis of the width of their head capsule (approximately 0.8 mm) and their body length (approximately 3.2-4.6 mm) (Hudon and Leroux, 1986a).

1.3.1.4 Antifeedant-Testing Compounds

AZA and AZA combined with PBO and or Citowett (1:1 AZA:PBO (v:v) and 0.1% Citowett) were tested as antifeedants. Acetone was used as solvent in the formulations since it evaporated quickly when applied to the leaf surface. PBO and Citowett were individually tested and also combined to observe any effect they might

have on feeding behaviour of the larvae. Three concentrations of AZA were used: 100, 10 and 1 ppm, corresponding to 7.2 ng ai mm^{-2} of leaf surface, 0.72 ng ai mm^{-2} and 0.072 ng ai mm^{-2} respectively. The compounds were applied to the exposed leaf surface in the feeding containers with a 10 μl Hamilton syringe. Accumulation of the compounds on the outer edges of the leaf surface was minimized by letting each drop of applied solution dry before adding more solution.

1.3.1.5 Evaluation of Feeding

Following the 24 hour testing period, the feeding containers were dismantled and the leaf sections were stored in methanol to preserve the tissues. The total area consumed was determined by placing the leaf section on a square mm grid, and under a dissecting microscope the number of squares that had been eaten could be counted. If an area of leaf tissue had only a portion of its thickness consumed, this was still counted as a fully consumed square.

1.3.1.6 Statistical Analysis of Data

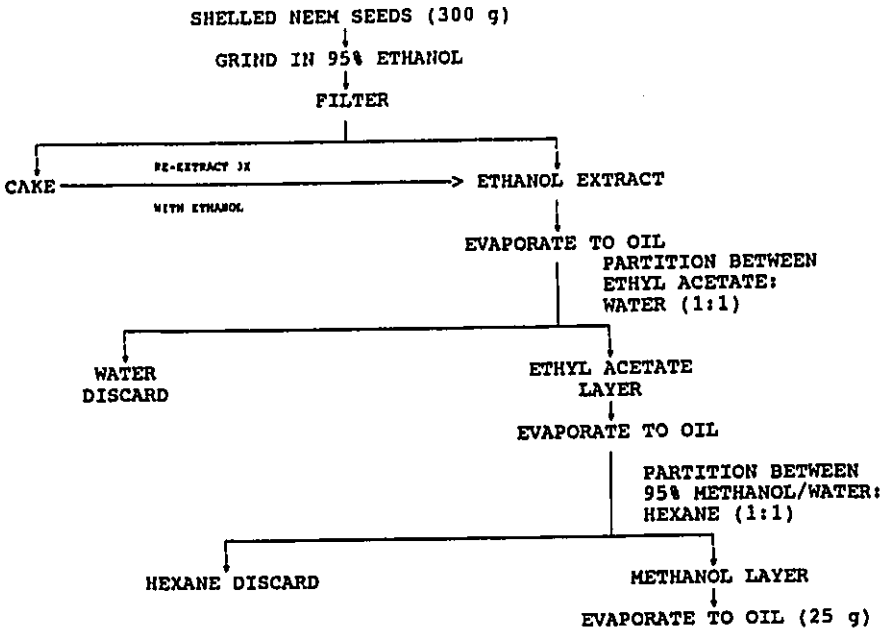
The feeding damage for each leaf section was expressed as a percent of the control (represented as 100% damage). The average ratios for each treatment were tested for normality and subjected to an ANOVA using Statgraphics 3.0 (Statistical Graphics Corporation) on an IBM personal computer. Comparisons among treatments were performed by Tukey's multiple range analysis at the p=0.05 level of significance.

FIGURE 7

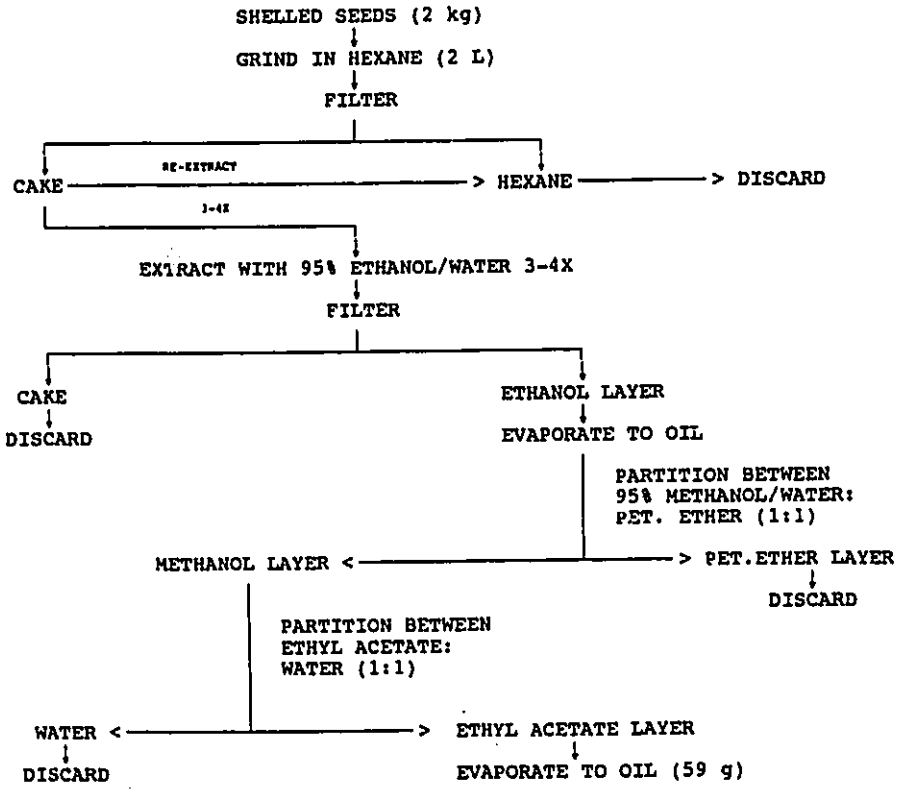
Extraction methods of NSKE from shelled neem seeds.

(1) Nakanishi's original 1975 procedure.
(2) Nakanishi and Schroeder's 1987 modified procedure.

NAKANISHI'S ORIGINAL PROCEDURE FOR NEEM EXTRACTION (1975)



NAKANISHI'S AND SCHROEDER'S NEW PROCEDURE FOR NEEM EXTRACTION (1987)



1.3.2 Growth and Development Studies with NSKE

1.3.2.1 The NSKEs

Two NSKEs were chosen to study the effect of neem components on the ECB larvae. Both NSKEs were prepared by J. Kaminski (Figure 7). One of the extracts was prepared following Nakanishi's original procedure for neem extraction from shelled seeds (1975), yielding a viscous dark brown paste, and the other, following a modified version of the procedure (Nakanishi and Schroeder, 1987) yielding dark brownish crystals. The modified procedure consisted in exhaustively defatting the ground neem seeds with hexane prior to extraction with 95% ethanol.

1.3.2.2 Preparation of Test Diets

The meridic diet used for the studies was prepared as described by Guthrie et al (1971) omitting the corn cob grits in order to enhance a homogeneous distribution of the NSKE (Appendix 1). For the first and second study, concentrations of the two NSKEs in the meridic diets were 1, 10, 100 and 1000 $\mu g\ g^{-1}$ wet weight, and 1, 3, 10 and 30 $\mu g\ g^{-1}$ wet weight, respectively.

The NSKEs stock solutions were prepared in 95% ethanol, forming a slurry. The diet with the NSKEs was prepared by adding no more than 1 ml of ethanol (<1%) to the mixture. Appropriate volumes of the stock solutions were placed in small beakers to which the cooled meridic diet (approximately 40°C) was added. The mixture was quickly and vigorously stirred and poured into 1 x 1 x 1 cm molds to cool. Each diet cube weighed approximately 2 g. The prepared diet was stored in a refrigerator.

1.3.2.3 Growth and Development Study Protocol

One hundred and thirty-five second and early third instar larvae (15 per NSKE concentrations) were chosen from the main culture. Each larva was weighed (range of

2.5 mg +/- 0.5 mg) and placed on one gram (one half of a cube) of the appropriate diet, in a 15 ml scintillation vial capped with a ball of cotton. During the course of the study, the larvae were weighed and their diet changed every 3 to 4 days. Mortality and growth parameters (pupation day, number of days in pupation, adult emergence) were monitored throughout the study. Pre-pupating larvae (they would usually be wrapped in the cotton stopper) were not weighed in order to avoid unnecessary stress. Once the insect had pupated, the vial was cleared of any diet and frass and the pupa was weighed. On the day of their emergence, adults were weighed and sexed.

1.3.2.4 Statistical analysis of Data

The weight data were subjected to an analysis of variance and Tukey's Multiple Range Test (Statgraphics 3.0). Kruskal-Wallis (nonparametric statistic) test was performed on all time measurements. The level of significance was p=0.05 for all tests.

2.0 Uptake and Systemic Movement of H_2-Azadirachtin and Azadirachtin in Sweet Corn Seedlings.

2.1 $^3H_2$-Azadirachtin Tracer Study

2.1.1 Preparation of $^3H_2$-Azadirachtin

Radiolabelled $^3H_2$-AZA was prepared by N. Werstiuk, by reducing AZA with pure tritium gas generated from the reactor at McMaster University. Approximately 40 mg of the ^{3}H-compound (2.0 Curie) were received in a large round-bottomed flask. Since the material could not be scraped from the container, it was diluted with methanol and separated into several smaller volumes of approximately 2 ml in 7 ml vials and stored at – 80°C; this would minimize any degradation of the tritiated material. One vial was kept as a stock solution.

2.1.2 Purity and Characterization of the ^{3}H-Compound

Because of the minimal amount of the highly radioactive compound, it could not be characterized by FAB or NMR. The purity and identity of the material was determined by HPLC and TLC. A 1/50 dilution (in 80:20 MeOH:H_2O v:v) of the stock solution was co-chromatographed with H_2-AZA.

2.1.2.1 HPLC Method

Twenty-five microliters of a solution containing the ^{3}H-material (12.5 μl) and H_2-AZA (12.5 μl) were injected into the HPLC system; the solvent system consisted of an isocratic elution with 40% acetonitrile/60% water. A series of fractions was collected as they eluted from the system. The fractions were collected every 30 seconds for a total time of 10 minutes. The run was repeated four times.

The volume of each fraction (approximately 2 ml) was brought up to 7 ml with scintillation fluid (Scintran Cocktail EX, BDH Chemicals Ltd.) miscible with water. The radioactivity was measured on a Beckman LS 3133P Liquid Scintillation counter. An appropriate quench curve was used to correct for chemical and colour quench. The samples were counted several times until the luminescence readings were nil in order to ensure a constant recording of decay per minute (dpm).

The amount of radioactivity for each fraction collected in the time sequence was plotted against the chromatogram of H_2-AZA to compare their relative retention times.

2.1.2.2 TLC Method

Each time a tracer study was set-up, the chemical nature of the compound was verified by TLC. The tritiated material was spotted on a plastic TLC plate along with the H_2-AZA and developed with different solvent systems. The H_2-AZA spot was visualized and the plate was subsequently cut into 5 mm wide strips. The strips were placed in 7

ml scintillation vials to which scintillation fluid was added and the amount of radioactivity was determined by LSC (liquid scintillation counting).

2.1.3 Preparation and Specific Activity of ^3H-Material

A stock solution of $^3H_2$-AZA+ H_2-AZA was prepared in MeOH:H_2O (25:75 v:v) and 0.1% Citowett. The specific activity of the 5 mg H_2-AZA ml^{-1} solution was 4.384 Ci $mole^{-1}$ $^3H_2$-AZA, where 10 μl (or 50 μg tritiated material) contained approximately 1 x 10^6 dpm.

2.1.4 Corn

Illini gold sweet corn was grown in the greenhouse of the University of Ottawa in Pearlite (purchased from Ritchie Feed and Seed), an inert material of volcanic origin. The seeds were watered regularly with distilled water until emergence of the coleoptile and were subsequently given a weekly dose of a modified Hoagland's solution (Appendix 2). At the V–2 stage of development, the seedlings were removed from the Pearlite and their roots were rinsed free of debris.

2.1.5 Hydroponics Set-up

Healthy corn seedlings at the same stage of growth (V2) and of the same height were selected. The roots of the corn seedlings were immersed in 45 ml Hoagland's solution in 50 ml test-tubes. A foam stopper was wrapped around the bottom part of the shoot to maintain the seedling in an upright position. A 2.5 mm inner diameter tubing was inserted between the outer edge of the foam stopper and the side of the test-tube to deliver air in order to ensure proper oxygenation of the roots. The test-tubes were placed together in metal racks under Vita Light^R high intensity fluorescent lamps (Duro-Test, Toronto, Ont., 50 wm^{-2}, 290-700 nm), in an environmental chamber. The temperature

under the Vita Light® was maintained constant at 23°C with a photoperiod of 16 hours light: 8 hours dark; the relative humidity was not controlled. The seedlings were left to acclimatize to the new conditions for a period of 48 hours before being treated.

2.1.6 Foliar Application of the ^3H-Compound

Ten μl of the prepared $^3H_2$-AZA solution were applied to the third leaf of each corn seedling with a Hamilton syringe. The volume was evenly spread on the upper surface of the upper third of the leaf, i.e. 2 cm from the tip of the leaf and no lower than 6 cm from the tip. The Citowett prevented any dripping from the area of application. The seedlings were placed under the UV lights once the formulation appeared dry; the area of application was clearly visible even when the solution had dried. The experiment was started with the 8 hour dark period to allow absorption of the intact molecules of H_2-AZA since it is known to easily degrade under UV lights.

In the first experiment, there were three replicates for three time periods; 24, 48 and 72 hours. The experiment was repeated and four replicates were used for each of the time periods.

2.1.7 Harvest

At the end of each time period, the treated leaf was cut directly below the site of application and rinsed, i.e. gently shaken for 30 seconds in approximately 4 ml of MeOH:H_2O (90:10 v:v) (this ensured recovery of 98 to 100% of the applied material). The leaf section was blotted dry and cut into small pieces which were placed in a 7 ml scintillation vial. The remaining part of the treated leaf as well as the 4 or 5 other leaves were cut at the level of the leaf collar of the first leaf. All harvested parts were cut into small pieces and placed in vials.

The "stem" included all remaining leafy material between the top of the first leaf

sheath and the beginning of the root system.

The germinated grain was included with the roots. The roots and seed were rinsed (gently shaken for 30 seconds) in 15–20 ml MeOH:H_2O (90:10 v:v), blotted dry, cut into small pieces and placed in a vial.

The Hoagland's solution was sampled after it was vigorously shaken. Four ml of the solution were pipetted into 20 ml scintillation vials; four replicates were taken.

2.1.8 Determination of Radioactivity in the Samples

All treated-leaf rinses and root-seed rinses were left to evaporate under a fumehood to approximately 0.5 ml. Scintillation fluid was added and samples were counted by LSC.

The amount of radioactivity in the 4 ml of Hoagland's solution was determined by LSC by simply adding 16 ml of scintillation fluid.

Extracting solvent (MeOH:H_2O 90:10 v:v) was added to the vials of all harvested material. The vials were placed on an Eberbach shaker for 12 to 14 hours. The solvent was then pipetted into a set of vials which were placed under the fumehood. The solvent was left to evaporate to less than 0.5 ml, the vial was filled with scintillation fluid and counted in the LSC. The vials with the leaf material were filled once again with the extracting solvent and placed for a second time on the shaker. This extraction procedure was repeated three times for all leaf material. Since H_2-azadirachtin is very soluble in all polar solvents, it was assumed that this procedure would extract all polar H^3-compounds from the tissues.

2.2 Systemic Movement of Azadirachtin: Feeding Bioassays

A choice test was performed on third instar ECB larvae to study the foliar uptake and systemic movement of AZA to new foliage of sweet corn seedlings.

2.2.1 Experimental set-up

Illini Gold seeds were planted in commercial earth in standard 5 in. diameter pots and grown in the greenhouses of the University of Ottawa. At V–2 stage of growth, seedlings of uniform size were chosen. The two newest leaves (second and third) of the seedlings were treated with varying amounts of azadirachtin. The seedlings were placed in a lighted room (approximately 5 watts m^{-2}, 16 hours light:8 hours dark) at 25°C (relative humidity was not controlled) for 24 or 48 hrs. Ten third instar ECB larvae from the main culture were subsequently placed in the whorl of each seedling. An inverted 1.5 litre Mason jar of which the bottom had been cut out and replaced with a fine mesh grid was carefully placed over the seedling to ensure adequate ventilation for the insects. The jars were returned to the above conditions and the larvae were left to feed for 72 hours, a time period which allowed noticeable feeding damage in the controls. Five replicates for each treatment were prepared for a total of 50 ECB larvae per treatment, as well as an appropriate control.

2.2.2 Preparation of Azadirachtin and its Application

AZA dissolved in acetone was applied to the treatment leaves (second and third leaf) using a 100 μl Hamilton syringe. A total of 1000, 100 or 10 μg (500, 50 or 5 μg on each leaf) were applied on the seedling, for an average of 280, 28 or 0.28 ng ai mm^{-2}, respectively. Acetone was used as carrying solvent in order to avoid build up of the wetting agent Citowett used in the tracer experiments since the volumes of the solution to be applied to the leaves were considerable (500, 250 and 25 μl of stock solution).

Acetone had not been found to affect the feeding pattern of the ECB larvae, and the solvent evaporated easily from the surface of the leaf thereby avoiding dripping of the solution into the whorl. The AZA solutions were carefully applied over approximately 10 cm of the leaf's length starting at the tip.

2.2.3 Evaluation of Feeding Damage

Following the 72-hour bioassay, the jar was removed and the remaining ECB larvae were collected and counted. All leaves were harvested (cut at the level of the sheath collar of the first leaf) and stored in methanol. The total surface area of each leaf was calculated by drawing the outline of the leaves on a square mm grid. The areas of feeding damage were then evaluated on a square mm grid under a dissecting microscope.

2.2.4 Statistical Analysis of Data

The leaf feeding damage for treated and untreated leaves was cumulated for each seedling and a percent of the control (represented as 100% damage) was calculated. The average ratios for each treatment were tested for normality, subjected to an ANOVA and Tukey's multiple range analysis at the p= 0.05 level of significance (Statgraphics 3.0).

D - RESULTS

1.0 Effects of Neem and Azadirachtin on Ostrinia nubilalis

1.1 Chemical Characteristics and Quantification of Neem Products

1.1.1 NSKE Yields

The 1975 Nakanishi method of extraction (Nakanishi, 1975) yielded 1.0 g NSKE from 10 g of neem kernels; this extract was used for the 1987 field study. In 1988 and 1989, the NSKE yield was 0.38 g 10 g^{-1} neem kernels and 0.39 g 10 g^{-1} neem kernels, respectively.

1.1.2 Chemical Characterization of Azadirachtin and H_2-Azadirachtin

J. Kaminski confirmed the identity of the extracted AZA by spectroscopy (FAB, NMR Appendix 3). The author performed TLC and HPLC on these compounds to determine R_f values, HPLC retention times and chromatography profiles (Table 9, Figure 8). Visualization of the TLC chromatograms of both compounds revealed one main spot with the R_f values for H_2-AZA (ex. R_f = 0.57, on aluminum TLC plate, chloroform:ethylacetate:methanol 4:4:1 v:v:v as solvent system) slightly less than those of AZA (R_f = 0.71, conditions as stated above), the former being more polar.

One main peak was observed in HPLC chromatograms of AZA or H_2-AZA (Figure 8). H_2-AZA eluted first (retention time = 6.2 min, water:acetonitrile 60:40 v:v) in the reverse-phase chromatography followed by AZA (retention time = 7.2 min). The 60% water:40% acetonitrile solvent system revealed a shoulder peak immediately preceding the main peak as well as a longer retention time, compared with the 70% methanol:30% water solvent system.

TABLE 9: Summary of chromatography (TLC and HPLC) of azadirachtin and H_2-azadirachtin.

TLC

COMPOUND	SOLVENT SYSTEM	TLC PLATE*	R_f VALUE
azadirachtin	100% ethyl acetate	200 μm aluminum	0.45
"		100 μm cellulose acetate	0.53
"	chloroform:ethyl acetate:methanol 4:4:1 v:v:v	200 μm aluminum	0.71
"	"	100 μm cellulose acetate	0.79
H_2-azadirachtin	100% ethyl acetate	200 μm aluminum	0.31
"	"	100 μm cellulose acetate	0.33
"	chloroform:ethyl acetate:methanol 4:4:1 v:v:v	200 μm aluminum	0.57
"	"	100 μm cellulose acetate	0.72

HPLC

COMPOUND	SOLVENT SYSTEM	RETENTION TIME (min)
azadirachtin	methanol:water 70:30 v:v	3.4
H_2-azadirachtin	"	3.0
azadirachtin	water:acetonitrile 60:40	7.2
H_2-azadirachtin	"	6.2

* silica gel thickness and type of TLC sheet

FIGURE 8

HPLC chromatograms of azadirachtin and H_2-azadirachtin.
Isocratic elution with 40% acetonitrile: 60% water
Flow rate = 1 mL min^{-1}
Detection wavelength λ = 210 nm

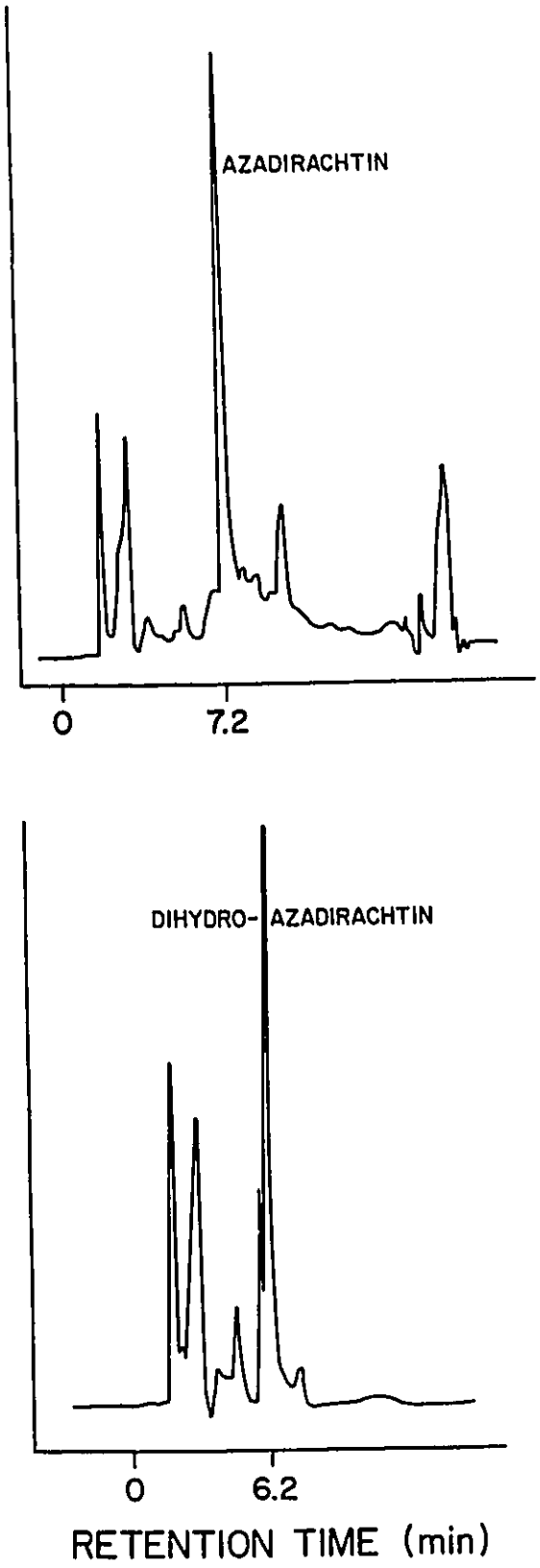

AZADIRACHTIN
0
7.2
DIHYDRO- AZADIRACHTIN
0
6.2
RETENTION TIME (min)

FIGURE 9

HPLC chromatograms of two NSKEs.

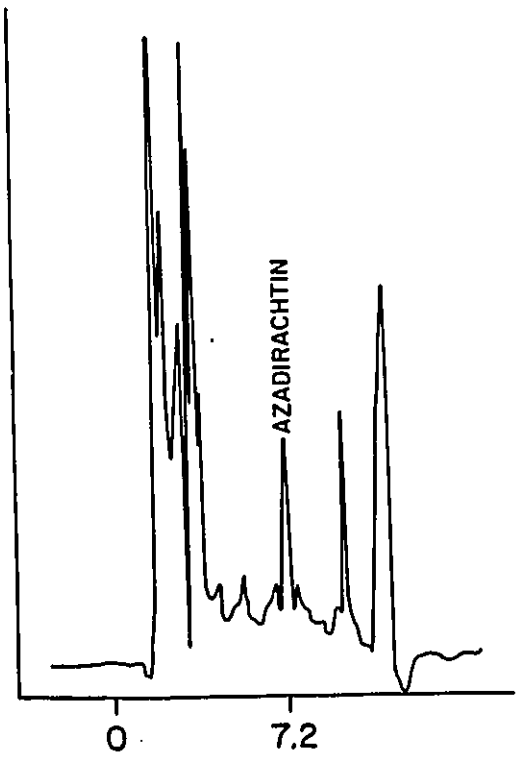
Isocratic elution with 40% acetonitrile: 60% water. Flow rate = 1 mL min^{-1}

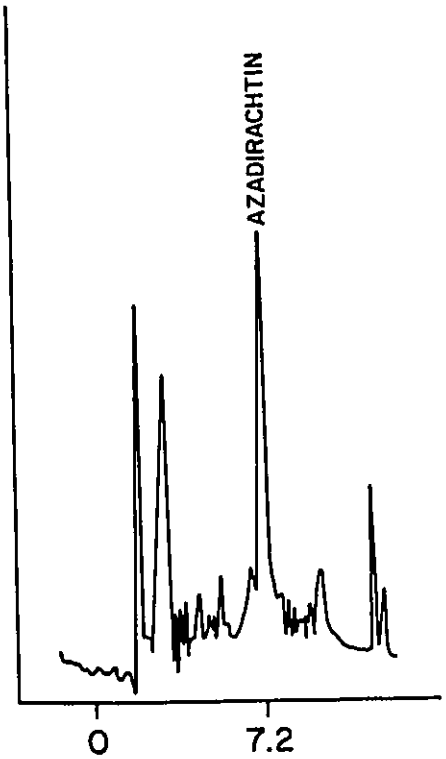
Detection wavelength λ = 210 nm

The NSKEs were purified using TLC to quantify azadirachtin.

Top chromatogram represents a purified fraction of NSKE obtained from Nakanishi's original procedure (1975).

Bottom chromatogram represents a purified fraction of NSKE obtained from Nakanishi and Schroeder's procedure (1987).





RETENTION TIME (min)

1.1.3 Quantification of Azadirachtin in the NSKE

J. Kaminski determined the AZA content of the NSKE (Nakanishi 1975 method of extraction) to be 2.5 mg (+/- 0.5 mg) ai g^{-1} NSKE. In HPLC chromatography of the two NSKEs (Figure 9) used in the growth and development studies, the AZA content of the NSKE extracted by Nakanishi and Schroeder's 1987 method was 5.1 mg (+/-0.5 mg) ai g^{-1} NSKE. The modified method of extraction yielded twice the amount of azadirachtin (10.2 mg (+/-0.5 mg) ai g^{-1} as compared to the original 1975 method of extraction).

1.2 Field Studies: 1987,1988 and 1989

1.2.1 Feeding Damage and Stalk Damage by ECB Larvae in Naturally versus Artificially Infested Corn

Natural infestation was low in all three field seasons as compared to artificially infested plots (Appendices 4–11). In 1987, the larvae found on artificially infested control cobs (1.8 larvae cob^{-1}) were 60 times more abundant than on the naturally infested ones (0.03 larvae cob^{-1}) and damaged on average 47.2 kernels cob^{-1} (8.3% of total kernels in a cob) as compared to only 2.0 kernels cob^{-1} naturally infested (0.4%). Stalk tunnelling, a measure of stalk damage, was 40X higher in artificially infested corn plants (ratio of 0.103 tunnels (cm) plant height $(cm)^{-1}$).

In 1989, the ratios of larvae in artificially infested: naturally infested cobs were of the order of 95X (3.8 versus 0.04 larvae cob^{-1}) and 57X (3.4 versus 0.06 larvae cob^{-1}) for fields 1 and 2 respectively, representing damage of 31.3 kernels cob^{-1} (5.4%):0.4 kernels cob^{-1} (0.07%) in field 1 and 42.6 kernels cob^{-1} (7.5%):0.3 kernels cob^{-1} (0.05%) in field 2. Stalk breakage ratings in fields 1 and 2 were 10X higher in artificially infested plants (5.8 versus 5.1) versus naturally infested ones (0.6 versus 0.5).

In 1988, a summer of unusually hot weather, natural infestation in field 1 was 10X (0.2 larvae cob^{-1}) higher than the 1987 and 1989 seasons, which were closer to the

climatic mean; the artificially infested cobs had 1.6X the number of larvae cob^{-1} (3.2 larvae cob^{-1}) compared to the naturally infested ones (0.2 larvae cob^{-1}). The artificially infested control cobs had on average 23.7 damaged kernels cob^{-1} (4.1%) while the naturally infested ones contained 2.6 damaged kernels cob^{-1} (0.5%). The number of larvae in naturally infested cobs from field 2 was similar to that of the other seasons with artificially infested cobs having 24X the number of larvae found in the naturally infested ones. The number of damaged kernels cob^{-1} averaged 33.5 (5.9%) in artificially infested plants and 1.1 (0.2%) in naturally infested ones. Stalk damage was not evaluated in the 1988 field season.

Since naturally infested plants showed very low damage and larval populations (statistical significance was minimal), the data are not presented in Figures 10–14 but are found in Appendices 5,7,8 and 11; only the results obtained from artificially infested plants are shown in Figures 10-14.

1.2.2 Effect of Azadirachtin on ECB Larval Populations, Feeding and Stalk Damage

The results of the 1987 to 1989 field seasons (Figures 10a, 10b, 11, 13a, 13b and Appendices 4, 5, 6, 8, 9) reveals that AZA effectively controlled the European corn borer. In artificially and naturally infested corn, the AZA formulations compared to non-treated controls reduced the number of ECB larvae found on harvested cobs, the number of damaged kernels cob^{-1} (Figure 10a, 11, 13a) and the amount of stalk damage (Figure 10b, 13b).

AZA was tested at two application rates, 10 g ha^{-1} and 100 g ha^{-1}, both formulations were applied twice at a 6 to 7 day interval. An examination of the difference between the two application rates showed that the higher AZA concentration, 100 g ha^{-1}, was generally more effective at reducing corn borer populations on the cobs

and borer damage (damaged kernels cob^{-1} and stalk damage) than the lower concentration, 10 g ha^{-1}.

In 1987, (Figures 10a, 10b and Appendix 4) the 100 g ha^{-1} AZA formulation reduced by 50% the larval population on the corn cobs (1.8 untreated versus 0.9 treated larvae cob^{-1}), and by 55% the number of damaged kernels cob^{-1} (47.2 untreated versus 21.3 treated damaged kernels cob^{-1}) in artificially infested plants. Stalk tunnelling (Figure 10b) was reduced by 33% (0.103 untreated versus 0.069 treated). Comparatively, the 10 g ha^{-1} formulation reduced the cob-larval population by 44% (1.8 untreated versus 1.0 treated larvae cob^{-1}), the kernel damage by 38% (47.2 untreated versus 29.2 treated damaged kernels cob^{-1}) and stalk damage by 22% (0.103 untreated versus 0.08 treated). There was no statistically significant difference between the two AZA concentrations in regards to the cob-larval population and kernel damage; however, the stalk damage was significantly different at the p=0.05 level.

The naturally infested cobs (Appendix 5) were significantly protected with both the 100 g ha^{-1} and 10 g ha^{-1} AZA treatments: the kernel damage was reduced by 86% (2.03 untreated versus 0.28 treated damaged kernels cob^{-1}) and 91% (2.03 untreated versus 0.18 treated damaged kernels cob^{-1}).

The results from the 1988 season (Figure 11, Appendix 6) showed that in artificially infested plants, the 100 g ha^{-1} AZA formulation reduced feeding damage by 12% (23.7 untreated versus 20.9 treated damaged kernels cob^{-1}) and the number of larvae cob^{-1} by 16% (3.2 untreated versus 2.7 treated) while the 10 g ha^{-1} concentration decreased the number of damaged kernels and larvae cob^{-1} by 7% (23.7 untreated versus 22.0 treated) and 44% (3.2 untreated versus 1.8 treated), respectively. The larval populations from the cobs of the two AZA treatments differed significantly one from another.

In the naturally infested 1988 plots (Appendix 8), there was a reduction of 27%

feeding damage (2.6 untreated versus 1.9 treated damaged kernels cob^{-1}) although the number of larvae cob^{-1} was 20% higher (0.20 untreated versus 0.25 treated) in plots treated with 100 g ha^{-1} AZA. The 10 g ha^{-1} formulation showed a kernel damage reduction of 46% (2.6 untreated versus 1.4 treated) with a lower borer population of 35% (0.2 untreated versus 0.13 treated). However, no statistically significant differences were detected between treated and untreated plants of these plots.

In 1989 (Figures 13a, 13b, Appendix 9), the results revealed that in artificially infested plants, corn borer population cob^{-1} and damaged kernels cob^{-1} were significantly lowered by 39% (3.8 untreated versus 2.3 treated) and 18% (31.3 untreated versus 25.8 treated) with the 100 g ha^{-1} AZA formulation, respectively. Stalk breakage was reduced by 14% (5.8 untreated versus 5.0 treated). The 10 g ha^{-1} AZA was not effective at lowering the number of larvae cob^{-1} (0% reduction, 3.8 larvae cob^{-1}) nor the number of damaged kernels cob^{-1} (0.3% reduction- 31.3 untreated versus 31.2 treated). A 12% decrease (5.8 untreated versus 5.1 treated) in stalk breakage was seen in treated plants. The 100 g ha^{-1} formulation significantly reduced the larval population on the cobs compared to the 10 g ha^{-1} formulation while the feeding damage and stalk breakage were not significantly different between the two formulations.

1.2.3 Effect of NSKE on ECB Larval Populations, Feeding and Stalk Damage

The results from the three field seasons showed that NSKEs were generally effective in significantly reducing ECB larvae cob^{-1}, kernel damage cob^{-1} and stalk damage in artificially and naturally infested plants as compared to the controls. A closer examination of the results will reveal if ECB control can be achieved more effectively by increasing NSKE concentrations, varying the formulations, increasing the number of 1.56 kg ha^{-1} applications, applying the NSKE prior to infestations or spraying at dusk rather than in full day-light.

FIGURE 10a

Field study 1987 - Evaluation of damage by ECB larvae on neem-treated corn, artificially infested with ECB larvae.

Histogram to the right of the origin represents the mean number of damaged kernels cob^{-1} (n=40) and standard error (s.e.). Histogram to the left of the origin represents the mean number of ECB larvae found on the cobs (n=40) and s.e.
* Means are significantly different from their respective control at p=0.05 (Wilcoxon rank-sum test).
Neem-products were applied 2X during the season.

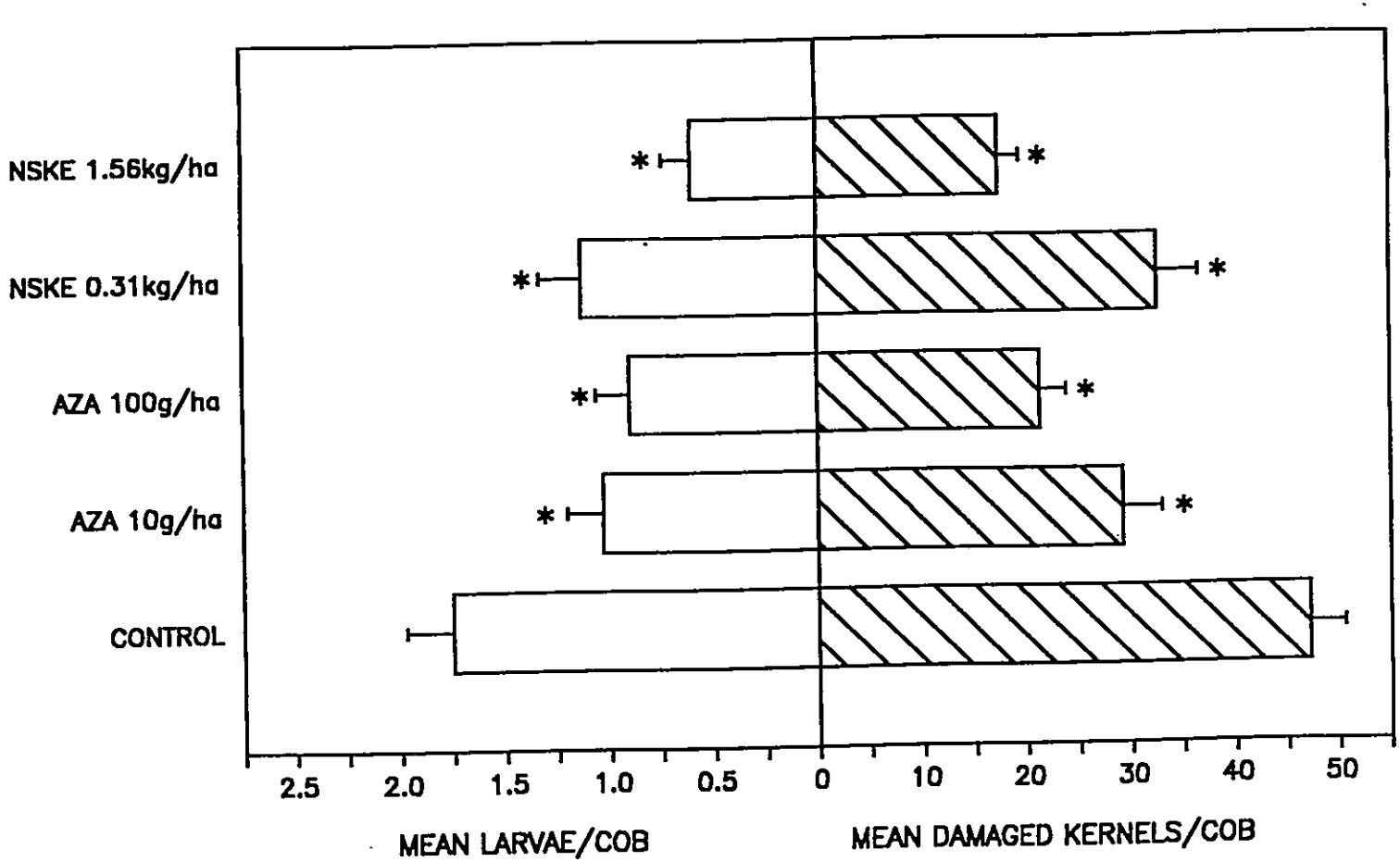

NSKE 1.56kg/ha
NSKE 0.31kg/ha
AZA 100g/ha
AZA 10g/ha
CONTROL
2.5
2.0
1.5
1.0
0.5
0
10
20
30
40
50
MEAN LARVAE/COB
MEAN DAMAGED KERNELS/COB

FIGURE 10b

Field study 1987 - Evaluation of damage by ECB larvae on neem-treated corn, artificially infested with ECB larvae.

Histogram to the right of the origin represents the mean tunnelling ratio (total length of stalk tunnels plant-height^{-1}) (n=40) and s.e. Histogram to the left of the origin represents the mean number of ECB larvae found on the cobs (n=40) and s.e.
* Means are significantly different from their respective control at p=0.05 (Wilcoxon rank-sum test).
Neem-products were applied 2X during the season.

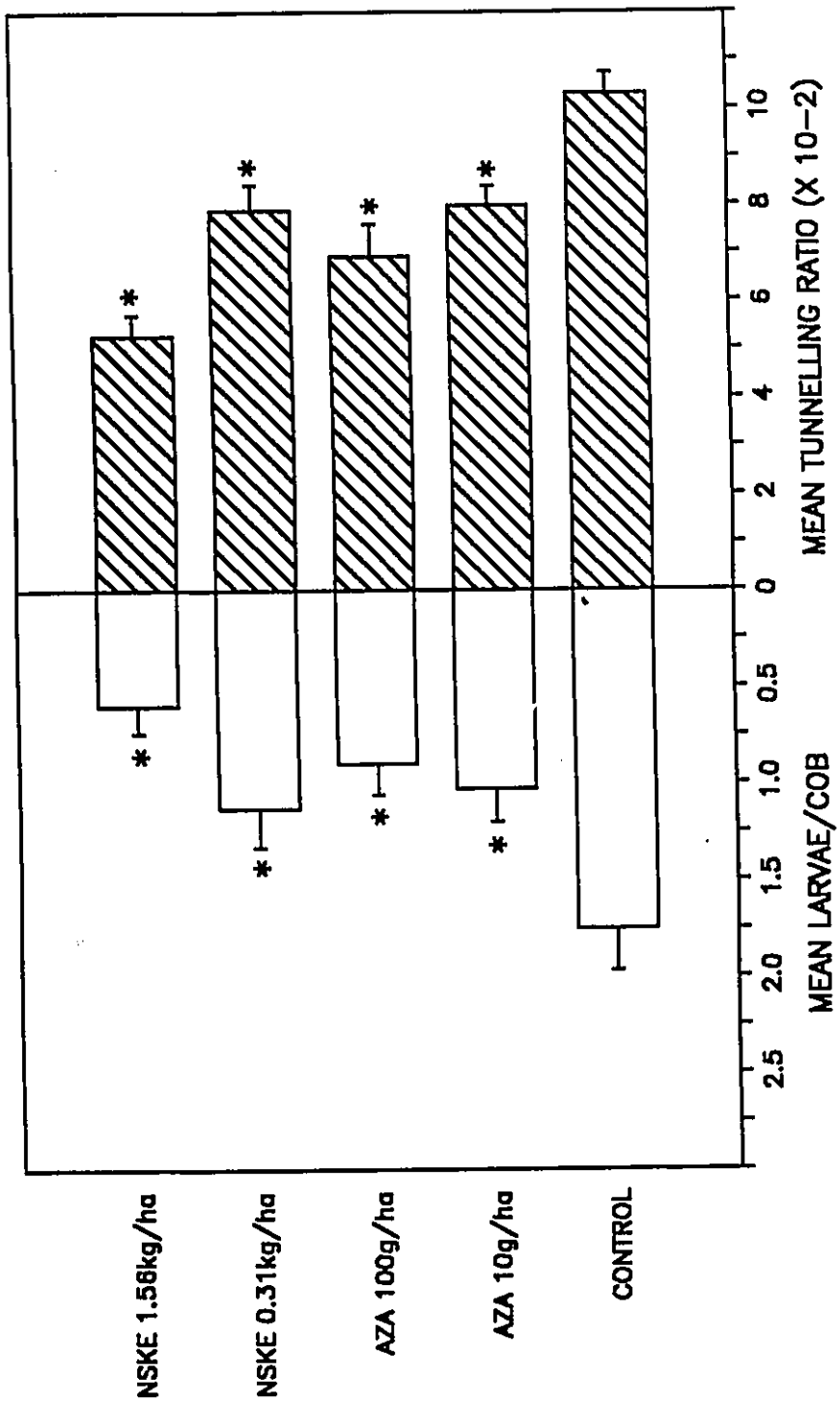

NSKE 1.58kg/ha
NSKE 0.31kg/ha
AZA 100g/ha
AZA 10g/ha
CONTROL
2.5
2.0
1.5
1.0
0.5
0
2
4
6
8
10
MEAN LARVAE/COB
MEAN TUNNELLING RATIO (X 10−2)

FIGURE 11

Field study 1988 - Field 1 - Evaluation of damage by ECB larvae on neem-treated corn, artificially infested with ECB larvae.

Histogram to the right of the origin represents the mean number of damaged kernels cob^{-1} (n=40) and s.e. Histogram to the left of the origin represents the mean number of ECB larvae found on the cobs (n=40) and s.e.

* Means are significantly different from their respective control at p=0.05 (Wilcoxon rank-sum test).

Neem-products were applied 2X during the season.

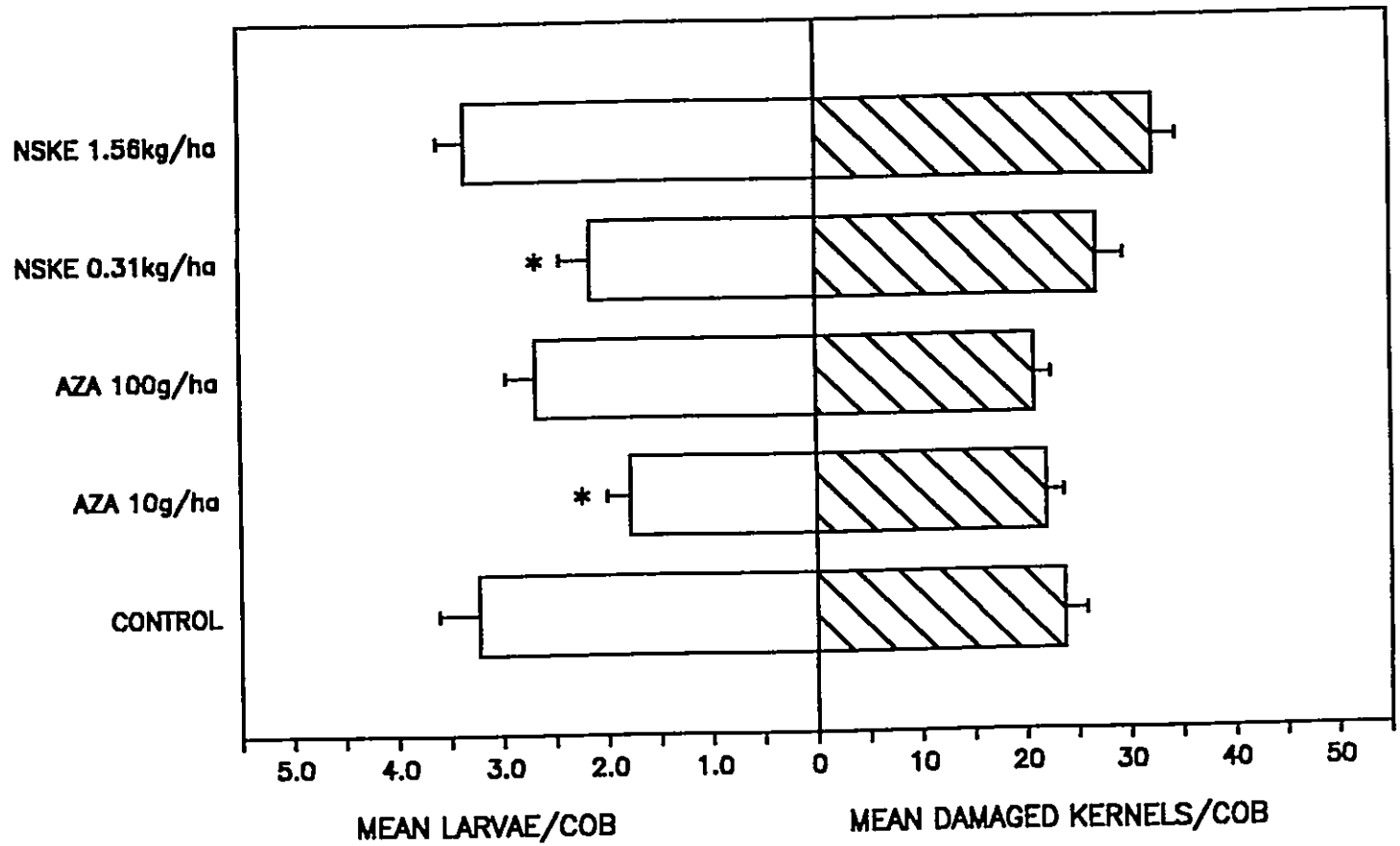

NSKE 1.56kg/ha
NSKE 0.31kg/ha
AZA 100g/ha
AZA 10g/ha
CONTROL
*
*
5.0
4.0
3.0
2.0
1.0
0
10
20
30
40
50
MEAN LARVAE/COB
MEAN DAMAGED KERNELS/COB

FIGURE 12

Field study 1988 - Field 2 - Evaluation of damage by ECB larvae on neem-treated corn, artificially infested with ECB larvae.

Histogram to the right of the origin represents the mean number of damaged kernels cob^{-1} (control n=45; EC 1.56kg ha^{-1} n=59; all others n=60) and s.e. Histogram to the left of the origin represents the mean number of ECB larvae found on the cobs (n=same as for damaged kernels) and s.e.

* Means are significantly different from their respective control at p=0.05 (Wilcoxon rank-sum test).

Neem-products were applied 2X during the season unless otherwise stated.

Rate of application of NSKE = 1.56kg ha^{-1} unless otherwise stated.

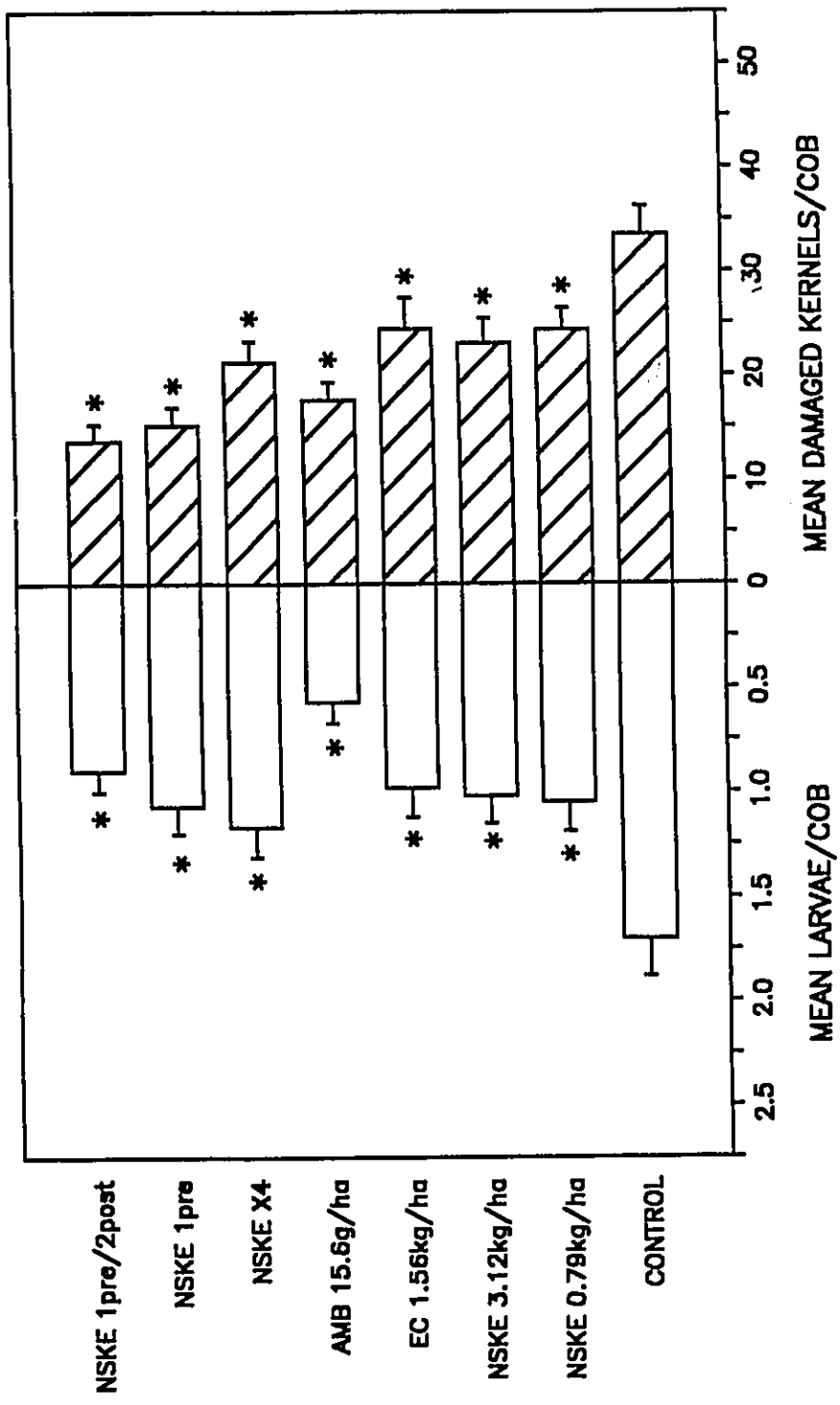

NSKE 1pre/2post
NSKE 1pre
NSKE X4
AMB 15.6g/ha
EC 1.56kg/ha
NSKE 3.12kg/ha
NSKE 0.79kg/ha
CONTROL
MEAN LARVAE/COB
2.5
2.0
1.5
1.0
0.5
0
10
20
30
40
50
MEAN DAMAGED KERNELS/COB

FIGURE 13a

Field study 1989 - Field 1 - Evaluation of damage by ECB larvae on neem-treated corn, artificially infested with ECB larvae.

Histogram to the right of the origin represents the mean number of damaged kernels cob^{-1} (n=80) and s.e. Histogram to the left of the origin represents the mean number of ECB larvae found on the cobs (n=80) and s.e.

* Means are significantly different from their respective control at p=0.05 (Wilcoxon rank-sum test).

SAF2 1.47kg ha^{-1} and SAF1 1.34kg ha^{-1} were applied 7X during the season; all other treatments were applied 2X.

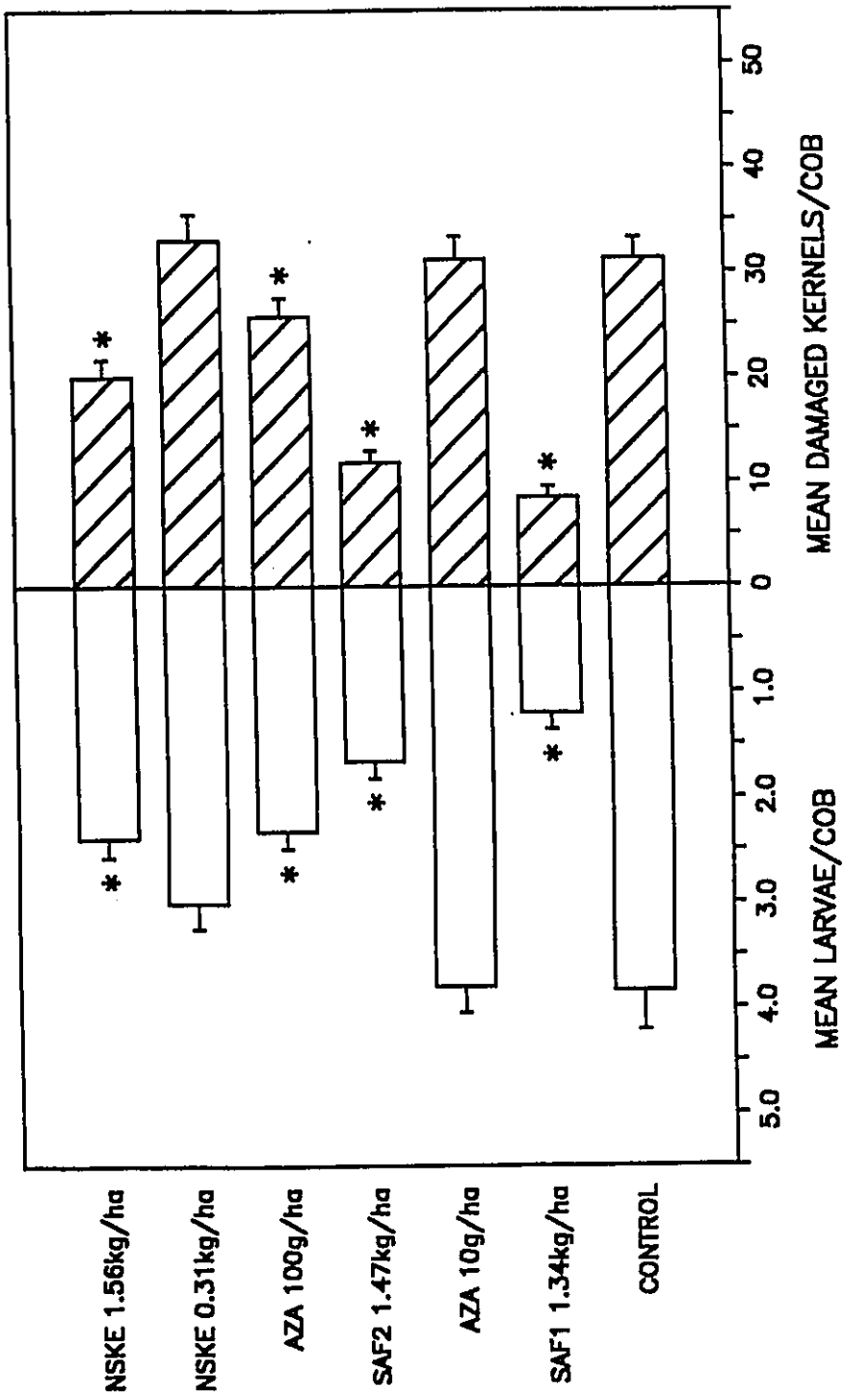

MEAN DAMAGED KERNELS/COB
MEAN LARVAE/COB
0
10
20
30
40
50
1.0
2.0
3.0
4.0
5.0
NSKE 1.56kg/ha
NSKE 0.31kg/ha
AZA 100g/ha
SAF2 1.47kg/ha
AZA 10g/ha
SAF1 1.34kg/ha
CONTROL

FIGURE 13b

Field study 1989 - Field 1 - Evaluation of damage by ECB larvae on neem-treated corn, artificially infested with ECB larvae.

Histogram to the right of the origin represents the mean stalk breakage rating of the corn plants (n=80) and s.e. Histogram to the left of the origin represents the mean number of ECB larvae found on the cobs (n=80) and s.e.

* Means are significantly different from their respective control at p=0.05 (Wilcoxon rank-sum test).

SAF2 1.47kg ha^{-1} and SAF1 1.34kg ha^{-1} were applied 7X during the season; all other treatments were applied 2X.

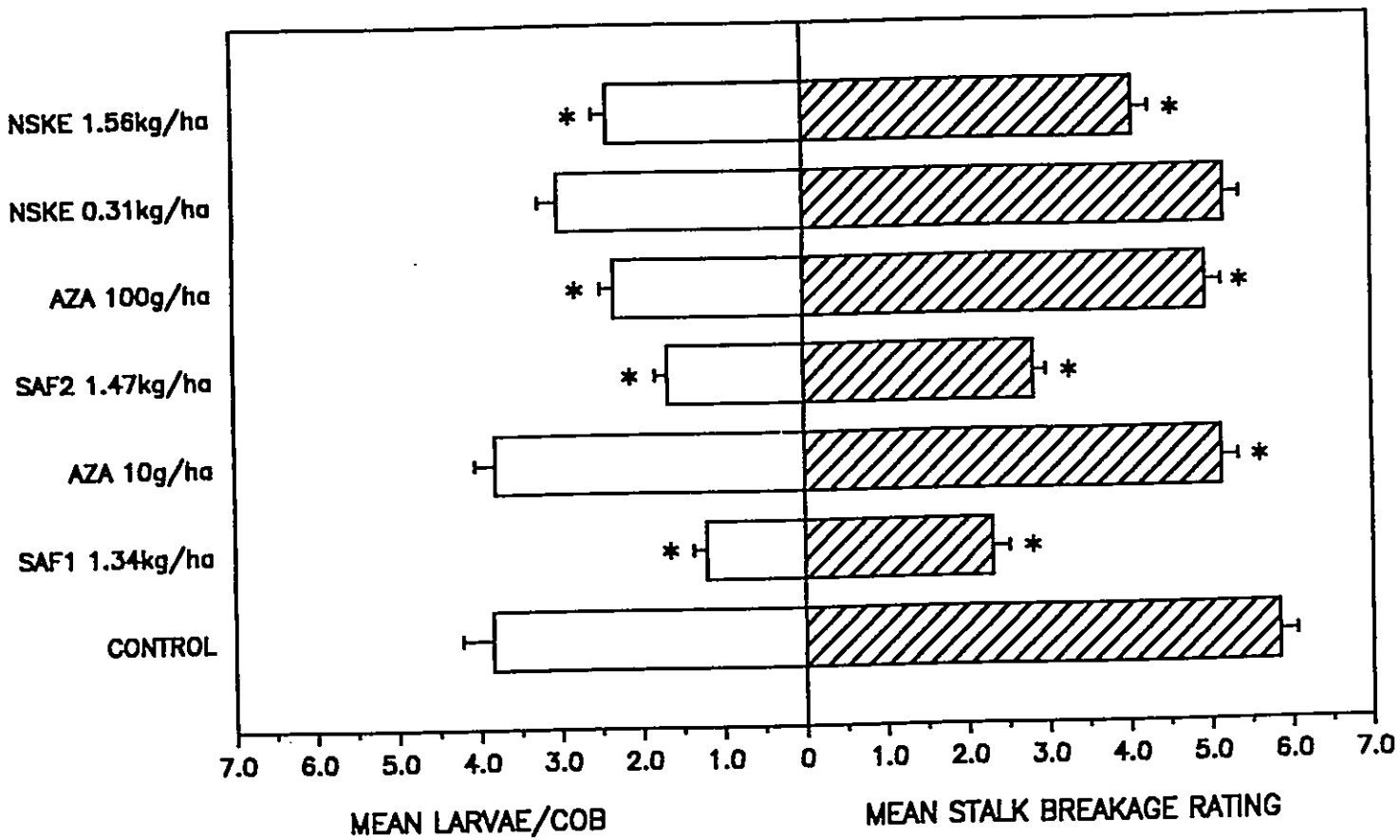

NSKE 1.56kg/ha
NSKE 0.31kg/ha
AZA 100g/ha
SAF2 1.47kg/ha
AZA 10g/ha
SAF1 1.34kg/ha
CONTROL
7.0 6.0 5.0 4.0 3.0 2.0 1.0 0 1.0 2.0 3.0 4.0 5.0 6.0 7.0
MEAN LARVAE/COB
MEAN STALK BREAKAGE RATING

FIGURE 14a

Field study 1989 - Field 2 - Evaluation of damage by ECB larvae on neem-treated corn, artificially infested with ECB larvae.

Histogram to the right of the origin represents the mean number of damaged kernels cob^{-1} (NSKE 1pre/3post n=71; NSKE 0.79kg ha^{-1} n=73; all others n=80) and s.e. Histogram to the left of the origin represents the mean number of ECB larvae found on the cobs (n=same as for damaged kernels) and s.e.

* Means are significantly different from their respective control at p=0.05 (Wilcoxon rank-sum test).

Neem-products were applied 2X during the season unless otherwise stated (table 5 in materials and methods).

Rate of application of NSKE = 1.56kg ha^{-1} unless otherwise stated.

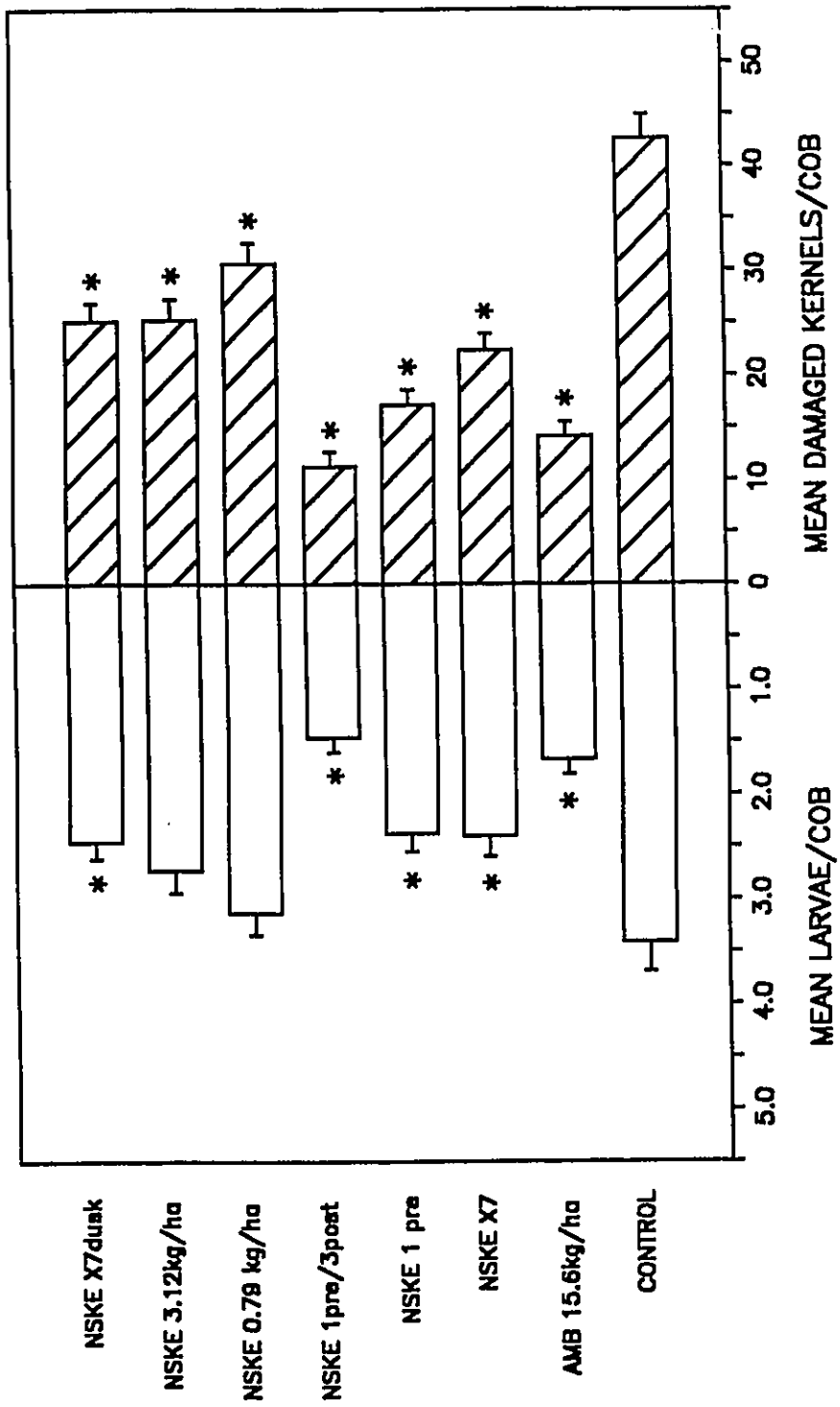
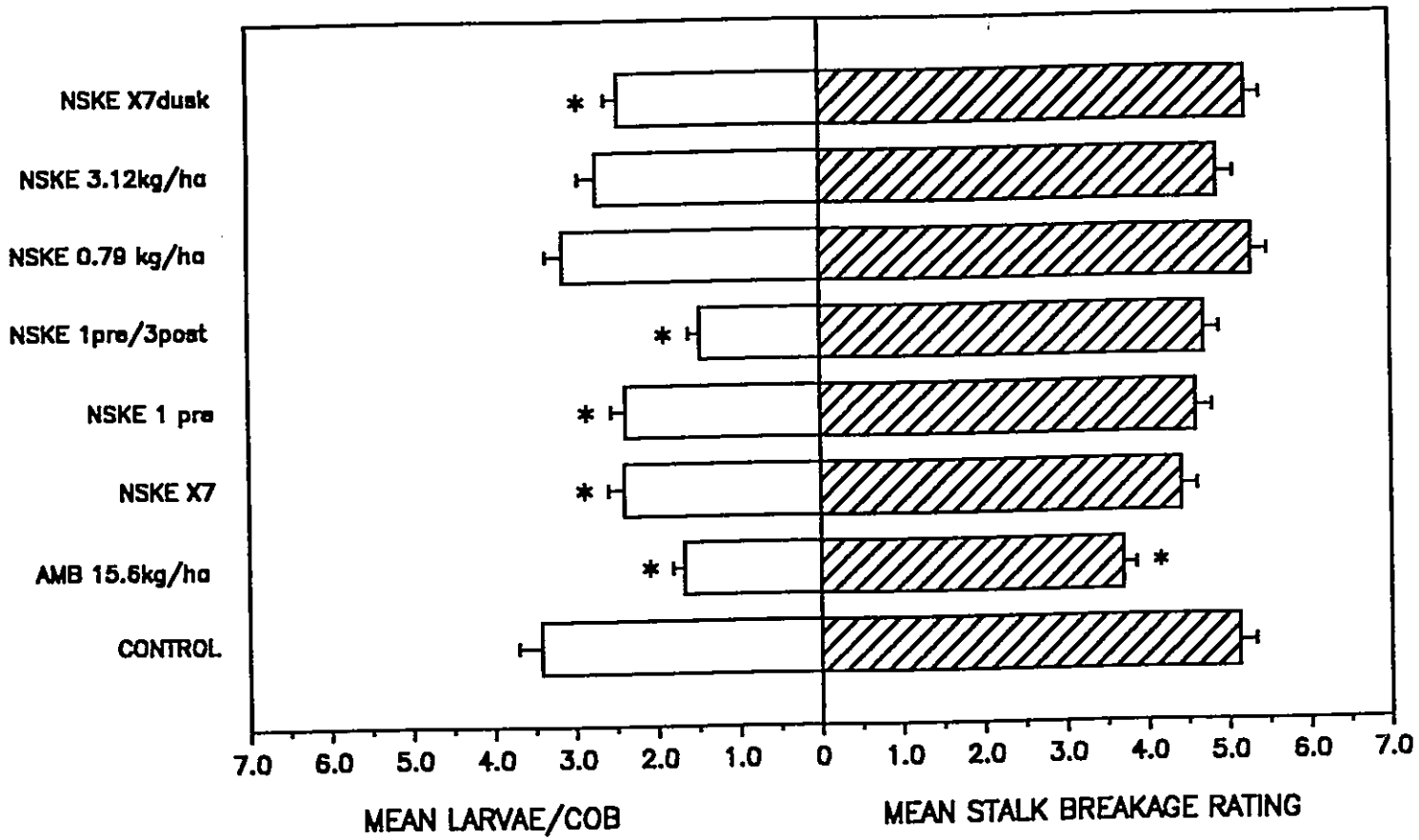

MEAN DAMAGED KERNELS/COB
MEAN LARVAE/COB
0
10
20
30
40
50
1.0
2.0
3.0
4.0
5.0
NSKE X7dusk
NSKE 3.12kg/ha
NSKE 0.78 kg/ha
NSKE 1pre/3post
NSKE 1 pre
NSKE X7
AMB 15.6kg/ha
CONTROL

FIGURE 14b

Field study 1989 - Field 2 - Evaluation of damage by ECB larvae on neem-treated corn, artificially infested with ECB larvae.

Histogram to the right of the origin represents the mean stalk breakage rating of the corn plants (n=80) (NSKE 1pre/3post n=71; NSKE 0.79kg ha^{-1} n=73; all others n=80) and s.e.
Histogram to the left of the origin represents the mean number of ECB larvae found on the cobs (n=same as for damaged kernels) and s.e.
* Means are significantly different from their respective control at p=0.05 (Wilcoxon rank-sum test).
Neem-products were applied 2X during the season unless otherwise stated (table 5 in materials and methods).
Rate of application of NSKE = 1.56kg ha^{-1} unless otherwise stated.


NSKE X7dusk
NSKE 3.12kg/ha
NSKE 0.79 kg/ha
NSKE 1pre/3post
NSKE 1 pre
NSKE X7
AMB 15.6kg/ha
CONTROL
7.0 6.0 5.0 4.0 3.0 2.0 1.0 0 1.0 2.0 3.0 4.0 5.0 6.0 7.0
MEAN LARVAE/COB
MEAN STALK BREAKAGE RATING

1.2.3.1 Application Rate

Several NSKE application rates were tested throughout the three field seasons; the formulations were applied twice at a 6 to 7 day interval. In the 1987 field season (Figures 10a, 10b and Appendix 4) the 1.56 kg ha^{-1} NSKE treatment reduced kernel damage cob^{-1} and the number of larvae cob^{-1} by 63% (47.2 untreated versus 17.4 treated) and 67% (1.8 untreated versus 0.6 treated), respectively, in artificially infested plants. Stalk tunnelling decreased by 49% (10.3 untreated versus 5.3 treated). The 0.31 kg ha^{-1} treatment was slightly less effective at reducing kernel damage cob^{-1} - 31% decrease (47.2 untreated versus 32.7 treated), number of larvae cob^{-1} - 39% (1.8 untreated versus 1.1 treated) and stalk tunnelling - 23% (10.3 untreated versus 7.9 treated). The 1.56 kg ha^{-1} treatment was significantly more effective at reducing feeding damage, borer population and stalk tunnelling than the 0.31 kg ha^{-1} treatment.

Naturally infested plots of the 1987 season were also protected with both NSKE treatments (Appendix 5): kernel damage decreased by 58% (2.03 untreated versus 0.85 treated) and 91% (2.03 untreated versus 0.18 treated) with 1.56 kg ha^{-1} and 0.31 kg ha^{-1}, respectively. The number of larvae cob^{-1} was unaltered in the 1.56 kg ha^{-1} treatment (0.03 larvae cob^{-1}) but reduced to 0 with 0.31 kg ha^{-1}. Stalk tunnelling was 55% higher (0.26 untreated versus 0.58 treated) in the 1.56 kg ha^{-1} treated plots but 54% lower (0.26 untreated versus 0.12 treated) in the 0.31 kg ha^{-1} treated plots. No statistically significant differences were found between the two treatments.

Both NSKE application rates were ineffective in reducing feeding damage by corn borer larvae in field 1 of the unusual 1988 season (Figure 11 and Appendix 6). Kernel damage in artificially infested plots treated with 1.56 kg ha^{-1} was 36% higher when compared to the control (23.7 untreated versus 32.3 treated) and the number of larvae cob^{-1} was 6% higher (3.2 untreated versus 3.4 treated). In plots sprayed with the lower NSKE application rate, the number of damaged kernels cob^{-1} was 14% higher than the

control (23.7 treated versus 26.9 untreated) while the larval population cob^{-1} was reduced by 31% (3.2 untreated versus 2.2 treated). Statistically, the kernel damage was the same for the two application rates while the borer population was significantly lower with the 0.31 kg ha^{-1} treatment.

In naturally infested plots (1988, Appendix 8) treated with the high NSKE application rate, the kernel damage was 85% higher (2.6 untreated versus 4.8 treated) while the number of larvae decreased by 50% (0.2 untreated versus 0.1 treated). In the plots treated with the lower NSKE application rate, kernel damage and larval population decreased by 50% (2.6 untreated versus 1.3 treated) and 60% (0.2 untreated versus 0.08 treated), respectively.

In field 2 (1988, Figure 12, Appendix 7), two additional NSKE application rates were tested on artificially infested plants: 3.12 kg ha^{-1} and 0.79 kg ha^{-1}, applied twice at one week intervals. Both application rates decreased feeding damage and larval numbers: number of damaged kernels cob^{-1} was 31% (33.5 untreated versus 23.1 treated) and 27% (33.5 untreated versus 24.3 treated) lower, and number of larvae cob^{-1} was 41% (1.7 untreated versus 1.0 treated) and 35% (1.7 untreated versus 1.1 treated) lower in the plots treated with 3.12 kg ha^{-1} and 0.79 kg ha^{-1}, respectively. Feeding damage and larval populations were not statistically different between the two application rates.

In field 1 of the 1989 season, 1.56 kg ha^{-1} and 0.31 kg ha^{-1} were tested on artificially and naturally infested plants. In artificially infested plots (Figure 13a, 13b, Appendix 9) kernel damage cob^{-1}, number of larvae cob^{-1} and stalk breakage were reduced in the 1.56 kg ha^{-1} sprayed plots by 36% (31.3 untreated versus 20.0 treated), 37% (3.8 untreated versus 2.4 treated) and 20% (5.8 untreated versus 4.1 treated), respectively. The 0.31 kg ha^{-1} treatment did not decrease feeding damage (6% increase-31.3 damaged kernels cob^{-1} untreated versus 33.1 treated), but the number of larvae cob^{-1} and stalk breakage were 21% (3.8 untreated versus 3.0 treated) and 10% (5.8 untreated

versus 5.2 treated) lower, respectively. The kernel damage and stalk breakage were significantly lower with 1.56 kg ha^{-1} versus 0.31 kg ha^{-1}.

In naturally infested plots (1989, Appendix 10), the 1.56 kg ha^{-1} treatment decreased kernel damage by 50% (0.4 untreated versus 0.2 treated) and stalk breakage by 33% (0.6 untreated versus 0.4 treated). In the 0.31 kg ha^{-1} treated plots, kernel damage was 75% higher (0.4 untreated versus 0.7 treated) and stalk breakage was 17% lower (0.6 untreated versus 0.5 treated). The number of larvae cob^{-1} remained unchanged (0.04) in plots treated with 1.56 kg ha^{-1} and was 25% higher in the ones treated with 0.31 kg ha^{-1} NSKE (0.04 untreated versus 0.05 treated).

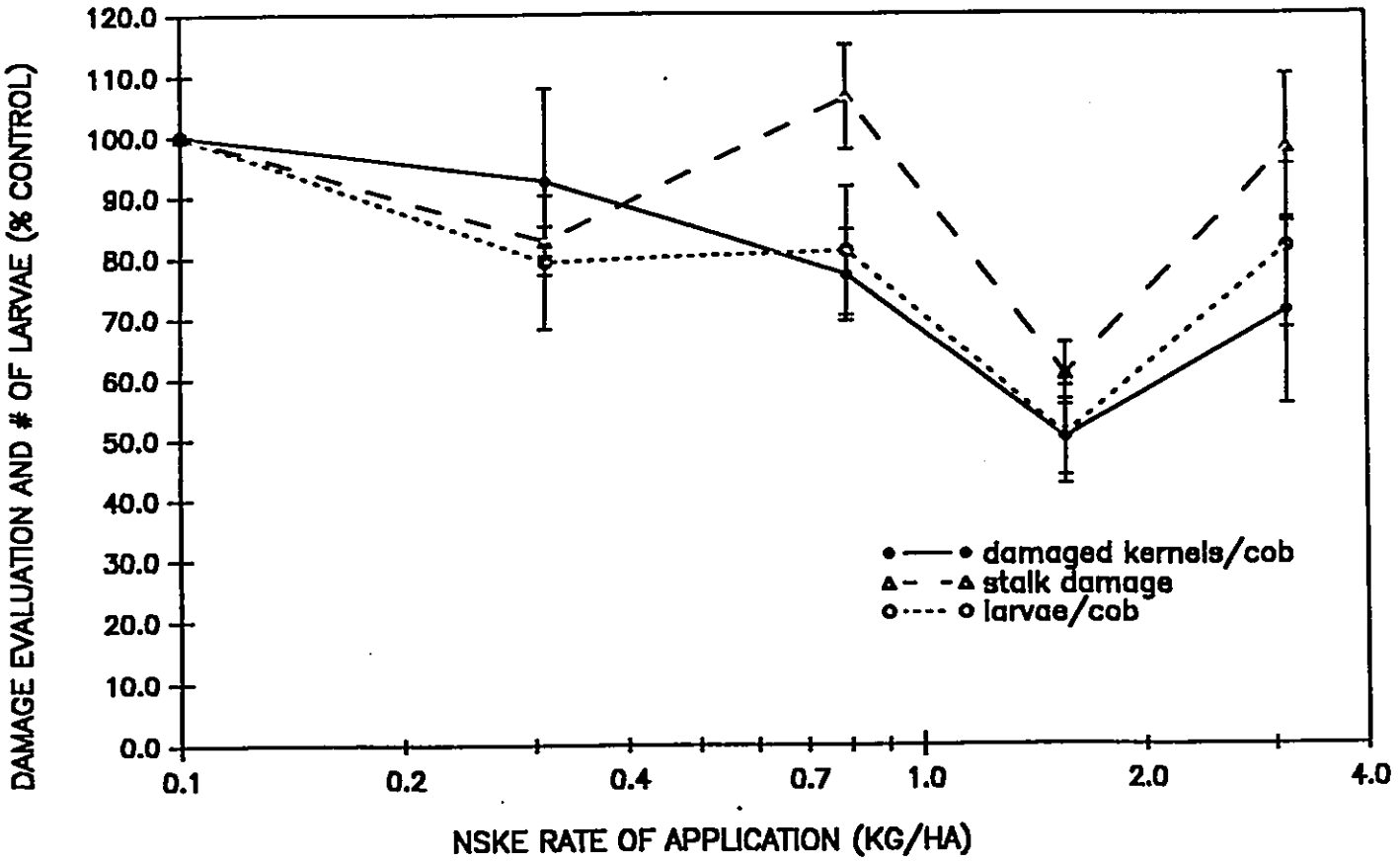
Both the 3.12 kg ha^{-1} and 0.79 kg ha^{-1} sprayings reduced feeding damage and cob-larval populations in field 2 (1989, Figures 14a, 14b and Appendix 10). The number of damaged kernels cob^{-1} was 41% (42.6 untreated versus 25.1 treated) and 28% (42.6 untreated versus 30.6 treated) lower in plots treated with 3.12 kg ha^{-1} and 0.79 kg ha^{-1}, respectively. The 3.12 kg ha^{-1} sprayings reduced the number of larvae cob^{-1} by 21% (3.4 untreated versus 2.7 treated) and the 0.79 kg ha^{-1}, by 9% (3.4 untreated versus 3.1 treated). Stalk breakage was 4% lower (5.1 untreated versus 4.9 treated) in plots sprayed with 3.12 kg ha^{-1} while those sprayed with 0.79 kg ha^{-1} showed an increase of 4% (5.1 untreated versus 5.3 treated).

Figure 15 shows that the most effective NSKE application rate for decreasing kernel damage cob^{-1}, number of larvae cob^{-1} and stalk damage (stalk tunnelling and breakage combined) is 1.56 kg ha^{-1}.

FIGURE 15

Effect of the NSKE application rate on number of ECB larvae and damage (damaged kernels and stalk damage (tunnelling ratio or stalk breakage ratio)) to sweet corn by ECB larvae.

Means of the damaged kernels cob^{-1}, larvae cob^{-1} and stalk damage for different NSKE application rates were compared to their respective controls and are represented as a % of their controls. A mean and the s.e. of these ratios over the 3 field seasons were obtained.


DAMAGE EVALUATION AND # OF LARVAE (% CONTROL)
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
0.1
0.2
0.4
0.7
1.0
2.0
4.0
NSKE RATE OF APPLICATION (KG/HA)
damaged kernels/cob
stalk damage
larvae/cob

1.2.3.2 Formulation

An emulsifiable concentrate (EC) of a NSKE prepared by Safer Corp. was tested in 1988 on artificially infested plants (Figure 12 and Appendix 7). The EC at 1.56 kg ha^{-1} (two applications at 6 day interval) effectively reduced feeding damage cob^{-1} and number of larvae cob^{-1} by 27% (33.5 untreated versus 24.5 treated) and 41% (1.7 untreated versus 1.0 treated), respectively. The efficacy of the EC was comparable to the 3.12 kg ha^{-1} and 0.79 kg ha^{-1} NSKE treatments which decreased feeding damage cob^{-1} by 31% and 27%, and number of larvae cob^{-1} by 41% and 35%, respectively.

Two products from Safer Corp. were tested on artificially infested plants in 1989 (Figure 13a, 13b and Appendix 9) at the highest number of applications i.e. 7 sprayings at 3 to 4 day interval. Both Saf1–1.34 kg ha^{-1} and Saf2- 1.47 kg ha^{-1} were the most effective neem products, reducing the number of damaged kernels cob^{-1} by 73% (31.3 untreated versus 8.5 treated) and 62% (31.3 untreated versus 11.8 treated), the number of larvae cob^{-1} by 68% (3.8 untreated versus 1.2 treated) and 55% (3.8 untreated versus 1.7 treated), stalk breakage by 60% (5.8 untreated versus 2.3 treated) and 52% (5.8 untreated versus 2.8 treated), respectively.

1.2.3.3 Number of Applications

Various numbers of NSKE applications at 1.56 kg ha^{-1} were tested over the three field seasons (Figures 10a, 10b, 12, 13a, 13b, 14a, 14b and Appendices 4, 7, 9, 10). The most protection against corn borer damage was achieved in 1987 with only 2 applications at a 1 week interval (Figures 10a, 10b and Appendix 4). This treatment decreased the number of damaged kernels cob^{-1} by 63% (47.2 untreated versus 17.4 treated), the number of larvae cob^{-1} by 67% (1.8 untreated versus 0.6 treated) and stalk tunnelling by 49% (10.3 untreated versus 5.3 treated). As previously mentioned, the two applications in 1988 did not decrease feeding damage or borer populations.

In 1989 (Figure 13a, 13b and Appendix 9), two sprayings offered significant protection with a decrease of 36% in kernel damage (31.3 untreated versus 20.0 treated), 29% in number of larvae cob^{-1} (3.8 untreated versus 2.4 treated), 29% in stalk breakage (5.8 untreated versus 4.1 treated).

In 1988 (Figure 12 and Appendix 7), 4 sprayings resulted in a similar protection as the 2 sprayings of 1989, with a 36% decrease in kernel damage (33.5 untreated versus 21.3 treated), and a 29% decrease in larvae cob^{-1} (1.7 untreated versus 1.2 treated).

By increasing the number of applications to 7 (1989, Figures 14a, 14b and Appendix 10), feeding damage was decreased by approximately 10% when compared to 2 applications, the number of larvae cob^{-1} was 8% higher and stalk damage was 15% greater. The number of damaged kernels cob^{-1} decreased by 47% (42.6 untreated versus 22.4 treated), the number of larvae cob^{-1} by 29% (3.4 untreated versus 2.4 treated) and stalk breakage by only 14% (5.1 untreated versus 4.4 treated).

Figure 16 shows that increasing from 2 to 4 or 7 the number of 1.56 kg ha^{-1} NSKE applications did not significantly reduce feeding damage or cob-borer populations but seems to increase stalk damage.

1.2.3.4 Time of Application

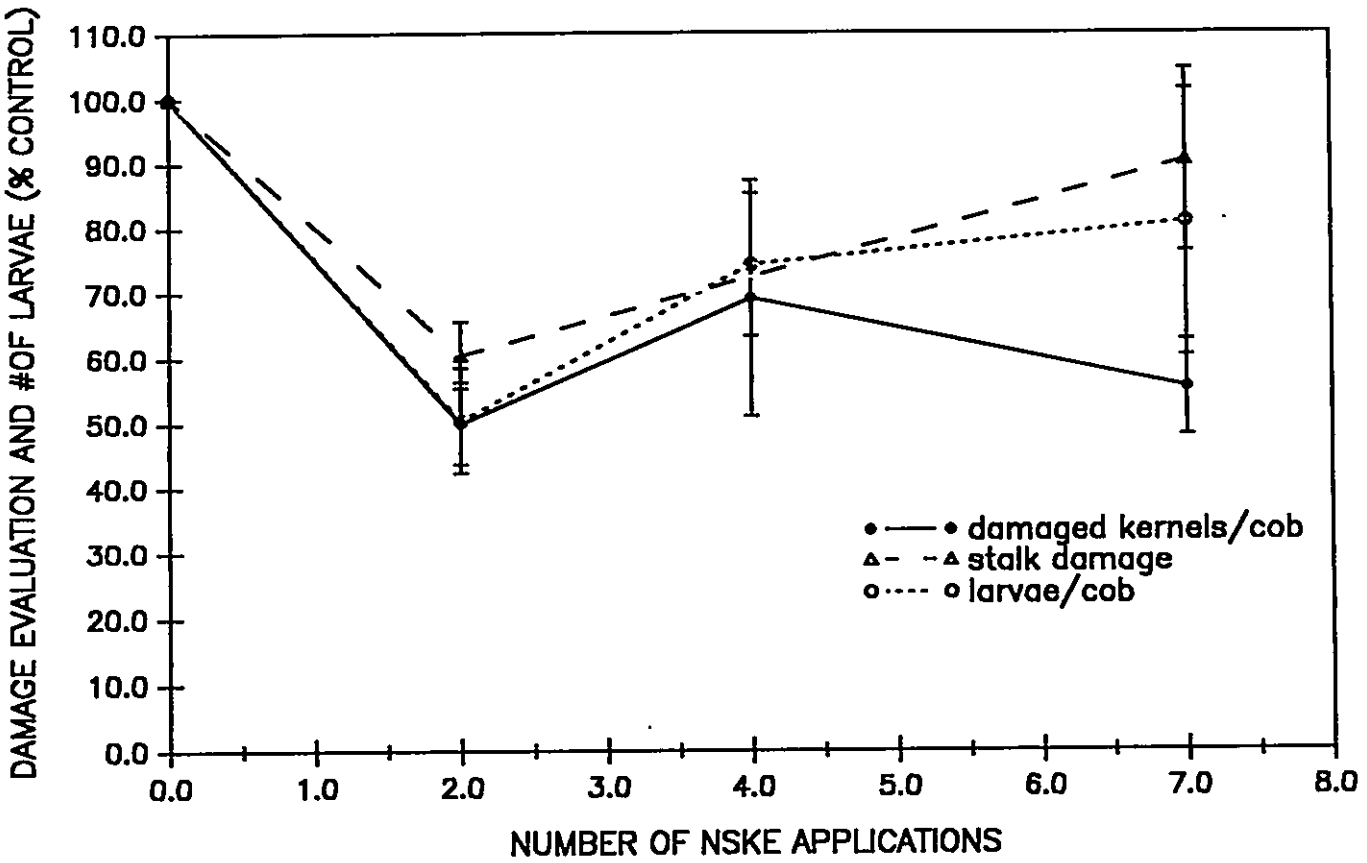
1.2.3.4.1 Pre-Infestation Sprayings

In 1988 and 1989, pre-infestation sprayings with NSKE followed by 2 or 3 post-infestation sprayings (1.56 kg NSKE ha^{-1} at 6 to 7 day interval) were tested and were found to be as effective as Safer's neem products in reducing the number of damaged kernels cob^{-1} (Figures 12, 14a, 14b and Appendices 7, 10).

FIGURE 16

Effect of number of NSKE applications on number of ECB larvae and damage (damaged kernels and stalk damage (tunnelling ratio or stalk breakage ratio)) to sweet corn by ECB larvae.

Means of the damaged kernels cob^{-1}, larvae cob^{-1} and stalk damage for different number of NSKE applications were compared to their respective control and are represented as a % of their controls. A mean and the s.e. of these ratios over the 3 field seasons were obtained.


DAMAGE EVALUATION AND #OF LARVAE (% CONTROL)
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
NUMBER OF NSKE APPLICATIONS
damaged kernels/cob
stalk damage
larvae/cob

In 1988 and 1989, one pre-infestation treatment i.e. one treatment preceding each of the two infestations, reduced feeding damage by 55% (33.5 untreated versus 15.2 treated) and 60% (42.6 untreated versus 17.1 treated), respectively (Figures 12, 14a, Appendices 7, 10). The number of larvae cob^{-1} decreased by 35% (1.7 untreated versus 1.1 treated) in 1988 and 29% (3.4 untreated versus 2.4 treated) in 1989. Stalk breakage was evaluated in 1989 only and was reduced by 10% (5.1 untreated versus 4.6 treated).

One pre-infestation spraying followed by 2 or 3 sprayings after the artificial infestation were effective at reducing feeding damage by 59% (33.5 untreated versus 13.7 treated) and 73% (42.6 treated versus 11.3 treated), the number of larvae cob^{-1} by 47% (1.7 untreated versus 0.9 treated) and 56% (3.4 untreated versus 1.5 treated) in 1988 and 1989, respectively (Figures 12, 14a, Appendices 7, 10). In 1989, stalk breakage was reduced by 8% (5.1 untreated versus 4.7 treated) (Figure 14b, Appendix 10). In both years, feeding damage was not significantly different between the pre-infestation and pre + post-infestation treatments but in 1989 the pre + post-infestation sprayings significantly reduced the number of larvae cob^{-1} when compared to the pre-infestation treatment alone.

1.2.3.4.2 Dusk versus Full Daylight Sprayings

Full daylight spraying was compared to dusk spraying in 7 applications of 1.56 kg ha^{-1} NSKE at 3 to 4 day intervals (Figures 14a, 14b and Appendix 10). Both treatments equally and significantly reduced kernel damage cob^{-1} and number of larvae cob^{-1}: 47% (42.6 untreated versus 22.4 treated) and 29% (3.4 untreated versus 2.4 treated) in full daylight sprayings, 41% (42.6 untreated versus 25.1 treated) and 26% (3.4 untreated versus 2.5 treated) in dusk sprayings. Stalk breakage was reduced by 14% (5.1 untreated versus 4.4 treated) in full daylight sprayings but was 2% higher than the control (5.1 untreated versus 5.2 treated) in dusk sprayings.

1.2.4 Ambush Compared to Azadirachtin and NSKE

To compare the efficacy of AZA and NSKEs to a commonly used commercial pesticide, Ambush, at the recommended rate of application, was tested on artificially and naturally infested plants. In 1988 and 1989 (Figures 12, 14a, 14b and Appendices 7, 10), Ambush significantly reduced the number of damaged kernels cob^{-1} in artificially infested plants by 48% (33.5 untreated versus 17.6 treated) and 67% (42.6 untreated versus 14.2 treated), the number of larvae cob^{-1} by 65% (1.7 untreated versus 0.6 treated), and 50% (3.4 untreated versus 1.7 treated), respectively. Stalk breakage decreased from the control by 28% (5.1 untreated versus 3.7 treated) in 1989. Feeding damage was also reduced in naturally infested plants of 1988 and 1989 (Appendices 8, 11) by 36% (1.1 damaged kernels cob^{-1} untreated versus 0.7 treated) and 33% (0.3 untreated versus 0.2 treated), respectively. The number of larvae cob^{-1} decreased to 0 in 1988 and by 50% (0.06 untreated versus 0.03 treated) in 1989, and stalk breakage by 60% (0.5 untreated versus 0.2 treated) in 1989.

The efficacy of AZA at 100 g ha^{-1} (two applications) and of NSKE at 1.56 kg ha^{-1} (two applications) in 1987 was comparable to that of Ambush at 15.6 g ha^{-1} (two applications) in 1988 and 1989. The feeding damage was 55% and 63% lower than the control with AZA and NSKE in 1987, and 48% and 67% lower with Ambush in 1988 and 1989. Larval populations and stalk damage were also comparable (Figures 10a, 10b, 12, 13a, 13b, 14a, 14b and Appendices 4, 7, 9, 10).

The results of field 2 in 1988 (Figure 12 and Appendix 7) indicate that Ambush at 15.6 g ha^{-1} and NSKE at 1.56 kg ha^{-1} pre-infestation treatments control equally and effectively the ECB. In both treatments, 1 pre + 2 post- and 1 pre-infestation NSKE 1.56 kg ha^{-1} sprayings, there were fewer damaged kernels cob^{-1} (13.7 and 15.2 respectively) than in the Ambush treatment (17.6); the kernel damage in the two NSKE treatments were not statistically different from the Ambush treatment. Compared to the control, feeding

damage in Ambush treated plants was reduced by 47%, the 1 pre + 2 post- infestation treatment decreased feeding damage by 59% and the 1 pre-infestation treatment by 55%. In 1989 (Figures 14a, 14b and Appendix 10), feeding damage in pre-infestation treated corn was not statistically different from the Ambush treated corn. The cobs treated with 1 pre + 3 post infestation sprayings were less damaged (11.3 damaged kernels cob^{-1}, representing a 73% damage reduction compared to the control) than those treated with Ambush (14.2, 67% reduction) while the ones treated with 1 pre-infestation spraying were slightly more damaged (17.1, 60% reduction).

In the 1988 Ambush treated corn, the number of larvae cob^{-1} was significantly lower (0.6 larvae cob^{-1}) than the corn treated with 1 pre + 2 post- (0.9 larvae cob^{-1}) and 1 pre- (1.1 larvae cob^{-1}) infestation sprayings of NSKE (1.56 kg ha^{-1}). The number of larvae cob^{-1} compared to the control was reduced by 64% in Ambush treated cobs, 47% and 35% in 1 pre + 2 post- and 1 pre-infestation treated cobs, respectively. In 1989, compared to Ambush, the number of larvae cob^{-1} was not statistically different in 1 pre + 3 post-infestation sprayings (1.7 versus 1.5) but was significantly higher in the 1 pre-infestation sprayings (2.4). Stalk breakage was evaluated in 1989. The damage was greatly reduced and significantly different in Ambush treated plants (rating of 3.7, 28% reduction compared to the control) compared to that of the 1 pre + 3 post- infestation sprayings of NSKE (1.56 kg ha^{-1}) (4.7, 8% reduction) and 1 pre-infestation spraying (4.6, 10% reduction).

Safer's products (Saf1 1.34 kg ha^{-1} and Saf2 1.47 kg ha^{-1}, two applications) offered better protection against the ECB than Ambush in the 1989 season (Figures 13a, 13b, 14a, 14b and Appendices 9, 10). Comparing the feeding damage cob^{-1}, larval populations cob^{-1} and stalk breakage in Safer's products and Ambush to the control reveals a 67% decrease in the number of damaged kernels cob^{-1} with Ambush versus 73% and 62% decrease with Saf1 and Saf2, a 50% decrease in the number of larvae cob^{-1} with Ambush

versus 68% and 55% with Saf1 and Saf2, a 27% decrease in stalk breakage ratings with Ambush versus 60% and 50% with Saf1 and Saf2.

1.2.5 NSKE Compared to Azadirachtin

An overview of the 1987, 1988 and 1989 results indicates that the NSKE at the application rates tested is generally more effective at controlling the ECB than AZA at 10 g ha^{-1} and 100 g ha^{-1} in artificially infested plants. Although the highest AZA application rate at 100 g ha^{-1} contained 800 mg L^{-1} of the active ingredient (ai) AZA, certain NSKE treatments at 1.56 kg ha^{-1} with 31.3 mg ai L^{-1} decreased cob-feeding damage, cob-larval populations and stalk damage equally or more effectively. For example, in 1987 (Figures 10a, 10b and Appendix 4), the number of damaged kernels cob^{-1}, number of larvae cob^{-1} and stalk tunnelling were not statistically different when comparing the two applications of the NSKE 1.56 kg ha^{-1} treatment (17.4 damaged kernels cob^{-1}, 0.6 larvae cob^{-1}, 0.053 stalk tunnelling) to the two applications of the AZA 100 g ha^{-1} treatment (21.3 damaged kernels cob^{-1}, 0.9 larvae cob^{-1}, 0.069 stalk tunnelling). In 1989 (Figures 13a, 13b and Appendix 9), the number of damaged kernels cob^{-1} and stalk breakage in the same two treatments were significantly different, the NSKE decreasing the two damage parameters more efficiently than AZA: 25.8 versus 20.0 damaged kernels cob^{-1} and 5.0 versus 4.1 stalk breakage rating for the AZA at 100 g ha^{-1} and the NSKE at 1.56 kg ha^{-1}, respectively. The cob-larval population was not significantly different between the AZA and NSKE treatments, the former having 2.3 larvae cob^{-1} and the latter, 2.4 larvae cob^{-1}. The 1988 field season (Figure 11 and Appendix 6) showed opposite results where the AZA at 100 g ha^{-1} was significantly more effective at reducing kernel damage cob^{-1} and cob-larval populations (20.9 damaged kernels cob^{-1}, 2.7 larvae cob^{-1}) compared to the NSKE at 1.56 kg ha^{-1} (32.3 damaged kernels cob^{-1}, 3.4 larvae cob^{-1}).

Although not as effective as the NSKE at 1.56 kg ha^{-1}, the NSKE at 3.12 kg ha^{-1} with 62.5 mg AZA L^{-1} and 0.79 kg ha^{-1} with 15.6 mg AZA L^{-1} had higher efficacy than AZA at 100 g ha^{-1} with 800 mg AZA L^{-1}. In 1988 (Figures 11, 12 and Appendices 6,7) the NSKE at 3.12 kg ha^{-1} and 0.79 kg ha^{-1} reduced kernel damage compared to the control by 31% and 27%, number of larvae cob^{-1} by 41% and 35%, respectively, while AZA at 100 g ha^{-1} decreased kernel damage cob^{-1} and cob-larval populations by only 12% and 16%, respectively. The 1989 season results revealed the same pattern of cob-feeding damage (Figures 13a, 13b, 14a, 14b and Appendices 9, 10): compared to the control, there was a 41% and 28% decrease in kernel damage cob^{-1} with the NSKE at 3.12 kg ha^{-1} and 0.79 kg ha^{-1}, 18% with AZA at 100 g ha^{-1}. However, the number of larvae cob^{-1} and stalk breakage rating were decreased by 39% and 14% respectively with the AZA treatment, 21% and 4% with NSKE at 3.12 kg ha^{-1} and 9% (larvae cob^{-1}) with NSKE at 0.79 kg ha^{-1} but stalk breakage was 4% higher than the control with the latter treatment.

A remarkable difference in efficacy was noted between the pre-infestation NSKE treatments (31.3 mg ai L^{-1}) and AZA (800 mg ai L^{-1}). The 1988 results (Figures 11, 12, Appendices 6, 7) show that AZA reduced kernel damage and number of larvae cob^{-1} by 12% and 16% while the NSKE treatment applied prior to infestation decreased kernel damage by 55% and the number of larvae cob^{-1} by 64%. Similarly, in 1989 (Figures 13a, 13b, 14a, 14b, Appendices 9, 10) kernel damage was reduced by 60% with pre-infestation NSKE treatments compared to 18% with the AZA treatments. However, the number of larvae cob^{-1} was reduced by 39% with AZA and 29% with the NSKE pre-infestation treatment; stalk breakage was slightly more reduced with AZA (14%) compared to NSKE pre-infestation treatments (10%).

Briefly looking at the naturally infested plots tested with AZA at 100 g ha^{-1} and NSKE at 1.56 kg ha^{-1} in 1987 and 1988 (Appendices 5, 8), AZA was more efficient at

controlling corn borer damage: in 1987, AZA decreased the number of damaged kernels cob^{-1} from 2.03 (untreated control) to 0.28 (86% reduction) and the cob- larval population to 0 compared to a 58% reduction with NSKE (0.85 damaged kernels cob^{-1} treated) with no cob-larval population decrease compared to the control. Stalk tunnelling was greater in both treatments compared to the control (0.0057-AZA, 0.0058-NSKE, 0.0026-untreated). In 1988, (Appendix 8) the number of damaged kernels cob^{-1} was reduced by 27% (2.6 untreated versus 1.9 treated) with AZA but was 46% higher (4.8 treated) with NSKE. Cob-larval populations were higher in AZA treated corn (0.20 larvae cob^{-1} untreated versus 0.25 treated) but lower in NSKE treated corn (0.10 treated).

1.2.6 Overall Effect of Neem Products on Larval Populations versus Kernel and Stalk damage.

The overall effects of all neem products tested over the three field seasons on the relation between cob-larval populations and kernel and stalk damage were examined. A calibration curve was generated using the calculated means of the number of damaged kernels cob^{-1} and stalk damage (tunnelling or rating) from non-treated control plots in naturally and artificially infested trials, plotted as a function of their mean cob-larval populations (Figures 17a–21b). The mean number of damaged kernels cob^{-1} for all treated plots were then plotted as a function of their mean number of larvae cob^{-1}. By expressing as a % of all treated plots the number of treated plots falling above or below the calibration curve, the kernel or stalk damage parameters were shown to be either enhanced by the neem treatments (a high % of plots above the calibration curve) or diminished (a low % of plots below the calibration curve) for a given cob-larval population.

FIGURE 17a

Field 1987 - Relation of mean number damaged kernels cob^{-1} to mean number larvae cob^{-1} in naturally and artificially infested plants treated with NSKE and azadirachtin.

Calculated regression line is shown for calibration data of non-treated control plants ($y = 7.26 + 1.20x$; $r=0.83$)
Each treatment is represented by 4 means (4 replicates)
Filled symbols: data obtained from artificially infested plants;
Blank symbols: data obtained from naturally infested plants.

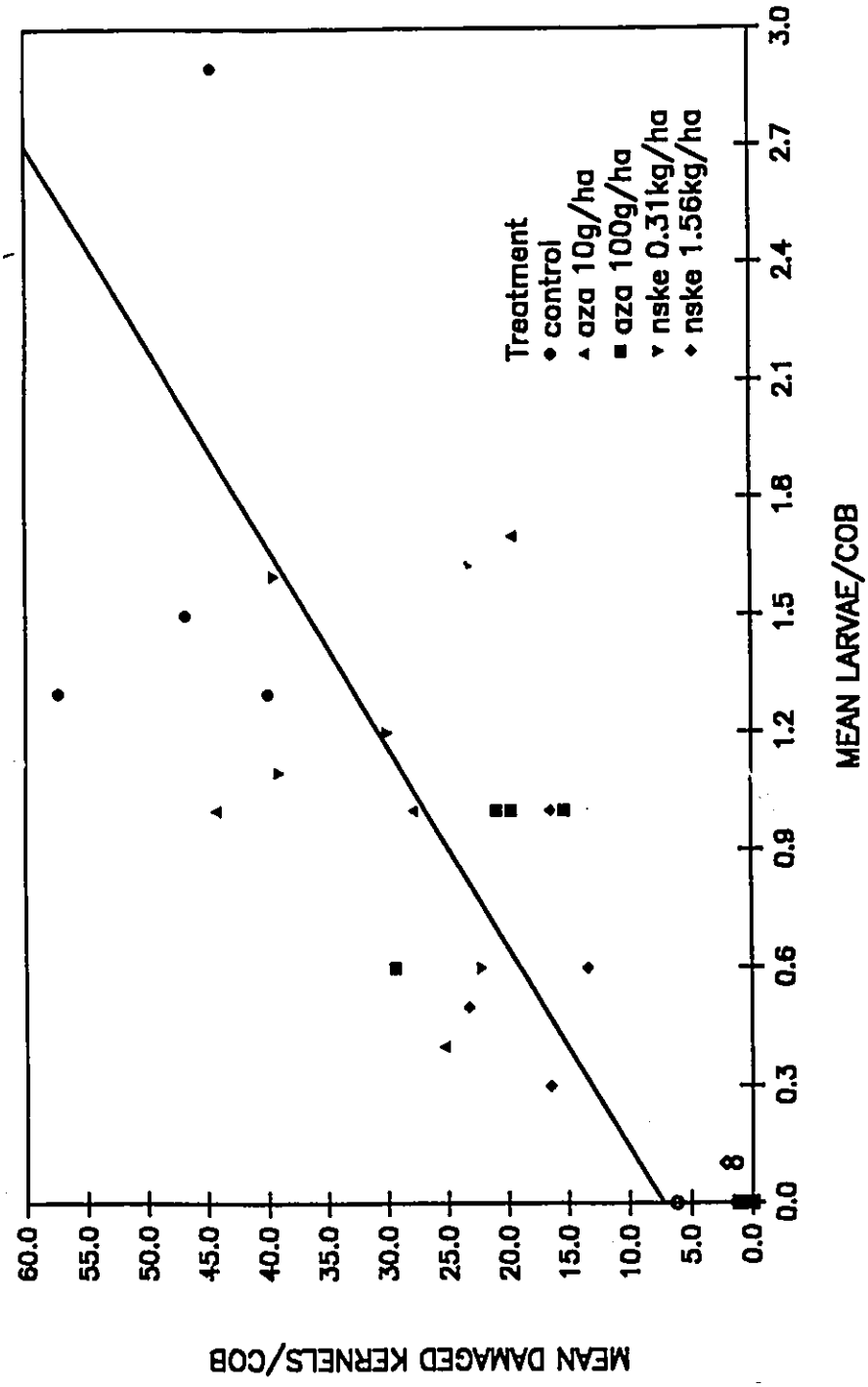

MEAN DAMAGED KERNELS/COB
60.0
55.0
50.0
45.0
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
0.0
0.3
0.6
0.9
1.2
1.5
1.8
2.1
2.4
2.7
3.0
MEAN LARVAE/COB
Treatment
control
aza 10g/ha
aza 100g/ha
nske 0.31kg/ha
nske 1.56kg/ha

FIGURE 17b

Field 1987 - Relation of mean tunnelling ratio to mean number larvae cob^{-1} in naturally and artificially infested plants treated with NSKE and azadirachtin.

Calculated regression line is shown for calibration data of non-treated control plants ($y = 1.33 + 4.45x$; $r=0.87$)
Each treatment is represented by 4 means (4 replicates)
Filled symbols: data obtained from artificially infested plants;
Blank symbols: data obtained from naturally infested plants.

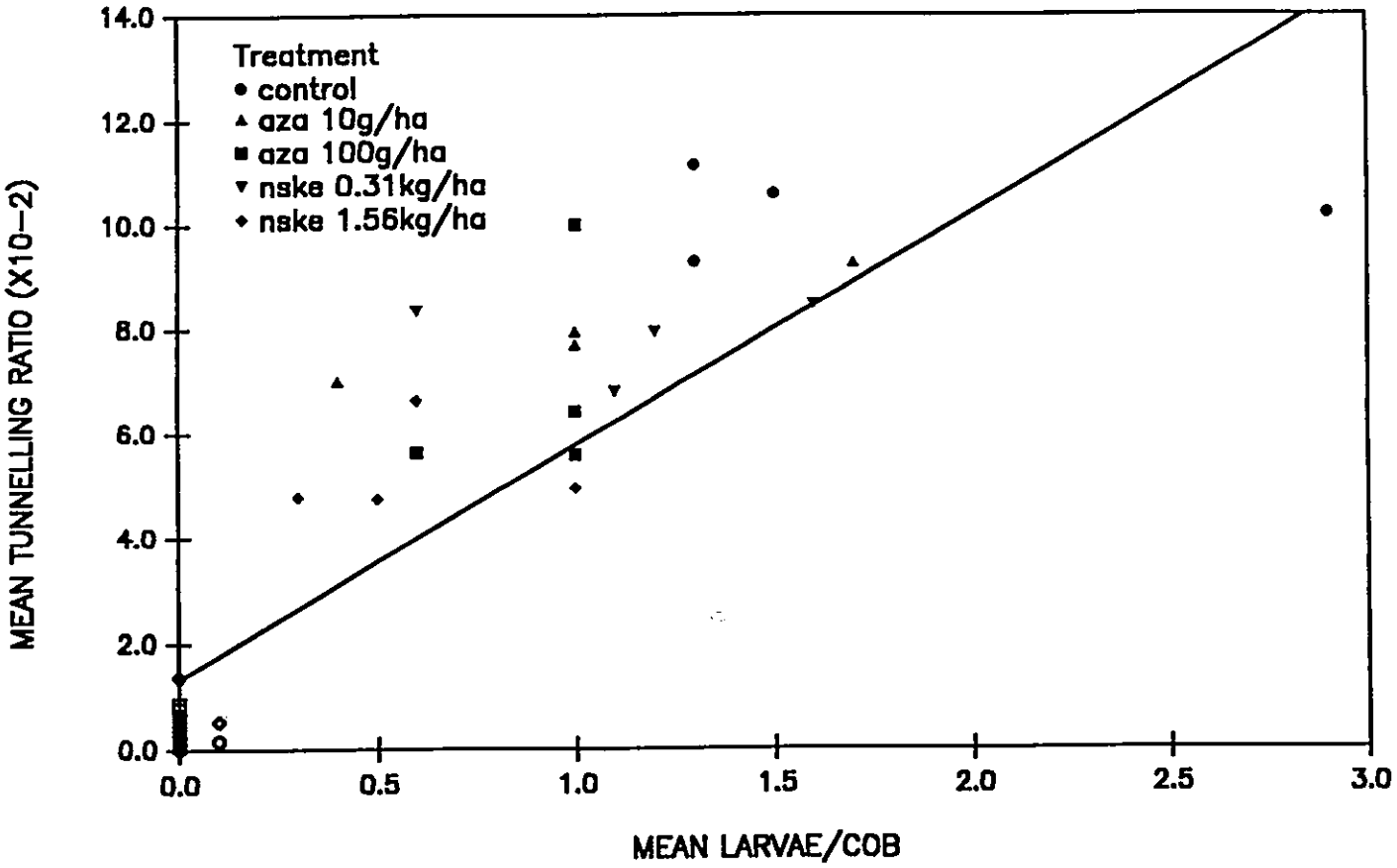

Treatment
control
aza 10g/ha
aza 100g/ha
nske 0.31kg/ha
nske 1.56kg/ha
MEAN TUNNELLING RATIO (X10−2)
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
0.0
0.5
1.0
1.5
2.0
2.5
3.0
MEAN LARVAE/COB

FIGURE 18

Field 1 - 1988 - Relation of mean number damaged kernels cob^{-1} to mean number larvae cob^{-1} in naturally and artificially infested plants treated with NSKE and azadirachtin.

Calculated regression line is shown for calibration data of non-treated control plants ($y= 3.54 + 5.61x$; $r=0.88$)
Each treatment is represented by 4 means (4 replicates)
Filled symbols: data obtained from artificially infested plants;
Blank symbols: data obtained from naturally infested plants.

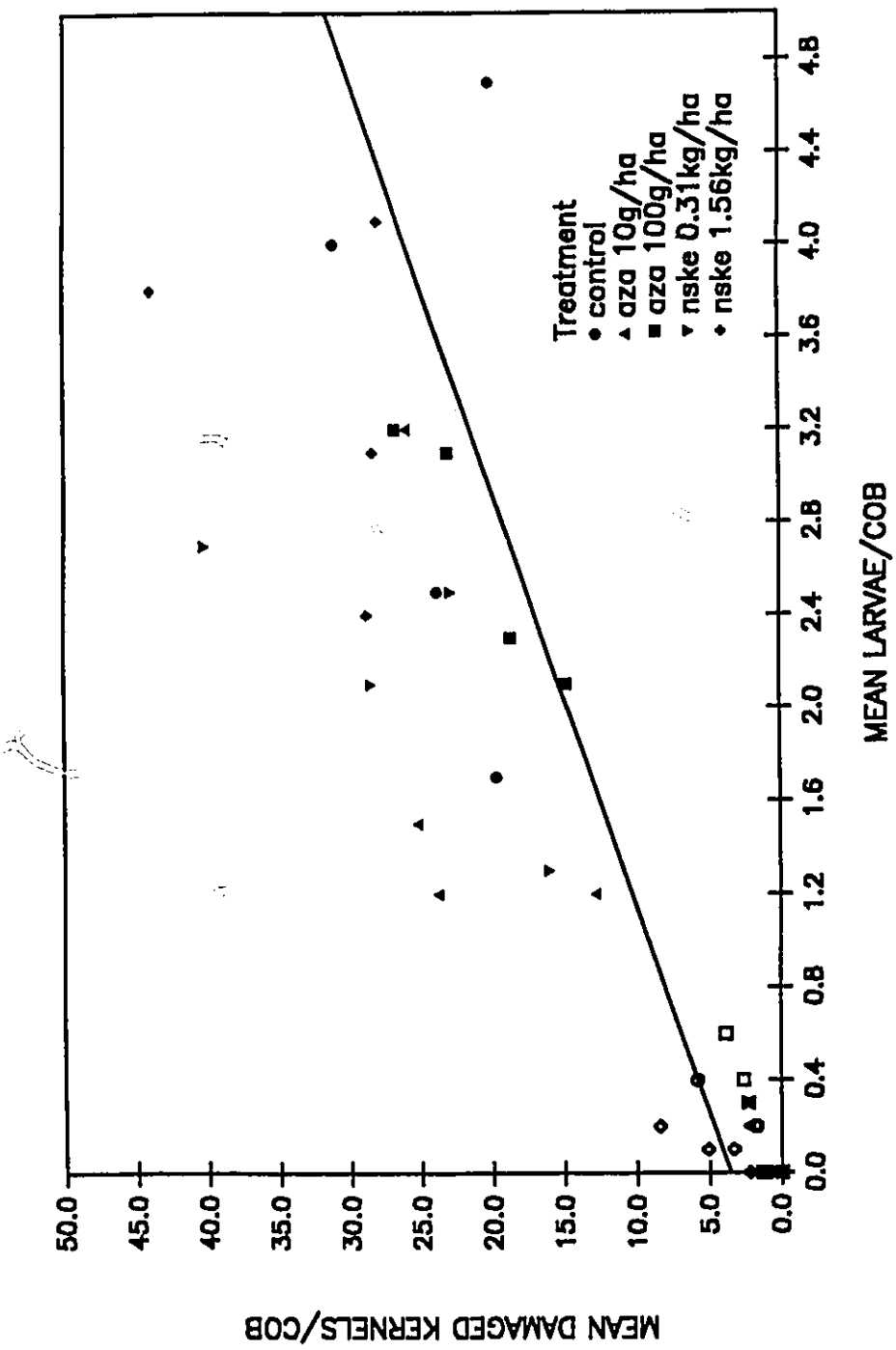

MEAN DAMAGED KERNELS/COB
50.0
45.0
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
0.0
0.4
0.8
1.2
1.6
2.0
2.4
2.8
3.2
3.6
4.0
4.4
4.8
MEAN LARVAE/COB
Treatment
control
aza 10g/ha
aza 100g/ha
nske 0.31kg/ha
nske 1.56kg/ha

FIGURE 19

Field 2 - 1988 - Relation of mean number damaged kernels cob^{-1} to mean number larvae cob^{-1} in naturally and artificially infested plants treated with NSKE and Ambush.

Calculated regression line is shown for calibration data of non-treated control plants ($y= 1.27 + 1.82x$; $r=0.96$)
Each treatment is represented by 4 means (4 replicates)
NI= naturally infested.

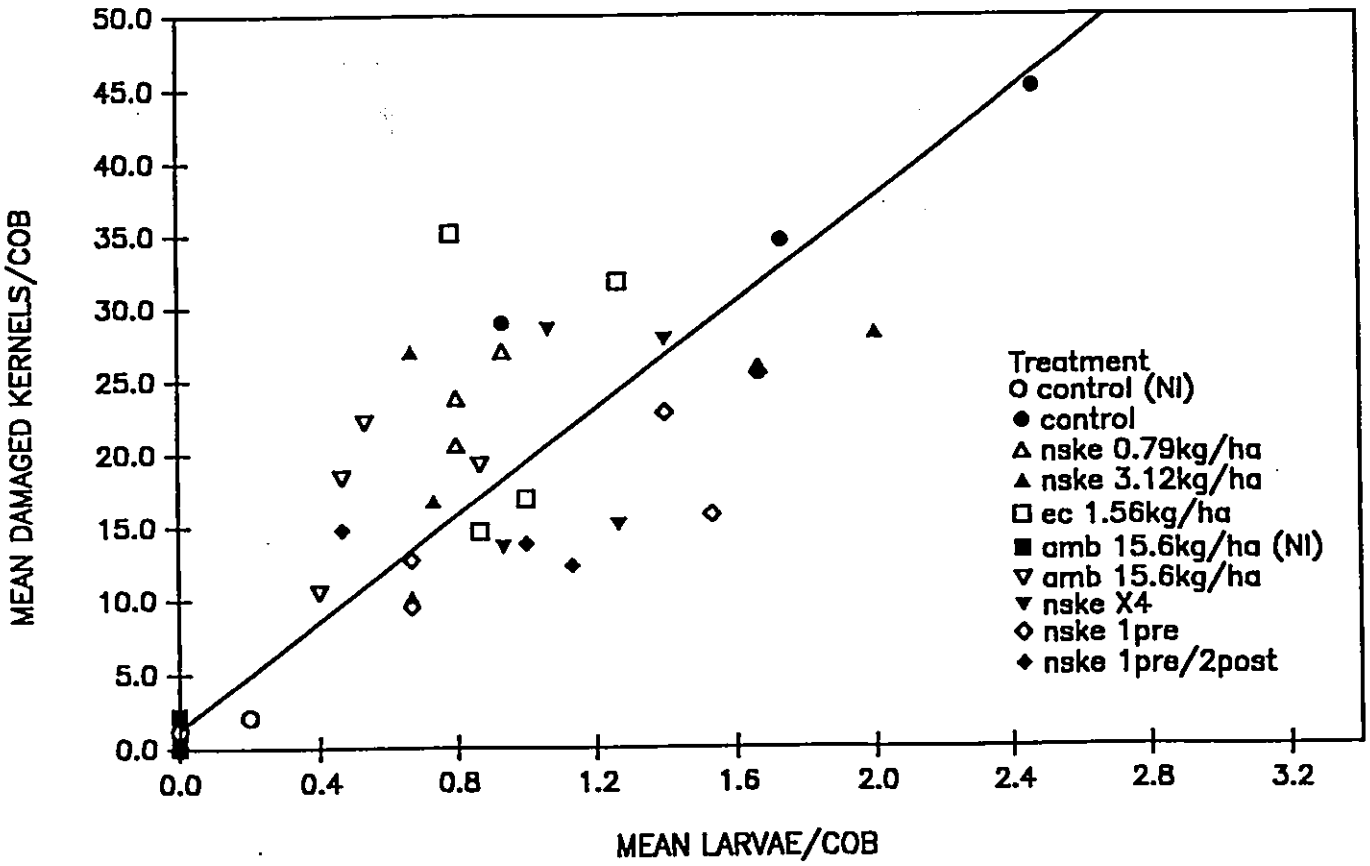

MEAN DAMAGED KERNELS/COB
50.0
45.0
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
0.0
0.4
0.8
1.2
1.6
2.0
2.4
2.8
3.2
MEAN LARVAE/COB
Treatment
control (NI)
control
nske 0.79kg/ha
nske 3.12kg/ha
ec 1.56kg/ha
amb 15.6kg/ha (NI)
amb 15.6kg/ha
nske X4
nske 1pre
nske 1pre/2post

FIGURE 20a

Field 1 - 1989 - Relation of mean number damaged kernels cob^{-1} to mean number larvae cob^{-1} in naturally and artificially infested plants treated with NSKE and azadirachtin.

Calculated regression line is shown for calibration data of non-treated control plants (y= 0.17 + 8.15x ; r=0.96)
Each treatment is represented by 4 means (4 replicates)
NI= naturally infested.

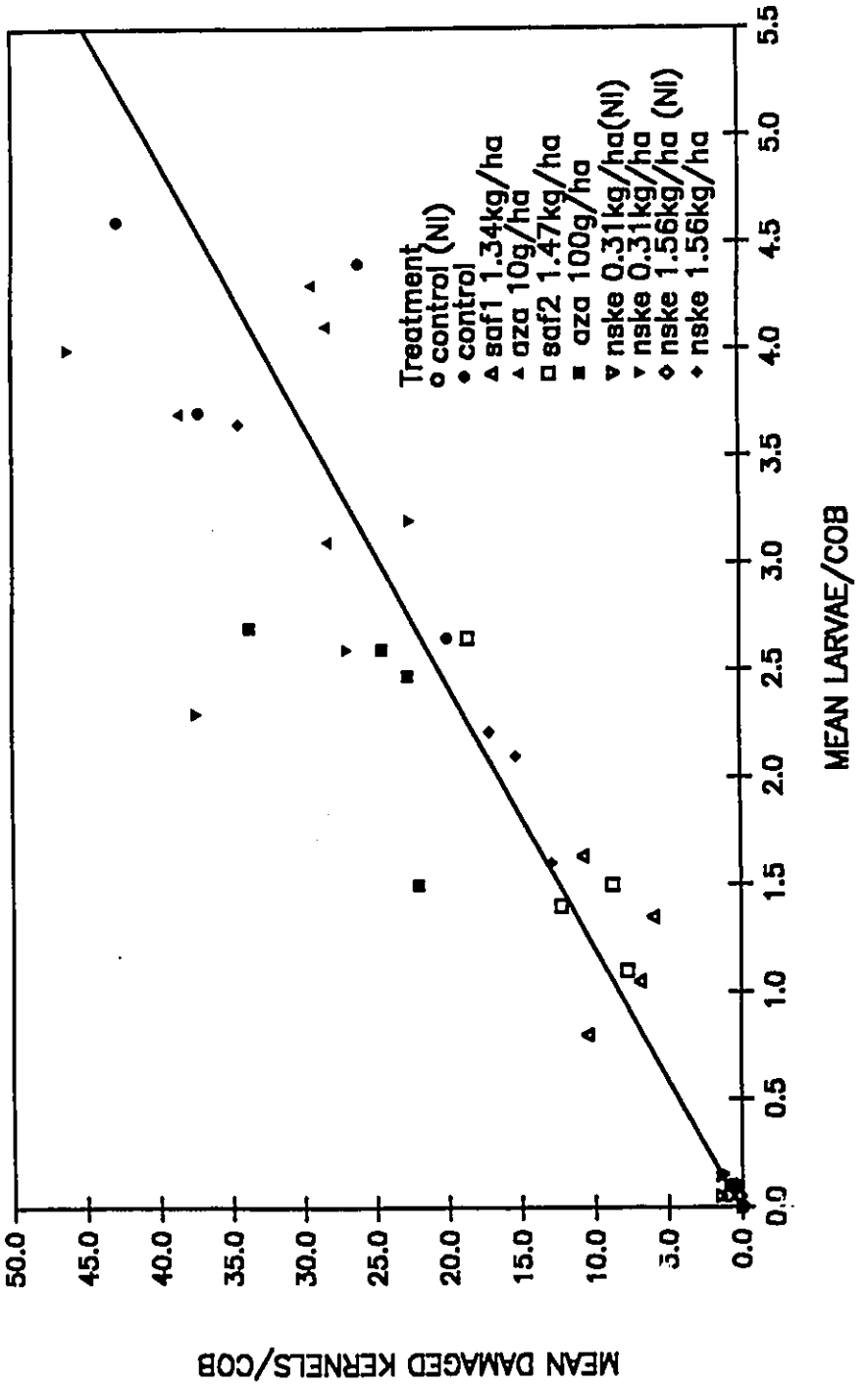

MEAN DAMAGED KERNELS/COB
MEAN LARVAE/COB
Treatment
control (NI)
control
saf1 1.34kg/ha
aza 10g/ha
saf2 1.47kg/ha
aza 100g/ha
nske 0.31kg/ha(NI)
nske 0.31kg/ha
nske 1.56kg/ha (NI)
nske 1.56kg/ha
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
50.0
45.0
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0

FIGURE 20b

Field 1 - 1989 - Relation of mean stalk breakage rating to mean number larvae cob^{-1} in naturally and artificially infested plants treated with NSKE and azadirachtin.

Calculated regression line is shown for calibration data of non-treated control plants ($y= 0.84 + 1.25x$; $r=0.94$)
Each treatment is represented by 4 means (4 replicates)
NI= naturally infested.

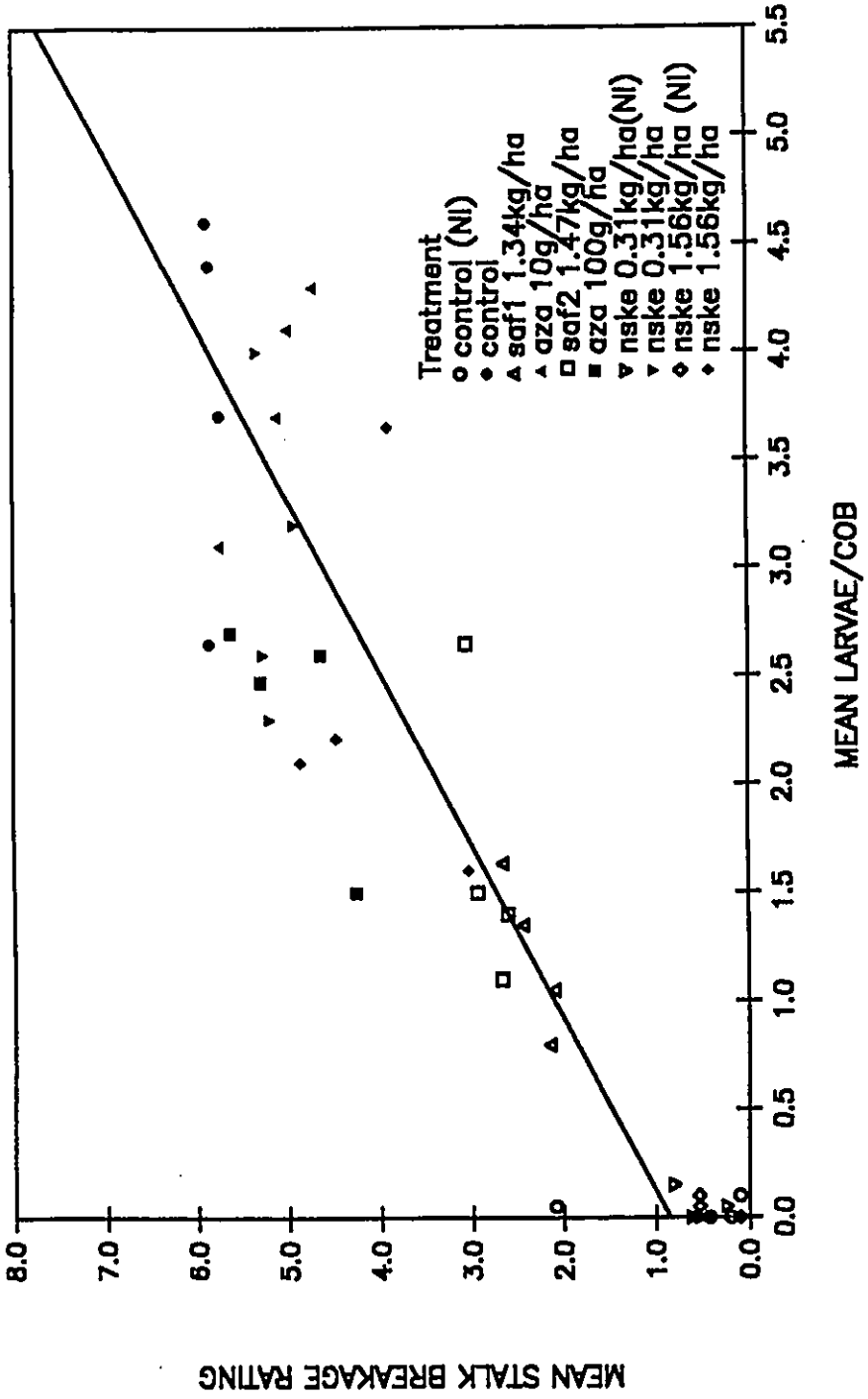

MEAN STALK BREAKAGE RATING
MEAN LARVAE/COB
Treatment
control (NI)
control
saf1 1.34kg/ha
aza 10g/ha
saf2 1.47kg/ha
aza 100g/ha
nske 0.31kg/ha(NI)
nske 0.31kg/ha
nske 1.56kg/ha (NI)
nske 1.56kg/ha
0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5

FIGURE 21a

Field 2 - 1989 - Relation of mean number damaged kernels cob^{-1} to mean number larvae cob^{-1} in naturally and artificially infested plants treated with NSKE and Ambush.

Calculated regression line is shown for calibration data of non-treated control plants ($y= 0.10 + 1.24x$; $r=1.0$)
Each treatment is represented by 4 means (4 replicates)
NI= naturally infested.

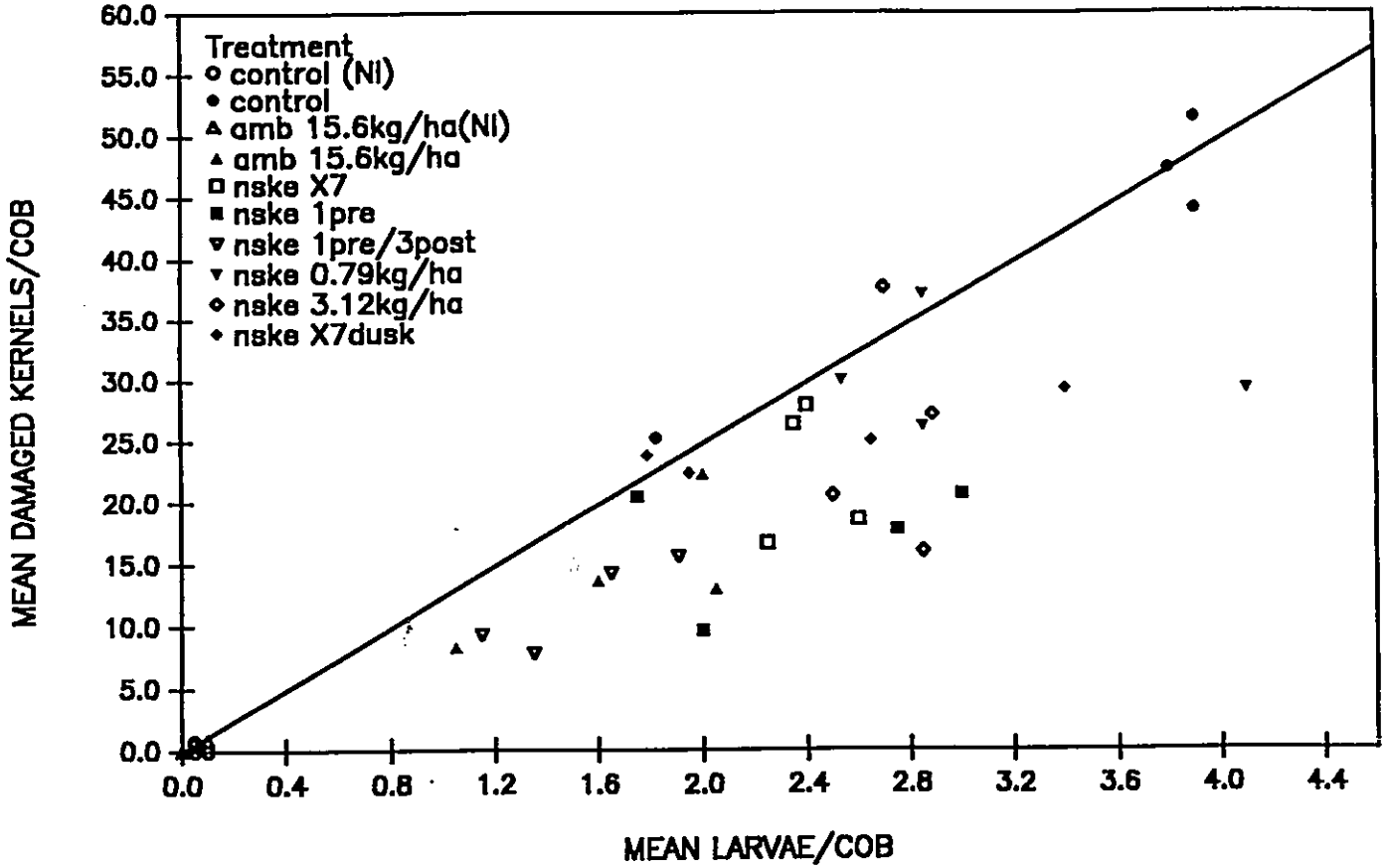
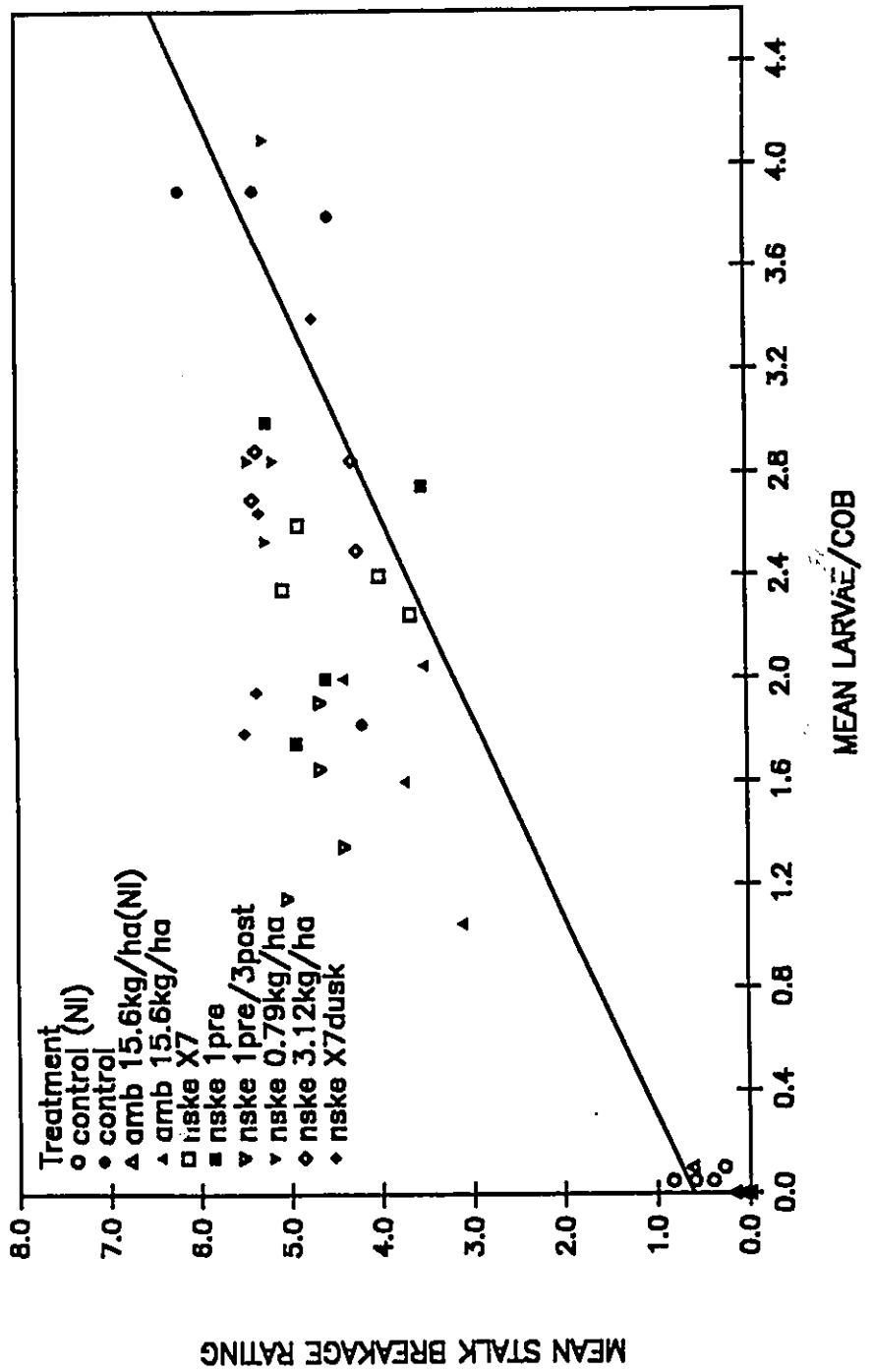

Treatment
control (NI)
control
amb 15.6kg/ha(NI)
amb 15.6kg/ha
nske X7
nske 1pre
nske 1pre/3post
nske 0.79kg/ha
nske 3.12kg/ha
nske X7dusk
MEAN DAMAGED KERNELS/COB
MEAN LARVAE/COB
60.0
55.0
50.0
45.0
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
0.0
0.4
0.8
1.2
1.6
2.0
2.4
2.8
3.2
3.6
4.0
4.4

FIGURE 21b

Field 2 - 1989 - Relation of mean stalk breakage rating to mean number larvae cob^{-1} in naturally and artificially infested plants treated with NSKE and Ambush.

Calculated regression line is shown for calibration data of non-treated control plants ($y= 0.61 + 1.28x$; $r=0.96$)
Each treatment is represented by 4 means (4 replicates)
NI= naturally infested.


Treatment
control (NI)
control
amb 15.6kg/ha(NI)
amb 15.6kg/ha
nske X7
nske 1pre
nske 1pre/3post
nske 0.79kg/ha
nske 3.12kg/ha
nske X7dusk
MEAN STALK BREAKAGE RATING
MEAN LARVAE/COB
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0.0
0.4
0.8
1.2
1.6
2.0
2.4
2.8
3.2
3.6
4.0
4.4

The plots of larval populations cob^{-1} to kernel damage cob^{-1} from 1987 to 1989 in artificially infested plants indicates that neem products decreased the number of damaged kernels cob^{-1} from the control for a given cob-larval population in 43.7% to 89.3% of treated plots (Figures 17a, 18, 19, 20a, 21a). In 1987, 43.7% of the treated plots fell below the calibration curve, 50% in field 2 of 1988, 54.2% and 89.3% in fields 1 and 2 of 1989. Exceptionally, in field 1 of 1988 only 6.2% of the treated plots had reduced kernel damage compared to the control plots for a given cob-larval population. In naturally infested plants, 100% of the treated plots in 1987 fell below the calibration curve, 87.5% of the plots in field 1 of 1988, and 75% of the plots in field 1 of 1989.

A look at the relation between larval population cob^{-1} and stalk damage in artificially infested plants of 1987 and 1989 reveals that although neem products generally decreased cob-larval populations, the amount of tunnelling or stalk damage increased (Figures 17b, 20b, 21b). In 1987, 87.5% of the treated plots were above the calibration curve. Similarly, 62.5% and 89.3% of the treated plots in fields 1 and 2 of 1989 were found to have higher stalk damage compared to the controls for given cob-larval populations. In naturally infested plants, 100% of the treated plots of 1987 and 1989 fell below the calibration curve.

1.3 Laboratory Studies

1.3.1 Azadirachtin Antifeedant No-Choice Bioassay

The laboratory leaf feeding bioassay indicated that AZA (7.2 ng AZA mm^{-2}) applied to segments of corn leaf surfaces reduced consumption by naive 3rd instar larvae (Figure 22). Examining the effect of the ai's concentration, AZA alone and AZA in a formulation with PBO and Citowett at 100 μg AZA ml^{-1} (7.2 ng AZA mm^{-2}) significantly decreased (p=0.05, Tukey's multiple range test) leaf feeding compared to their controls by 25% and 39%, respectively. However, the two lower concentrations of AZA alone or

in a formulation (10 and 1 μg ai ml^{-1} or 0.72 and 0.072 ng ai mm^{-2}) enhanced leaf consumption compared to their controls.

The formulation composed of AZA, PBO and Citowett was more effective at protecting the leaf-disks than AZA alone. At 7.2 ng AZA mm^{-2}, the formulation increased the efficacy of AZA by 14%.

The ingredients of the formulation when tested alone generally increased leaf consumption (Appendix 12). PBO combined with Citowett enhanced feeding in all three trials (average of 22% increase) when compared to the non-treated control. Leaf consumption increased in the second and third trials when the leaf disks were treated with Citowett while PBO alone slightly reduced leaf damage in two of the trials.

1.3.2 Effect of NSKE on the Growth and Development of O. nubilalis

The results of two growth studies showed that NSKEs reduced mean ECB larval, pupal and adult weights, increased the mean number of days to pupation and adult emergence, and modified sex ratios at a minimum of 3 μg NSKE g^{-1} diet (Tables 10–13). NSKE was larvicidal at 10, 30, 100 and 1000 μg g^{-1} diet. The results also demonstrated that the method of extracting the NSKE developed by Nakanishi in 1987 was more effective at altering the development of the corn borer than the 1975 method.

Since larvae of trial 1 weighed significantly more than those of trial 2 throughout the growth studies, the results were analyzed separately. However, expressing the mean larval weights as a % of their control at day 12 of the studies clearly showed the weight reducing effect of increasing NSKE concentrations and the higher efficacy of the 1987 method of extracting the NSKE (Figure 23).

FIGURE 22

Mean leaf-disk feeding as a % of the control versus Azadirachtin concentration.

AZA alone and AZA in a formulation with PBO and Citowett were tested for their antifeedant properties on early 3rd instar ECB larvae in Illini Gold sweet corn leaf-disk (total area= 138 mm^2) bioassays.
100 $\mu g\ mL^{-1}$= 7.2 ng azadirachtin mm^{-2}
10 $\mu g\ mL^{-1}$= 0.72 ng azadirachtin mm^{-2}
1 $\mu g\ mL^{-1}$= 0.072 ng azadirachtin mm^{-2}
Mean (s.e.) leaf-feeding (3 trials, 10 replicates each) is expressed as a % of the respective controls (1st two bars of histogram).
Means with different letters are significantly different at p=0.05
level of significance using Tukey's multiple range test.

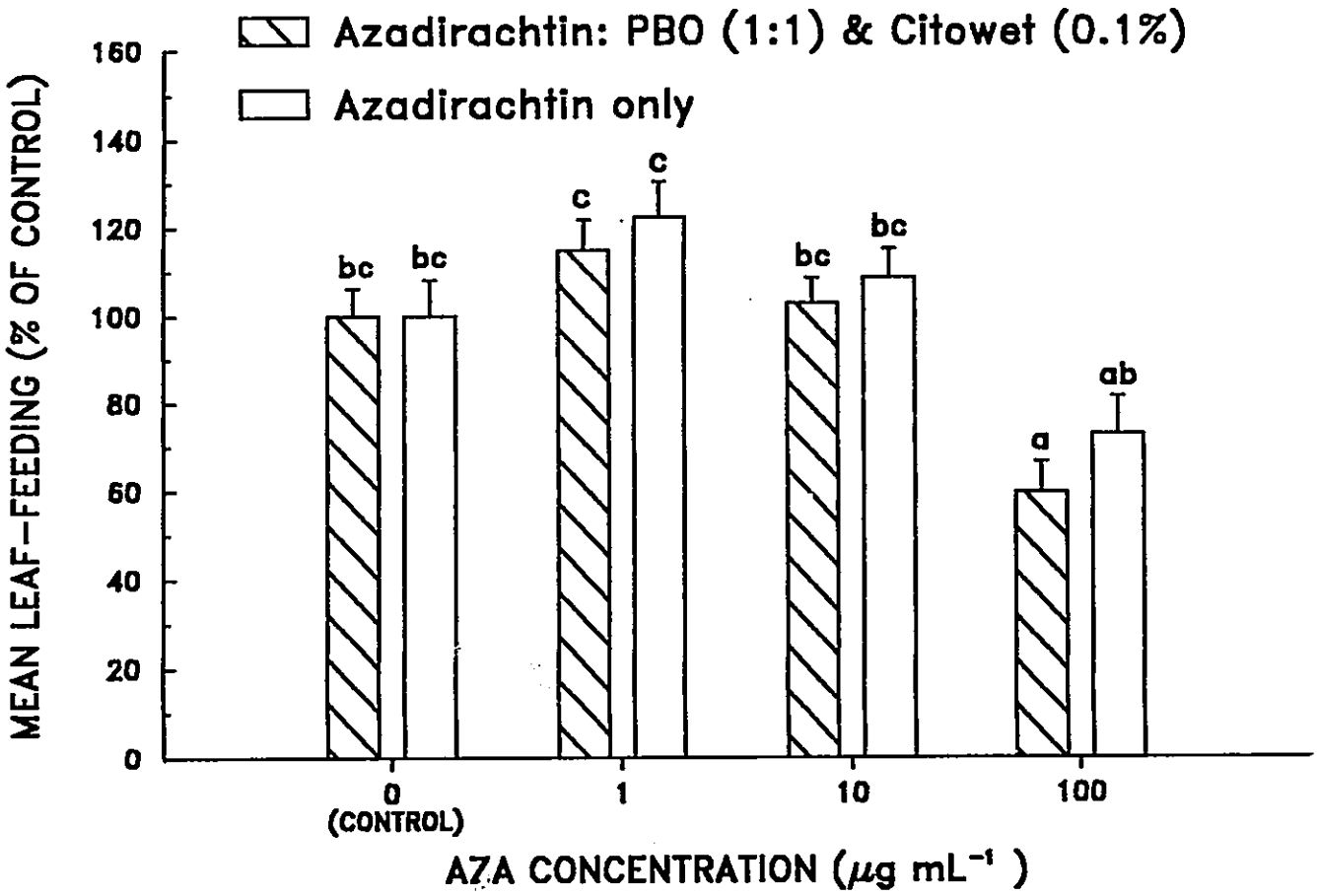

Azadirachtin: PBO (1:1) & Citowet (0.1%)
Azadirachtin only
MEAN LEAF-FEEDING (% OF CONTROL)
160
140
120
100
80
60
40
20
0
bc
bc
c
c
bc
bc
a
ab
0
(CONTROL)
1
10
100
AZA CONCENTRATION (μg mL⁻¹)

TABLE 10: Effect of NSKE concentrations and methods of extraction on ECB weights. Trials (1) and (2) were carried out at different times.

NSKE CONCENTRATION (µg/g diet)	MEAN LARVAL WEIGHT (mg)									
	DAY 1		DAY 5		DAY 8		DAY 12		DAY 15	
	(1)[1]	(2)[2]	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
Trial (1)										
0	2.63 a		14.91 d		33.91 bc		84.40 bc		61.36 bc	
1	2.69 a	2.76 a	16.70 d	13.49 cd	39.79 c	30.70 bc	103.09 c	73.37 bc	99.42 c	87.01 c
10	2.94 a	2.83 a	13.58 cd	12.28 bcd	31.25 bc	24.92 b	52.01 bc	34.05 ab	61.09 bc	38.40 ab
100	2.81 a	2.57 a	8.00 abc	6.87 ab	9.32 a	7.60 a	9.25 a	7.46 a	8.43 a	6.84 a
1000	2.70 a	2.83 a	5.25 a	5.14 a	5.31 a	5.30 a	4.68 a	5.10 a	4.42 a	5.18 a
Trial (2)										
0	2.28 a		5.26 a		11.05 ab		27.16 de		44.61 e	
1	2.50 a	2.56 a	5.51 a	5.10 a	9.74 ab	12.65 b	18.20 bcd	29.01 e	28.30 bcd	46.07 e
3	2.37 a	2.52 a	5.98 a	4.97 a	11.34 ab	9.75 ab	22.74 cde	16.84 abc	35.56 cde	25.80 bc
10	2.45 a	2.29 a	4.79 a	4.39 a	9.87 ab	7.25 a	15.96 abc	10.79 ab	23.89 abc	13.77 ab
30	2.45 a	2.35 a	4.93 a	5.17 a	9.47 ab	7.09 a	13.21 abc	7.86 a	18.71 ab	8.57 a

Trials (1) and (2) were carried out at different times.
Mean weights were subjected to Tukey's multiple range test. Means in each block followed by different letters are significantly different at p=0.05 level of significance.

[1] Nakanishi method of extracting NSKE - 1975
[2] Nakanishi method of extracting NSKE - 1987

FIGURE 23

NSKE concentrations in diet (μg g^{-1} diet) versus mean larval weight at day 12 (% of control).

The NSKE obtained from the two Nakanishi methods of extraction (1975 and 1987) were compared for their effect on the growth and development of ECB larvae. 2nd instar larvae were placed on the diets at day 1 and their weights were monitored throughout their development.
Mean (s.e.) larval weight (2 trials, 15 replicates each) is expressed as a % of the respective controls.

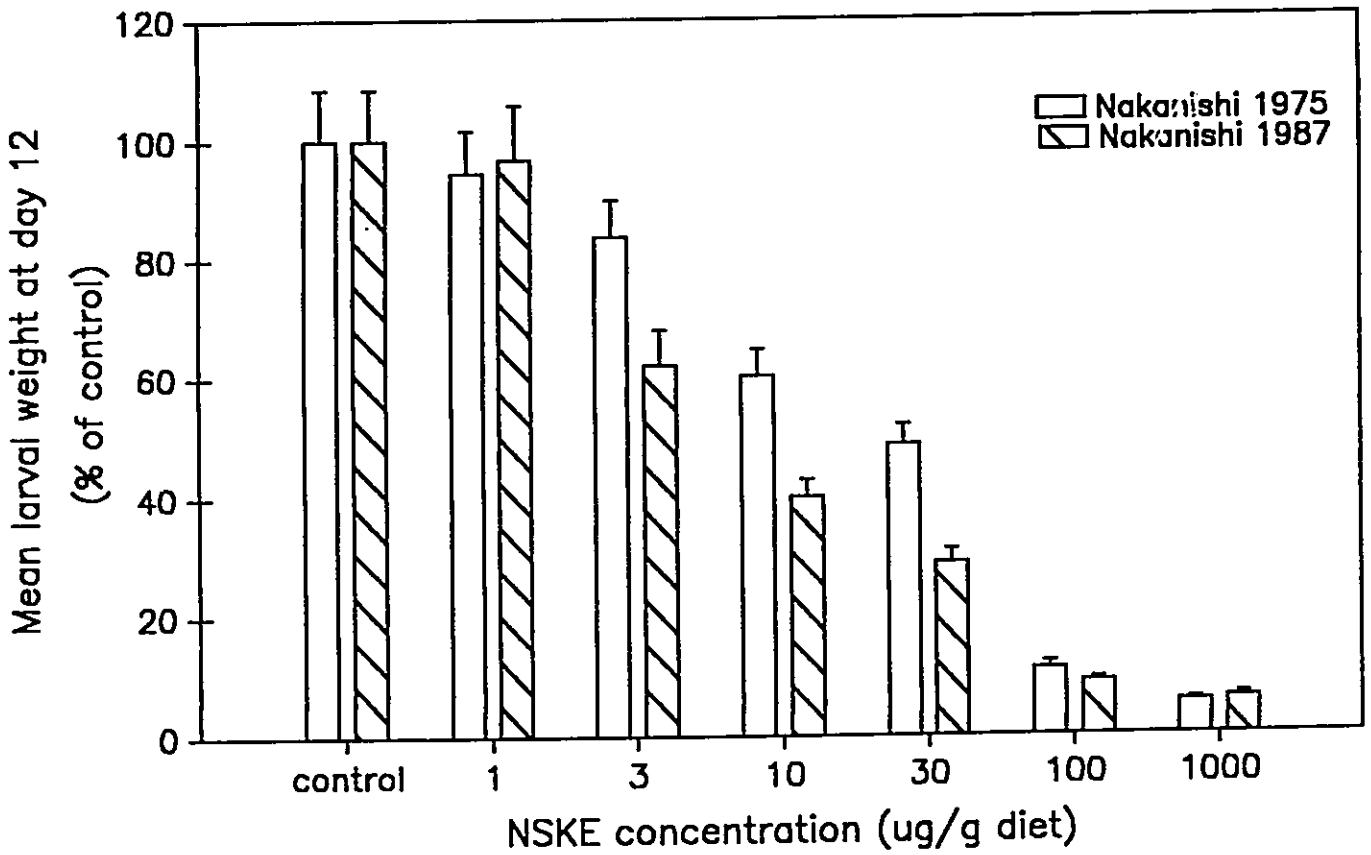

120
100
80
60
40
20
0
Mean larval weight at day 12
(% of control)
Nakanishi 1975
Nakanishi 1987
control
1
3
10
30
100
1000
NSKE concentration (ug/g diet)

TABLE 11: Effect of NSKE concentrations and methods of extraction on ECB growth parameters and mortality.

NSKE CONCENTRATION (µg/g diet)	MEAN TIME TO PUPATION (days) (1)[1] (2)[2]	SURVIVAL TO PUPATION % (1) (2)	MEAN TIME TO ADULT EMERGENCE (days) (1) (2)	SURVIVAL TO ADULT EMERGENCE % (1) (2)	SEX RATIO OF SURVIVING ADULTS (F:M) (1) (2)
Trial (1)					
0	17.7	73.3	25.0	66.7	1:0.7
1	22.3* 26.8*	93.3 66.7	33.2* 33.8*	60.0 26.7	1:2 1:1
10	-- --	0 0	-- --	0 0	-- --
100	-- --	0 0	-- --	0 0	-- --
1000	-- --	0 0	-- --	0 0	-- --
Trial (2)					
0	24.4	80.0	33.3	80.0	1:1
1	26.3 25.4	73.3 73.3	36.1 34.3	60.6 66.7	1:0.3 1:1.4
3	25.6 42.9*	80.0 60.0	35.5 45.3	73.3 20.0	1:1.8 1:0
10	36.3* --	60.0 0	37.0 --	13.3 0	1:1 --
30	-- --	0 0	-- --	0 0	-- --

Trials (1) and (2) were carried out at different times.
Mean days to pupation and adult emergence were subjected to Kruskal-Wallis test. Means in each block followed by * are significantly different from the control at p=0.05 level of significance.

[1] Nakanishi method of extracting NSKE - 1975
[2] Nakanishi method of extracting NSKE - 1987

TABLE 12: Effect of NSKE concentrations and methods of extraction on ECB pupal and adult weights.

NSKE CONCENTRATION (μg/g diet)	MEAN PUPAL WEIGHT (mg) (1)[1]	(2)[2]	MEAN ADULT WEIGHT (mg) (1)	(2)
Trial (1)				
0	84.5		52.5 b	
1	70.1	78.2	34.9 a	43.3 ab
10	49.1	--	--	--
100	--	--	--	--
1000	--	--	--	--
Trial (2)				
0	66.6 c		37.6	
1	56.5 bc	55.7 bc	34.4	31.1
3	51.5 abc	40.1 ab	24.3	25.5
10	35.5 a	--	21.3	--
30	--	--	--	--

Trials (1) and (2) were carried out at different times.
Mean weights were subjected to Tukey's multiple range test. Means in each block followed by different letters are significantly different at p=0.05 level of significance.

[1] Nakanishi method of extraction, 1975
[2] Nakanishi method of extraction, 1987

1.3.2.1 Larval Weight

Concentrations of 3, 10, 30, 100 and 1000 μg NSKE g^{-1} diet reduced growth rates of ECB larvae (Table 10). Larval weights, from day 12 and on of the experiments, were significantly lower (p=0.05, Tukey's multiple range test) than the controls with 3, 10, 100 and 1000 μg NSKE g^{-1} diet (NSKE extracted by the 1987 Nakanishi method). Weight gain was minimal at 1000, 100 and 30 μg NSKE g^{-1} diet. For example, on day 12 of growth study 2, the weight of larvae feeding on 30 μg NSKE g^{-1} diet (1987 method) had

TABLE 13: Effect of NSKE concentrations and methods of extraction on ECB male, female pupal and adult weights.

NSKE CONCENTRATION (μg/g diet)	MEAN PUPAL WEIGHT (mg)				MEAN ADULT WEIGHT (mg)			
	M		F		M		F	
	(1)[1]	(2)[2]	(1)	(2)	(1)	(2)	(1)	(2)
Trial (1)								
0	73.0		92.3		39.8		60.9	
1	68.2	65.5	88.0	92.1	30.8	33.0	43.0	53.5
10	--	--	--	--	--	--	--	--
100	--	--	--	--	--	--	--	--
1000	--	--	--	--	--	--	--	--
Trial (2)								
0	56.1		77.1 b		29.2		45.9 c	
1	38.8	47.1	61.0 ab	69.5 ab	23.0	22.6	37.7 abc	42.9 bc
3	45.4	--	60.1 ab	44.3 a	20.0	--	31.9 abc	25.5 ab
10	--	--	42.5 a	--	--	--	21.3 a	--
30	--	--	--	--	--	--	--	--

Trials (1) and (2) were subjected to Tukey's multiple range test. Means in each block followed by different letters are significantly different at p=0.05 level of significance.

[1] Nakanishi method of extraction, 1975
[2] Nakanishi method of extraction, 1987

increased only by an average of 5.51 mg (day 1- 2.35 mg, day 12- 7.86 mg) whereas the control group had gained on average 24.88 mg (day 1- 2.28 mg, day 12- 27.16 mg). A concentration of 1 μg NSKE g^{-1} diet increased larval weight compared to the control group (day 12) in trial 1 with the NSKE- 1975 extraction method and in trial 2 with the NSKE- 1987 extraction method.

Larval weights were lower in groups feeding on the NSKE- 1987 extraction method compared to those in the NSKE- 1975 extraction method throughout the larval

period. The weights in the two groups were not significantly different from each other.

1.3.2.2 Developmental Parameters

The pupation and adult emergence parameters recorded for the two trials are summarized in Table 11. No larvae placed on diet containing 1000, 100 and 30 μg NSKE g^{-1} diet (1975 and 1987 method of extraction) pupated. With 10 μg NSKE g^{-1} diet, only the larvae of trial 2 (1975 method) were able to pupate in 36.3 days. This was significantly different (p=0.05, Kruskal-Wallis test) from the control group's ability to pupate in 24.4 days. Larvae fed 1 and 3 μg NSKE g^{-1} diet (1975 and 1987 method) had longer larval period compared to the control. In trial 1, both NSKEs at 1 μg g^{-1} diet significantly increased the number of days to pupation compared to the control. In trial 2, the groups feeding on 1 μg NSKE g^{-1} diet (1975 and 1987 method) had prolonged larval periods and at 3 μg NSKE g^{-1} diet (1987 method), the number of days spent in the larval stage increased significantly by 18.5 compared to the control. In trial 1 at 1 μg NSKE g^{-1} diet and trial 2 at 3 μg NSKE g^{-1} diet the mean time to pupation was higher with the NSKE–1987 method than with the NSKE- 1975 method.

Similar trends were noted in the number of days to adult emergence. The NSKE-1987 method, prolonging the time to a greater degree compared to the NSKE- 1975 method. The greatest time increase in adult emergence was noted in larvae fed 3 μg NSKE g^{-1} diet (1987 method); larvae feeding on the treated diet became adults in 45.3 days compared to the control group who emerged as adults in 33.3 days.

1.3.2.3 Pupal and Adult Weights

Pupal weights were affected by both NSKEs at 1, 3 and 10 μg NSKE g^{-1} diet (Tables 12, 13). Pupal weights of both sexes were decreased in all treated groups but only the mean pupal weights of females and not of males were significantly lower when

compared to the control. By considering separately female and male weight parameters, it was observed that only female larvae of trial 2 in the 1 μg NSKE g^{-1} diet (1987 method), 3 and 10 μg NSKE g^{-1} diet (1975 method) pupated. The most considerable weight decrease was seen in female larvae treated with 10 μg NKSE g^{-1} diet (1975 method); control pupae weighed on average 77.1 mg whereas the treated ones weighed 42.5 mg, a reduction of 45%.

Data for adult weights showed similar decreases with NSKEs as described for pupal weights.

1.3.2.4 Survivorship

When survivorship statistics for pupae were examined (Table 11), the NSKE- 1987 method of trials 1 and 2 decreased the number of survivors at 1 (trial 1- 66.7%, trial 2- 73.3%) and 3 μg g^{-1} diet (trial 2- 60%) compared to the control (trial 1- 73.3%, trial 2- 80.0%). No larvae survived at 10, 100 or 1000 μg NSKE g^{-1} diet in trial 1 nor, in trial 2, at 30 μg NSKE g^{-1} diet (1975 and 1987 method) and 10 μg g^{-1} diet (1987).

With the NSKE- 1975 method, survivorship increased with 1 μg NSKE g^{-1} diet in trial 1 (93.3%) by 20% when compared to the control (73.3%). In trial 2, 1 μg NSKE g^{-1} diet (1975 method) reduced survivorship to 73.3% compared to 80% in the control group. The 3 μg NSKE g^{-1} diet (1975 method) did not adversely effect survivorship (80%). There was an equal number of survivors with the 10 μg NSKE g^{-1} diet (1975 method) as with the 3 μg NSKE g^{-1} diet (1987 method); survival to pupation was 60% in both of those groups compared to 80% in the control group.

Survival to adult emergence was reduced by both NSKEs at 1 μg g^{-1} diet in trials 1 and 2; compared to 66.7% adult emergence in the control group of trial 1, only 26.7% of the NSKE- 1987 method group successfully emerged and 60% of the NSKE- 1975 method group. In trial 2, 80% of the control group emerged as adults but 66.7% of the

NSKE–1987 method groups successfully survived to adulthood and 60.6% of the NSKE–1975 method group. At 3 μg NSKE g^{-1} diet, survivorship was further reduced to 20% with the NSKE- 1987 method but was higher with the NSKE- 1975 method (73.3%) compared to the 1 μg NSKE g^{-1} diet treatment (60.6%). At 10 μg NSKE g^{-1} diet (1975 method), survival to adult emergence was only 13.3%, a difference of 66.7% compared to the control group.

1.3.2.5 Sex Ratios

Although the calculated sex ratios of the surviving adults were not consistent between trials 1 and 2 with the two NSKEs at 1 μg g^{-1} diet, they were greatly altered (Table 11). In trial 1, the number of males was twice that of females with the NSKE-1975 method while the NSKE- 1987 method resulted in the number of males equalling that of females. At 3 μg NSKE g^{-1} diet, there were 1.8 males for one female with the 1975 extraction method while there were no males with the 1987 method.

The sex ratios were reversed in trial 2 with 1 μg NSKE g^{-1} diet where only 0.3 male per female were observed (1975 method) and 1.14 male per female with the 1987 method. With 10 μg NSKE g^{-1} diet (1975 method), the number of males and females was equal.

2.0 Uptake and Systemic Movement of H_2-Azadirachtin and Azadirachtin in Sweet Corn Seedings.

2.1 $^3H_2$-Azadirachtin Tracer Study

2.1.1 Characterization of the ^{3}H-Compound

The chemical identity of the tritiated compound believed to be $^3H_2$-AZA was supported by the co-chromatography of the material with H_2-AZA using high pressure liquid chromatography (Figure 24). The fraction collected between 6.0 and 6.5 min

following injection of the compound into the HPLC system contained 38% of the total injected radioactive counts (dpm); this represented the highest percentage in the fractions collected over a 10 minute period. This fraction coincided with the main peak of the HPLC tracing of H_2-AZA under the same chromatographic conditions. Chromatography of the non-radioactive H_2-AZA indicated a retention time of 6.2 min.

Examining the chromatography profiles of the ^{3}H-compound and H_2-AZA strongly suggests that the radioactive material was the tritiated form of H_2-AZA. However, the tritiated compound was found to have a shoulder peak (14% of total dpm in the 6.5 to 7.0 min fraction) immediately following the main peak of radioactivity while the non-radioactive H_2-AZA had a small shouider (5.8 min retention time) immediately preceding its main peak.

Co-chromatography of the radioactive and non-radioactive H_2-AZA on TLC revealed equivalent R_f values of 0.71 (R_f band with highest % dpm =0.72 on plate #1 and 0.70 on plate #2) under the same developing conditions (Appendices 13a, 13b).

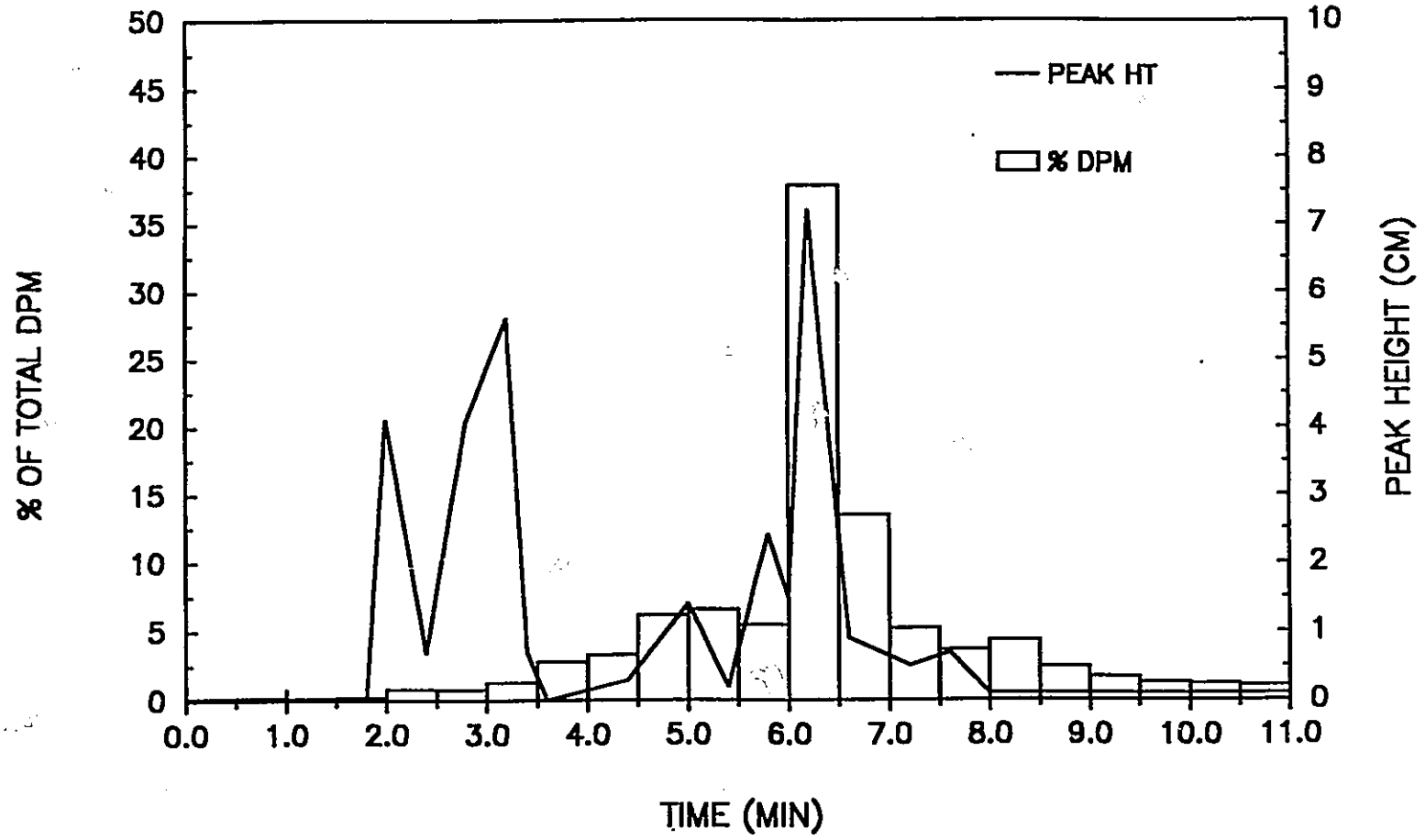
2.1.2 Uptake and Systemic Movement of $^3H_2$-Azadirachtin

Distribution of radioactivity in the corn plants following topical leaf application of $^3H_2$-AZA (s.a.= 4.384 Ci/mole) at young seedling stage is shown in Appendix 14. Approximately 90% of the applied radioactivity was recovered in the rinse of the treated leaf section (leaf 3) following the three time periods in trials 1 and 2). A calculation of the mass balance of each replicate revealed that an average of 89.3% (trial 1) and 94.6% (trial 2) of the applied radioactivity could be accounted for after a 24 hour period, 89.0% (trial 1) and 95.4% (trial 2) following a 48 hour period and 86.6% (trial 1) and 89.5% (trial 2) after 72 hours (Appendix 14).

FIGURE 24

HPLC tracing of H_2-azadirachtin and profile of radioactive counts (dpm) from collected fractions of the ^3H-labelled compound, following HPLC separation.

The H_2-AZA peak (retention time= 6.2 min) corresponds to the highest radioactive count of the fraction collected between 6.0 and 6.5 min.


PEAK HT
% DPM
% OF TOTAL DPM
PEAK HEIGHT (CM)
TIME (MIN)
0
5
10
15
20
25
30
35
40
45
50
0
1
2
3
4
5
6
7
8
9
10
0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
10.0
11.0

Examining the total adsorbed/absorbed tritiated material after 24, 48 and 72 hours revealed no increase in ad/absorption with time. However, the levels of adsorption/absorption were higher in trial 2 as compared to trial 1 (Appendix 14). The plants adsorbed/absorbed 1.82% (trial 1) and 3.13% (trial 2) of the applied radioactivity from leaf 3 after a period of 24 hours, 1.32% (trial 1) and 3.07% (trial 2) after 48 hours and 1.33% (trial 1) and 2.37% (trial 2) after a 72 hour period.

The amount of translocated label was computed by subtracting the radioactivity recovered from the treated section of leaf 3 (following the rinse) which represented a portion of the adsorbed/absorbed tritiated material, from the total counts recovered in the plant parts. The translocated material represented 44.4% (trial 1) and 55.8% (trial 2) of the ad/absorbed label after a 24 hour period (0.8%-trial 1, 1.7%-trial 2 of the total applied $^{3}H_{2}$-AZA), 17.7% (trial 1) and 10.4% (trial 2) after 48 hours (0.2%-trial 1, 0.3%-trial 2 of the total), 21.2% (trial 1) and 34.1% (trial 2) following a 72 hour period (0.3%-trial 1 and 0.8%-trial 2 of the total).

The distribution of the translocated label in the corn seedlings following the three time periods is graphically represented in Figures 25a and 25b). The highest percentage of the translocated material in trial 2 was found in the root medium (84.6%, 42.5% and 86.1%) and the roots (12.5%, 26.5% and 22.4%) of all three time periods. However, in trial 1, a high amount of the translocated label was found in mature leaves 1 and 2 (59.4%–24 hrs, 11.9%-48 hrs, 18.7%-72 hrs) compared to trial 2 (0.5%–24 hrs, 4.8%-48 hrs, 1.6%-72 hrs).

Combining the % translocated label from the roots and the root medium in trial 2 shows that 97.1% (24 hours), 69.0% (48 hours) and 87.0% (72 hours) of the total extracted translocated label moved systemically to the root area. In trial 1, 15.2% (24 hours), 62.4% (48 hours) and 33.6% (72 hours) of the translocated label was found in the root area.

FIGURE 25a

Translocation of absorbed ^{3}H-label to various parts of V-2 corn seedlings after 24, 48 and 72 hours following application of ^{3}H-azadirachtin to the 3rd leaf. Trial 1.

The % of total extracted translocated label is represented by means and s.e. of 3 replicates.
Leaf 3 includes the untreated section only.
Leaves 1, 2, 3 and 4 were cut at the level of the 1st leaf collar.
The "stem" includes all leafy material between the 1st leaf collar and the remaining seed.
The % translocated label to the root medium was recovered from the total volume of remaining medium.
The roots include nodal roots, the radicle, seminal roots, the remaining seed and the root rinse.

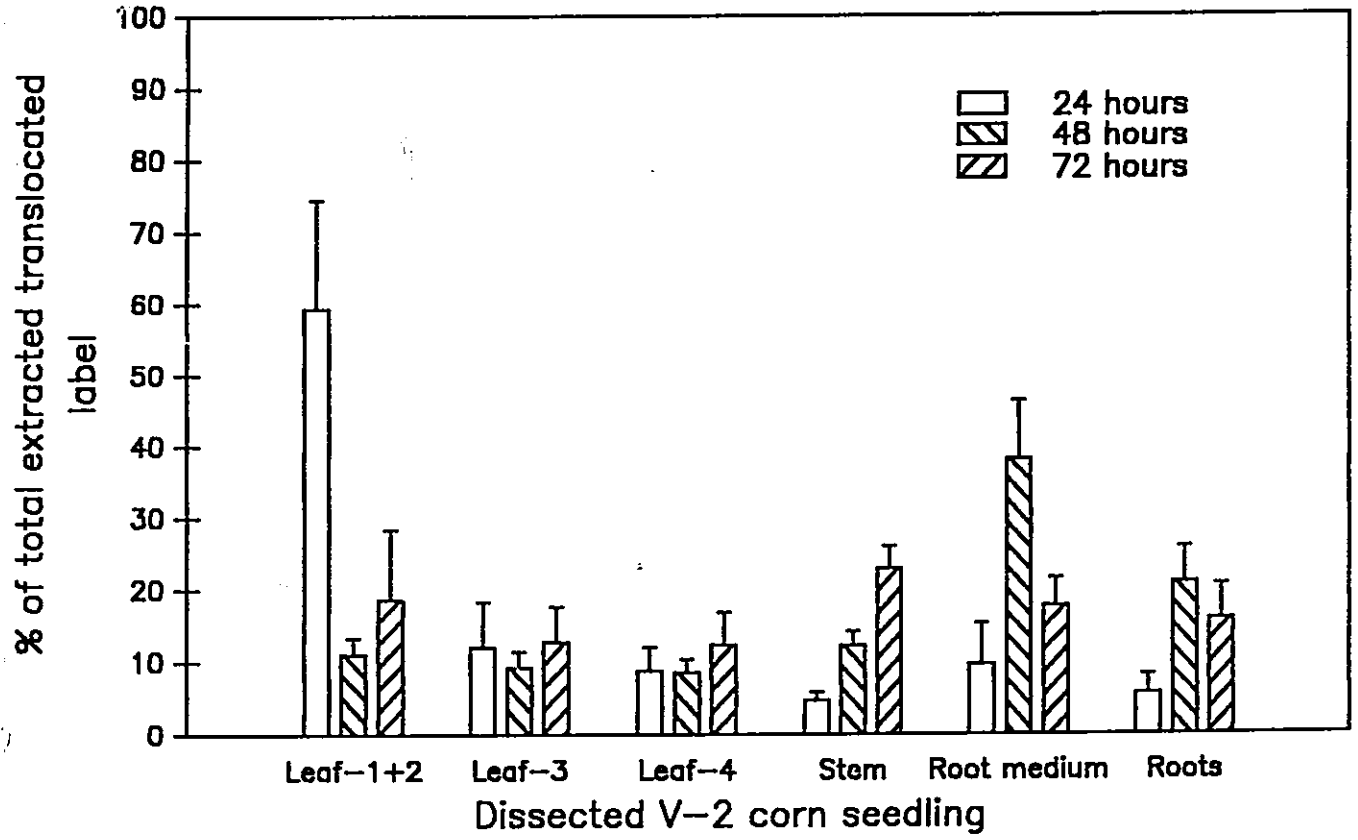
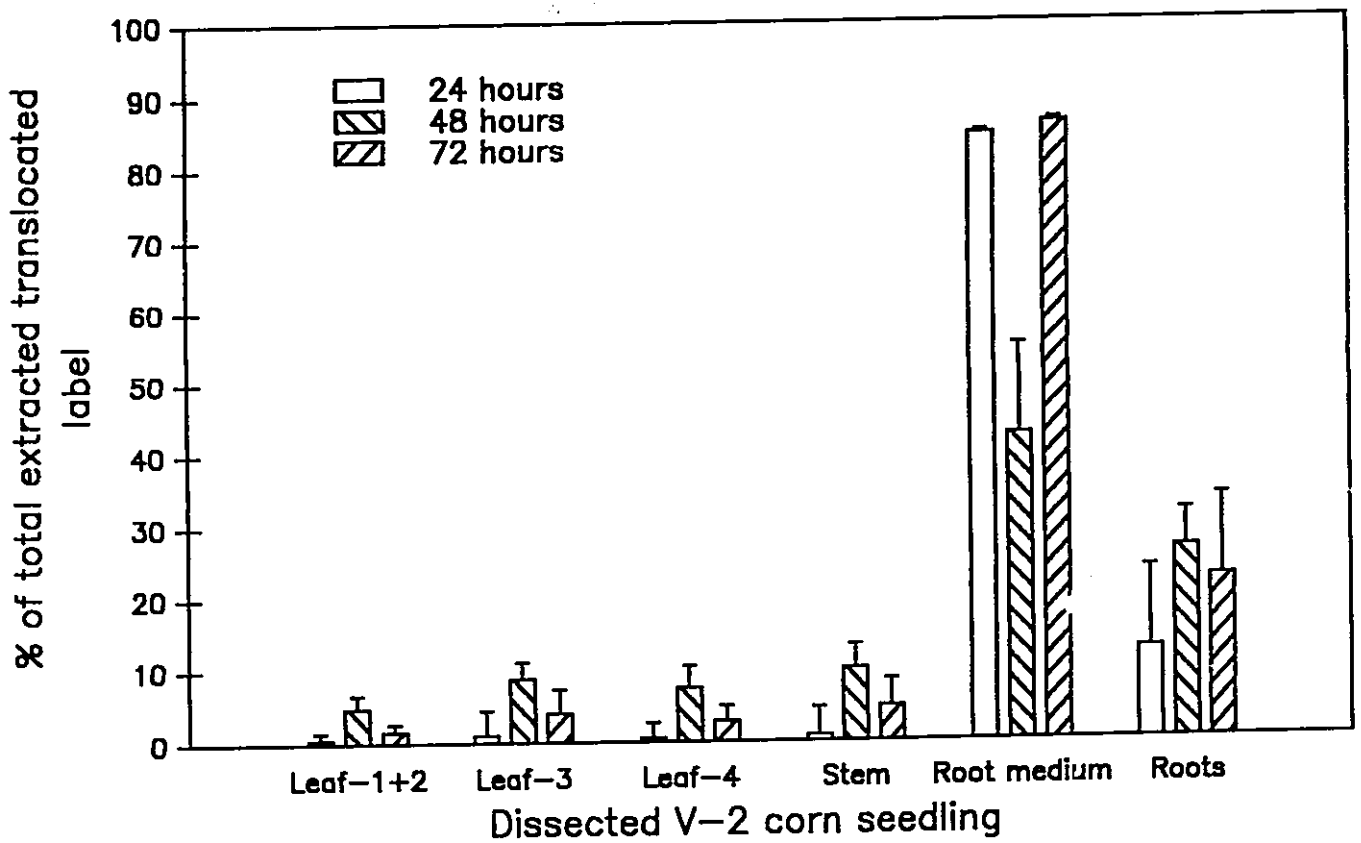

% of total extracted translocated label
100
90
80
70
60
50
40
30
20
10
0
24 hours
48 hours
72 hours
Leaf–1+2
Leaf–3
Leaf–4
Stem
Root medium
Roots
Dissected V–2 corn seedling

FIGURE 25b

Translocation of absorbed ^{3}H-label to various parts of V-2 corn seedlings after 24, 48 and 72 hours following application of ^{3}H-dihydro-azadirachtin to the 3rd leaf. Trial 2.

The % of total extracted translocated label is represented by means and s.e. of 4 replicates.
Leaf 3 includes the untreated section only.
Leaves 1, 2, 3 and 4 were cut at the level of the 1st leaf collar.
The "stem" includes all leafy material between the 1st leaf collar and the remaining seed.
The % translocated label to the root medium was recovered from the total volume of remaining medium.
The roots include nodal roots, the radicle, seminal roots, the remaining seed and the root rinse.


24 hours
48 hours
72 hours
% of total extracted translocated label
100
90
80
70
60
50
40
30
20
10
0
Leaf–1+2
Leaf–3
Leaf–4
Stem
Root medium
Roots
Dissected V–2 corn seedling

The label distribution in the 24 and 72 hour time periods was approximately 5 to 10% higher in trial 1 than in trial 2 for the untreated leaves 3, the developing leaf 4 and the stem. The 48 hour time period in both trials was comparable in all the dissected parts of the corn seedling.

2.2 Uptake and Systemic Movement of Azadirachtin in Corn Seedlings: Feeding Bioassays.

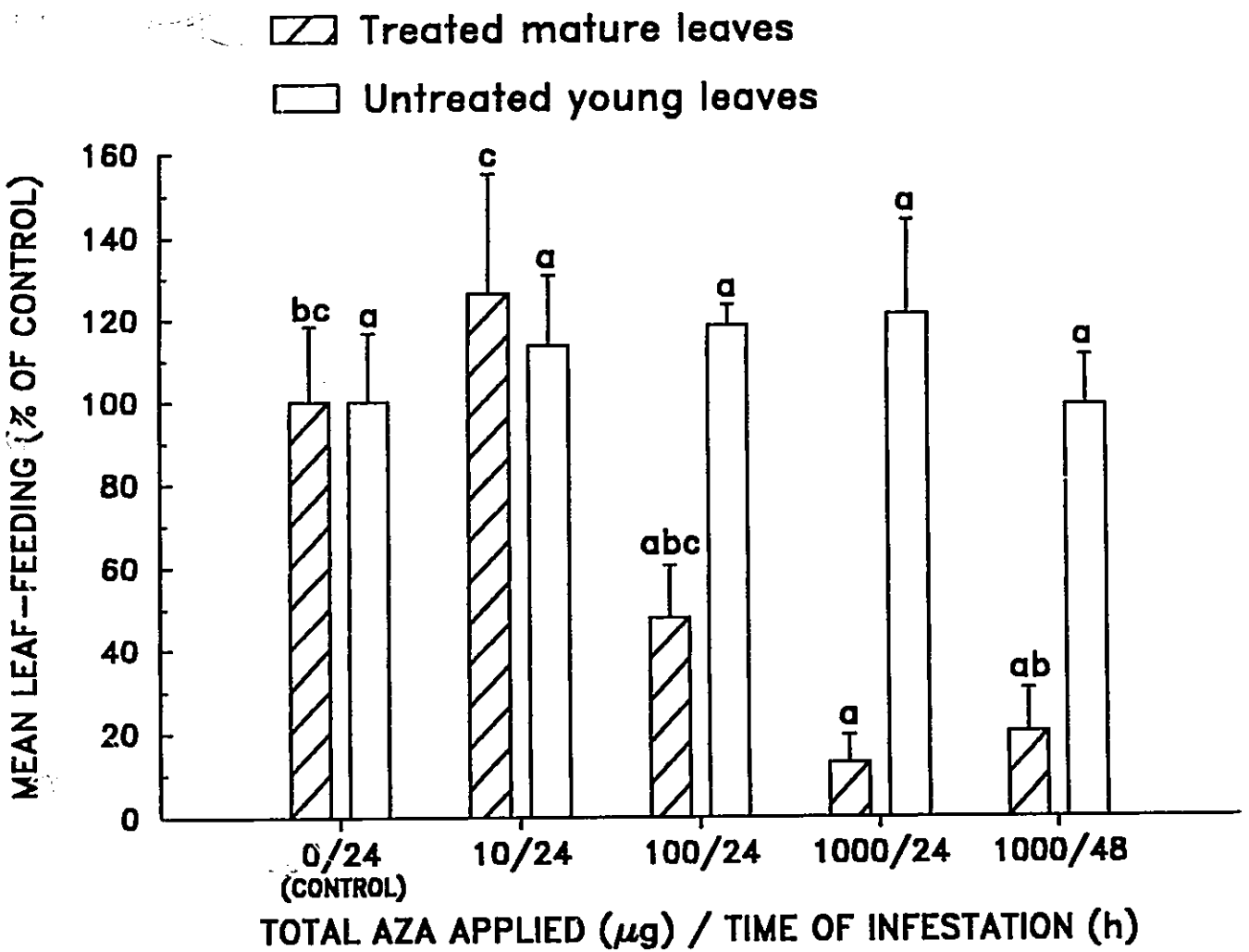
The results from the feeding choice tests did not demonstrate the systemic movement of AZA from mature to young developing leaves in corn seedlings (Figure 26). In all pre-infestation treatments where AZA (in various concentrations) was applied to two mature leaves and the corn seedlings were infested following a period of 24 and 48 hours, the untreated mature and growing leaves remained unprotected from feeding damage by naive 3rd instar larvae. Statistical analysis revealed no significant differences between the control's untreated young leaves and the AZA-test-seedlings' untreated young leaves.

The larvae markedly preferred the untreated young leaves to the treated mature leaves in the 1000 and 100 μg AZA treatments (Appendix 15). In the 1000 μg AZA/48 hour pre-infestation treatment, untreated developing leaves comprised 80% of the total area consumed by the larvae, and in the 1000 μg AZA/24 hour pre-infestation treatment, the untreated young leaves totalled 89% of the consumed area. With lower AZA concentrations, the treated mature leaves progressively became as palatable as the untreated young leaves; with 100 μg AZA/24 pre-infestation treatment 67% of the total eaten area was from the untreated young leaves and with the 10 μg treatment, this amount decreased to 42% which is approximately equivalent to control level (44%).

FIGURE 26

Mean leaf-feeding of treated and untreated leaves as a % of the control versus total azadirachtin applied to mature leaves only of young sweet corn seedlings.

AZA was tested for possible systemic movement in sweet corn seedlings (V-2 stage) after periods of 24 and 48 hrs of application, using ECB 3rd instar larvae (10 per plant, 5 replicates) in a feeding bioassay.
1000 μg= 282 ng azadirachtin mm^{-2} of leaf surface treated.
100 μg= 28.2 ng azadirachtin mm^{-2} of leaf surface treated.
10 μg= 2.82 ng azadirachtin mm^{-2} of leaf surface treated.
Means with different letters are significantly different at p=0.05 level of significance using Tukey's multiple range test.
Treated mature leaves and untreated young leaves were analyzed independently.


Treated mature leaves
Untreated young leaves
MEAN LEAF-FEEDING (% OF CONTROL)
160
140
120
100
80
60
40
20
0
bc
a
c
a
a
abc
a
a
a
ab
a
0/24
(CONTROL)
10/24
100/24
1000/24
1000/48
TOTAL AZA APPLIED (μg) / TIME OF INFESTATION (h)

The significant effects noted in these experiments were the antifeedant properties of AZA applied to leaf surfaces of corn seedlings. Examining the feeding damage of the treated mature leaves, 282 ng AZA mm^{-2} reduced mean leaf-feeding by 80% and 75% in the 24 and 48 hour pre-infestation treatments, respectively. The 28.2 ng AZA mm^{-2} treatment decreased mean leaf-feeding by 50%. However, at the lower AZA concentration (2.82 ng AZA mm^{-2}) feeding was enhanced by 30% when compared to the control.

The mean number of larvae remaining on the corn seedlings following the 72 hour feeding period was constant throughout the different treatments. Out of 10 naive 3rd instar larvae placed on each seedling, an average of 6 (+/- 1) larvae were found on the plant at the end of the experiment.

E - DISCUSSION

1.0 Field Studies: Chemical Aspects, Formulations and Efficacy of Neem Products in Control of Ostrinia nubilalis in sweet corn.

The field trials conducted between 1987 and 1989 revealed that foliage spraying of neem-based products can be an effective means of controlling sweet corn damage by the polyphagous lepidopteran Ostrinia nubilalis (Hubner). These studies also revealed important factors which appear to influence the success of neem products for controlling the ECB in sweet corn production, namely field and weather conditions, and time of application.

These trials and other related studies have revealed that the efficacy of the neem-based products depends on their phytochemical characteristics. Factors that affect the concentrations of the active principles in a neem preparation are the source of the neem kernels (type of tree from which the seeds are collected and local climatic conditions) (Ermel et al, 1987; Singh, 1987; Isman et al, 1990), the extraction procedure of the NSKE or oil (Schneider et al, 1987; Nakanishi, 1975; Nakanishi and Schroeder, 1987) and the nature of the neem formulation (Parmar et al, 1987; Isman et al, 1990). Isman et al (1990) found that AZA content was responsible for 72% to 90% of the bioactivity variations of neem preparations and could be used as a quality criterion for neem used as crop protection. Therefore, in the present study, the NSKEs used in field and lab studies were chemically analyzed for their AZA content.

1.1 Phytochemical Characteristics of Neem Products

1.1.2 Efficiency of Analytical Method

High pressure liquid chromatography was the most precise tool to determine the presence of AZA in NSKEs and its quantity or to monitor if AZA or H_2AZA crystals

were stable in storage. Isocratic elution with 60% water/40% acetonitrile as solvent system at a flow rate of 1 ml min^{-1} and absorbance measured at 220 nm permitted a clear separation of AZA from other compounds in the NSKEs (Figure 9). This solvent system, compared to 70% methanol/30% water lengthened the retention times of pure AZA and H_2-AZA to 7.2 min and 6.2 min respectively, thereby assuring a clear distinction of the two compounds.

1.1.3 Azadirachtin Content of NSKEs

Nakanishi's extraction method (Nakanishi, 1975) yielded a NSKE to be used in the field studies containing 2500 ppm (+/- 500 ppm) AZA, which can be qualified as a highly bioactive extract. Such an AZA content is comparable to the most active neem oils tested by Isman et al (1990). Isman et al found that oils containing between 3000-4000 ppm AZA were consistently the most active in the three types of bioassays using Peridroma saucia (larval growth inhibition and antifeedant activity bioassays) and Oncopeltus fasciatus (most disruption activity bioassay).

The AZA content of NSKE doubled when Nakanishi and Schroeder's procedure (Nakanishi and Schroder, 1987) was used. Exhaustive defatting of the ground neem seeds with hexane prior to the ethanolic extraction concentrated the active ingredients, yielding 5100 ppm AZA in the NSKE. The bioactivity of this extract proved to be much higher than the NSKE containing 2500 ppm in a growth and development study of Ostrinia nubilalis (discussed in a later section).

1.2 Formulating Azadirachtin and NSKE for Field Trials.

The adopted 25% methanol/75% water spray formulation of AZA and NSKE effectively blended the ingredients and ensured that the mixture was homogeneous. However, at higher NSKE concentrations (25 g NSKE L^{-1}), oily deposits would

accumulate on the surface of the mixture and the sides of the container. The emulsifiable concentrates of NSKE such as the ones developed by Safer Limited offered a consistently homogeneous product which could be easily prepared and would probably be the most compatible with a grower's operations.

1.2.1 Effect of the Environment on Azadirachtin

AZA is a sensitive, easily degradable molecule. The efficacy of the formulations used in the field might have been affected by ultraviolet radiation (Stokes and Redfern, 1982; Ermel et al 1987; Barnby et al, 1989) or by contact with water in the formulation (personal communication, Isman), the pH of the solution (Larson, 1989), and environmental factors such as temperature, rainfall, or pH of treated plant parts (Schmutterer, 1990a). Some of these factors were controlled, such as maintaining all formulations in darkened and refrigerated conditions prior to spraying, but weather conditions and exposure to sunlight following the application could not be avoided.

1.2.2 Piperonyl Butoxide

The use of piperonyl butoxide (PBO) in the AZA and neem formulations was based on reports showing that the growth disruption effects of NSKEs could be effectively synergized with PBO (Lange, 1984; Lange et al, 1984; Sombatsiri et al, 1986). Lange (1984) found that an increase in mortality of 90% could be attributed to PBO added to a NSKE (ratio 5:1 PBO:NSKE) when 4th instar Plutella xylostella L. larvae were allowed to feed on treated leaf discs for 24 hours only. However, the synergistic effect was apparent after approximately 8 days and short term feeding damage was not reduced by the addition of PBO. Similarly, in the present study, the 24 hour antifeedant trials with AZA in combination with PBO and Citowett performed on 3rd instar O. nubilalis larvae did not statistically improve the effectiveness of AZA (Figure 22). Since PBO inhibits

the enzymes responsible for the degradation of toxicants, Lange (1984) proposed that PBO might also inhibit the enzymes responsible for the degradation of growth regulators like AZA. The synergistic effect of PBO appears to lie in the long term growth disrupting activity of AZA and NSKE; this hypothesis warrants further investigation.

1.3 Artificial Infestation

The uninfested sweet corn plants suffered little damage from naturally occurring corn borers. The kernel damage cob^{-1}, the larvae found on each cob and the stalk damage were all found to be very low when compared to artificially infested plants. Thus artificial infestation was necessary to assess efficacy of field trials.

Over the 3 year period artificial infestation of corn plants was consistent. Evaluations of corn plant damage were generally consistent within similar treatments and artificially infested control plants were heavily damaged at harvest in all three seasons. However, field 1 in 1988 presented anomalies when compared to the 1987 and 1989 results. The larval population in the naturally infested cobs was ten times more abundant than 1987 and 1989 while artificially infested plants had similar populations. The population ratio of artificially to naturally infested plants was much lower than in 1987 and 1989 and field 2 of 1988 indicating that the artificial infestation was probably unsuccessful, or that the site had been contaminated from the previous season's artificial infestation. The same site was used for field 1 in 1987 and 1988, therefore the corn borer population, having overwintered as larvae in the remaining stalks, might have reinfested the newly planted corn.

1.3.1 Rationale for the Method of Infestation

Artificially infesting each plant once in the whorl and once at the ear zone resulted in a heavy borer infestation reaching up to 95 times the population level of naturally

infested plants. This technique simulated a first brood infestation (egg masses in the whorl) and more importantly for sweet corn, a second brood infestation (egg masses between the developing ear and the stalk) (Andrew and Carlson, 1976; Carlson and Andrew, 1976) since the second generation borers cause the most damage to the cobs (Hudon and Leroux, 1986b).

1.3.2 Evaluation Method

Natural infestation levels in a sweet corn field would probably never reach the levels obtained by artificially infesting each cob of each corn plant. The evaluation of the neem treatments could thus not be based on the number of intact, larvae-free marketable cobs, which is normally the producer's concern. It was necessary to evaluate the efficacy of a treatment by the damage suffered on each cob and each plant. The number of larvae found on each cob was also an important factor to evaluate the efficacy of a treatment. If the number of larvae on treated cobs remained the same as the control but damage was lowered, then an antifeedant action of the compound could be suspected. If the number of larvae was lowered, the compound could have had an insecticidal action or might have modified the behaviour of the insect causing the insect to move away from the treated site to an untreated part of the plant.

The stalk damage ratings are somewhat less important for sweet corn growers but can nevertheless reveal major effects of neem product treatments on the life cycle of the borers (section 1.6 of discussion).

Two methods used in 1987 and 1989 to evaluate stalk damage i.e. the tunnelling ratio and a stalk breakage rating were found to be highly correlated (Reid, 1988) and were therefore used interchangeably. Reid suggests that plant breakage may be due to corn borer tunnelling and stalk rot but borer tunnelling appears to be the major factor because of a stronger correlation.

1.4 Protection of Sweet Corn from ECB Damage by Azadirachtin

There are few reports of the efficacy of pure AZA used in the field to protect crops from pest damage. The reason for this remains the high cost of its extraction from neem kernels or its syntheses in the laboratory (Ley et al, 1989). Because of the high cost of AZA, the use of the pure compound as a foliage spray is not recommended.

1.4.1 Rationale for the Choice of Azadirachtin Concentrations

The present experiment with pure AZA was undertaken to determine if the putative active compound performs in field trials in a similar way to NSKE. The choice of the AZA concentrations (0.08 g ai L^{-1} or 0.008% and 0.8 g ai L^{-1} or 0.08%) was based on laboratory studies conducted by Meisner et al (1986) who found that concentrations ranging from 0.006% to 0.5% AZA completely inhibited pupation of larvae fed on treated leaves of corn seedlings. In a study of residue, pupation rate was 0 when plants were infested two days after the treatment, 11% after four days and 16% after six days following application, compared to pupation rates ranging from 48% to 53% in control plants.

1.4.2 Efficacy of Azadirachtin

The 1987 field studies revealed that pure AZA (100 g ha^{-1}, 0.8 g L^{-1}) offered some protection against the corn borer in artificially infested sweet corn (the number of damaged kernels cob^{-1}, the number of larvae cob^{-1} and stalk tunnelling were decreased by 55%, 50% and 33%, respectively, when compared to control levels). The protective action of the AZA treatments was much less dramatic in the two following years. Yearly differences in results could be attributed to varying weather conditions (1988 was an unusually dry and hot summer with frequent wind storms), or varying methods and techniques in 1987 (field study conducted by J. Apablaza). The Chapin compressed air sprayer

used in 1987 may have provided better spray coverage resulting from higher spray pressure (Radcliffe and Lagnaoui, 1990) compared to the hand-held sprayer used in 1988 and 1989.

1.4.3 Azadirachtin versus Ambush

The commercial insecticide, Ambush, applied at the recommended dosage (2X, 0.125 g permethrin L^{-1}, 15.6 g ha^{-1}, Ontario Ministry of Agriculture and Food, 1988, 1989 and 1990) controlled kernel damage and larval populations significantly better than AZA treatments in 1988 and 1989. The quick knockdown ability of permethrin reduced the number of ECB larvae in the corn plants (low number of larvae cob^{-1}, low stalk breakage ratings) thereby protecting the kernels from feeding damage. AZA offered some protection against corn borer damage via its antifeedant and growth regulating action, but the immediate knockdown effect that commercial pesticides such as the pyrethroids offer is not present with antifeedants and IGR compounds.

1.4.4. Residual Effect of Azadirachtin

AZA has low residual effects which might also explain some of the low efficacy data obtained in some of the field trials. In a field study, corn plants (whorl stage) were sprayed with solutions of up to 600 mg AZA L^{-1} (Wood, 1990). Leaves were collected from the plants 24 hours and 72 hours after treatment and were bioassayed for feeding deterrence against fall armyworm larvae. The 24 hour-treatment leaves contained enough AZA residues to prevent larval feeding but the leaves harvested after 72 hours did not deter larval feeding. The effectiveness of AZA in the field might also be reduced because of environmental factors such as photodegradation (Barnby, 1989).

An antifeedant is effective if it is taken up by the plant and systemically moved to untreated parts of the plant. Although it has been reported that when AZA is applied

to the roots of corn seedlings, bioactivity will be expressed in the shoots of the seedlings (Meisner et al, 1986), uptake through foliage is minimal. The decrease in feeding and larval populations seen in the 1987 and 1989 field studies would probably not be caused by continuous exposure to the active compounds as would be the case if they were systemically moved, but could be explained by a short period where feeding was deterred when the larvae came in direct contact with the active compound shortly after application, or ingestion of a bioactive compound causing reduced feeding, intoxication and inhibition of growth and development (Schmutterer, 1990a).

1.5 Protection of Sweet Corn from ECB Damage by NSKE

In the present study, some NSKE treatments proved to be as effective as the commercial insecticide Ambush; these results paralleled field studies of others. For example, in a study from western Ecuador, a neem seed water extract (50 g L^{-1}) and seed powder (2 g $funnel^{-1}$ in a funnel treatment of corn) were found to control all major pests of three crops with the same efficacy as Ambush: S. frugiperda and Heliocoverpa zea on corn, Mocis latipes and Oebalus ornatus on rice and Anticarsia gammatalis and Stegasta bosquella on peanut (Schmutterer, 1990b).

1.5.1 Rationale for the Choice of NSKE Concentrations

As with the AZA concentrations, NSKE concentrations (0.25% and 1.25%) were chosen on the basis of Meisner's laboratory findings. Following NSKE sprayings, sweet corn seedlings were infested with ECB larvae; after 13 days, survival was 0 on 1.0, 0.5 and 0.25% NSKE treated seedlings. A study of resdiues showed that survival of larvae feeding on 2 to 6 day old residues was significantly reduced compared to controls.

1.5.2 Effect of Application Rate and Number of Applications

No significant effect on the control of corn borer damage (Figures 15 and 16) was found by increasing the rate of application of NSKE higher than 15.6 kg ha^{-1} (1.25%) or the number of applications. Two applications 6 to 7 days apart, at a rate of 1.56 kg ha^{-1} (12.5 g NSKE L^{-1}) was as effective as two applications at a rate of 3.12 kg ha^{-1} (25 g NSKE L^{-1}).

It appears that younger larvae exposed to the antifeedant and growth regulator are the most affected while later sprayings had no significant bearing on feeding behaviour or development. Earlier sprayings might increase the chances of the borers coming in direct contact with the NSKE since the young larvae have not yet burrowed into the cob. In fact, studies have shown that early instars of Lepidoptera are the most sensitive to xenobiotics or plant allelochemicals (Brattsten, 1979; Ahmad, 1986).

A higher concentration of NSKE does, under laboratory conditions, increase feeding deterrence and growth regulation (section 3.0 of discussion). However, it seems that under field conditions, doubling the rate of application of NSKE from 1.56 kg ha^{-1} to 3.12 kg ha^{-1} does not result in better damage control.

There was significant reduction in kernel damage, borer population and stalk damage when NSKE rate of application was increased from 0.31 kg ha^{-1} to 1.56 kg ha^{-1}. A possible explanation may be that the highest NSKE concentration used did not emulsify adequately; the highly viscous material settled on the inside of the sprayer. The amount of active ingredients actually sprayed might have been less than expected.

1.5.3 Effect of Formulation

The high damage control using Safer's formulations in 1989 emphasized the importance of formulating neem products to provide proper emulsification for spray applications. The active ingredients in both formulations were adequately delivered to the

plant since there was total emulsification. Both products contained less AZA (19 mg L^{-1}) than most NSKE-formulations tested (6.25 mg to 62.5 mg L^{-1}) but were significantly more effective at reducing borer damage. This further supports the results indicating that, under field conditions, a higher active ingredient concentration does not necessarily increase efficacy of the product.

No large differences were found between the neem formulation which contained 19 mg AZA L^{-1} and the neem + pyrethrin formulation with 19 mg Aza + 650 mg pyrethrin L^{-1}; in both treatments, damage ratings were significantly lower than controls. Perhaps differences in efficacy between these two formulations would appear at different concentrations.

1.5.4 Effect of Exposure to Sunlight

In order to minimize the UV exposure of the NSKE formulation following application, a treatment consisting of dusk sprayings was tested; efficacy might possibly be increased if uptake and translocation of the active compounds occurred before daylight. Results indicated no improvement in damage control. Sunlight does not seem to be a significant factor contributing to reduced efficacy of the NSKE although numerous reports show a decrease in bioactivity of residual compounds exposed to UV radiation in laboratory trials.

1.5.5 Azadirachtin Versus NSKE

The 1987 and 1989 results clearly indicate that NSKE was equally or more effective at controlling corn borer damage than pure AZA. The most effective NSKE application rate (1.56 kg ha^{-1}, 12.5 g L^{-1}) containing 31.3 mg AZA L^{-1} reduced kernel damage, borer population and stalk damage more effectively than 800 mg AZA L^{-1}. NSKE contains AZA, the most important active principle, as well as a considerable

number of other compounds (Jones et al, 1989) showing antifeedant activity as well as growth regulation. It is probable that these compounds act synergistically or additively. The oils in the extract may also contribute to stabilize the active ingredients through antioxidants and UV- absorbing substances (Isman et al, 1990).

However it is also clear that pure AZA acts in a similar way to the NSKE which confirms its role as a principal active agent in NSKEs.

1.5.6 Ambush Versus NSKE

Conventional application of Ambush (15.6 g ha^{-1}, 125 mg permethrin L^{-1}) and NSKE (1.56 kg ha^{-1}, 12.5 g L^{-1}) at their optimal rate of application, consisted of 2 sprayings at 6 to 7 day intervals following the observation of pinhole feeding by ECB larvae on corn leaves. Both treatments significantly reduced borer damage compared to their respective controls; Ambush was slightly more effective than the NSKE in protecting sweet corn corn from the ECB. A look into various properties of the two pesticides will shed some light on advantages and disadvantages to the use of both products.

1) The different modes of action of the two pesticides might explain the observed differences in efficacy: the ai of Ambush, permethrin, is a contact neurotoxin (Pedigo, 1989) while the ai of NSKE, AZA, is an antifeedant and IGR. The contact insecticide quickly kills the insect while the antifeedant/IGR requires a longer period of time to affect the insect thereby permitting a longer feeding period on NSKE treated corn.

2) Both NSKE and Ambush have low mammalian toxicity. The acute oral LD_{50} of Margosan-O (a registered neem product) in rats is greater than 5 ml kg^{-1} body weight, the LD_{50} of methanolic NSKE extract in rats in doses of 5000-8750 mg kg^{-1} body weight is nil as well as an AZA enriched neem extract of 5000 mg kg^{-1} body weight (Larson, 1990). The oral LD_{50} values for permethrin in rats are 430-4000 mg kg^{-1} body weight (Meister, 1991). The acute dermal toxicity test (LD_{50}) on albino rats for Margosan-O was in excess of 2 mg kg^{-1} while an acute inhalation study on albino rats (LC_{50}) was in excess

of 43.9 mg L^{-1} h^{-1}, the limit of the test (Larson, 1990). Acute dermal toxicity (LD_{50}) for permethrin is reported to be >4000 mg kg^{-1} in rats while acute inhalation (LC_{50}) in rats is >23.5 mg L^{-1} of air (Meister, 1991).

3) Environmental guidelines for using Ambush warn that permethrin is highly toxic to fish and bees in laboratory tests but low in field tests; it is also "practically nontoxic to birds" (Meister, 1991). Margosan-O, in similar studies, showed low toxicity to fish: LC_{50} in Rainbow trout= 8.8 ml L^{-1} water, and the 96 hour of no-observed effect was 5 mg L^{-1}; 96 hour LC_{50} in Bluegill sunfish= 37 mg L^{-1} and a no-observed-effect 96 hour level of 20 mg L^{-1}. Margosan-O administered as a direct contact chemical using field dosages up to 4478 ppm ai ha^{-1} was found to be harmless to honeybees, a dosage well above the recommended level of 20 ppm for a common pest, the gypsy moth (Larson, 1990). Avian dietary LC_{50} for Bobwhite quail and Mallard ducks were in excess of 7000 ppm. The ducks and quails, given a basal diet with additions of Margosan-O ranging from 1000-7000 ppm, remained active and healthy during the test and recovery period.

4) Pesticide residue studies of both NSKEs and permethrin have shown that they remain active for approximately 5 to 7 days (Meisner et al, 1981; Meisner et al, 1985; Worthing and Walker, 1987; Caunter, personal communication; Ontario Ministry of Agriculture and Food, 1988, 1989, 1990).

5) Resistance build-up to targeted insect pest occurs with the use of pyrethroids; it is a well-known phenomenon observed in the Colorado potato beetles (Radcliffe and Lagnaoui, 1990). Margosan-O has shown no resistance build-up to targeted insects in over 2 years of continuous testing. The wide diversity of active ingredients in NSKEs is most probably reponsible for delaying or stopping resistance build-up; it is more difficult for an insect species to acquire or develop detoxifying mechanisms to a diversity of toxic compounds than to only one active ingredient such as permethrin in Ambush.

The use of NSKEs and neem products such as Margosan-O over the use of pyrethroids, such as Ambush, seems justified when long-term factors are considered. The sweet corn grower would profit from the use of Ambush over NSKEs on short-term basis; his crop-yield would probably be higher. But if factors other than strictly economical ones are considered, such as the effect of a product on non-target organisms and resistance build-up in a target pest, the use of neem products is justified.

1.5.7 Effect of Time of Application of NSKE

Traditionally, most sweet corn growers apply insecticides on a weekly basis to control ECB starting in mid-June, shortening the spray interval to 3-4 days in August. They do not take into account whether adults are present (Ferro and Fletcher-Howell, 1985). Because of the public's growing concern about the indiscriminate use of pesticides, reducing the amount of pesticides applied to sweet corn while effectively controlling corn borer damage has been the goal of integrated pest management programs. The Ontario Ministry of Agriculture and Food (1988, 1989, 1990) recommends the initiation of a spray program based on the presence of pinhole feeding and detected adults through field inspections.

However, leaf feeding indicates that eggs have hatched and larvae are starting the migration towards the cob. Ideally, if approximate times of the ECB adult flights could be indicated in regions of heavy corn borer infestations, pesticide applications could be reduced; this can now be achieved based on behavioral studies. Numerous studies have shown that flight and ovipositional activity of adult moths could be efficiently monitored and used in pest management (Hudon et al, 1989). More recent behavioral studies on adult flight and mating have shown that peak oviposition on corn is closely associated with peak mating which can be determined based on the colour and degree of depletion of the spermatophore and depletion of the ovaries (Showers et al, 1976).

NSKE was tested as a "pre-ovipositional" treatment by spraying the corn plants prior to the infestation; the emerging larvae might then come immediately in contact with the compounds, reducing feeding and altering growth and development at the earliest instar. Mortality of newly hatched ECB larvae can vary from 1-41% while the annual incidence of mortality from all factors averages 98.7% (Hudon and Leroux, 1986b); any factor increasing early instar mortality will have a significant effect on decreasing later instar populations.

Results strongly support the high efficacy of NSKE as pre-ovipositional treatments since kernel damage control and larval populations on the cobs of plants treated with 1.56 kg NSKE ha^{-1} in pre-infestation sprayings were comparable to Ambush treated corn in both 1988 and 1989 trials. Compared to controls, feeding damage was reduced up to 73% in 1989 and the number of larvae per cob by 56%; with Ambush treated corn, the feeding damage was reduced by 67% and the number of larvae per cob by 50%. Adding 2 or 3 conventional post-infestation sprayings did not markedly improve the efficacy of the treatments. In a similar study with Colorado potato beetle, Radcliffe and Lagnaoui (1990) found that mortality levels were high in larval populations hatching on neem-treated foliage. The timing of the NSKE application therefore seems to be the most critical factor in efficient control of the ECB with a minimal number of spray applications.

1.5.8 The 1988 Season

In the 1988 field study (a repetition of the 1987 study), the NSKE treatments (1.56 kg ha^{-1} and 0.31 kg ha^{-1}) did not provide corn borer control. The poor efficacy of the NSKE might reflect the significant effects of exceptional environmental conditions (high temperatures, dry conditions) on the activity of neem compounds. The soil conditions of the field might not have been optimal thereby increasing the susceptibility of the sweet corn to corn borer damage; the neem products might have reduced feeding but the effects

were masked by the plant's higher susceptibility. The inexperience of the researcher and assistants might also have contributed to poor results: unequal infestation, dissimilar techniques in evaluating kernel damage.

1.6 General Effects of Neem Products on Kernel and Stalk Damage in Relation to Larval Populations.

1.6.1 Rationale for the "Calibration Curve" Method

A qualitative approach to the effects of neem products on the ECB in sweet corn may shed some light on the dynamics of its use in crop protection (Figures 17(a)–21(b)). A technique developed by Blau et al (1978) was modified to evaluate the feeding inhibition and toxic action of neem products in the field. In their laboratory study, Blau et al wanted to distinguish between feeding inhibition and toxic action of a glucosinolate on three Lepidopterans. A "calibration curve" was obtained for the effect of varying consumption rate on larval growth rate in the absence of supplementary glucosinolate. They reasoned that plots of growth rate versus consumption rate for larvae fed supplementary glucosinolate in a diet would fall below the calibration line if the compound was toxic but if the compound inhibited feeding, the data point should fall on the calibration line.

From the present field data, the following pairs of variables: mean larvae cob^{-1} and mean damaged kernels cob^{-1}, mean larvae cob^{-1} and mean tunnelling ratio, mean larvae cob^{-1} and mean stalk breakage, were highly correlated and the regression line obtained from control plots could parallel Blau's "calibration curve".

1.6.2 Effects of NSKE and Azadirachtin on Feeding and Stalk Damage

The antifeedant action of both AZA and NSKE may be responsible for lowered kernel damage/cob seen in 44% to 89% of the treatments (field 1 in 1987, field 2 in 1988,

fields 1–2 1989) for a certain larval population (i.e. treatments falling below the calibration curve). However, unlike Blau's study, feeding inhibition cannot be distinguished from toxicity of the different neem product treatments. The reduction in feeding may not only be due to feeding deterrence but also to toxic effects of the compounds.

A lowering of the number of larvae found on the cobs compared to the controls may be the result of insecticidal action or deterrence. For a same low number of larvae found on the treated cobs, stalk damage (tunnelling ratio or stalk breakage rating) is increased compared to control plants (treatments above the "calibration curve"). It is highly probable that some of the neem product treatments modified the feeding behaviour of the ECB larvae by deterring them from entering the cob and precipitating an early migration to the stalk. Since systemic movement of the compounds from the treated areas (whorl, cobs) to the stalk is probably minimal, feeding can resume in the untreated stalk if the toxic effect of the neem product is not persistent or if larvae were exposed to only a small amount of the active compound.

Stalk damage by ECB larvae is not critical in the production of sweet corn, unlike grain corn where harvesting by combine becomes impossible because of stalk breakage. Sweet corn is harvested earlier than grain corn (before ECB migration towards the stalk) and protecting the cobs is the most important factor. Utilization of neem products in the production of grain corn would not be recommended since stalk protection is an important aspect of its production.

1.6.3 Phagostimulant Effect of Neem Products

An important aspect to consider when using feeding inhibitors in crop protection is a possible reversal of the desired action by a compound. The effect of a treatment will depend on the concentration of material used and in some cases low concentrations are

phagostimulatory rather than inhibitory (Chapman, 1974). This effect seemed prevalent in field 1 of the 1988 season. Only 6% of all treatments protected the corn cobs from corn borer damage; 94% of the treatments fell above the calibration curve indicating increased feeding. This phenomena might be due to a phagostimulant effect. Because of the unusual environmental conditions, the amount of residual active compounds might have been substantially lowered compared to the other field sites.

2.0 Laboratory Leaf-Feeding Bioassay of Azadirachtin as an Antifeedant against O. nubilalis

2.1 Efficiency of the Method

The petri plate system used in the no-choice leaf-feeding bioassays permitted observation of differences between the effects of low concentrations of AZA. There are a number of advantages to this technique for testing the antifeedant effect of compounds;
1) The insect is offered a natural substrate comparable to the one encountered in the field,
2) The exposed leaf surface does not include a wounded area. Lewis and Van Emden (1986) warns that wound reactions occurring in a leaf disk can give results quite different from those obtained for whole leaves.
3) The leaf section remains turgid and palatable (no desiccation).
4) A known amount of compound can be applied over a known surface area. Results obtained from this method can be replicated but variability remains high. Appendix 12 shows the high variability among the replicates of each trial. Although care was taken to select larvae of the same weight and instar, and similar leaf material, feeding patterns will vary because of genetic or behavioral differences in the corn borer population.

2.2 Antifeedant Effect of Azadirachtin

The term antifeedant refers to a compound which will inhibit feeding temporarily

or permanently after an insect has tasted it, or the insect, without any contact, will be repelled by the compound (Klocke et al, 1988). It does not distinguish between deterrence (the insect, having bitten and tasted the material, will be deterred from further feeding) and toxicity (the insect will avoid feeding following ingestion because of intoxication).

Although mean leaf-feeding by early 3rd instar larvae on 100 ppm AZA (or 7.2 ng AZA mm^{-2}) was statistically different from the control, the leaf was only protected at 40% (60% mean leaf-feeding as a % of the control). O. nubilalis larvae might not be as sensitive to AZA as other insect pests. For example, the most sensitive insect is the desert locust (Schistocerca gregaria). Feeding by 5th instar larvae is completely inhibited when offered sucrose filter paper treated with 0.04 ppm AZA, corresponding to approximately 1 ng cm^{-2} (Butterworth et al, 1971). In a 48 hour leaf disk bioassay with 3rd instar larvae of the fall armyworm (Spodoptera frugiperda), Kubo and Klocke (1982) reported a PC_{95} value (concentration at which the limonoid offers 95% protection of the plant material) of less than 1 μg AZA $disk^{-1}$. The sensitivity of tropical insects to AZA may be due to selective pressures leading these species to develop sensory mechanisms to avoid feeding on plants containing toxic limonoids. Since European and North American species like O. nubilalis have not evolved with such plants which are restricted to tropical and sub-tropical areas, the insects would not have been under any selective pressure to develop avoidance mechanisms (Arnason et al, 1985).

A feeding study (Arnason et al, 1985) established a PC_{50} value of 24 ppm and a PC_{95} value of 790 ppm for 3rd instar O. nubilalis. The leaf-disk method used might account for the discrepancies with the present study. In Arnason et al's study (1985), leaf-disks were cut from the leaf, AZA was applied on both sides, and the disk was placed with one larva in a vial. The leaf-disk might not have been as palatable because of wound reactions or desiccation. Arnason et al (1985) also found neonate larvae more

sensitive to the antifeedant effects (PC_{50} = 3.5 ppm) which would support field results where low kernel damage and larval populations were obtained with early (pre-infestation) sprayings.

The low sensitivity of the test might also be a result of poor detection of the antifeedant. Canney and Gardner (1988) suggest that when larvae are reared on a meridic diet, their taste sensillum pores become occluded by a thick substance which they exude in profusion compared to wild or corn-reared larvae.

The increased consumption at 1 ppm AZA (or 0.072 ng AZA mm^{-2}) might indicate a phagostimulatory response (Figure 22).

2.3 Effect of PBO and Citowett

When tested separately, PBO, a polysubstrate monooxygenase (PSMO) inhibitor (Casida, 1970; Metcalf, 1967; Liu et al, 1984), and Citowett, a sticker, had no substantial effect on feeding behaviour of O. nubilalis 3rd instar larvae. PBO combined with Citowett seemed to enhance feeding slightly (Appendix 12).

The treatment of AZA in combination with PBO and Citowett showed a slight increase (not statistically significant) in feeding deterrence compared to AZA alone. However, it is unlikely that a synergistic effect of PBO with AZA would be responsible for the decrease in feeding after only a 24 hour period. Lange (1983) found that a synergistic effect of PBO with neem extracts tested on P. xylostella became apparent only after 8 days.

The use of stickers such as Citowett is often recommended, especially in the case of antifeedant compounds to prevent wash-off by rain. However, they should be used with caution since some stickers have been shown to reduce the effectiveness of the inhibitors (Chapman, 1974).

3.0 Neem Extracts: Their Growth Regulating and Insecticidal Properties.

Of all the insect species affected by neem products, lepidopterans are probably the most sensitive in terms of the growth regulating properties of the phytochemical (Schmutterer, 1990a). In the present study, a concentration of 10 μg NSKE g^{-1} diet fed continuously to O. nubilalis was larvicidal and concentrations as low as 3 μg g^{-1} diet significantly reduced larval, pupal and adult weights, increased the larval period, the pupal period, decreased adult emergence and caused wing deformities. In similar studies, 5th instar larvae of the tobacco hornworm Manduca sexta fed on a diet containing 5-50 ppm neem seed extract turned dark and died within 7 days while those on diet with concentrations of 1 and 2 ppm fed less actively and did not gain weight, had delayed metamorphosis and disturbances during moults (Haasler, 1984). The cabbage looper, Trichoplusia ni, and the beet armyworm, Spodoptera exigua, fed a diet with neem seed extract at 0.02, 0.2 and 2.0% showed prolonged development, inhibition of pupal formation and mortality in all larval stages (Prabhaker et al, 1986).

3.1 Azadirachtin Content of NSKE: A Critical Factor

The AZA content of different NSKEs will vary greatly and should be specified since biological activity of the NSKE depends mostly on this factor (Isman et al, 1990). The exhaustive defatting in Schroeder and Nakanishi's method (1987) of extracting NSKE from neem kernels with organic solvents concentrated AZA in the final extract and doubled the amount of the active ingredient compared to Nakanishi's initial extracting method (Nakanishi, 1975) (Figure 9). O. nubilalis larvae feeding on diet containing the higher concentration of the active ingredient in the NSKE (1987 method) at 3, 10, 30, 100 and 1000 μg mL^{-1} containing 16.6, 50, 166.6, 500, 5000 ng AZA mL^{-1}, respectively, consistently showed less weight gain than the ones feeding on diet with the lower NSKE-AZA content (1975 method) at the same concentrations but containing 8.3, 25, 83.3, 250,

2500 ng AZA mL^{-1}. Mortality as well as larval and pupal periods were all increased in larvae fed diet with the more concentrated NSKE.

3.2 Importance of Other Bioactive Compounds Present in NSKE

AZA has been the most investigated compound extracted from neem for its effect on the insect life cycles and physiology (Arnason et al, 1985; Rembold et al, 1984; Rembold et al, 1987; Koul et al, 1987; Schluter et al, 1985; Rembold, 1987; Barnby and Klocke, 1990; Penner et al, 1988; Dorn et al, 1987; Rembold, 1989; Sieber and Rembold, 1983; Bidmon et al, 1987) However, a variety of other limonoids can be found in neem extracts and have been shown to reduce feeding, growth and development in Lepidopteran species (Schoonhoven, 1982; Kubo and Klocke, 1986; Arnason et al, 1987). These compounds probably play a considerable role in the insecticidal properties of neem extracts or in modifying larval growth and development. AZA incorporated in the diet at 10 μg g^{-1} diet caused 100% mortality in O. nubilalis larvae (Arnason et al, 1985) while 10 μg NSKE g^{-1} diet with 25 ng AZA g^{-1} diet also caused 100% mortality in the same insect under the same test conditions. The combination of AZA with the bioactive limonoids may have an additive or possibly a synergistic effect.

Arnason found that the consumption index was not altered by AZA (Arnason et al, 1985) or two limonoids (Arnason et al, 1987) in nutritional indices studies on O. nubilalis and postulated that the antifeedant effect of the compounds might be masked by the meridic diet. Antifeedant compounds of NSKE might be present and may add to the IGR activity since other bioactive ingredients are present in neem seeds. This can possibly be supported by the quick onset of minimal weight gain compared to controls in larvae feeding on 100 μg NSKE g^{-1} diet with an AZA content of 250 ng g^{-1} diet after only 4 days. In Arnason's study with pure AZA, control larvae and those fed 1 μg AZA g^{-1} diet were of identical weight after 5 days. Balandrin et al (1988) identified 25 volatile

organosulfur compounds from neem seeds and showed that di-n-propyl disulfide, the major volatile constituent of neem seeds (75.7%) was larvicidal to three species of insects. An antifeedant effect may possibly be attributed to the presence of these volatile sulphur compounds responsible for the strong, garlicky odour of neem seeds.

3.3 Effect of NSKE on Larval Growth

Reduced larval growth could then be attributed to reduced feeding due to a possible antifeedant effect in the first days of the experiment, and toxicity in later stages of larval growth. Arnason's nutritional indices had shown that AZA probably affected metabolism following digestion (Arnason et al, 1985). Reduced weight gain has been recorded in many insect species fed neem products. For example, tobacco hornworm larvae feeding on a diet containing 1 and 2 ppm neem extract showed reduced feeding activity and weight gain (Haasler, 1983). O. nubilalis fed a sublethal dose of AZA (0.1 ppm) in the diet had a maximal larval weight of 104.5 mg compared to control larvae weighing 110.8 mg (Arnason et al, 1985).

3.4 Effect of NSKE on Pupae and Adults

A sublethal dose of 3 μg NSKE g^{-1} diet increased the time to pupation and adult emergence, decreased pupal and adult weight, altered sex ratios, caused pupal deformities, wing malformation in adults and an inability in adults to emerge from the pupal case. Such effects of neem products have been noticed in many insect species. For example, Epilachna varivestis (the mexican bean beetle) fed neem products could not emerge from the pupal skin (Schmutterer, 1990a). Manduca sexta larvae feeding on diets containing 1 and 2 ppm neem extract did not pupate or moulted into malformed, nonviable pupae. Neem extract (1000 ppm) topically applied to M. sexta larvae resulted in malformed pupae or pupae that developed into adults with crippled wings and loose proboscis

(Haasler, 1984). Injection of AZA into larvae of the blowfly, Calliphora vicina increased the time to pupation, reduced pupal and adult weights, and inhibited adult emergence (Bidmon et al, 1987).

The effects of NSKE on the larval development of O. nubilalis suggest an influence of the bioactive compound on the insect's hormonal balance. The exact mode of action of neem derivatives has not yet been determined but based on the results of the numerous studies, AZA, the main active ingredient influences the hormonal system (Schmutterer, 1990a). A study of the effect of AZA on the endocrine system of Bombyx mori has suggested more than one site of action; the effect might be on the central nervous system and would "depend on the balance of developmental hormones at the time of treatment" (Koul et al, 1987). It must be mentioned, however, that starvation due to the ingestion of a toxic compound or a feeding deterrent can be factor that will affect the hormonal system in insects (Slama, 1978).

3.5 Insecticidal Properties of NSKE

Different biological effects of NSKE on O. nubilalis depend on the NSKE concentration in the diet:

1) A lethal effect if fed in concentrations of 10 $\mu g\ g^{-1}$ diet or more, with an AZA concentration of more than 25 $ng\ g^{-1}$ diet.

2) A sublethal effect marked by developmental abnormalities if fed in concentrations of 10 $\mu g\ g^{-1}$ diet or less with an AZA concentration of less than 25 $ng\ g^{-1}$ diet. The lethal effect of NSKE may possibly be attributed to the combined effects of partial or total starvation and toxicity following ingestion (Schmutterer, 1988; Arnason et al, 1985) since AZA has been described to act first as a stomach poison (Schmutterer, 1988). Starvation might result from the phagodeterrency of NSKEs' bioactive compounds such as organosulfur compounds combined to the toxic action on the insect's metabolism.

4.0 Uptake and Translocation Studies

4.1 H_2-Azadirachtin and $^3H_2$-Azadirachtin

The derivative H_2-AZA has been shown to have the same antifeedant potency, to elicit similar metamorphosis-inhibiting and growth regulating activities as AZA (Barnby et al, 1989; Rembold, 1989; Yamasaki and Klocke, 1987; Rembold, 1989). The hydrogenation of the 22,23-double bond present in natural AZA can even result in a slight increase in biological activity (Rembold, 1987). For example, the LC_{50} values for AZA and 22,23-H_2-AZA in a bioassay using first instar H. virescens were 0.80 (0.46–1.39) ppm and 0.47 (0.32-0.67) ppm, respectively, while the EC_{50} values were 0.07 (0.05-0.10) ppm for AZA and 0.08 (0.04-0.15) ppm for 22,23-H_2-AZA (Yamasaki and Klocke, 1987). With Epilachna varivestis bioassay, the LC_{50} values for AZA and 22,23-H_2-AZA were 1.66 ppm and 1.26 ppm, respectively (Rembold, 1989).

It is now well established that the hydrogenated form of AZA is a much more stable molecule (Barnby et al, 1989) and will retain biological activity for longer periods of time. Barnby et al found that the derivative 2^1, 3^1, 22, 23-tetrahydro AZA was highly stable after 200 hours of exposure to UV light and prevented pupation whereas non-hydrogenation analogues lost their activity.

The chemical structure of the two molecules varies only by the addition of two hydrogen atoms at the carbon-carbon double bond at C–22,23. Chromatography (HPLC and TLC) of AZA and H_2-AZA revealed a slight increase in the polarity of the latter: R_{fAZA} = 0.71, $R_{fH2\text{-}AZA}$ = 0.57; Retention time AZA = 7.2 min, H_2-AZA= 6.2 min). This factor might influence the uptake of the molecules through the epicuticular and cuticular layers of leaf surfaces and their translocation to other parts of the plant (Baker and Hunt, 1988). Although AZA and H_2-AZA are close analogues, their fate in foliar uptake and systemic movement might differ.

Chromatography (HPLC and TLC) of the tritiated form of 22,23-H_2-AZA suggests

that both radioactive and non-radioactive compounds had similar purity and chemical behavior (Figure 24). HPLC retention time and chromatograph profiles, as well as R_f values (Appendix 13) were identical for the radioactive and non-radioactive H_2-AZA. The use of the tritiated compound 22,23 $^3H_2$-AZA as a tracer for H_2-AZA is thus justified.

4.2 Foliar Uptake of ^{3}H-Compound

Uptake of a chemical via the leaves of a plant following foliar application refers to the proportion of the applied chemical which is not recovered following a wash with an organic solvent (Baker and Hunt, 1988). In the present study, uptake of the tritiated material following application of $^3H_2$-AZA to the third leaf of sweet corn seedlings was minimal. More than 85% of the applied radioactivity was recovered in the methanol/water rinse 24, 48 or 72 hours post-application.

4.2.1 Factors Affecting Foliar Uptake of Pesticides

Factors that affect the uptake of the active ingredient (ai) by plant foliage can be divided into two major groups, the chemical aspects of the ai and the structure and morphology of the leaf surface (Baker and Hunt, 1988; Price and Anderson, 1985; Stevens and Baker, 1987). Although these factors must be considered separately, they are nevertheless closely related in determining uptake of pesticides.

4.2.1.1 Chemical Aspect of the Active Ingredient

Results from published reports are conflicting. Stevens and Baker (1987) and Baker and Hunt (1988) found relationships between uptake patterns, water solubility and lipophilicity of the ai while Price and Anderson (1985) reported weak correlations with molecular properties of the molecules. For example, Price and Anderson tested eight pesticides covering a wide range of lipophilicity and found all of them to be well taken

up by maize leaves while Baker and Hunt (1988) found varying uptake rates by maize leaves of pesticides with different partition coefficients and water solubility. Stevens and Baker (1987) reported low uptake rates for apolar 2,4-D and prochloraz (<17%) as well as for polar compounds like glyphosate (<6%) in maize.

Uptake of the polar maleic hydrazide (a herbicide with growth regulating properties) in maize leaves was less than 2% following 24, 48 and 72 hours periods (Baker and Hunt, 1988). Uptake of the $^{3}H_{2}$-AZA tracer was less than 3.13% over the same time periods of 24, 48 and 72 hours (Appendix 14, label ab/adsorbed by plant, % of total) The low uptake of the $^{3}H_{2}$-AZA tracer supports Baker's findings that water soluble compounds have lowest uptake rates. H_{2}-AZA can be qualified as a polar molecule since it is highly soluble in water and all polar solvents.

Baker and Hunt (1988) suggest that uptake might be low for water soluble compounds because of the formation of large needle (5 to 20 μm) as the compound crystallizes on the surface of the leaves.

The greater uptake of lipophilic versus water soluble pesticides suggests that if two routes of uptake through the cuticle exist for polar and apolar molecules, a polar porous pathway and a lipophilic diffusion route, the former is relatively inefficient.

4.2.1.2 The Leaf Surface

The composition of the leaf surface is likely to have an influence on foliar uptake of pesticides. The low uptake of the polar $^{3}H_{2}$-AZA might also be due to the morphology of the epicuticular wax layer on the surface of maize leaves.

Studies have revealed that uptake of pesticides is always greater in a waxy leaf (ex. strawberry and rape) compared to non-waxy leaves (ex. sugar beet, maize) (Baker and Hunt, 1988; Stevens and Baker, 1987). Stevens and Baker (1987) have compared the properties of adaxial leaf surfaces of eleven plant species. Maize leaves were described

as glaucous with an epicuticular wax thickness of 3.8 μg cm^{-2}, a relatively low amount compared to a waxy leaf like strawberry (17.8 μg cm^{-2}), and an amorphous cuticle less than 10 nm thick (a very thin cuticle compared to all the 10 remaining species studied which had cuticles more than 25 nm thick). The highest uptake rates were found to be in leaves with the heaviest epicuticular wax deposits and thickest cuticle. Thus, lipophilic leaf surfaces are probably major sinks for apolar chemicals, but regardless of their thickness, they become barriers to the absorption of polar compounds. The thin cuticle of maize leaves, which is relatively more polar than the epicuticular layer might have contributed to the small amount of uptake of the polar ^{3}H-tracer that did occur.

Different areas of the leaf have been reported to have varying uptake rates. These areas of varying lipophilicity might have contributed to the small amount of uptake of the ^{3}H-tracer. For example, the mid-rib of maize leaves is less hydrophobic than most of the leaf surface (Stevens and Baker, 1987) and would be a preferential site of the penetration of a polar compound such as H_2-AZA, but by applying the solution to the upper part of the leaves, the greater part of the mid-rib was avoided. Stomata have also been found to be a site of increased absorption, regardless of the pesticide's solubility properties. This increase in absorption may be related to a thinner cuticle and/or reduced wax deposits overlying the guard cells (Dunleavy et al, 1982). Since the highest proportion of stomata are found on the lower surface of leaves (Bidwell, 1979) and the H_2-AZA + $^3H_2$-AZA solution was applied on the upper surface of the leaves, increased absorption at the stomatal site was not observed.

4.2.2 Effect of Aqueous Solvents and Adjuvants on Uptake of Pesticides

The solvents and adjuvants in which an ai is dissolved and spreads on a leaf surface affects foliar absorption. The total surface area over which the ai is spread might

influence its crystallization or dissolution of the ai The components of a formulation might also affect the structural surface of the leaf and thereby affect absorption.

The use of aqueous methanol (25:75 MeOH:$H_2$0) enabled complete dissolution of the ai H_2-AZA and its tracer $^3H_2$-AZA. The volatile MeOH evaporated quickly from the leaf surface following application of the H_2-AZA + $^3H_2$-AZA with a microsyringe. Stevens (1987) reported that aqueous solvents did not enhance the uptake of either glyphosate, metalaxyl or fenarimol compared to their respective commercial formulations; this might suggest that solvents used in the present study such as methanol or acetone had no effect on the uptake of the ai.

The use of Citowett in the formulation effectively spread the H_2-AZA + $^3H_2$-AZA over the leaf surface while preventing the solution from dripping. However, the addition of adjuvants can influence the uptake of the ai. Studies show that surfactant and wetting agents like Citowett can increase the uptake of the ai up to 27 times. For example, in maize, the uptake of prochloraz increased by 10 fold with surfactant and in sugar beet (similar epicuticular structure as maize but with a thicker cuticle), uptake of 2,4-D and prochloraz was enhanced by a surfactant (Stevens and Baker, 1987). However, Baker and Hunt (1988) had found that in maize, uptake of polar maleic hydrazide was actually lower in the presence of surfactant. The ability of surfactants to increase uptake of an ai can be attributed to several factors: increasing the surface area of contact between the ai and the leaf surface. A greater area of contact would permit wider diffusion of the ai on the leaf surface, solubilize the ai, maintain a high level of moisture on the area of application, or disrupt the epicuticular wax layer (Stevens and Baker, 1987).

The uptake of pesticides is a complex process involving the interaction of many factors. Uptake of a pesticide not only varies among plant species but probably also under laboratory versus field conditions. Caution must be taken when formulating an ai such as AZA in a NSKE for foliage spraying to ensure a maximum uptake by the leaves;

systemic movement of the ai is an important factor in its efficacy, especially in the case of antifeedants, or growth regulators that must be ingested by the insect pest.

4.3 Translocation of the ^{3}H-Compound

Translocation of a compound in a plant can be defined as "the proportion of applied chemical recovered in the area surrounding the treated tissue" (Baker and Hunt, 1988). Translocation of the tritium label following application of H_2-AZA + $^{3}H_2$-AZA formulation varied between 10.4% and 55.8% (Appendix 14, calculated as the % of the ad/absorbed radioactivity) over the three time periods (24, 48 and 72 hours). The translocated label was found mostly in the roots of the corn seedlings and root medium, where the percentage of the total translocated label varied between 15.2% and 97.1%. Results varied somewhat between the first and second trials; a greater percentage of the translocated label was found in the leaves in trial 1 compared to trial 2 where 69.0% to 97.1% of the translocated label was in the roots and root medium.

The translocated material suggests that the tracer may be transported from the leaf via the phloem, towards the roots and other terminal sinks, or there may be some leakage into the xylem and the label is transported back towards the leaves (Goulgler and Geiger, 1981; Bidwell, 1979). The translocation process via the phloem of the labelled compound implies that the material enters the symplast (that "portion of the cell lying within the boundary of the outer differentially permeable membrane of the cell" (Bidwell, 1979)) (Goulgler and Geiger, 1981). In order to reach the phloem of the leaf's veins, the labelled compound must be permeable to the mesophyll and bundle sheath cells. Reports have shown that the process is probably one of passive diffusion (Goulgler and Geiger, 1981).

The presence of tritium in untreated leaves suggests that xylem transport may also be occurring but to a lesser degree. Peterson et al (1978) described this dual movement

of a pesticide. A compound that has penetrated the phloem elements might leak out into the xylem. Once the compound has reached the root system or other terminal areas where transport slows down and stops, the chemical can slowly leak out of the phloem and penetrate the xylem where it will be carried with the transpiration stream.

There is indirect evidence of systemic movement of neem products via the phloem. The movement of bioactive compounds on rice plants sprayed with neem oil was studied using N. virescens (Saxena and Khan, 1985). Phloem feeding was significantly less on the treated plants when compared to acetone treated plants. Xie et al (1991) found that mortality of Northern corn rootworm larvae increased when maize leaves were sprayed with AZA, suggesting that a bioactive compound was absorbed through the leaf surface and translocated to the root area. The movement of an active compound from the leaf to the roots also suggests phloem transport.

Published reports on the uptake and translocation of bioactive compounds from neem after foliar application have so far been baed on indirect evidence with many conflicting results, as will be discussed in the following section. The uptake and systemic movement of a tritiated compound following foliar application of H_2-AZA + $^3H_2$-AZA is the first direct evidence of the phenomenon.

4.4 Antifeedant Bioassays

Results from the bioassays with ECB larvae suggest that when AZA is applied to leaves of young corn seedlings, AZA or other antifeedant compounds have not moved to untreated mature or growing leaves in amounts detectable by the larvae. The primary antifeedant action of translocated AZA or any active metabolites was not detectable in this bioassay. While AZA-treated leaves (1000, 100 ppm) remained practically untouched 24 or 48 hours after application, nearby untreated control leaves were completely consumed. Results from the tracer study suggests that small amounts of H_2-AZA or a

metabolite are translocated; a growth and development study of larvae feeding on the untreated leaves might reveal the presence of a bioactive compound.

The results of the bioassay combined with the tracer study are comparable to some of the published reports. For example, Larew (1988) found that a 1% solution of NSKE (2,300 ppm AZA) used against Liriomyza trifolii did not increase toxicity of untreated chrysanthemum leaves positioned above or below the sprayed leaves. However, in bean leaves, results indicated that a bioactive compound had moved from treated leaves to untreated leaves but the toxic effect was 50% of that obtained with treated leaves.

In an antifeedant bioassay with Popillia japonica adults, where half-leaves of Sassafras were painted with 0.25–10% NSKE + surfactant + water, while the other halves were painted with surfactant + water only, the NSKE-treated half-leaves were completely protected and the control halves were totally consumed 24 or 48 hours following application (Ladd et al, 1978). In another study, toxins in a NSKE and Margosan-O were found to move from the treated leaves into other areas of the plant and affect development of leafminer (Larew et al, 1987).

Reports showing systemic movement of neem products from the roots to the foliage are more numerous. However, it is still indirect evidence, where insects are used to detect the presence of bioactive compounds. A significant study by Meisner et al (1986) had shown systemic activity of neem against O. nubilalis in corn seedlings 4 to 6 days after incorporation of aqueous neem extract into the soil. Larval weights and pupation were significantly reduced in larvae feeding on treated plants where seedlings were infested 6 days after soil treatment compared to control larvae. In another study, roots of 3 week old corn seedlings were treated with AZA (0-50 mg) and the plants were infested with third instar larvae. The primary antifeedant effect was obvious after 48 hours when feeding was reduced by 98% with the 50 ppm AZA solution.

Evidence strongly supports that neem products can be translocated from the

treatment site to other parts of the plant, either from the leaves to roots and other leaves, or from roots to leaves. In view of the factors that affect foliar uptake of compounds, and the mode of action of neem products, the search for a formulation maximizing uptake and translocation of neem's bioactive material is of utmost importance.

E - CONCLUSIONS

The present study was initiated to determine the efficacy of neem-based products, under field conditions, as a means of protecting sweet corn against Ostrinia nubilalis. Laboratory studies were conducted to determine the efficacy of AZA as an antifeedant, the growth regulating effects of NSKEs on the corn borer, and the systemic action of H_2-AZA in sweet corn seedlings.

All the original objectives of the study have been met.

1 - Studies on the effect of neem-based products on the ECB.

1.1 Three field seasons have revealed the following:

(a) Foliage spraying of neem-based products such as NSKE was an effective means of controlling sweet corn damage by O. nubilalis, and were comparable to Ambush, a recommended pyrethroid-based pesticide.

(b) AZA acted in a similar way to NSKEs in protecting sweet corn from corn borer damage, confirming and extending its activity as the active ingredient in NSKEs, in laboratory trials by Isman, to the field.

(c) The most effective rate of application of the NSKE, when conventionally sprayed twice in the growing season, was found to be 1.56 kg extract ha^{-1}. Kernel damage, borer population and stalk damage were reduced by approximately 50% as compared to non- treated controls.

(d) The NSKE's efficacy was greatly enhanced when applied prior to egg infestation. Feeding damage was reduced up to 73% as compared to non-treated controls while larval populations were reduced by 56%.

(e) The efficacy of a neem extract was highly dependent on its formulation. Two neem formulations provided by Safer Ltd. were significantly more efficient at reducing borer damage than most NSKE treatments. Proper emulsification of the active ingredients

in a formulation for spray application is indispensable.

(f) Weather conditions such as high temperatures, and dry conditions may be detrimental to the efficacy of neem products.

(g) Neem products may modify the feeding behavior of ECB larvae by deterring them from entering the cob and precipitating an early migration to the stalk thereby increasing stalk breakage.

1.2 Laboratory antifeedant bioassays with azadirachtin have revealed the following:

(a) AZA at 7.2 ng mm^{-2} inhibited feeding by 3rd instar larvae in no-choice leaf-disk feeding bioassays. Chemicals (PBO and Citowett) used in the field spray formulation did not seem to affect the antifeedant action of AZA.

(b) At low concentrations, AZA may act as a phagostimulant.

1.3 Growth studies of the ECB fed NSKEs have revealed the following:

(a) The NSKE used in the field studies are effective IGRs and can be larvicidal. 3 μg NSKE g^{-1} diet (16.6 ng AZA g^{-1} diet) significantly reduced larval, pupal and adult weights, increased larval and pupal periods, decreased adult emergence and caused wing deformities, while 10 μg NSKE g^{-1} diet (50 ng AZA g^{-1} diet) was larvicidal.

(b) AZA content of NSKE was an important factor in the efficacy of the extract as an IGR or larvicide. However, there are other active compounds in the NSKEs which may act synergistically to increase the activity of AZA.

2 - Studies on the uptake and translocation of H_2-AZA and AZA.

2.1 Uptake and translocation studies of $^3H_2$-AZA have revealed the following:

(a) Foliar uptake of the tritiated form of H_2-AZA by sweet corn seedlings was

minimal (<15%) 24, 48 or 72 hours following application.

(b) The small amount of absorbed ^{3}H-compound was mostly translocated to the roots of corn seedlings and their bathing medium. It was also translocated to the leaves, suggesting a dual translocation route via the phloem and possibly the xylem.

2.2 The feeding bioassays using sweet corn seedlings were not sensitive enough to detect the movement of antifeedant compounds from an AZA-treated leaf to young developing leaves.

Future Work

The development of a neem-based pesticide for foliar application requires a formulation that can stabilize and protect the sensitive AZA molecule, as well as ensure a constant concentration range of the active compound. Environmental conditions affecting the efficacy of neem products in the field need to be assessed in order to develop optimal pest management strategies. Field studies which combine the use of neem products with other means of controlling insect pests (chemical, biological, mechanical) need to be conducted to optimize pest control.

Since foliar uptake of the tritiated form of H_2-AZA is minimal, research towards a means of increasing the permeability of the leaf surface to the antifeedant AZA is imperative. The fate of the AZA molecule once it is absorbed by a plant and translocated remains to be determined. Is the molecule translocated intact or is it metabolized by the plant? Direct evidence of translocation of an active compound and its identity is still not available. Root uptake of AZA remains to be studied. Direct evidence of this phenomenon using labelled material might warrant the development of a soil-drench formulation.

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APPENDICES

Appendix 1: Meridic diet used in rearing of Ostrinia nubilalis (Adapted from Guthrie, 1971).

INGREDIENTS	QUANTITY (for 800 g diet)
Distilled water	433.0 mL (for agar)
Agar	14.90 g
Distilled water	172.0 mL (for cooling)
Dry mix *	98.50 g
Methyl-p-hydroxybenzoate	4.0 ml
Propionic acid	4.6 ml
Formaldehyde	0.38 ml
Fumidil B	0.40 g
Corn cob grits **	2.20 g
Distilled water	87.0 ml (for mixing)
Wheat germ	520.0 g
Dextrose	400.0 g
Casein	440.0 g
Cholesterol	32.0 g
Salt mixture (wesson)	144.0 g
Vitamin Mix (Vanderzant Adkisson)	92.0 g
Ascorbic acid	120.0 g
Aureomycin	27.0 g
Sorbic acid	80.0 g

* Dry mix (for 1855.0 g)

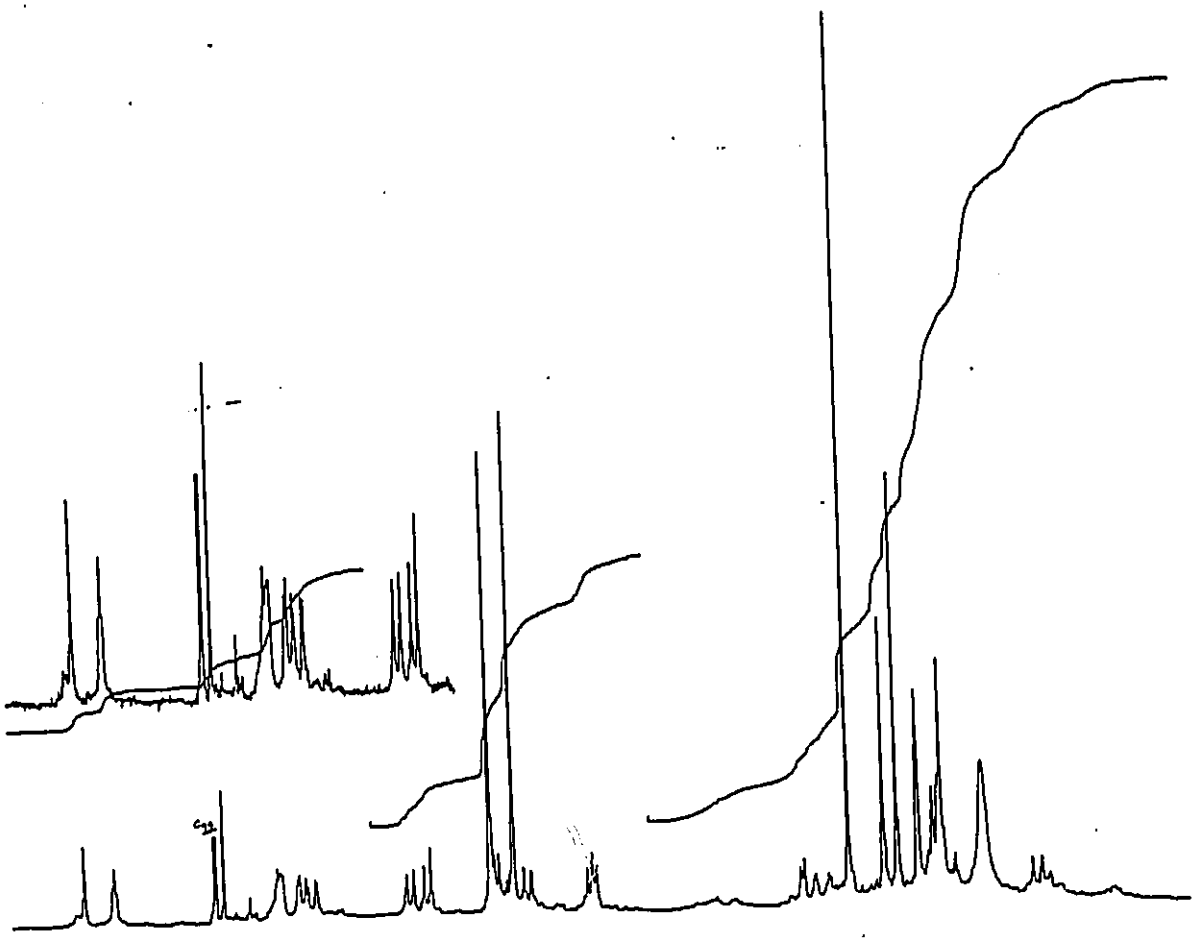
** Corn cob grits are omitted when preparing test diets

Appendix 2: Hoagland's nutrient solution for hydroponically grown corn.

CHEMICAL	g for 100 ml stock solution	MOLARITY	ml for 4 L solution
Macronutrients			
$Ca(NO_3)_2.4H_2O$	47.2	2.0 M	10.0
KNO_3	20.2	2.0 M	10.0
$MgSO_4$	12.04	1.0 M	8.0
KH_2PO_4	13.61	1.0 M	4.0
FeEDTA (Fe(330)Sequestrene)	50.0	89 mM	8.0
Micronutrients			
$MnCl_2.4H_2O$	1.8	9.0 mM	
H_3BO_3	0.029	5.0 mM	
$ZnSO_4.7H_2O$	0.022	0.78 mM	8.0
$CuSO_4.5H_2O$	0.008	0.32 mM	
$(NH_4)_6Mo_7O_{24}.4H_2O$	0.002	0.01 mM	

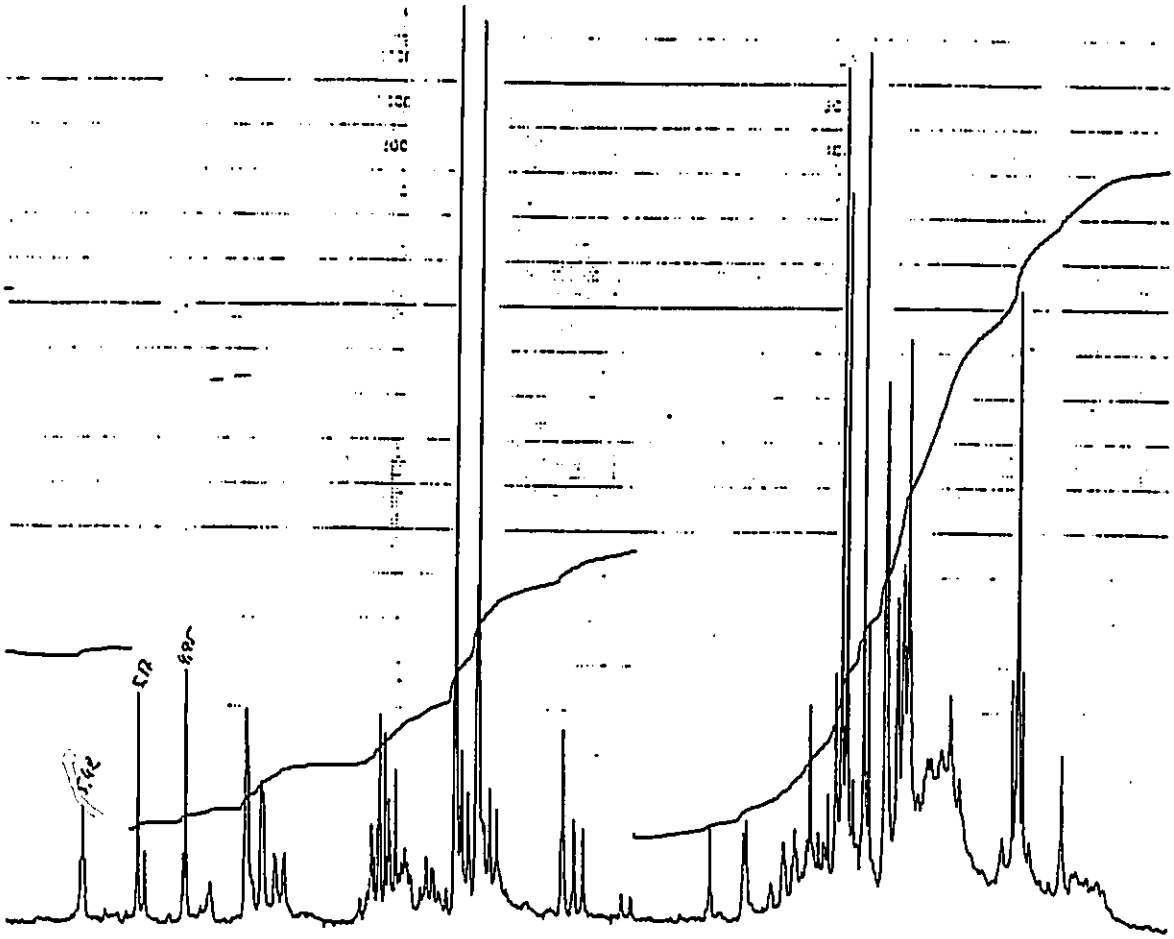
Adapted from Hoagland and Arnon (1940), by University of Carleton for greenhouse grown corn.

Appendix 3a: NMR spectrum of azadirachtin.



Solvent: Cd-chloroform

Appendix 3b: NMR spectrum of H_2-azadirachtin.



Solvent: Cd-chloroform

Appendix 4: Field study 1987 - Comparison matrix of mean damaged kernels cob^{-1}, larvae cob^{-1} and tunnelling ratio for neem-product treatments in artificially infested plots.

MEAN (STD DEV)	CONTROL	AZA 10g/ha 2X	AZA 100g/ha 2X	NSKE 0.31kg/ha 2X
DAMAGED KERNELS/COB	47.2 (21.9)	29.2 (23.5)	21.3 (16.1)	32.7 (25.0)
LARVAE/COB	1.8 (1.4)	1.0 (1.1)	0.9 (1.0)	1.1 (1.2)
TUNNELLING RATIO ($X10^{-2}$)	10.3 (2.8)	8.0 (2.6)	6.9 (4.3)	7.9 (3.3)
NSKE 1.56kg/ha 2X				
17.4 (13.1)	**	*	-	*
0.6 (0.9)	**	-	-	*
5.3 (2.8)	**	**	-	*
NSKE 0.31kg/ha 2X				
32.7 (25.0)	**	-	*	
1.1 (1.2)	**	-	-	
7.9 (3.3)	**	-	-	
AZA 100g/ha 2X				
21.3 (16.1)	**	-		
0.9 (1.0)	**	-		
6.9 (4.3)	**	*		
AZA 10g/ha 2X				
29.2 (23.5)	**			
1.0 (1.1)	*			
8.0 (2.6)	**			

Comparisons between means were performed using the Wilcoxon 2-sample test.
** means are significantly different at p=0.01
* means are significantly different at p=0.05
- means are not significantly different.

Appendix 5: Field study 1987 - Comparison matrix of mean damaged kernels cob^{-1}, larvae cob^{-1} and tunnelling ratio for neem-product treatments in naturally infested plots.

MEAN (STD DEV)	CONTROL	AZA 10g/ha 2X	AZA 100g/ha 2X	NSKE 0.31kg/ha 2X
DAMAGED KERNELS/COB	2.03 (5.57)	0.18 (1.11)	0.28 (1.45)	0.18 (1.12)
LARVAE/COB	0.03 (0.16)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
TUNNELLING RATIO ($X10^{-2}$)	0.26 (0.49)	0.30 (0.80)	0.57 (0.94)	0.12 (0.59)
NSKE 1.56kg/ha 2X				
0.85 (3.48)	-	-	-	-
0.03 (0.16)	-	-	-	-
0.58 (1.46)	-	-	-	-
NSKE 0.31kg/ha 2X				
0.18 (1.12)	*	-	-	
0.00 (0.00)	-	-	-	
0.12 (0.59)	*	-	**	
AZA 100g/ha 2X				
0.28 (1.45)	*	-		
0.00 (0.00)	-	-		
0.57 (0.94)	-	*		
AZA 10g/ha 2X				
0.18 (1.11)	*			
0.00 (0.00)	-			
0.30 (0.80)	-			

Comparisons between means were performed using the Wilcoxon 2-sample test.
** means are significantly different at p=0.01
* means are significantly different at p=0.05
- means are not significantly different.

Appendix 6: Field study 1988 - Field 1 - Comparison matrix of mean damaged kernels cob^{-1}, larvae cob^{-1} for neem-products treatments in artificially infested plots.

MEAN (STD DEV)	CONTROL	AZA 10g/ha 2X	AZA 100g/ha 2X	NSKE 0.31kg/ha 2X
DAMAGED KERNELS/COB LARVAE/COB	23.7 (13.58) 3.2 (2.4)	22.0 (10.7) 1.8 (1.4)	20.9 (10.4) 2.7 (1.8)	26.9 (16.2) 2.2 (1.9)
NSKE 1.56kg/ha 2X				
32.3 (14.2) 3.4 (1.7)	* -	** **	** *	- **
NSKE 0.31kg/ha 2X				
26.9 (16.2) 2.2 (1.9)	- *	- *	- -	
AZA 100g/ha 2X				
20.9 (10.4) 2.7 (1.8)	- -	- *		
AZA 10g/ha 2X				
22.0 (10.7) 1.8 (1.4)	- **			

Comparisons between means were performed using the Wilcoxon 2-sample test.
** means are significantly different at p=0.01
* means are significantly different at p=0.05
- means are not significantly different.

Appendix 7: Field study 1988 - Field 2 - Comparison matrix of mean damaged kernels cob^{-1}, larvae cob^{-1} for neem-products treatments in artificially infested plots.

MEANS (STD DEV)	CONTROL	NSKE 0.79kg/ha 2X	NSKE 3.12kg/ha 2X	EC 1.56kg/ha 2X	AHB 15.6g/ha 2X	NSKE 1.56kg/ha X4	NSKE 1.56kg/ha 1 pre
DAMAGED KERNELS/COB	33.5 (21.3)	24.3 (16.3)	23.1 (18.2)	24.5 (22.9)	17.6 (13.7)	21.3 (15.5)	15.2 (13.4)
LARVAE/COB	1.7 (1.4)	1.1 (1.1)	1.0 (1.0)	1.0 (1.1)	0.6 (0.7)	1.2 (1.0)	1.1 (1.0)
NSKE 1.56kg/ha 1 pre/2 post							
13.7 (12.2)	**	**	*	**	-	*	-
0.9 (0.8)	**	*	-	-	*	*	-
NSKE 1.56kg/ha 1 pre							
15.2 (13.4)	**	*	*	**	-	*	
1.1 (1.0)	**	-	-	-	**	-	
NSKE 1.56kg/ha X4							
21.3 (15.5)	**	-	-	-	-		
1.2 (1.0)	*	-	-	*	**		
AHB 15.6g/ha 2X							
17.6 (13.7)	**	*	*	*			
0.6 (0.7)	**	**	**	**			
EC 1.56kg/ha 2X							
24.5 (22.9)	**	-	-				
1.0 (1.1)	**	-	-				
NSKE 3.12kg/ha X2							
23.1 (18.2)	**	-					
1.0 (1.0)	**	-					
NSKE 0.79kg/ha X2							
24.3 (16.3)	*						
1.1 (1.1)	**						

Comparisons between means were performed using the Wilcoxon 2-sample test.
** means are significantly different at p=0.01
* means are significantly different at p=0.05
- means are not significantly different.

Appendix 8: Field study 1988 - Fields 1 and 2 - Comparison matrix of mean damaged kernels cob^{-1}, larvae cob^{-1} for neem-products treatments in naturally infested plots.

MEAN (STD DEV)	CONTROL	AZA 10g/ha 2X	AZA 100g/ha 2X	NSKE 0.31kg/ha 2X
DAMAGED KERNELS/COB LARVAE/COB	2.6 (5.2) 0.20 (0.41)	1.4 (3.4) 0.13 (0.33)	1.9 (4.6) 0.25 (0.74)	1.3 (3.6) 0.08 (0.47)
NSKE 1.56kg/ha 2X				
4.8 (7.9) 0.10 (0.30)	- -	- -	- -	- -
NSKE 0.31kg/ha 2X				
1.3 (3.6) 0.08 (0.47)	- -	- -	- -	
AZA 100g/ha 2X				
1.9 (4.6) 0.25 (0.74)	- -	- -		
AZA 10g/ha 2X				
1.4 (3.4) 0.13 (0.33)	- -			

MEAN (STD DEV)	CONTROL
DAMAGED KERNELS/COB LARVAE/COB	1.1 (4.9) 0.07 (0.45)
AMB 15.6g/ha 2X	
0.7 (2.5) 0.00 (0.00)	- -

Comparisons between means were performed using the Wilcoxon 2-sample test.
** means are significantly different at p=0.01
* means are significantly different at p=0.05
- means are not significantly different.

Appendix 9: Field study 1989 - Field 1 - Comparison matrix of mean damaged kernels cob^{-1}, larvae cob^{-1} and stalk breakage ratio for neem-products treatments in artificially infested plots.

MEAN (STD DEV)	CONTROL	SAF1 1.34kg/ha 2X	AZA 10g/ha 2X	SAF2 1.47kg/ha 2X	AZA 100g/ha 2X	NSKE 0.31kg/ha X2
DAMAGED KERNELS/COB	31.3 (17.3)	8.5 (9.4)	31.2 (19.8)	11.8 (9.7)	25.8 (15.9)	33.1 (21.9)
LARVAE/COB	3.8 (3.3)	1.2 (1.4)	3.8 (2.2)	1.7 (1.4)	2.3 (1.5)	3.0 (2.1)
STALK BREAKAGE	5.8 (2.3)	2.3 (2.3)	5.1 (2.2)	2.8 (1.8)	5.0 (2.3)	5.2 (2.1)
NSKE 1.56kg/ha X2						
20.0 (15.1)	**	**	**	**	*	**
2.4 (1.6)	**	**	**	**	-	-
4.1 (2.3)	**	**	**	**	*	**
NSKE 0.31kg/ha X2						
33.1 (21.9)	-	**	-	**	*	
3.0 (2.1)	-	**	-	**	*	
5.2 (2.1)	**	**	-	**	-	
AZA 100g/ha X2						
25.8 (15.9)	*	**	-	**		
2.3 (1.5)	**	**	**	**		
5.0 (2.3)	**	**	-	**		
SAF2 1.47kg/ha 2X						
11.8 (9.7)	**	*	**			
1.7 (1.4)	**	*	**			
2.8 (1.8)	**	-	**			
AZA 10g/ha 2X						
31.2 (19.8)	-	**				
3.8 (2.2)	-	**				
5.1 (2.2)	**	**				
SAF1 1.34kg/ha X2						
8.5 (9.4)	**					
1.2 (1.4)	**					
2.3 (2.3)	**					

Comparisons between means were performed using the Wilcoxon 2-sample test.
** means are significantly different at p=0.01
* means are significantly different at p=0.05
- means are not significantly different.

Appendix 10: Field study 1989 - Field 2 - Comparison matrix of mean damaged kernels cob^{-1}, larvae cob^{-1} and stalk breakage ratio for neem-products treatments in artificially infested plots.

MEAN (STD DEV)	CONTROL	AMB 15.6g/ha X2	NSKE 1.56kg/ha X7	NSKE 1.56kg/ha 1 pre	NSKE 1.56kg/ha 1 pre/3 post	NSKE 0.79kg/ha X2	NSKE 3.12kg/ha X2
DAMAGED KERNELS/COB	42.6 (20.5)	14.2 (12.3)	22.4 (13.7)	17.1 (13.2)	11.3 (12.3)	30.6 (16.8)	25.3 (17.7)
LARVAE/COB	3.4 (2.4)	1.7 (1.2)	2.4 (1.7)	2.4 (1.6)	1.5 (1.2)	3.1 (1.8)	2.7 (1.9)
STALK BREAKAGE RATIO	5.1 (2.2)	3.7 (1.8)	4.4 (2.2)	4.6 (2.2)	4.7 (2.0)	5.3 (2.1)	4.9 (2.1)
NSKE 1.56kg/ha X7 DUSK							
25.1 (15.5)	**	**	-	*	**	*	-
2.5 (1.5)	*	*	-	-	**	-	-
5.2 (2.0)	-	**	*	*	-	-	-
NSKE 3.12kg/ha X2							
25.3 (17.7)	**	**	-	*	**	*	
2.7 (1.9)	-	**	-	-	**	-	
4.9 (2.1)	-	**	-	-	-	-	
NSKE 0.79kg/ha X2							
30.6 (16.8)	**	**	*	**	**		
3.1 (1.8)	-	**	-	-	**		
5.3 (2.1)	-	**	**	*	*		
NSKE 1.56kg/ha 1 pre/3 post							
11.3 (12.3)	**	-	**	-			
1.5 (1.2)	**	-	*	*			
4.7 (2.0)	-	**	-	-			
NSKE 1.56kg/ha 1 pre							
17.1 (13.2)	**	-	*				
2.4 (1.6)	**	**	-				
4.6 (2.2)	-	**	-				
NSKE 1.56kg/ha X7							
22.4 (13.7)	**	*					
2.4 (1.7)	**	**					
4.4 (2.2)	*	*					
AMB 15.6g/ha X2							
14.2 (12.3)	**						
1.7 (1.2)	**						
3.7 (1.8)	**						

Comparisons between means were performed using the Wilcoxon 2-sample test.
** means are significantly different at p=0.01
* means are significantly different at p=0.05
- means are not significantly different.

Appendix 11: Field study 1989 - Fields 1 and 2 - Comparison matrix of mean damaged kernels cob^{-1}, larvae cob^{-1} and stalk breakage ratio for neem-products treatments in naturally infested plots.

MEAN (STD DEV)	CONTROL	NSKE 0.31kg/ha X2
DAMAGED KERNELS/COB	0.4 (1.9)	0.7 (3.3)
LARVAE/COB	0.04 (0.19)	0.05 (0.22)
STALK BREAKAGE	0.6 (1.8)	0.5 (1.6)
NSKE 1.56kg/ha X2		
0.2 (1.0)	-	-
0.04 (0.25)	-	-
0.4 (1.2)	-	-
NSKE 0.31kg/ha X2		
0.7 (3.3)	-	
0.05 (0.22)	-	
0.5 (1.6)	-	

MEAN (STD DEV)	CONTROL
DAMAGED KERNELS/COB	0.3 (2.0)
LARVAE/COB	0.06 (0.24)
STALK BREAKAGE	0.5 (1.7)
AMB 15.6g/ha X2	
0.2 (1.9)	-
0.03 (0.22)	-
0.2 (1.0)	-

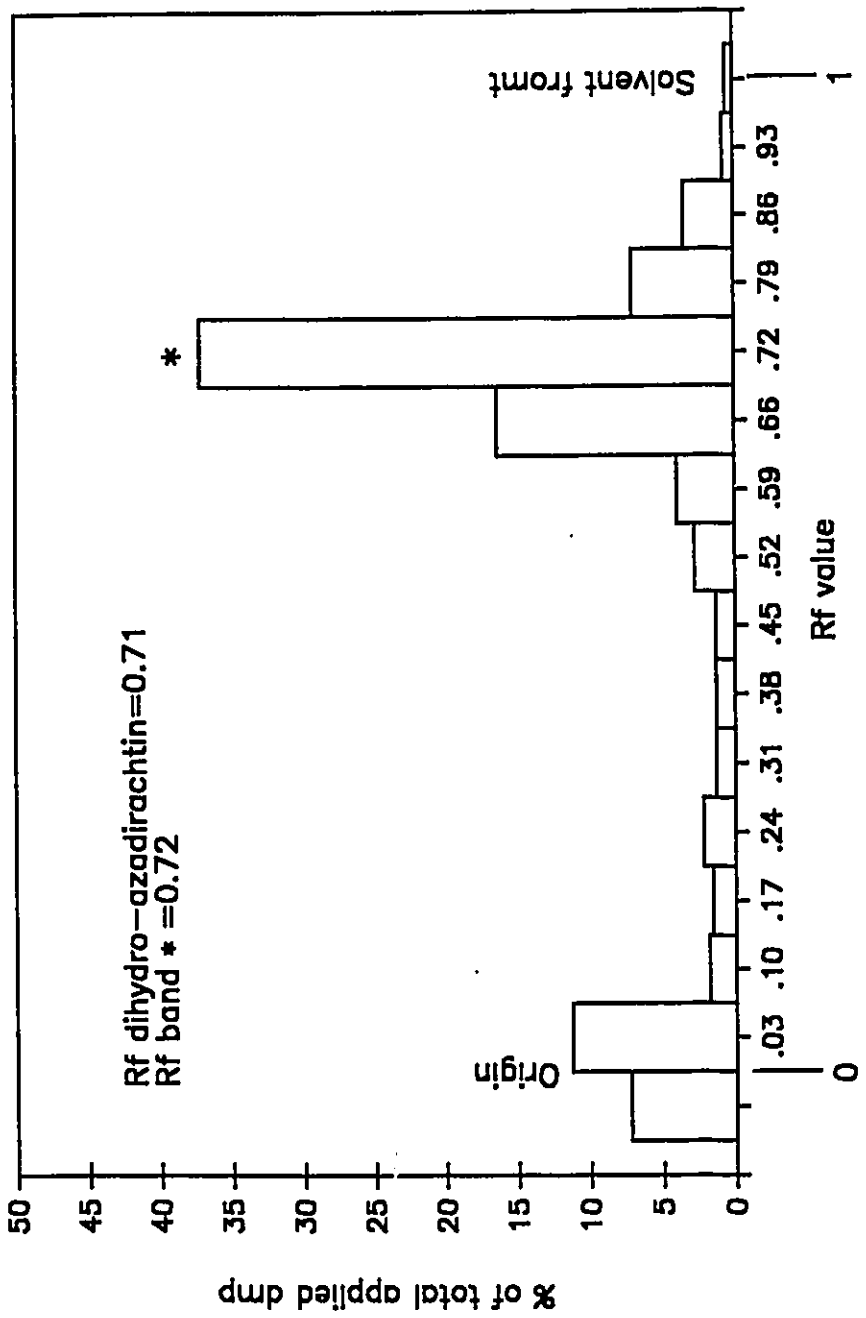
Comparisons between means were performed using the Wilcoxon 2-sample test.
** means are significantly different at p=0.01
* means are significantly different at p=0.05
- means are not significantly different.

Appendix 12: Leaf-feeding bioassay with AZA alone and AZA in a formulation.

TRIALS	TREATMENTS									
	CONTROL	APC 100	APC 10	APC 1	A 100	A 10	A 1	PC	C	P
	Area of leaf-disk consumed by larvae (mm^2)									
1	52	63	85	84	100	97	69	71	30	44
	56	21	122	75	102	61	86	125	56	64
	116	0	101	88	55	89	77	120	86	99
	66	25	47	85	49	80	20	93	99	37
	78	34	125	69	65	77	48	63	108	125
	55	83	87	61	57	79	89	71	60	35
	53	32	115	107	16	120	111	78	72	125
	91	55	120	58	70	74	106	79	74	125
	70	76	77	125	44	66	78	96	40	94
	72	40	80	101	25	78	125	123	38	102
Mean	70.9	42.9	95.9	85.3	58.3	82.1	80.9	91.9	66.3	85.0
s.d.	19.1	24.7	23.9	20.0	26.5	15.9	29.2	22.2	25.0	35.1
2	100	71	93	23	62	40	113	119	30	11
	65	3	117	113	40	69	121	35	93	98
	62	18	42	99	53	83	122	49	103	65
	23	35	72	113	63	9	53	118	71	54
	7	50	89	91	98	51	29	45	40	11
	10	51	98	128	80	90	70	117	96	90
	109	99	56	88	14	66	71	48	124	35
	66	92	67	61	5	104	105	57	114	114
	90	86	109	89		56	86	53	101	20
	73		85				99	65	55	17
Mean	60.5	56.1	82.8	89.4	51.9	63.1	86.9	70.6	82.7	51.5
s.d.	34.4	31.7	22.3	29.7	29.4	26.9	29.3	31.9	30.4	36.7
3	38	56	85	65	30	29	30	69	56	44
	67	16	65	85	63	82	74	43	48	36
	42	26	43	110	12	23	65	94	91	79
	30	55	71	111	5	64	64	43	60	81
	73	30	35	102	11	67	42	61	78	58
	47	63	73	82	62	70	70	59	85	98
	96	68	57	70	53	90	82	92	63	27
	71	34	73	85	54	73	82	75	77	43
		20	27		2	60	54	66	82	53
		7	65		16	51	71	84		61
Mean	58.0	37.5	59.4	88.8	30.8	60.9	63.4	68.6	72.2	58.0
s.d.	20.9	20.3	17.7	16.2	23.5	20.3	16.0	17.1	13.0	21.1

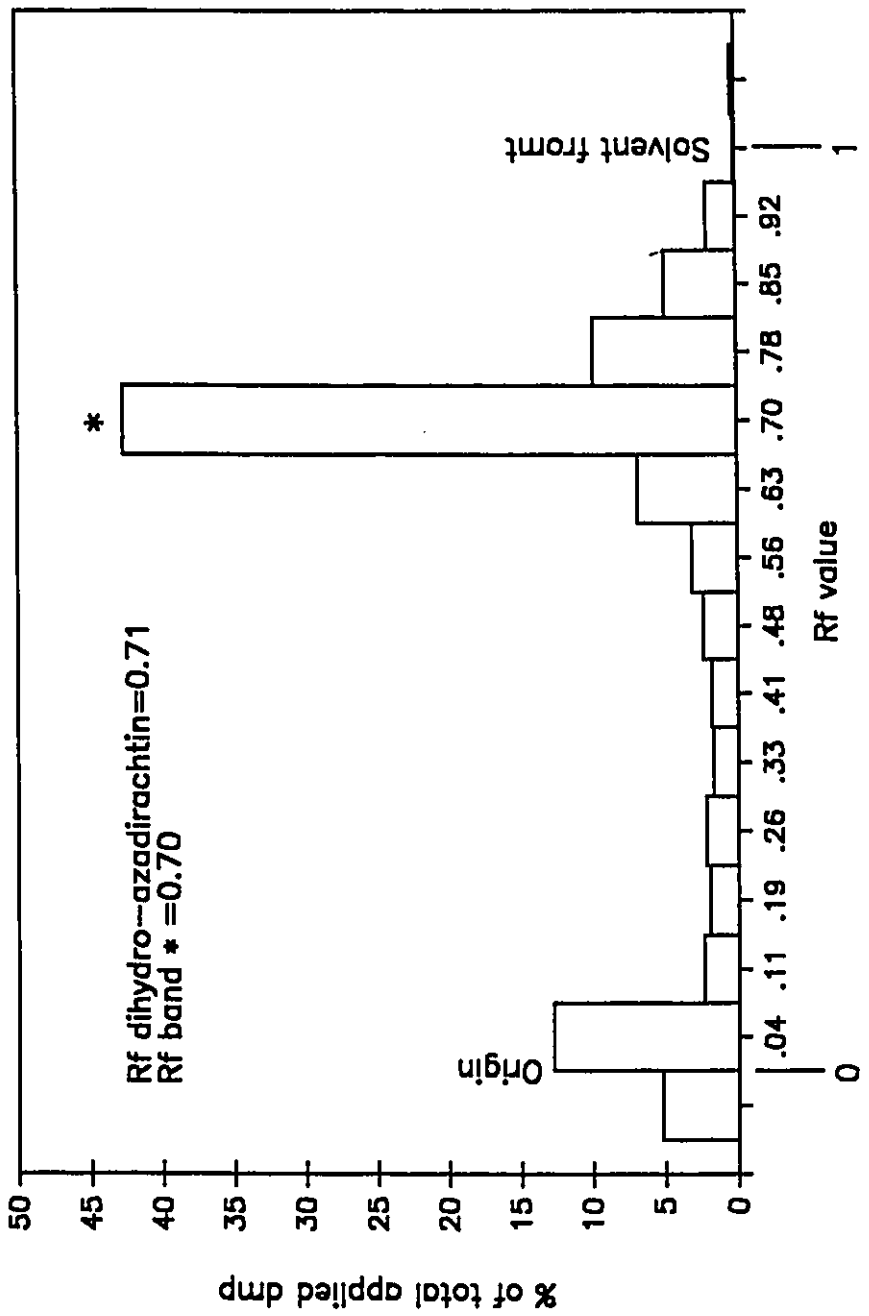
Three early 3rd instar ECB larvae were left to feed for 24 hours.
A = azadirachtin
P = Piperonyl butoxide in a 1:1 (v:v) ratio with AZA
C = Citowett, 0.1% of the solution applied
100 = 100 µg mL^{-1} AZA solution (7.2 ng AZA mm^{-2} leaf)
10 = 10 µg mL^{-1} AZA solution (0.72 ng AZA mm^{-2} leaf)
1 = 1 µg mL^{-1} AZA solution (0.072 ng AZA mm^{-2} leaf)
Total leaf-disk area exposed = 138 mm^2

Appendix 13a: Thin layer chromatography of H_2-azadirachtin and $^3H_2$-azadirachtin. Trial 1



Solvent system - chloroform:ethylacetate:methanol 4:4:1 (v:v:v).

Appendix 13b: Thin layer chromatography of H_2-azadirachtin and $^3H_2$-azadirachtin. Trial 2



Solvent system - chloroform:ethylacetate:methanol 4:4:1 (v:v:v).

Appendix 14: Uptake of $^{3}H_{2}$-azadirachtin and translocation of ^{3}H-label by sweet corn seedlings.

TIME REPS	RINSE-TREATED LEAF 3 % OF TOTAL µg IN RINSE		EXTRACTED FROM TREATED LEAF 3 % OF RECOVER	ng LABEL	ng LABEL/mg	EXTRACTED FROM LEAF 1 & 2 % OF RECOVER	ng LABEL	ng LABEL/mg
24HRS								
1.00	88.72	44.36	0.86	390.99	134.82	0.581	263.36	39.31
2.00	86.92	43.46	1.04	461.37	124.69	0.894	396.71	61.03
3.00	87.43	43.72	0.98	432.82	123.66	0.138	60.98	8.71
AVG	87.69	43.85	0.96	428.39	127.73	0.54	240.35	36.35
+/- STD	0.76	0.38	0.07	28.90	5.04	0.31	138.02	21.46
4.00	89.31	44.66	1.04	478.09	170.75	0.014	6.55	0.78
5.00	91.92	45.96	1.75	845.74	169.15	0.004	1.85	0.18
6.00	93.54	46.77	1.12	539.22	131.52	0.006	2.79	0.27
7.00	91.86	45.93	1.40	658.07	156.68	0.005	2.39	0.38
AVG	91.66	45.83	1.33	630.28	157.02	0.01	3.40	0.40
+/- STD	1.51	0.76	0.28	140.22	15.70	0.00	1.85	0.23
48HRS								
1.00	88.46	44.23	0.89	396.47	120.14	0.019	8.44	1.51
2.00	86.89	43.44	1.06	466.54	122.77	0.040	17.65	2.85
3.00	88.17	44.09	1.30	582.87	161.91	0.019	8.38	1.37
AVG	87.84	43.92	1.08	481.96	134.94	0.03	11.49	1.91
+/- STD	0.68	0.34	0.17	76.87	19.10	0.01	4.36	0.67
4.00	90.83	45.42	5.19	2492.45	607.92	0.022	10.61	1.33
5.00	92.74	46.37	2.58	1232.51	385.16	0.011	5.02	0.68
6.00	93.85	46.93	1.66	792.72	180.16	0.005	2.31	0.24
7.00	92.56	46.28	1.75	827.24	153.19	0.007	3.26	0.35
AVG	92.49	46.25	2.79	1336.23	331.61	0.01	5.30	0.65
+/- STD	1.08	0.54	1.43	689.58	183.02	0.01	3.21	0.42
72HRS								
1.00	85.85	42.93	1.11	483.78	146.60	0.241	105.37	15.73
2.00	83.94	41.97	1.00	424.11	151.47	0.015	6.21	0.96
3.00	86.47	43.24	0.94	411.15	132.63	0.012	5.37	0.70
AVG	85.42	42.71	1.02	439.68	143.57	0.09	38.98	5.79
+/- STD	1.08	0.54	0.07	31.63	7.98	0.11	46.94	7.02
4.00	94.31	47.15	1.44	698.91	145.61	0.010	4.97	0.80
5.00	93.79	46.90	1.12	534.39	133.60	0.004	1.81	0.24
6.00	66.45	33.22	2.03	700.99	179.74	0.005	1.88	0.26
7.00	95.41	47.71	1.17	565.01	166.18	0.002	1.06	0.17
AVG	87.49	43.74	1.44	624.83	156.28	0.01	2.43	0.37
+/- STD	12.16	6.08	0.36	75.91	17.87	0.00	1.50	0.25

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TIME REPS	EXTRACTED FROM UNTREATED LEAF 3 % OF RECOVER	ng LABEL	ng LABEL/mg	EXTRACTED FROM LEAF 4 % OF RECOVER	ng LABEL	ng LABEL/mg	EXTRACTED FROM STEM % OF RECOVER	ng LABEL	ng LABEL/mg
24HRS									
1.00	0.298	134.95	18.24	0.174	79.08	14.38	0.066	29.86	2.84
2.00	0.020	8.68	1.28	0.026	11.45	1.71	0.025	11.09	0.98
3.00	0.034	15.15	1.97	0.032	14.16	2.02	0.020	8.90	0.75
AVG	0.12	52.92	7.16	0.08	34.89	6.04	0.04	16.62	1.53
+/- STD	0.13	58.06	7.84	0.07	31.26	5.90	0.02	9.41	0.94
4.00	0.008	3.86	0.64	0.009	3.93	0.30	0.013	5.83	0.62
5.00	0.068	33.06	3.24	0.006	3.08	0.41	0.013	6.40	0.42
6.00	0.011	5.19	1.02	0.007	3.22	0.44	0.012	5.96	0.60
7.00	0.009	4.41	0.75	0.008	3.84	0.80	0.013	5.88	0.56
AVG	0.02	11.63	1.41	0.01	3.52	0.49	0.01	6.02	0.55
+/- STD	0.03	12.38	1.06	0.00	0.37	0.19	0.00	0.23	0.08
48HRS									
1.00	0.022	9.70	1.67	0.018	8.00	1.43	0.023	10.30	1.32
2.00	0.027	11.77	1.51	0.013	5.60	0.60	0.040	17.70	1.55
3.00	0.013	5.99	0.88	0.027	12.22	1.72	0.021	9.53	0.99
AVG	0.02	9.15	1.35	0.02	8.61	1.25	0.03	12.51	1.29
+/- STD	0.01	2.39	0.34	0.01	2.74	0.47	0.01	3.68	0.23
4.00	0.031	14.75	2.34	0.019	9.24	2.05	0.043	20.77	1.81
5.00	0.030	14.53	2.85	0.011	5.36	1.03	0.023	11.11	1.28
6.00	0.009	4.50	0.56	0.010	4.75	0.78	0.016	7.45	0.64
7.00	0.014	6.74	1.14	0.020	9.51	1.29	0.013	6.35	0.47
AVG	0.02	10.13	1.72	0.02	7.21	1.29	0.02	11.42	1.05
+/- STD	0.01	4.58	0.92	0.00	2.17	0.48	0.01	5.68	0.53
72HRS									
1.00	0.022	9.56	1.41	0.020	8.73	0.83	0.146	63.79	4.04
2.00	0.026	11.00	1.62	0.033	14.05	1.06	0.053	22.36	1.53
3.00	0.025	11.03	1.62	0.019	8.49	0.47	0.021	9.19	0.54
AVG	0.02	10.53	1.55	0.02	10.43	0.79	0.07	31.78	2.04
+/- STD	0.00	0.69	0.10	0.01	2.57	0.24	0.05	23.26	1.47
4.00	0.007	3.57	0.79	0.010	4.68	0.92	0.011	5.38	0.39
5.00	0.006	2.69	0.50	0.006	2.96	0.62	0.008	3.72	0.40
6.00	0.010	3.47	0.61	0.008	2.73	0.64	0.015	5.32	0.49
7.00	0.007	3.20	0.63	0.004	2.05	0.50	0.008	3.64	0.38
AVG	0.01	3.23	0.63	0.01	3.11	0.67	0.01	4.52	0.42
+/- STD	0.00	0.34	0.11	0.00	0.97	0.15	0.00	0.83	0.05

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TIME REPS	EXTRACTED FROM ROOT MEDIUM % OF RECOVER	ng LABEL	ng LABEL/mL	ROOT RINSE % OF RECOVER	ng LABEL	EXTRACTED FROM ROOTS % OF RECOVER	ng LABEL	ng LABEL/mg
24HRS								
1.00	0.11	49.00	1.07	0.008	3.44	0.027	12.29	0.15
2.00	0.00	2.12	0.06	0.006	2.48	0.023	10.31	0.11
3.00	0.06	28.63	0.67	0.007	3.24	0.028	12.56	0.15
AVG	0.06	26.58	0.60	0.01	3.05	0.03	11.72	0.14
+/- STD	0.04	19.20	0.41	0.00	0.41	0.00	1.00	0.02
4.00	1.18	538.21	11.70	0.008	3.46	0.175	79.90	0.89
5.00	2.67	1288.97	29.98	0.007	3.24	0.324	156.51	2.10
6.00	1.48	713.69	16.22	0.007	3.31	0.235	113.01	1.49
7.00	0.81	383.10	8.24	0.007	3.50	0.114	53.71	1.09
AVG	1.54	730.99	16.53	0.01	3.38	0.21	100.78	1.39
+/- STD	0.70	342.72	8.26	0.00	0.11	0.08	38.43	0.46
48HRS								
1.00	0.04	17.66	0.39	0.008	3.39	0.045	20.23	0.19
2.00	0.11	48.70	1.06	0.008	3.46	0.029	12.77	0.15
3.00	0.13	58.81	1.30	0.008	3.42	0.041	18.51	0.18
AVG	0.09	41.73	0.92	0.01	3.42	0.04	17.17	0.17
+/- STD	0.04	17.51	0.38	0.00	0.03	0.01	3.19	0.01
4.00	0.06	30.18	0.74	0.006	3.09	0.042	20.03	0.24
5.00	0.21	98.53	2.14	0.007	3.46	0.089	42.69	0.37
6.00	0.26	122.15	2.63	0.007	3.50	0.056	26.85	0.26
7.00	0.02	10.11	0.22	0.007	3.50	0.047	22.00	0.19
AVG	0.14	65.24	1.43	0.01	3.39	0.06	27.89	0.27
+/- STD	0.10	46.41	0.99	0.00	0.17	0.02	8.89	0.07
72HRS								
1.00	0.16	69.52	1.48	0.008	3.54	0.036	15.95	0.20
2.00	0.03	12.40	0.26	0.008	3.54	0.023	9.81	0.09
3.00	0.02	6.65	0.14	0.008	3.54	0.021	9.14	0.10
AVG	0.07	29.52	0.63	0.01	3.54	0.03	11.63	0.13
+/- STD	0.06	28.38	0.60	0.00	0.00	0.01	3.06	0.05
4.00	0.92	444.63	9.88	0.007	3.39	0.101	48.97	0.55
5.00	0.77	367.93	7.91	0.007	3.50	0.085	40.89	0.32
6.00	1.47	506.65	12.21	0.009	3.12	0.206	71.20	0.77
7.00	0.00	0.00	0.00	0.007	3.16	0.020	9.71	0.11
AVG	0.79	329.80	7.50	0.01	3.29	0.10	42.69	0.44
+/- STD	0.52	196.65	4.59	0.00	0.16	0.07	22.04	0.25

.../cont

...cont

TIME REPS	% MASS BALANCE	LABEL AB/AD-SORBED BY PLANT % OF TOTAL	ng LABEL	ng LABEL/mg	TRANSLOCATED LABEL % OF AD/AB	% TOTAL	ng LABEL	ng LABEL/mg
24HRS								
1.00	90.65	2.12	962.96	8.42	59.40	1.14	571.98	5.00
2.00	88.73	2.04	904.20	6.99	48.98	0.89	442.84	3.42
3.00	88.58	1.30	576.43	4.67	24.91	0.29	143.61	1.16
AVG	89.32	1.82	814.53	6.70	44.43	0.77	386.14	3.20
+/- STD	0.94	0.37	170.07	1.54	14.44	0.36	179.42	1.57
4.00	91.55	2.45	1119.83	8.65	57.31	1.28	641.74	4.96
5.00	96.60	4.84	2338.84	19.08	63.84	2.99	1493.10	12.18
6.00	96.32	2.88	1386.40	12.28	61.11	1.69	847.18	7.50
7.00	94.09	2.37	1114.90	13.75	40.97	0.91	456.83	5.63
AVG	94.64	3.13	1489.99	13.44	55.81	1.72	859.71	7.57
+/- STD	2.03	1.01	502.24	3.75	8.87	0.78	390.88	2.82
48HRS								
1.00	89.41	1.06	474.21	3.49	16.39	0.16	77.74	0.57
2.00	88.06	1.33	584.20	4.82	20.14	0.24	117.65	0.97
3.00	89.57	1.56	699.71	5.15	16.70	0.23	116.85	0.86
AVG	89.01	1.32	586.04	4.49	17.74	0.21	104.08	0.80
+/- STD	0.68	0.20	92.07	0.72	1.70	0.04	18.63	0.17
4.00	96.04	5.42	2601.12	22.17	4.18	0.22	108.67	0.93
5.00	95.56	2.96	1413.21	9.79	12.79	0.36	180.70	1.25
6.00	95.78	2.01	964.23	6.73	17.79	0.34	171.51	1.20
7.00	94.33	1.88	888.71	5.68	6.92	0.12	61.47	0.39
AVG	95.43	3.07	1466.82	11.09	10.42	0.26	130.59	0.94
+/- STD	0.65	1.42	684.90	6.57	5.27	0.10	48.59	0.34
72HRS								
1.00	87.37	1.74	760.23	6.28	36.36	0.55	276.45	2.28
2.00	84.95	1.19	503.49	3.37	15.77	0.16	79.38	0.53
3.00	87.40	1.06	464.55	3.19	11.50	0.11	53.40	0.37
AVG	86.58	1.33	576.09	4.28	21.21	0.27	136.41	1.06
+/- STD	1.15	0.29	131.17	1.41	10.86	0.20	99.59	0.87
4.00	96.74	2.51	1214.49	9.83	42.45	1.03	515.58	4.17
5.00	95.71	2.00	957.89	6.08	44.21	0.85	423.50	2.69
6.00	69.04	3.75	1295.38	10.40	45.89	1.19	594.39	4.77
7.00	96.59	1.22	587.83	4.87	3.88	0.05	22.82	0.19
AVG	89.52	2.37	1013.90	7.80	34.11	0.78	389.07	2.96
+/- STD	11.83	0.92	275.75	2.37	17.49	0.44	219.94	1.77

Appendix 15: Systemic movement of azadirachtin feeding bioassay.

	TREATMENT- TOTAL AZADIRACHTIN APPLIED/TIME OF ECB INFESTATION FOLLOWING APPLICATION														
	CONTROL/ 24 HRS (0 Mg AZA)			10 µg AZA/24 HRS			100 µg AZA/24 HRS			1000 µg AZA/24 HRS			1000 µg AZA/48 HRS		
	AREA OF TREATED AND UNTREATED LEAVES CONSUMED BY LARVAE, # ECB REMAINING ON SEEDLING AFTER 72 HRS FEEDING														
REPLICATES	TREATED	UNTREATED	#ECB	TREATED	UNTREATED	#ECB	TREATED	UNTREATED	#ECB	TREATED	UNTREATED	#ECB	TREATED	UNTREATED	#ECB
1	112	166	5	276	195	9	130	147	6	51	176	9	2	138	4
2	151	78	9	224	146	6	98	175	6	10	57	5	92	85	1
3	145	93	8	338	212	8	24	143	6	0	143	6	6	155	4
4	287	200	9	157	86	7	35	175	7	0	204	3	55	11	5
5	140	137	7	58	127	7	112	158	8	46	236	6	13	178	9
MEAN	167	135	6	211	153	7	80	160	7	21	163	6	34	133	5
STD DEV	62	45	2	97	46	1	42	14	1	23	61	2	35	33	3

Ten 3rd instar larvae were placed on V-2 corn seedlings following a period of 24 and 48 hours (1000 µg AZA treatment only) post-application of AZA.

Treated mature leaves and young untreated leaves were evaluated for feeding damage following a 72 hrs feeding period.