Laboratory and field evaluations of imidacloprid against the migratory grasshopper, Melanoplus sanguinipes (F.), and the cereal leaf beetle, Oulema melanopus (L.), on small grains
by Cecil I Tharp

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Entomology
Montana State University

Abstract:
The toxicity of imidacloprid to the migratory grasshopper, Melanophis sanguinipes (Fabricius) and cereal leaf beetle, Oulema melanopus (Linneaus), was measured under laboratory and field conditions. Mortality, damage, and lethal dosage values were determined from artificial and natural infestations of M. sanguinipes at various growth stages of winter and spring wheat. All rates of imidacloprid tested caused < 60% mortality to 4th instar M. sanguinipes. A high LD50 confirmed that imidacloprid had low toxicity, however, 100% sickness was observed at concentrations ≤ 1 ppm. Combining imidacloprid with insect pathogens or another insecticide may significantly improve control.

All rates of imidacloprid as a seed treatment caused > 90% mortality to cereal leaf beetle larvae when eggs were applied at the 2-leaf and 4-leaf stage, but were ineffective when eggs were applied at the flag-leaf and early tillering stages of barley. This window of high toxicity played a role in field trials where peak larval emergence did not occur until the early tillering stage of barley. The resulting mortality never exceeded 30%. Foliar imidacloprid, however, caused > 90% mortality in the field, and may be another option in the management of the cereal leaf beetle.

LABORATORY AND FIELD EVALUATIONS OF IMIDACLOPRID AGAINST THE MIGRATORY GRASSHOPPER, *MELANOPLUS SANGUINIPES* (F.), AND THE CEREAL LEAF BEETLE, *OULEMA MELANOPUS (L.)*, ON SMALL GRAINS

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APPROVAL

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Cecil I. Tharp

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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ABSTRACT

The toxicity of imidacloprid to the migratory grasshopper, *Melanoplus sanguinipes* (Fabricius) and cereal leaf beetle, *Oulema melanopus* (Linneaus), was measured under laboratory and field conditions. Mortality, damage, and lethal dosage values were determined from artificial and natural infestations of *M. sanguinipes* at various growth stages of winter and spring wheat. All rates of imidacloprid tested caused < 60% mortality to 4th instar *M. sanguinipes*. A high LD_{50} confirmed that imidacloprid had low toxicity, however, 100% sickness was observed at concentrations ≥ 1ppm. Combining imidacloprid with insect pathogens or another insecticide may significantly improve control.

All rates of imidacloprid as a seed treatment caused > 90% mortality to cereal leaf beetle larvae when eggs were applied at the 2-leaf and 4-leaf stage, but were ineffective when eggs were applied at the flag-leaf and early tillering stages of barley. This window of high toxicity played a role in field trials where peak larval emergence did not occur until the early tillering stage of barley. The resulting mortality never exceeded 30%. Foliar imidacloprid, however, caused > 90% mortality in the field, and may be another option in the management of the cereal leaf beetle.

CHAPTER 1

INTRODUCTION

Organophosphate, carbamate, and organochlorine insecticides have offered excellent control and residual activity in pest management through the past. However, in the past 40 years more than 364 species of arthropods have developed resistance towards chemicals in these classes (Vidal 1993, Georghiou and Taylor 1977). Resistance is a major threat to public health and agriculture worldwide (Georghiou and Saito 1983). Foliar applications of these chemicals also cause high mortality to insect predators and/or parasitoids, further increasing the potential of secondary pest resurgence (Trichilo and Wilson 1993). New chemistries and application methods can be combined with biological and cultural control methods to improve pest management and reduce negative impacts of pesticide use.

Imidacloprid, having a different mode of action, is formulated as a foliar, seed, and soil treatment (Lentz and Austin 1994). While organophosphate, carbamate, and organochlorine chemicals inhibit acetylcholine esterase, imidacloprid binds to nicotinergic acetylcholine receptors in the postsynaptic region of the insect nerve (Bai et al. 1991, Schroeder and Flattum 1984). This novel mode of action enables many insects resistant to traditional insecticides to be susceptible.

The exposure of nontarget species is minimal when imidacloprid is applied as a seed treatment (Gaucho 480F) or soil application (Admire 2.0). These formulations avoid

nontarget impact by mainly affecting pests that consume leaf tissues and plant fluids (Mizell and Sconyers 1992). This is a high priority for pest management programs that promote biocontrol and stringent environmental standards. Imidacloprid has been documented as being highly effective against sucking pests, such as leaf hoppers, plant hoppers, aphids, thrips, and whiteflies. However, studies on many insects are incomplete or lacking (Pawar et al. 1993, Schmeer et al. 1990, Elbert et al. 1990).

The migratory grasshopper, *Melanoplus sanguinipes* (Fabricius), is an important pest of cereals throughout North America. This pest has caused extensive damage in the central and north central states as early as the 1800's (Capinera and Sechrist 1982). Infestations in 1944 destroyed 70 - 80% of the grasses in the open range of British Columbia causing $28 million in damage. Pickford and Mukerji (1974) indicated that an average of two grasshoppers per plant could achieve such a yield loss.

To control outbreaks of *M. sanguinipes*, poison arsenite baits carried on sawdust were used in the early 1900's (Shotwell 1942, Paul 1942). However, the use of baits diminished because organochlorine, organophosphate, carbamate, and pyrethroid insecticides increased in popularity. Aqueous formulations of these chemicals offered a high degree of control of grasshoppers with carbofuran and carbaryl being widely used for managing infestations (Fuller et al. 1992). However, Mukerji et al. (1981) reinitiated the study of baits against grasshoppers because of the problems of nontarget impact with foliars. This study indicated that host specific carbamate baits failed to achieve > 71% mortality at acceptable rate-doses in the field.

Although the carbamate baits were unsuccessful at attaining acceptable mortalities in

the field (Mukerji et al. 1981), imidacloprid as a seed treatment may cause sufficient mortality while also reducing the nontarget impact problem. Blodgett et al. (1995) evaluated grasshopper damage to winter wheat seed treated with imidacloprid. Treated plots had significantly less grasshopper damage than untreated plots. Although these results suggest successful protection of plants treated with imidacloprid, a more comprehensive study analyzing the qualitative and quantitative responses of grasshoppers against imidacloprid was needed.

In 1962 the cereal leaf beetle, *Oulema melanopus* (Linneaus), was identified in Michigan (Haynes and Gage 1981). Since then it has spread throughout the U.S., and has been present in Montana since 1989 (Morrill et al. 1992). This pest causes economic yield losses in wheat, barley, and oats. Studies have documented a 56% yield loss in barley at 2.6 larvae per stem, and a 38% yield loss in barley at 1.6 larvae per stem (U.S. Animal & Plant Health Inspection Service 1994, Webster and Smith 1979). In some cases farmers have abandoned crops when heavy infestations eliminated a profitable yield.

Beetle infestations were quarantined and treated with insecticides before an extensive host plant resistance program was initiated. By 1966, over 1.6 million acres received at least one blanket spray of carbaryl, but this application failed to control the spread of this pest (Haynes and Gage 1981). A study was initiated that would attempt to use host plant resistance to lower the high yield loss from this pest. Pubescent varieties of wheat, specifically trichrome length, were documented as a source of resistance towards cereal leaf beetle larvae (Webster and Smith 1979). However, developing host resistance was

time consuming and may be overcome by beetles in a short amount of time (Moffitt et al. 1993). There are no commercial varieties of wheat and barley available that incorporate this trait that will provide resistance to the cereal leaf beetle.

Biocontrol agents are thought to keep beetle populations under control in Europe where this pest is widespread. Consequently, a rearing program was established by the USDA Federal Plant Protection and Quarantine (PPQ) Division in Niles, Michigan, to develop and implement biocontrol measures to suppress beetle populations. Following the release of the parasitoid *Anaphes flavipes* (Foerester) in Michigan, it was hard to find one beetle in areas that researchers use to collect 10,000 a day (Wellso 1982). Because parasitoids take years to become established, insecticides are an important tool for short term control of this pest. This is especially true in areas where beetle populations were still expanding. Foliar applications of carbaryl, endosulfan, and carbofuran provided excellent control of this pest (Blodgett and Tharp 1996) but are broad spectrum and do not discriminate between this pest and its natural enemies. Minimizing non-target effects will be important to encourage development of biocontrol programs. If high rates of mortality could be achieved using a seed or soil treatment, nontarget impact would be minimized. Imidacloprid as a seed and/or soil treatment may be an excellent tool for these future infestations.

To determine if imidacloprid could be a viable alternative to traditional foliar treatments of cereal leaf beetle and grasshopper infestations, a study was designed to assess the toxicological effects on each pest after being fed imidacloprid-treated cereals in the laboratory and field. The overall objectives of this study were to determine the

mortality and foliar damage resulting from artificial and natural infestations of cereal leaf beetles and artificial infestations of grasshoppers feeding on 2-leaf (growth stage 12), 4-leaf (growth stage 14), early tillering (growth stage 22) and flag-leaf stage (growth stage 37; Zadok et al. 1974) barley, spring, and winter wheat. Cereals were treated with foliar imidacloprid and imidacloprid as a seed treatment. In addition to this, the oral and topical debilitation, $LD_{50's}$, and $LD_{90's}$ of grasshoppers vs. imidacloprid would be determined. Results obtained from imidacloprid treatments would then be compared to carbofuran as a standard.

CHAPTER 2

LITERATURE REVIEW

Migratory Grasshopper

History

The migratory grasshopper, *Melanoplus sanguinipes* F., causes more crop damage than any other grasshopper in the United States. High densities may damage or destroy fields of oats, barley, wheat, alfalfa, clover, corn, and vegetables (Pfadt 1994). In the 1800's, large swarms forced settlers to leave their homes convinced that profitable agriculture was not possible (Capinera and Sechrist 1982). Similar infestations in 1944 destroyed 70 - 80% of the grasses in the open range of British Columbia. Pickford and Mukerji (1974) quantified the damage caused by the migratory grasshopper from 1972 - 1973. They documented an 81% yield loss when two grasshoppers per spring wheat plant were present.

Life Cycle

The migratory grasshopper hatches from mid-late May dependent on temperature. Nymphs may hatch near crop borders, or within a field if the crop has been previously infested. They pass through five to six instars in 35 to 55 days before becoming adults in mid-late summer. Upon reaching maturity, females undergo a preoviposition period of two to three weeks in which they increase in weight, mate, and mature their first batch of

18 - 24 eggs. Eggs are deposited in a curved 2.5 cm by 0.3 cm egg pod that is deposited 2.5 cm - 5.0 cm below the surface of the ground. Pods may be located near fence rows or around the base of wheat stubble and alfalfa. A female may produce as many as 20 egg pods during her lifetime (Pfadt 1994, Capinera and Sechrist 1982).

The high reproductive rate may result in infestation levels that can lower a crop yield by: 1) completely destroying crops, 2) depressing yield by stress, 3) stem breakage leaving the grain head unharvestable. Damage is exacerbated by the migratory behavior of this pest. Grasshoppers may migrate when temperatures exceed 29^{0}C, and densities are > 32 per m^2. They will fly long distances, before descending to destroy crops (Pfadt 1994). Flights usually begin in midmorning and may continue until late afternoon when the grasshoppers stop to feed and rest. Densities may increase to several hundred per square meter after the swarm descends (Capinera and Sechrist 1982). The longest migration recorded in 1938 was made by a swarm flying from northeastern South Dakota to the southeastern corner of Saskatchewan, a distance of 920 kilometers (Pfadt 1994). These swarms, which were common through the 1800's and early 1900's, were thought to be the Rocky Mountain locust *Melanoplus spretus* (Walsh). However, *M. spretus* is now believed to be a swarming phase of *M. sanguinipes* (Capinera and Sechrist 1982). Weather, insecticides, and pathogens are believed to be responsible for keeping densities of *M. sanguinipes* at levels lower than necessary to produce migratory behavior in recent years. However, *M. sanguinipes* is believed to maintain the potential for aggregative swarming if conditions become optimum.

Rearing

To understand and develop control measures, researchers initiated a grasshopper rearing program. A successful program depended on scientists determining optimum rearing temperatures and age related forage utilization. This information enabled scientists to efficiently produce a supply of grasshoppers that could be used for insecticide and behavioral trials during months when natural infestations were unavailable. This increased the efficiency and volume of research that could be attempted on this pest in one year.

Visscher et al. (1979) investigated the effects of different rearing temperatures on grasshoppers. This study indicated an optimum rearing temperature of 30°C and 24°C with a corresponding photoperiod of 16L:8D h for day and night, respectively. Cooler rearing temperatures reduced the fecundity, egg viability, and longevity of grasshoppers (Visscher et al. 1979). Hewitt (1979) confirmed that lower temperatures caused higher mortality and reduced feeding at temperatures below 21°C. Consequently, temperature is an important factor when assessing grasshopper mortality in the laboratory or field.

Age-related forage utilization became increasingly important as studies isolating the damage potential of certain instars became more common. Onsager (1983) initiated such a study which included the relationships between survival rates, forage utilization, and instars of *M. sanguinipes*. Onsager concluded that the first three instars utilized only 15 to 20% of the total forage potential (consumed + waste). Later instars and adults were responsible for both the greatest rate of forage utilization and also for the greatest proportion of forage utilization (Onsager 1983). Since the first three instars were not

responsible for a large proportion of damage, they may be less important when grasshopper feeding aspects on biomass or yield are studied.

Insecticides

High densities of grasshoppers on range or croplands have frequently required the use of insecticides. During the 1930's and early 1940's, sodium arsenite bait, using sawdust as carriers, as a common and effective tool for managing grasshopper outbreaks (Shotwell 1942, Paul 1942). The use of baits virtually disappeared with the introduction of organochlorine insecticides such as DDT. During the last four decades aqueous sprays of organochlorine, organophosphate, and carbamate insecticides have been the primary tool for managing grasshoppers (Mukerji et al. 1981). Consequently, the results of insecticide trials which evaluated the toxicities of many different foliar treatments were used for developing decision making guidelines. Fuller et al. (1992) indicated carbofuran at 421 gai/ha, and all labeled rates of carbaryl to significantly lower grasshopper numbers below the economic threshold of eight grasshoppers per m^2. These findings indicated the high toxicities of carbaryl and carbofuran towards grasshoppers. The high toxicity of carbofuran was also evident when Onsager and Muzuranich (1986) calculated the oral and topical lethal dosages against grasshoppers . They determined the oral and topical LD_{50} for carbofuran to be 1.7 and 0.3 ppm, respectively.

Currently dimethoate, disulfoton, carbofuran, chlorpyrifos, malathion, methyl parathion, ethyl parathion, methyl parathion, semaspore bait, carbaryl, phorate, and lamda-cyhalothrin are labeled for control of grasshoppers on wheat in Montana. Carbofuran, malathion, methyl parathion, ethyl parathion, methyl parathion, and

Nosema locustae bait are labeled for use on barley (Hendrickson and Johnson 1998). Foliar applications of insecticides offer a high degree of pest control, but spraying may be expensive both in terms of investment and environmental risk (Mukerji et al. 1981). Alternative control methods such as biocontrol and/or seed treatments eliminate the need for reapplication, while being very host specific and reducing negative nontarget effects. Research on these methods of pest management may be another option for pest control in the future.

Cereal Leaf Beetle

History

The cereal leaf beetle, *Oulema melanopus* (L.), is indigenous to Europe, with the beetle reaching its greatest damage potential in eastern and southeastern Europe. This pest is also found in Hungary, Yugoslavia, Rumania, North Africa, Central Asia and the southern parts of Kharkov (Haynes and Gage 1981, Spears 1964). The insect was first discovered in North America in Michigan in June 1962. However, the beetle was believed to be present in low densities from the mid 1950's, due to reports of producers spraying an unidentified pest for years prior to its identification (Haynes and Gage 1981). The cereal leaf beetle then began a slow but continuous dispersal throughout the U.S., driven by the availability of food sources, favorable environmental conditions, and appropriate weather patterns (Haynes and Gage 1981). Infestations reached just past the Mississippi River by 1981 (Battenfield and Gage 1982), and were identified in Yellowstone County, Montana in 1989 (Morrill et al. 1992). Initially it seemed to infest

counties along the Yellowstone River system, but by 1997 this pest was found in 11 out of 12 counties in southeastern Montana. The insect now occurs in Connecticut, Delaware, Georgia, Idaho, Illinois, Indiana, Iowa, Kentucky, Maine, Maryland, Massachusetts, Michigan, Minnesota, Missouri, Montana, North Carolina, New Jersey, New York, New Hampshire, Ohio, Pennsylvania, South Carolina, Tennessee, Utah, Vermont, Virginia, West Virginia, and Wisconsin (Moffitt et al. 1993). More than 25% of the small grains grown in the U.S. are in areas infested with the cereal leaf beetle (Haynes and Gage 1981). This is expected to increase with the anticipated dispersal into the Dakotas and the Pacific Northwest .

Life Cycle and Damage

The cereal leaf beetle is univoltine and overwinters in any location protected from temperature extremes such as grain stubble, grass crowns, forest litter, and under bark (Castro et al. 1965, Yun 1967). Adults emerge in Montana when temperatures exceed 13°C (Morrill et al. 1992), and begin feeding on native grasses, winter grains, and eventually spring grains. Mating occurs from April to June in Montana. Females deposit up to 150 yellowish eggs individually or in pairs near the base of the plant (Helgesen and Haynes 1972). Larvae pass through four instars while feeding for two to three weeks on a wide range of host plants, including wheat, oats, barley, rye, corn, and certain grasses (Castro 1965, Yun 1967). They feed on the upper leaf surface, leaving cereals with a frosted appearance. Larvae drop to the ground to pupate, and adults emerge in two to three weeks. Adults feed for three weeks then seek shelter and become quiescent. Beetles search for overwintering sites in the fall when temperatures fall below 10°C. The

adults overwinter until the following spring when they reemerge and repeat the cycle (Hilterhaus 1965, Wellso et al. 1975).

This pest causes economic yield losses in wheat, barley, and oats. One larva per flag leaf may cause a five to six bushel loss of grain per acre (U.S. Animal & Plant Health Inspection Service 1994). Webster and Smith (1979) documented a 56% yield loss in barley at 2.6 larvae per stem, and a 38% yield loss in barley at 1.6 larvae per stem. High fecundity in combination with a high potential for damage has forced many farmers to abandon crops when extensive damage renders a crop unprofitable. The initial response in Michigan was containment and eradication, which was followed by an intensive program on host plant resistance, and finally a biocontrol effort of questionable success (Haynes and Gage 1981).

Quarantine and Eradication

Several approaches have been taken to reduce the economic losses that the cereal leaf beetle has forced upon many producers. In 1962, 20 townships in Michigan, and five in Indiana were quarantined, thus forcing all small grains being shipped out of these counties to be treated with insecticides (Dysart et al. 1973). By 1966, over three million bales of hay and straw, five million bushels of small grains, and large amounts of corn were treated with carbaryl. In conjunction with quarantines, a large scale eradication effort was implemented. These efforts were aimed at eradicating the cereal leaf beetle from sites in North America. By 1966, over 1.6 million acres received at least one application of carbaryl in the quarantined area (Haynes and Gage 1981). This provided temporary crop protection from cereal leaf beetle infestations, but did not halt the spread

of this insect. Since the pest continued to spread despite the large scale eradication program, it was decided to discontinue these attempts and focus on other management methods.

Host Plant Resistance

Many studies have examined host plant resistance for cereal leaf beetle management. Gallun et al. (1966) found pubescent wheat to be the least preferred for oviposition and feeding, due to the number of trichomes per unit area of leaf surface. Ringlund and Everson (1968) indicated a high correlation between larval mortality and leaf pubescence. However, the biological response of beetles to the length of trichomes was not fully understood. Hoxie and Wellso (1974) examined the effects of trichome length by introducing beetles to short and long trichomes. Larval survival decreased as density and trichome length increased. Although trichome length was the definitive factor for host resistance, a study which assessed all the varieties of wheat, oats, and barley was needed. The USDA-ARS, Michigan State University, and Purdue University jointly pursued a large scale resistant program, which analyzed over 30,000 varieties of wheat, oats, and barley. Resistance to cereal leaf beetles was found to occur in certain varieties of pubescent wheat and was related to trichome length and density (Haynes and Gage 1981). Although host plant resistance can be an effective tool for managing infestations, breeding resistant germplasm into agronomically acceptable small grain cultivars is a long term process. Beetles also may compromise plant resistance through development of resistance in a short amount of time (Moffitt et. al. 1993). Therefore, a biocontrol program became the focus of long term control of the cereal leaf beetle.

Biocontrol / Rearing

In 1963 the USDA-APHIS began searching for native parasitoids of the cereal leaf beetle in Eurasia. Scientists found four wasps in Europe which eventually became established in the U.S. The most effective, *Anaphes flavipes* (Foerster), injects its eggs into beetle eggs. *Tetrastichus julis* (Walker), *Lemophagus curtus* Townes, and *Diasparsis temporalis* (Linnaeus), inject their eggs into larvae where they hatch and feed, eventually killing the larvae. By 1966 the USDA APHIS Federal Plant Protection Quarantine (PPQ) Division established the cereal leaf beetle parasite rearing program at Niles, Michigan (Dysart et al. 1973). This station dispersed parasitoids to suppress beetle populations. Grain plants infested with beetle eggs and larval parasitoids, *A. flavipes*, were provided to farmers, extension service workers, and researchers to control beetle infestations. This program made it hard to find one beetle in areas that researchers use to collect 10,000 a day (Wellso 1982). Once the parasitoids were established in Michigan, beetle densities were reduced by 60%, and grain losses to <1%.

Although the biocontrol program successfully controlled the cereal leaf beetle in Michigan, continual supplies of biocontrol agents were difficult to maintain. This was due to an incomplete understanding of the period of diapause that the cereal leaf beetle must go through to survive. Connin and Hoopingarner (1971) determined that adult females require a period of diapause, while adult males were sexually mature regardless of cold storage time. Hoxie and Wellso (1983) later investigated temporal effects on fecundity and survivorship to cereal leaf beetles in cold storage at 27°C with 70%RH. As time in cold storage increased from 7 - 235 days there was a simultaneous decrease in

mortality, feeding days to oviposition and females not feeding. A successful rearing program for the cereal leaf beetle would have to mitigate the 10 - 17 week period of diapause that was necessary for adequate rates of survival.

Even with a more complete understanding of diapause requirements for the cereal leaf beetle, other conditions such as humidity, temperature needs and pupal recovery were additional barriers to establishing a rearing program. Castro (1964) partially solved this problem by rearing cereal leaf beetles using glass lantern globe cages. The cages were able to isolate cereal leaf beetles, while boosting humidity to levels that were necessary for beetle rearing. This method enabled the rearing of limited numbers of beetles, but complications arose recovering pupae from the soil. Connin et al. (1966) later solved this problem with the use of plaster of paris. The plaster aided in pupal recovery by preventing larvae from entering soil. The larvae were forced to pupate in sand that was applied over the plaster. Using this method, pupae were easily collected by sifting sand through a sieve, providing optimal survival.

With this additional information about temperature and humidity requirements, a program was developed which solved the problems associated with rearing the cereal leaf beetle. This program enabled the rearing of large numbers of all life stages which were used for insecticide trials, biocontrol studies, and other research projects. The rearing procedure used separate chambers for each life stage, with beetles being collected, transferred, and placed into refrigeration every three to four days. A photoperiod of 16:8 (L : D) h and temperatures of 28°C and 27°C, respectively, were used for rearing. A RH greater than 60% was also optimum for survival of the cereal

leaf beetle. This program produced more than 10,000 beetles per month which were used to meet all research needs for many years (Connin et. al. 1968). However, due to the time biocontrol takes to become established, insecticides continue to be an important tool for providing short term control of this pest.

Insecticides

Insecticide studies have been conducted since the pest was first identified in 1962, but the search for an insecticide which would effectively control beetle populations while causing a minimum impact on parasitoids did not occur until 1971. Ruppel and Stehr (1972) reported that spray timing provides the greatest protection of small grains from the pest, and also is recommended as a means of reducing exposure of the cereal leaf beetle parasite, *T. julis*, to the insecticides. Carbaryl and endosulfan offered a high level of protection for over two weeks, while acephate, and methidathion provided short-term mortality, with beetles becoming reestablished weeks after application (Ruppel 1973).

Much of the data on cereal leaf beetle insecticide trials were of limited use in Montana because of local climate and environmental conditions. Blodgett and Tharp (1996) investigated the effects of a variety of insecticides on the cereal leaf beetle in Montana. Chlorpyrifos, lamda-cyahalothrin, carbofuran, carbaryl, and malathion were found to offer excellent protection from this pest. Although this was true, many of the insecticides either were not labeled or were canceled on this pest in Montana as of 1998.

Insecticides currently available for cereal leaf beetle management in Montana are limited. As of May 1998 methomyl, malathion, carbaryl, and lambda-cyahalothrin are labeled for use against the cereal leaf beetle on wheat in Montana, while methomyl,

malathion, and endosulfan are labeled for use against the cereal leaf beetle on barley (Hendrickson and Johnson 1998). These options are even more limited due to restrictions regarding preharvest grazing and livestock feeding.

Imidacloprid

The search for new insecticides to aid in pest management has led to the discovery of heterocyclic nitromethylenes by Soloway et al. (1978). Imidacloprid, synthesized from this chemical group, can be formulated as a seed (Gaucho), soil (Admire), and foliar (Provado) insecticide treatment. This chemical has a novel mode of action, low toxicity towards mammals, and favorable environmental characteristics (Mullins 1993).

Resistance Management

Because resistance has developed by many insects to traditional insecticides, a search for a new insecticide became a priority (Elbert et al. 1990). Imidacloprid was found to offer excellent control of resistant pests such as planthoppers *Nephotettix cincticeps* Uhler, *Nilaparvata lugens* Stahl, *Laodelphax striatellus* (Fallen) and green peach aphids, *Myzus persicae* (Sulzer), due in part to its novel mode of action. Carbamates and organophosphates inhibit acetylcholine esterase, while pyrethroids trigger the sodium-ion channel (Leicht 1994). Imidacloprid, however, differs by binding to the nicotinergic acetylcholine receptors of the post synaptic region of the insect nerve. This binding leads to an opening in the sodium ion channel, thus producing uncontrolled muscle reflexes (Bai et al. 1991, Schroeder and Flattum 1984). Imidacloprid is degraded by acetylcholine

esterase much slower than acetylcholine (Mullins 1993). In many cases this action leads to the death of an insect.

Nontarget Organisms

Toxicological studies have indicated imidacloprid to have low mammalian toxicity. In addition, all imidacloprid based insecticides in the U.S. are proposed as Category III or "Caution" category products (Mullins 1993). Mammals are less sensitive to imidacloprid than insects because insects have a high proportion of nicotinergic acetyl choline receptors (imidacloprid binding site) in contrast to mammals (Bomann 1989). In acute toxicity tests, imidacloprid shows no eye or dermal irritation, and no mutagenic effects. Leaching is minimal because of a combination of a relatively short half life (< 150 days), and residues not reported below 30.5 cm of soil. If present in water, imidacloprid has little impact on water quality due to its half life of 1.4 days. This is primarily caused by the degradation of imidacloprid in water from sunlight. These aspects indicate the minimal effects that imidacloprid has on mammalian drinking water.

Pfluger and Schmuck (1991) analyzed the ecotoxicological profile of imidacloprid. Imidacloprid did not impair activity of soil microbes even at high doses. This, in combination with imidacloprid's predominantly systemic nature, causes a limited effect on beneficial insects such as lady bird beetles, syrphid flies, lacewings, and rove beetles (Heimbach 1986). However, imidacloprid was found to be very toxic towards birds and honey bees (*Apis mellifera* Linnaeus) in acute reproductive toxicity tests (Mullins 1993). This is minimized in practice due to imidacloprid's repellent effect, which reduces the danger towards these species (Schmidt 1989, Zeller 1990).

Efficacy Trials

Imidacloprid has a number of registered uses in agriculture. As a result, studies have been conducted on the compatibility of imidacloprid with fungicides. Pike et al. (1993) investigated the effects of combining seed treated with imidacloprid and fungicides (carboxin-thiram, triadimenol-captan, and tebuconazole-thiram) on the Russian wheat aphid. This study indicated no compatibility problems, and showed excellent control of aphids 27 - 85 days after planting. Imidacloprid was also found to be highly effective against leafhoppers, planthoppers, thrips, whiteflies, rice stem borers, rice leaf beetles, and rice water weevils (Schmeer et al. 1990, Elbert et. al. 1990, Pawar et al. 1993). Imidacloprid as a seed treatment provided excellent residual control suppressing green peach aphid populations from 60 - 80 days after planting (Sloderbeck et al. 1996). Foliar applications, however, required that aphids be exposed for up to five days before reaching maximum mortality. Topical applications may therefore induce sickness which causes increased mortality over time (Boiteau et al. 1997). Although imidacloprid is a broad spectrum insecticide, tests on many species are continuing.

Reports of grasshoppers being controlled with the use of imidacloprid have been received by Montana extension personnel periodically through the 1993 - 1995 field seasons (G. Johnson, Department of Entomology, Montana State University, Bozeman, personal communication). Information, however, was based on anecdotal reports and not replicated tests. Consequently, Blodgett et al. (1995) initiated a study evaluating imidacloprid applied as a winter wheat seed treatment for control of adult grasshoppers. They found that imidacloprid treated plots had significantly less grasshopper damage

than untreated plots which suggest an effect on grasshoppers. The degree of mortality and/or repellency was still unknown.

Sublethal Effects

Studies have indicated sublethal doses of imidacloprid to occasionally cause insects to become debilitated. Rates of debilitation were often inversely correlated with mortality (Kaakeh et al. 1997, Lagadic and Ludovic 1993, Schroeder and Flattum 1984). To increase mortality, researchers have often combined imidacloprid with other chemicals and/or pathogens to promote an acceptable level of mortality.

Lagadic and Ludovic (1993) reported imidacloprid to cause sublethal effects when used against tobacco budworms and Egyption cotton leafworms. Mortality was cumulative up to 96 h, but larvae ceased feeding after 10 - 20 minutes of consumption of imidacloprid treated foliage. Restricted feeding was believed to be due to paralysis of the mouthparts. This effect was also observed when house flies ingested nitromethylene heterocyclic insecticides (structural group that imidacloprid originates from) in laboratory assays (Schroeder and Flattum 1984). This caused rapid 100% knockdown (immobility) of flies, but a high percentage of recovery occurred. When houseflies were pretreated with sesamex, an inhibitor of microsomal mixed function oxidase, knockdown doses became lethal.

High rates of knockdown and recovery were also observed in a study conducted on the root weevil, *Diaprepes abbreviatus* (Linnaeus), when using imidacloprid. To solve this problem, Quintela and McCoy (1997) combined imidacloprid with the pathogens, *Metarhizium anisopliae* (Merschnikoff) and *Beuvaria bassiana* (Balsomol). Each

combination significantly increased mortality from 40 - 50% over imidacloprid alone, to 90 - 100% at > 100 ppm. Kaakeh et al. (1997) also assessed the synergism of *M. anisopliae* and imidacloprid, on the German cockroach. Cockroaches exhibited significantly higher mortality after feeding on imidacloprid after a topical application of spore suspensions of *M. anisopliae*, than compared with imidacloprid alone. Each study indicated that the loss of mobility brought about by imidacloprid may have prevented first instars from removing conidia which eventually caused death.

Each study indicates the strong possibility of enhancing mortality through the use of chemicals and/or pathogens when sublethal effects are present. Therefore, the presence of debilitated insects is an important factor when analyzing the effects of imidacloprid on various pests, even when mortality seems very low.

CHAPTER 3

GRASSHOPPER STUDIES

Materials/Methods

Two thousand 4th instar migratory grasshoppers, *M. sanguinipes*, were obtained from the USDA-ARS Rangeland Insect Laboratory at MSU-Bozeman. These grasshoppers were used for all trials using spring wheat. Three hundred adult *M. sanguinipes* were collected from field sweeps on rangeland near Three Forks, Gallatin County, MT. These grasshoppers were used for all winter wheat studies. Field collected grasshoppers were placed in acetate tubes with lettuce and bran before being placed in a greenhouse bay with a photoperiod of 16:8 (L : D) h and temperatures of 30°C:24°C. Procedures used for rearing grasshoppers were developed by the USDA-ARS Rangeland Insect Laboratory in Bozeman, MT (J. Onsager, USDA-ARS, Sidney, MT, personal communication).

Greenhouse Trials.

Greenhouse experiments were conducted at the Montana State University Plant Growth Center at Bozeman, MT. A photoperiod of 16:8 (L : D) h and temperatures of 30°-24°C were maintained in the greenhouse. The first trial consisted of determining the effects of imidacloprid on *M. sanguinipes* infesting 4-leaf (growth stage 14) spring wheat, *Triticum aestivium* Linneaus (cv. 'Newana'). Twenty seven 51.0 cm by 40.0 cm by 6.5 cm plastic trays were planted with spring wheat. Seed was treated with

imidacloprid at 0.60 or 0.90 gai/kg (Gaucho 480F, Gustafson, Plano, TX). Both treatments and an untreated control were each planted in two rows of ten seeds per row, 13 cm apart on 18 Jan 1996. Three treatments were arranged in a randomized block design with nine replications. Twenty seven aluminum framed screen cages, each measuring 35 cm by 35 cm by 89 cm, were placed over the trays when the wheat reached the 4-leaf stage. Two centimeters of soil were deposited around the base of each cage before adding seven 4th instar grasshoppers. A second greenhouse trial assessed the effects of grasshoppers applied to 4-leaf stage spring wheat treated with imidacloprid at twice the recommended labeled rate. Spring wheat seed was treated with imidacloprid at 1.3 and 1.9 gai/kg. These two treatments and an untreated control were planted in four rows 2.5 cm apart with ten seeds per row. Procedures used in the previous trial were followed.

For each greenhouse experiment, grasshopper mortality and leaf defoliation were assessed daily until control plants were entirely consumed. Mortality was corrected using Abbott's formula (Abbott 1925). Leaf defoliation was assessed visually using a numerical rating from 0-5, where 0 = no leaf defoliation, 1 = 1-25%, 2 = 26-50%, 3 = 51-75%, 4 = 76-99%, and 5 = 100%. An average defoliation was obtained by rating each stem for the entire cage (Olfert et al. 1995).

Bioassays

A Conviron incubator model PGR15 (Conviron Company, Winnipeg, Manitoba), with a photoperiod of 16:8 (L : D) h and temperatures of 30°C:24°C was used for grasshopper bioassays. A mean weight of 30 *M. sanguinipes* was used to determine dosages (active

ingredient per unit of grasshopper body weight) for each trial. Dosages were obtained by diluting imidacloprid with distilled water in four 30 ml flasks.

Oral Applications. Selected concentrations of imidacloprid were applied with a Burkard microapplicator (Burkard Industries, Rickmansworth, England) to 60 lettuce disks that were cut using an 0.4 cm hole punch (Table 1). Fifty to sixty grasshoppers, starved for 24 h, were trapped under a 120 ml specimen container with a treated lettuce disk. Grasshoppers which consumed the entire lettuce disk within four hours were collected and placed in four 7.5 cm by 61 cm acetate tubes containing untreated lettuce and bran. The tubes were then placed in the incubator. Syringes were washed with a 70% ethanol solution and rinsed with distilled water between each application. Every 24 h fresh lettuce and bran were added to each tube (J. Onsager, USDA-ARS, Sydney, MT, personal communication).

Topical Applications. Dosages of imidacloprid were applied onto the wingbuds of each grasshopper with the microapplicator (Table 1). Procedures outlined for the oral application bioassay were followed.

Table 1. Selected concentrations used for oral and topical bioassays.

Application	Trial #	Date	Sample Size	Concentrations (ppm)
Oral	1	5 April, 96	40	0.1, 1.0, 10.0, 100.0
	2	12 April	40	8.0, 18.0, 27.0, 50.0
	3	22 April	40	20.0, 38.0, 70.0, 130.0
	4	27 April	50	46.0, 63.0, 90.0
Topical	1	1 June	40	1.0, 10.0, 100.0, 200.0
	2	6 June	50	50.0, 500.0, 1000.0

Recording Results. The time period used for analyzing mortality was 72 h. This was due to a large percentage of recovery up to 72 h. Mortality was corrected using Abbott's correctional formula with corrected mortalities between 20% and 80% used for the analysis. Values within this range have the most weight in a probit analysis (J. Onsager, USDA-ARS, Sidney, MT, personal communication). Debilitation was determined at 0.08, 0.17, 0.5, 1, 3, 24, 48, 72, 96, and 120 h intervals after treatment. Debilitation was determined by gently rolling grasshoppers on their side. If they could not reposition within ten seconds, but still exhibited leg and antennal movements, they were designated debilitated (J. Onsager, USDA-ARS, Sidney, MT, personal communication).

Field Trials.

All field trials were conducted at the MSU Central Agricultural Research Center, Moccasin, Judith Basin County, MT. Field trials were separated into spring wheat, *T. aestivium* (cv. 'Fortuna'), and winter wheat, *T. aestivium* (cv. 'Tiber'), experiments to analyze imidacloprid efficacy as influenced by season such as weather, host variety, and/or maturity of pest.

Spring Wheat. Three plots, 20 m by 5 m, were planted with seed treated with imidacloprid at 0.90 gai/kg. Nine plots were planted with untreated (for carbofuran, foliar imidacloprid, and control) spring wheat. Plots were seeded at 14 kg/ha using a 2.5 m wide John Deere double disk drill (John Deere Corporation, Waterloo, Iowa). Treatments (foliar and seed treatments of imidacloprid, carbofuran, and a control) were arranged in a randomized block design with three replications. A 2.5 m by 2.5 m section

was flagged and labeled flag-leaf stage, and the remainder of the plots were labeled 4-leaf stage.

Foliar treatments of imidacloprid (Provado 1.6F, Miles Inc., Kansas City, MO), and carbofuran (Furadan 4F, FMC Corporation, Princeton, NJ) were applied when spring wheat reached the 4-leaf (growth stage 14) and flag-leaf (growth stage 37). Carbofuran at 110.50 gai/ha and foliar imidacloprid at 51.50 gai/ha were applied on 24 June and 11 July with a CO_2-powered backpack sprayer equipped to deliver 280 ml/15 sec. at 14 kgsi with four Teejet model XR800VS nozzles (Spraying Systems, Wheaton, Illinois). At 24 h post application, eight 4th instar *M. sanguinipes* were placed inside two 19 liter plastic bucket cages on each plot. Cages had six centimeters of screen along the base, and a 14 cm screen over the top to provide air circulation.

Winter Wheat. Nine plots measuring 2.0 m by 6.6 m plots were planted with seed treated with imidacloprid at 0.90, 0.60, 0.30 gai/kg; nine plots were planted with untreated winter wheat. Plots were seeded at 8.1 kg/ha with a 2.0 m wide model 8000 hoe drill (Haybuster, Fargo, ND). Six treatments (three rates of imidacloprid seed treatment, foliar imidacloprid, carbofuran, and untreated) were arranged in a randomized block design with three replications. A 3.3 m by 1 m section or half of each plot was flagged and labeled 2-leaf stage while the other half was flagged and labeled early tillering stage.

Foliars insecticides were applied when wheat reached the 2-leaf (growth stage 12) and early tillering stages (growth stage 22). Carbofuran at 220 gai/ha and foliar imidacloprid at 51.50 gai/ha were applied to the plots on 26 August and 8 September

using a CO_2-powered backpack sprayer equipped to deliver 280 ml/15 sec. at 14 kgsi with four Teejet XR800VS nozzles. The following day, two 19 liter bucket cages were placed on each plot before placing five adult *M. sanguinipes* inside.

For both spring and winter wheat trials weekly mortality and leaf defoliation estimates were recorded from enclosures using methods previously described.

Statistics

Enclosures. Treatment effects over time were analyzed using PROC ANOVA with time as a repeated measures (P=0.05; SAS Institute 1989). If treatment and/or interaction effects were significant, treatment effects for each time period were analyzed using the Tukeys studentized range test (SAS Institute 1989).

Bioassays. Corrected mortality for the different doses was analyzed using PROC PROBIT (SAS Institute 1989). Debilitation values were transformed to a natural log + 0.0001 scale and analyzed using time as the independent variable and debilitation as the dependent variable.

Results

Greenhouse Trials

Repeated measures analysis indicated no significant treatment or treatment by time interaction effects present for mortality or defoliation when grasshoppers were placed on spring wheat seed treated with imidacloprid at 0.60 gai/kg (F = 1.71; df = 2, 64; P =0.21) or 0.90 gai/kg (F = 0.81; df = 8, 64; P = 0.59). Mortality did not exceed 12% for either imidacloprid treatment. Seed treated wheat plants were totally consumed by

day 5. Although leaf defoliation was severe, there were no significant defoliation differences or interactions between either rate of imidacloprid and the untreated ($F = 0.11$; df = 2, 64; $P = 0.90$; $F = 0.55$; df = 8, 64; $P = 0.81$, respectively).

At the 1.30 and 1.90 gai/kg rates, differences between treatments ($F = 5.03$; df = 2, 144; $P = 0.02$), and treatment by time interaction effects were significant for grasshopper mortality using repeated measures analysis ($F = 2.36$; df = 18, 144; $P = 0.002$). Mortality, although low, was significantly different for each rate of imidacloprid compared to the untreated by day 8 (Table 2). Mortality of grasshoppers in the 1.90 gai/kg treatment increased to 31% by day 14, while the 1.30 gai/kg rate increased to 16% at 12 days post treatment, then fell to 5% (Table 2).

Overall leaf defoliation differences and interactions effects were significant between the 1.30, 1.90 gai/kg rates and the untreated control using repeated measures analysis ($F = 6.61$; df = 2, 144; $P = 0.008$; $F = 4.77$; df = 18, 144; $P = 0.0001$, respectively). Leaf defoliation in both treated rates was significantly less than the untreated ($P < 0.03$) by day 8, and remained significantly lower than the untreated through the remainder of the experiment. By day 14 leaf defoliation ratings averaged 3.6, 4.0, and 5.0 for the 1.90, and 1.30 gai/kg treatments, and the untreated, respectively (Table 3). The experiment was terminated by day 14 because foliage was consumed in all controls.

Bioassays

Oral Applications. Within minutes of ingestion of lettuce disks treated with $\geq$ 1 ppm of imidacloprid, 100 % of the grasshoppers exhibited leg flexing, abdominal quivering, and tremors. Grasshoppers then became motionless and appeared dead. Knockdown

was temporary; > 70% of grasshoppers recovered after 24 h of ingestion at concentrations ≤ 10 ppm. Grasshoppers fed 100 ppm treated lettuce disks remained debilitated throughout the experiment (Fig. 1).

The Pearsons' goodness of fit test indicated a significant fit of observed vs. predicted values using probit analysis ($X^2 = 0.96$). This model predicted an oral LD_{50} of 53.38 ppm. Mortality >90% did not occur until concentrations reached 100 ppm (Table 4).

Topical Applications. All grasshoppers treated topically with imidacloprid had difficulty staying upright, jumping, and/or holding onto perches within 24 h, although grasshoppers remained partially mobile (Fig. 2). One hundred percent of grasshoppers were debilitated at 24 h post ingestion at all doses and remained constant at all doses ≥ 100 ppm throughout the five day period. However, percent debilitation was reduced to 40% at 10 ppm (Fig. 2).

A significant fit of observed vs. predicted values was found when grasshopper mortality was analyzed with probit analysis ($X^2 = 0.38$). This statistical model predicted a topical LD_{50} of 86.12 ppm. Although 50% mortality was reached at < 100ppm, topical applications of imidacloprid did not increase mortality beyond 90% at any concentration (Table 4).

Field Trial

Spring Wheat. Grasshopper mortality and/or leaf defoliation from 4-leaf stage wheat treated with foliar and seed applied imidacloprid and foliar carbofuran applications indicated no significant ($P > 0.05$) insecticide or insecticide by time interaction with the

repeated measures analysis (Table 5). Grasshopper mortality reached 20% after feeding on plants treated with carbofuran and foliar imidacloprid. Wheat seed treated with imidacloprid at the 0.90 gai/kg rate caused no grasshopper mortality 21 days post treatment.

Mortality was significantly different between foliar applied imidacloprid, carbofuran and untreated samples after grasshoppers were placed on flag leaf treated wheat using the repeated measures analysis ($F = 97.92$; df = 3, 18; $P = 0.0001$). By day 7, 93% mortality was recorded in carbofuran treated plots and 38% mortality was recorded in foliar imidacloprid treated plots. Mortality in the foliar imidacloprid plots increased to 54% by day 21. However, seed treatment mortality never exceeded 6% (Table 6).

Repeated measures analysis indicated significant differences in leaf defoliation at the flag-leaf stage between treatments ($F = 19.59$; df = 3, 18; $P = 0.001$). Differences were recorded between carbofuran and untreated plots by day 7 (Tukey Test; $P < 0.05$). The defoliation index remained at approximately 2.1 in carbofuran treated enclosures throughout the experiment compared to 4.1 in the untreated by day 21. Defoliation differences were not present between foliar and seed treatments of imidacloprid and untreated plots at day 21 (Table 7).

Winter Wheat. Repeated measures indicated significant differences in grasshopper mortality between treatments ($P = 0.04$) of foliar and seed treatments of imidacloprid and carbofuran after being applied to 2-leaf stage winter wheat. Mortality ranged from 92 - 96% in carbofuran plots and was significantly different than the untreated throughout the experiment. Foliar imidacloprid caused 43% mortality by day 21, but

mortality was not significantly different than the untreated ($P < 0.05$). Both seed treated rates of imidacloprid caused approximately 30% mortality, although neither rate was significantly different from the other (Table 8).

Significant differences in defoliation were present between treatments at the 2-leaf stage of winter wheat ($P = 0.03$) using repeated measures analysis. Defoliation was significantly lower in carbofuran treated plots (0.5), compared to the untreated (4.7) by day 14. Seed and foliar imidacloprid treatments contained less leaf defoliation than the untreated although differences were not significant (Table 9).

When grasshoppers were placed on early tillering winter wheat (growth stage 22), repeated measures analysis indicated significant differences in mortality between treatments ($F = 12.07$; df = 4, 16; $P = 0.001$), while a significant treatment by time interaction was absent ($P > 0.05$). Carbofuran caused 100% mortality by day 7, compared to 17% in the foliar treatment of imidacloprid. Seed treated with imidacloprid at 0.30 and 0.90 gai/kg caused 46% and 38% mortality by day 21, respectively (Table 10).

Leaf defoliation was significantly different between treatments ($F = 11.17$; df = 4, 16; $P = 0.002$) at the early tillering stage when analyzed using repeated measures. Carbofuran treated plots contained significantly less defoliation at each time interval when compared to the untreated. The mean defoliation rating of 0.5 was recorded in carbofuran treated plots as compared to 2.3 in untreated plots by day 21. Defoliation in plots treated with the 0.90 gai/kg rate of imidacloprid seed treatment (1.4) was significantly less than in the untreated plots by day 21.

However, foliar imidacloprid treated plots provided little protection from grasshoppers, with no significant defoliation differences recorded at any time interval (Table 11).

Table 2. Corrected grasshopper mortality ± SE after being applied to 4-leaf stage spring wheat planted with imidacloprid treated seed at 1.30 and 1.90 gai/kg under greenhouse conditions.

Treatment	Rate (gai/kg)	Days Post Grasshopper Application									
		1	2	3	4	5	6	8	10	12	14
Imidacloprid	1.30	4 ± 3	4 ± 3	4 ± 3	7 ± 5	9 ± 5	9 ± 5	11± 5*	16± 6*	16± 5	5 ± 4
Imidacloprid	1.90	4 ± 3	4 ± 3	4 ± 3	9 ± 4	9 ± 4	9 ± 4	11± 4*	18± 4*	28± 5*	31± 6*
F-Statistic		1.00	1.00	1.00	2.08	2.29	2.29	3.57	5.24	8.90	3.65
n		27	27	27	27	27	27	27	27	27	27
P-value		0.39	0.39	0.39	0.16	0.13	0.13	0.05	0.01	0.002	0.05

Means within columns followed by * are significantly different than the untreated (Tukey Test; P=0.05).

Table 3. Leaf defoliation (0-5) ± SE by grasshoppers applied to 4-leaf stage spring wheat that was seed treated with imidacloprid at 1.30 and 1.90 gai/kg.

Treatment	Rate (gai/kg)	Days Post Grasshopper Application									
		1	2	3	4	5	6	8	10	12	14
Imidacloprid	1.30	1.0±0.0a	1.2±0.1a	1.2±0.1a	1.2±0.1a	1.1±0.1a	1.3±0.1a	1.8±0.3b	3.0±0.5b	3.3±0.4b	4.0±0.4ab
Imidacloprid	1.90	1.0±0.0a	1.1±0.1a	1.1±0.1a	1.0±0.0a	1.1±0.1a	1.3±0.2a	1.8±0.3b	2.4±0.4b	2.9±0.4b	3.6±0.4b
Untreated		1.0±0.0a	1.1±0.1a	1.1±0.1a	1.2±0.2a	1.3±0.1a	1.7±0.1a	2.7±0.3a	4.3±0.4a	4.8±0.1a	5.0±0.0a
F-Statistic		Infinite	0.73	1.93	1.21	1.53	3.41	4.08	5.17	7.18	6.20
n		27	27	27	27	27	27	27	27	27	27
P-value		> 0.99	0.50	0.18	0.32	0.24	0.06	0.03	0.02	0.005	0.01

Means within columns followed similar letters are not significantly different (Tukey Test; P=0.05).

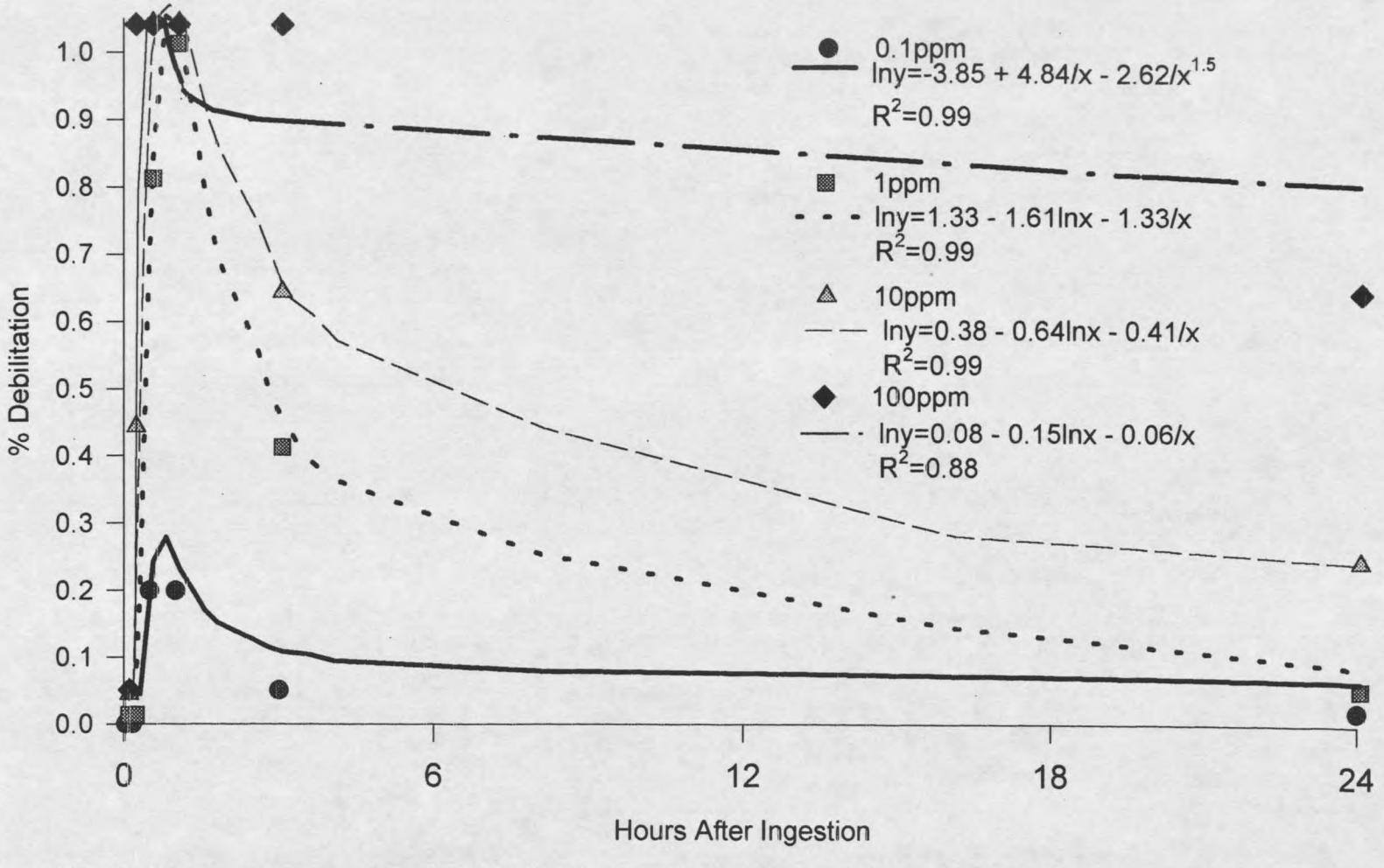

Fig. 1. Observed vs. predicted debilitation hours after imidacloprid ingestion by 4th instar *M. sanguinipes* (Symbols represent observed, while lines represent predicted debilitation values).

Table 4. LD_{50} and LD_{90} values obtained after various concentrations of imidacloprid were applied to 4th instar *M. sanguinipes* (Probit Analysis).

Application	Intercept ± SE	Slope ± SE	n	LD_{50}(ppm)	LD_{90}(ppm)	Chi-Square
Oral	-9.12 ± 1.40	5.27 ± 0.78	213	53.38	93.32	0.96*
T statistic	42.31	44.78				
P-value	0.0001	0.0001				
Topical	-0.78 ± 0.28	-0.40± 0.13	237	86.12	128,824.95	0.38*
T statistic	7.78	10.08				
P-value	0.005	0.001				

* Predicted slope not significantly different from actual at $P<0.05$.

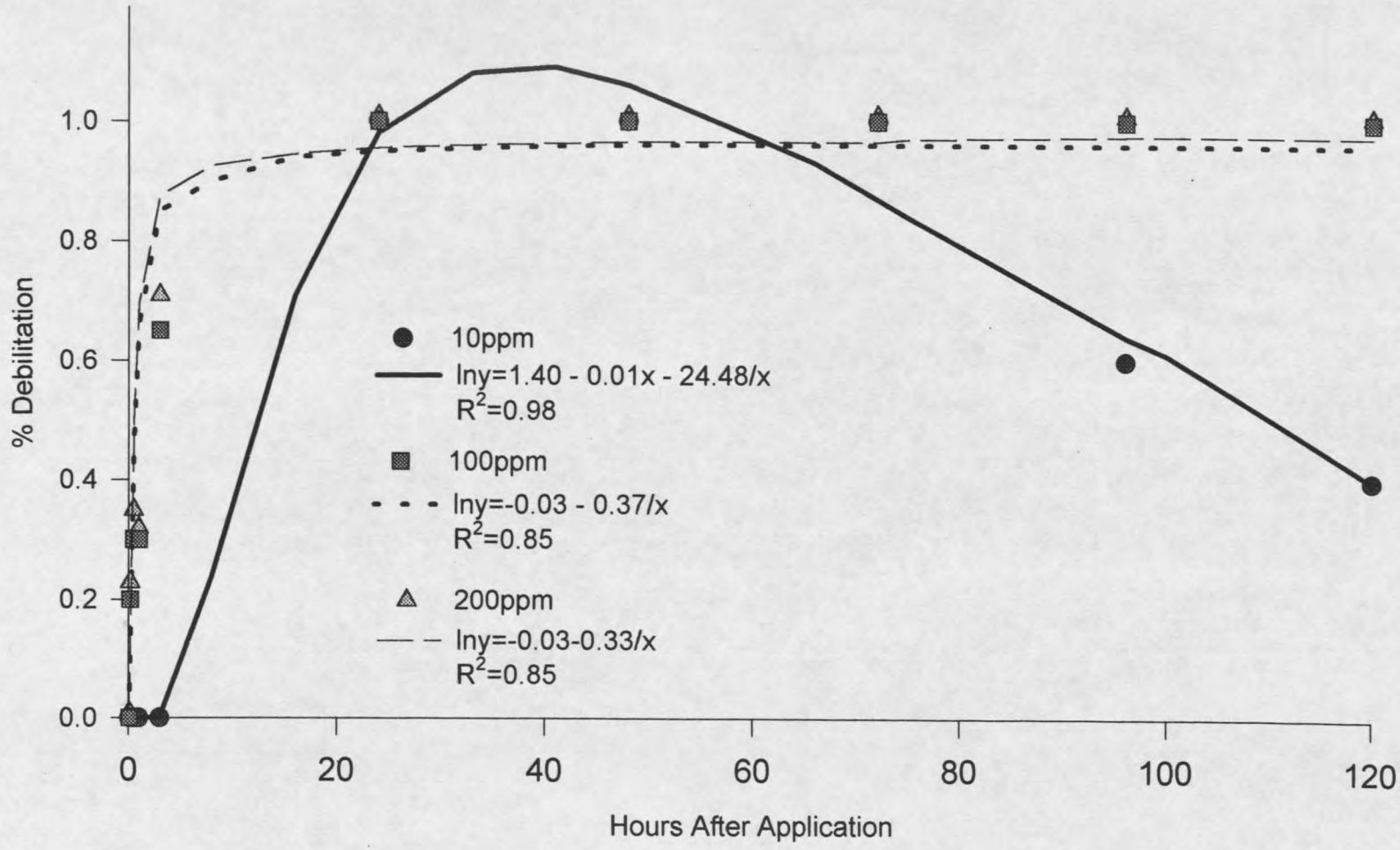

Fig. 2. Observed vs. predicted debilitation at various time intervals after imidacloprid was topically applied to 4th instar *M. sanguinipes (*Symbols represent observed, while lines represent predicted debilitation values).

Table 5. Summary of repeated measures analysis of mortality and leaf defoliation after grasshoppers infested 4-leaf stage spring wheat treated with foliar and seed treatments of imidacloprid, and carbofuran near Moccasin,MT.

Mortality	DF	F-Statistic	Pr>F
Trt	3	0.57	0.65
Time	2	10.39	0.002*
Trt by Time	6	0.84	0.56
Leaf Defoliation			
Trt	3	0.93	0.48
Time	2	133.55	0.0001*
Trt by Time	6	0.31	0.91

* Represents values significant at $P<0.05$.

Table 6. Corrected grasshopper mortality ± SE after being applied to flag-leaf stage spring wheat that was treated with foliar and seed treatments of imidacloprid, and foliar carbofuran in plots near Moccasin, MT.

Treatment	Rate (gai/ha)	Days Post Grasshopper Application		
		7	14	21
Imidacloprid (Seed)	0.90 (kg)	4 ± 5	3 ± 5	6 ± 13
Foliar Imidacloprid	51.50	38 ± 5*	46 ± 3*	54 ± 7*
Carbofuran	210.50	93 ± 7*	93 ± 7*	90 ± 7*
F-Statistic		88.27	99.70	12.97
n		12	12	12
P-value		0.0001	0.0001	0.004

Means within columns followed by * are significantly different than the untreated (Tukey Test; P=0.05).

Table 7. Leaf defoliation (0-5) ± SE by grasshoppers applied to flag-leaf stage spring wheat that was treated with foliar and seed treatments of imidacloprid, and foliar carbofuran in plots near Moccasin, MT.

Treatment	Rate (gai/ha)	Days Post Grasshopper Application		
		7	14	21
Imidacloprid (Seed)	0.90 (kg)	2.9 ± 0.1ab	3.6 ± 0.3a	3.8 ± 0.2a
Foliar Imidacloprid	51.50	2.9 ± 0.3ab	1.9 ± 0.2b	3.5 ± 0.4a
Carbofuran	210.50	2.1 ± 0.3b	1.7 ± 0.2b	2.1 ± 0.3b
Untreated		3.2 ± 0.1a	3.6 ± 0.2a	4.1 ± 0.1a
F-Statistic		4.38	28.04	14.39
n		12	12	12
P-value		0.05	0.0006	0.003

Means within columns followed by similar letters are not significantly different (Tukey Test; P=0.05).

Table 8. Corrected grasshopper mortality ± SE after being applied to 2-leaf stage winter wheat that was treated with foliar and seed treatments of imidacloprid, and foliar carbofuran in plots near Moccasin, MT.

Treatment	Rate	Days Post Grasshopper Application	
	(gai/ha)	7	14
Imidacloprid	0.30 (kg)	33 ± 31	28 ± 31
Imidacloprid	0.90 (kg)	30 ± 9	28 ± 11
Foliar Imidacloprid	51.50	19 ± 9	43 ± 7
Carbofuran	210.50	92 ± 3*	96 ± 3*
F-Statistic		3.78	3.45
n		150	15
P-value		0.05	0.05

Means within columns followed by * are significantly different than the untreated (Tukey Test; P=0.05).

Table 9. Leaf defoliation (0-5) ± SE by grasshoppers applied to 2-leaf stage winter wheat that was treated with foliar and seed treatments of imidacloprid, and foliar carbofuran in plots near Moccasin, MT.

Treatment	Rate	Days Post Grasshopper Application	
	(gai/ha)	7	14
Imidacloprid	0.30 (kg)	3.3 ± 0.4ab	3.8 ± 0.2ab
Imidacloprid	0.90 (kg)	3.0 ± 1.3ab	3.1 ± 1.6ab
Foliar Imidacloprid	51.50	3.3 ± 0.9ab	3.5 ± 0.8ab
Carbofuran	210.50	0.5 ± 0.3b	0.6 ± 0.7b
Untreated		4.5 ± 0.5a	4.7 ± 0.7a
F-Statistic		5.55	4.20
n		15	15
P-value		0.01	0.04

Means within columns followed by similar letters are not significantly different (Tukey Test; P=0.05).

Table 10. Corrected grasshopper mortality ± SE after being applied to early tillering stage winter wheat treated with foliar and seed treatments of imidacloprid, and foliar carbofuran in plots near Moccasin, MT.

Treatment	Rate	Days Post Grasshopper Application		
	(gai/ha)	7	14	21
Imidacloprid	0.30 (kg)	17 ± 6	50 ± 10	46 ± 10
Imidacloprid	0.90 (kg)	33 ± 6	33 ± 3	38 ± 3
Foliar Imidacloprid	51.50	17 ± 15	18 ± 13	27 ± 7
Carbofuran	210.50	100 ± 0*	100 ± 0*	100 ± 0*
F-Statistic		10.65	3.89	5.53
n		15	15	15
P-value		0.002	0.04	0.01

Means within columns followed by * are significantly different than the untreated (Tukey Test; P=0.05).

Table 11. Leaf defoliation (0-5) ± SE by grasshoppers applied to early tillering stage winter wheat that was treated with foliar and seed treatments of imidacloprid, and foliar carbofuran in plots near Moccasin, MT.

Treatment	Rate	Days Post Grasshopper Application		
	(gai/ha)	7	14	21
Imidacloprid	0.30 (kg)	1.8 ± 0.3ab	1.8 ± 0.2ab	1.7 ± 0.2ab
Imidacloprid	0.90 (kg)	1.5 ± 0.5b	1.5 ± 0.3ab	1.4 ± 0.2b
Foliar Imidacloprid	51.50	1.0 ± 0bc	2.1 ± 0.4ab	2.0 ± 0.3ab
Carbofuran	210.50	0.5 ± 0c	0.7 ± 0.3b	0.5 ± 0c
Untreated		2.5 ± 0.3a	2.2 ± 0.3a	2.3 ± 0.2a
F-Statistic		6.95	3.80	16.87
n		15	15	15
P-value		0.01	0.04	0.0006

Means within columns followed by similar letters are significantly different (Tukey Test; P=0.05).

Discussion

Greenhouse Trials.

There was extremely low *M. sanguinipes* mortality and feeding deterrence on 4-leaf spring wheat treated with imidacloprid at 0.60 and 0.90 gai/kg. However, this experiment was terminated as early as day five due to controls being totally consumed. A study conducted by Boiteau et al. (1997) indicated that imidacloprid treated foliage may cause increasing toxicity over longer periods of time. They reported that mortality increased for over five days after five species of aphids were applied to imidacloprid treated plants. This suggests that imidacloprid may accumulate in the insects body over a period of time, and may explain the low mortality observed on grasshoppers in this trial which ran for a shorter period of time. An experiment conducted over a longer time interval would consider cumulative mortality in the evaluation of this chemical. To attain this, enclosures would need fewer grasshoppers and more plant biomass. This would prevent controls from being defoliated quickly.

A low level of mortality and leaf protection as recorded when grasshoppers fed for 14 days on wheat seed treated at twice the labeled rate of imidacloprid. Although mortality was <35% in each rate of imidacloprid by day 14, it was significantly different than the untreated (Table 2). Elbert et al. (1990) reported >90% mortality of *Schizaphis graminum* (Rondani) on wheat for up to 40 days post sowing. High mortality recorded by Elbert et al. (1990) indicates the low grasshopper toxicity achieved when imidacloprid is used on *M. sanguinipes* compared to *S. graminum*. This level of mortality predictably

caused a low level of plant protection from defoliation (Table 3). Pike et al. (1993) reported <3% of all plants received any damage from Russian wheat aphids for up to 21 days after sowing wheat treated with imidacloprid. This is 75% less damage than that achieved in this trial.

Mortality and feeding deterrence by grasshoppers in either trial may indicate differences in insect feeding (chewing vs. sucking), metabolic breakdown, and/or tolerances of imidacloprid between aphids and grasshoppers that may render the chemical ineffective. This may be due to imidacloprid being concentrated in the xylem/phloem of the plant when applied as a seed treatment (Leicht 1994). When chewing insects consume plant tissues as well as fluid in the xylem/phloem they may get a relatively lower concentration of imidacloprid. Sucking insects may extract a higher concentration of insecticide directly from the xylem/phloem. This could explain the low toxicity observed after grasshoppers consumed seed treated plants. When aphid mortality and deterrence is compared with grasshopper mortality, it becomes obvious that an acceptable level of mortality or plant protection was not reached.

Bioassays

Debilitation and recovery within 24 hr resulted in low mortality of grasshoppers from oral and topical applications of imidacloprid (Table 4 and Fig. 2). However, topical mortality did not exceed levels reached from similar doses in the oral assay. Previous studies have indicated oral applications of imidacloprid to be ten times more toxic than topical applications (Leicht 1993). A previous bioassay by Onsager and Muzuranich (1986) determined an oral and topical LD_{50} of carbofuran to be 1.7 ppm and 0.3 ppm,

respectively. This is significantly lower than the oral and topical LD_{50} determined for imidacloprid in this study and suggests that this chemical is not highly toxic to *M. sanguinipes* (Table 4).

Debilitation and recovery results in this bioassay were similar to those results found when imidacloprid was ingested by the German cockroach, *Blattella germanica* L., house fly, *Musca domestica* L., and the root weevil, *Diaprepes abbreviatus* L. (Kaakeh et al. 1997, Schroeder and Flattum 1984, Quintela and McCoy 1997).

In each study, imidacloprid was combined with fungal pathogens or other chemicals to increase mortality to higher levels. Schroeder and Flattum (1984) indicated that by pretreating cockroaches with sesamex, an inhibitor of microsomal mixed-function oxidase, doses causing debilitation became lethal. This was due to blocking nerve transmission at cholinergic synapses, thus causing a reduced breakdown of imidacloprid in nervous tissue and reduced ability to recover. A similar synergistic reaction was observed by Kaakeh et al. (1997) when imidacloprid and the fungi, *Metarhizium anisopliae*, were combined on cockroaches. Cockroach mortality was increased with this combination compared to imidacloprid used alone. Quintela and McCoy (1997) used the pathogen, *B. bassiana,* to achieve higher levels of mortality on the root weevil, *D. abbreviatus;* low mortality was produced by imidacloprid alone. In each study using fungal pathogens, difficulty coordinating muscles of the legs and antennae limited preening activities. This prevented removal of fungal conidia from the insect body and increased the susceptibility to infection and death. Grasshopper mortality may be increased by combining imidacloprid with sesamex or fungal pathogens.

The pathogen-imidacloprid synergism may further increase mortality if used under field conditions. Temperature fluctuations, weather, and/or humidity extremes may stress these pests, causing higher mortality than if the chemical/pathogen combination itself were used alone. Furthermore, thermoregulation may be impeded due to loss of mobility, resulting in higher mortality under appreciatively more diverse temperatures in the field.

Field Trials.

Significant differences in leaf defoliation or mortality were not recorded with the use of carbofuran and/or foliar imidacloprid when applied at the 4-leaf stage of spring wheat (Table 5). Toxicity in carbofuran treated plots is believed to be much higher due to this insecticide causing high mortality to grasshoppers in all field trials on spring and winter wheat in this study (Table 6). Fuller et al. (1992) and Onsager and Muzuranich (1986) also reported carbofuran to cause higher mortality to grasshoppers. Low grasshopper mortality in all foliar applied plots may have been due to 2 cm of precipitation 2 h posttreatment. This may have rinsed foliar applied deposits from leaves, reducing the efficacy of carbofuran and foliar imidacloprid treated plots to < 20%.

Foliar treatments did provide significant grasshopper control when applied at the flag-leaf stage of spring wheat and the 2-leaf and early tillering stage of winter wheat. Mortality reached approximately 93% and 60% in carbofuran and imidacloprid treated plots, respectively (Table 6). Foliar imidacloprid caused 38% mortality at day 7 and increased to 54% by day 21. Similar trends in mortality were recorded when foliar imidacloprid was applied to five aphid species in a study by Boiteau et al. (1997). They

recorded foliar imidacloprid to cause <35% mortality before increasing to 50 - 75% over a five day period. This indicates that foliar imidacloprid is slow acting in killing many hosts including aphids and grasshoppers. Mortality may increase over time because of chronic debilitation observed in bioassays as well as other studies (Schroeder and Flattum 1984, Kaakeh et al. 1997, Quintela and McCoy 1997). Symptoms of debilitation include paralysis of mouthparts, inability to thermoregulate, or inability to move to an appropriate area to feed (Lagadic and Ludovic 1993). This may explain the increasing grasshopper mortality recorded in the foliar imidacloprid plots. Debilitation did not reduce the leaf defoliation from that of the untreated by day 21 (Table 7). Increasing mortality and a nonsignificant reduction in defoliation implies that the insects were debilitated. However, effects on feeding deterrence (paralysis of mouthparts and inability to move or thermoregulate) may have been minimal.

Seed treated with imidacloprid at the labeled rate did not provide acceptable levels of mortality at the 4-leaf and flag-leaf stage of spring wheat or the 2-leaf and early tillering stage of winter wheat. Mortality did not increase over time as it did in plots treated with foliar imidacloprid. This suggests that grasshoppers were not receiving high enough doses of imidacloprid when applied as a seed treatment at the labeled rates. Since grasshopper infestations are not likely to infest wheat plants prior to the 2-leaf stage, imidacloprid would be an ineffective choice for controlling grasshoppers. This may be due to imidacloprid's period of highest toxicity is in the first 30 - 60 days of a plants growth (Elbert et al. 1990, Pawar et al. 1993, Sloderbeck et al. 1996). Each of these

studies recorded sufficient protection from aphids, whiteflies, and thrips in the first 30 - 60 days of a treated plants growth, but toxicity decreased sharply after that time period.

Defoliation was minimally reduced in some plots seed treated with imidacloprid at all stages of spring or winter wheat (Table 9 and 11). This correlates with laboratory findings earlier in this study as well as a previous study (S. Blodgett et al., Department of Entomology, Montana State University, unpublished research). This study reported imidacloprid treated cereals to have significantly less grasshopper damage than that of the untreated. Each study suggests that although wheat seed treated with imidacloprid may deter pests, the level of protection is minimal. When compared to defoliation in carbofuran treated plots (0.5), it becomes obvious that the level of protection that this treatment offers (1.4) is minimal. This implies that economically acceptable rates of control will not be reached, regardless of plant maturity, host variety, and maturity of grasshoppers using this seed treatment.

CHAPTER 4

CEREAL LEAF BEETLE STUDIES

Materials / Methods

Rearing.

Sixty diapausing cereal leaf beetles were collected on 22 August 1996 at the USDA APHIS-PPQ field insectary near Huntley, Yellowstone County, MT. Beetles were deposited in paper sacks and placed in a cooler at 10^0C, before being transported back to MSU - Bozeman. Beetles were placed in a 25 cm by 16 cm by 8 cm plastic refrigerator carton containing folded wax paper. A sponge saturated with 0.05% sodium hypochlorite solution was added to the carton to maintain RH at 100% and inhibit molds (Hoxie and Wellso 1983). To break diapause, the cartons were placed in a cold storage room for a minimum of 10 wk with a 24 h dark photoperiod and temperature range between 3^0-5^0C (Wellso 1982, Hoxie and Wellso 1983).

Twenty 10 cm pots were planted with 20-25 barley seeds, *Hordeum vulgare* Linnaeus (cv. 'Harrington') on 21 November 1996. The pots were placed in a 51 cm by 40 cm by 6 cm plastic tray filled with 2.5 cm of water to raise the RH to 80-90%. The tray containing the barley seedlings was placed in an 106 cm by 91 cm by 76 cm plastic oviposition cage when plants reached the 2-leaf stage. Beetles emerging from diapause were placed in the oviposition cage and allowed to begin feeding and mating. Eggs were

obtained for subsequent experiments by removing egg-infested plants every eight days. Eggs were placed into cartons held in cold storage and taken out when needed for an experiment.

Greenhouse Trials.

Three sets of twenty 11.4 cm pots were seeded with barley, *H. vulgare* (cv. 'Harrington'), with four seeds per pot. Sets were randomly assigned to three growth stages, 2-leaf (growth stage 12), 4-leaf (growth stage 14), or flag-leaf stage (growth stage 37, Zadok et al. 1974). Treatments were imidacloprid seed treatment (Gaucho 480F, Gustafson, Plano, TX) at 0.30, 0.60, 0.90 gai/kg and untreated. The treatments were replicated five times in a greenhouse bay with a photoperiod of 16:8 (L:D) and fluctuating temperature regime of 26°-22°C. One pot was placed in each corner of fifteen 51 cm by 60 cm by 6.5 cm plastic trays filled with water.

Eggs harvested from rearing procedures were placed on leaves of 2-leaf, 4-leaf, and flag-leaf barley on 9 January, 18 January, and 3 February, respectively. Eight eggs were distributed on leaves of 2-leaf stage barley; 12 eggs were applied to 4-leaf and flag-leaf stage plants. Eggs were applied at the base of each stem with a fine paintbrush. To increase humidity to 80 - 90%, one 60 by 20 cm acetate tube with a nylon top, was placed over each pot.

Larval mortality and leaf defoliation were assessed every 48 h. Mortality was corrected using Abbott's formula (Abbott 1925). Leaf defoliation was assessed visually using a numerical rating from 0-5, where 0 = no leaf defoliation, 1 = 1-25%, 2 = 26-50%,

3 = 51-75%, 4 = 76-99%, and 5 = 100%. An average was obtained by rating each stem in the entire cage (Olfert et al. 1995).

Field Trials.

A field for studying the effects of imidacloprid on cereal leaf beetles was located 11 km east of Huntley, MT. Three 8 m by 4 m plots were seeded with barley, *H. vulgare* (cv. 'C22'), treated with imidacloprid at 0.45 gai/kg. Nine plots designated for carbofuran, foliar imidacloprid, and no treatment were planted with untreated barley. Plots were seeded at 55 kg/ha with a 4 m wide International model 5100 drill (International, Burlington, IA). Foliar and seed treatments of imidacloprid, carbofuran, and untreated were arranged in a randomized block design with three replications. Plots were split into 4 m by 4 m subplots for treatment infestations at the 2-leaf and 4-leaf stage.

Eggs were applied to subplot treatments at 2-leaf growth stage on 8 May, and 4-leaf growth stage on 15 May. Two 90 cm by 20 cm dowel framed, screen cages were placed over 15 cereal leaf beetle eggs. Eggs were previously placed on each of six barley plants within each plot. Foliar applications of carbofuran (Furadan 4F, FMC Corporation, Princeton, NJ) at 220 gai/ha and imidacloprid (Provado 1.6F, Miles Inc., Kansas City, MO) at 51.51 gai/ha were made the day after eggs were placed on the 2-leaf barley. Foliar insecticide applications were delayed for one week after eggs were deposited on 4-leaf stage barley because of low levels of larvae emerging in the field. Foliar applied insecticides were sprayed using a 2.2 m CO_2-powered backpack sprayer calibrated to

deliver 280 ml/15 sec. at 14kgsi (30 psi) with four Teejet model XR 8002VS nozzles (Spraying Systems, Wheaton, Illinois).

Mortality and leaf defoliation were recorded at four day intervals. Estimates were recorded using methods previously described. Plant damage and larval densities were recorded from natural infestations adjacent to enclosures. Damaged tillers and larval densities in the field were assessed by randomly taking three 0.33 m samples from a row within each plot.

Statistics

Enclosures. Treatment effects over time were analyzed using PROC ANOVA with time as a repeated measure (P=0.05; SAS Institute 1989). If treatment and/or interaction effects were significant, treatment effects for each time period were analyzed using Tukeys studentized range test (SAS Institute 1989).

Field Samples - (Natural Infestations) Treatment effects of these values were analyzed using only the Tukeys studentized range test for each time period (SAS Institute 1989).

Results

Rearing

Within 2-3 h after removal of beetles from cold storage, 35 adult beetles were becoming mobile and initiating feeding. The adults were observed mating by day 5 and ovipositing 12 days later. Oviposition took place over an 81 day period, with a mean

29 eggs per adult deposited from 18 - 27 days after emergence. Oviposition decreased while mortality increased over the next 55 days.

Greenhouse Trials

The repeated measures model indicated treatment effects for mortality and leaf defoliation to be significant ($F = 86.09$; df = 3, 60; P = 0.0001, $F = 45.22$; df = 3, 60; $P = 0.0001$, respectively) when cereal leaf beetle eggs were placed on 2-leaf barley seed treated with 0.30, 0.60, and 0.90 gai/kg rates of imidacloprid. More than five larvae per cage were recorded in the untreated by day 8. Larvae were not found in any seed treatment after day 8 (Table 12). All rates of the seed treatment protected plants from leaf defoliation. A mean defoliation rating of 0.0 and 3.6 was recorded in the untreated and treated rates, respectively, by day 16 (Table 13).

When eggs were placed on 4-leaf barley seed treated with imidacloprid at 0.30, 0.60, and 0.90 gai/kg, significant differences in mortality were present among treatments using repeated measures ($F = 66.01$; df = 3, 75; $P = 0.0001$). Interactions were also observed when analyzing mortality over time ($P < 0.0001$). Approximately seven larvae per cage emerged in the untreated by six days. On this day all rates of imidacloprid significantly increased mortality to 90 - 100% (Table 14).

Significant differences in defoliation were present among treatments at the 4-leaf stage ($F = 55.72$; df = 3, 75; $P = 0.0001$), along with a significant treatment by time interaction ($P<0.0001$) when defoliation was assessed using the repeated measures model. As expected, all rates of the seed treatment had significantly less plant

defoliation (<0.4) from that of the untreated (3.4) by day 16 (Table 15). Significant differences were observed by day 4.

The repeated measures model indicated significant differences in mortality between treatments when cereal leaf beetle eggs were applied to flag leaf barley seed treated with imidacloprid at 0.30, 0.60, and 0.90 gai/kg ($F = 6.00$; df = 3, 75; $P = 0.009$). A significant treatment by time interaction was also observed ($F = 3.31$; df = 15, 75; $P = 0.0005$) throughout the experiment. At day 6 the 0.90 gai/kg rate of imidacloprid treated plots had 57% mortality. However, no significant differences in mortality were recorded at any rate after this day. The 0.30, 0.60, and 0.90 gai/kg rates caused 22, 26, and 52% mortality by day 12, respectively (Table 16).

Significant differences in defoliation were observed between treatments ($F = 5.95$; df = 3, 75; $P = 0.01$) when eggs were applied to flag-leaf barley using the repeated measures model. However, a treatment by time defoliation interaction was not present ($F = 0.89$; df = 15, 75; $P = 0.58$). Defoliation differences between treatments were not observed until day 4. The 0.90 gai/kg rate provided the greatest protection, with a defoliation index of 2.6 at day 12 compared to the untreated with 4.0 (Table 17).

Field Trial

When eggs were applied to 2-leaf barley treated with carbofuran, foliar and seed treatments of imidacloprid, the repeated measures model indicated no significant differences between treatments or interaction effects for mortality, leaf defoliation, damaged tillers, or field densities (Table 18). A maximum of three larvae per cage were

recorded four days after application of eggs. A maximum 18 larvae per m^2 were recorded from natural infestations in the plots at day 12.

The repeated measures model indicated 4-leaf barley treated with carbofuran and foliar and seed treatments of imidacloprid had significantly different larval mortality between treatments (Table 19). All foliar treatments resulted in significantly greater mortality than the untreated throughout the experiment. A mean of 95% mortality was recorded in foliar imidacloprid plots, and 100% in carbofuran plots 12 days after treatment. Plots treated with the seed treatment did not significantly increase mortality from that of the untreated (Table 20).

Leaf defoliation in enclosures was significantly different between treatments using the repeated measures model, with a significant treatment by time interaction when eggs were placed on 4-leaf barley (Table 19). From day four through day 12 all foliars offered protection from cereal leaf beetles. Significant differences occurred by day 4. A mean defoliation index of 0.1 was recorded in plots treated with either foliar application compared to 2.2 in the untreated plots. However, the seed treatment did not significantly reduce defoliation from that of the untreated for the duration of the trial (Table 21).

Natural field densities present at the 4-leaf stage were significantly different between treatments over the span of the experiment (Table 19). By day 8, significantly lower larval densities were present in plots treated with either foliar applied insecticide (6.3 per m^2), compared to the untreated (62.1 per m^2) (Table 22). Plots treated with the seed treatment contained 83.7 larvae per m^2 by day 12, and were not different from the untreated for the trials duration.

Field densities had a significant effect on damaged tillers in the various treated plots using repeated measures analysis (Table 19). Foliar imidacloprid and carbofuran treatments significantly reduced damage to tillers from that of the untreated by day 8. At day 12, foliar imidacloprid, carbofuran, and untreated plots had 23, 26, and 81% damaged tillers, respectively. Plots treated with the seed treatment were not significantly different than the untreated throughout the experiment (Table 23).

Table 12. Corrected mortality ± SE after *O. melanopus* larvae emerged from eggs applied to 2-leaf stage barley seed treated with imidacloprid under greenhouse conditions.

Treatment	Rate (gai/kg)	Days Post Egg Application							
		2	4	6	8	10	12	14	16
Imidacloprid	0.30	34± 5	92 ± 3*	85 ± 5*	100 ± 0*	100 ± 0*	100 ± 0*	100 ± 0*	100 ± 0*
Imidacloprid	0.60	87± 2	100 ± 0*	100 ± 0*	100 ± 0*	100 ± 0*	100 ± 0*	100 ± 0*	100 ± 0*
Imidacloprid	0.90	100± 0	100 ± 0*	100 ± 0*	100 ± 0*	100 ± 0*	100 ± 0*	100 ± 0*	100 ± 0*
F-Statistic		1.48	27.71	51.89	22.73	22.73	21.00	14.55	6.70
n		20	20	20	20	20	20	20	20
P-value		0.25	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.003

Means within columns followed by * are significantly different than the untreated (Tukey Test; P=0.05).

Table 13. Leaf defoliation (0-5) ± SE after *O. melanopus* larvae emerged from eggs applied to 2-leaf stage barley seed treated with imidacloprid under greenhouse conditions.

Treatment	Rate (gai/kg)	Days Post Egg Application							
		2	4	6	8	10	12	14	16
Imidacloprid	0.30	0.6± 0.2a	0.4± 0.2b	0.6± 0.2b	0.3± 0.2b	0.1± 0.1b	0.0± 0.0b	0.0± 0.0b	0.0± 0.0b
Imidacloprid	0.60	0.4± 0.2a	0.0± 0.0b	0.0± 0.0b	0.0± 0.0b	0.0± 0.0b	0.0± 0.0b	0.0± 0.0b	0.0± 0.0b
Imidacloprid	0.90	0.0± 0.2a	0.0± 0.0b	0.0± 0.0b	0.0± 0.0b	0.0± 0.0b	0.0± 0.0b	0.0± 0.0b	0.0± 0.0b
Untreated		0.3± 0.2a	1.3± 0.2a	1.4± 0.2a	2.1± 0.3a	2.5± 0.4a	2.7± 0.5a	3.1± 0.6a	3.6± 0.7a
F-Statistic		1.56	15.03	18.53	27.20	45.14	33.91	24.96	29.79
n		20	20	20	20	20	20	20	20
P-value		0.23	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

Means within columns followed by similar letters are not significantly different (Tukey Test; P=0.05).

Table 14. Corrected mortality ± SE after *O. melanopus* larvae emerged from eggs applied to 4-leaf stage barley seed treated with imidacloprid under greenhouse conditions.

Treatment	Rate (gai/kg)	Days Post Egg Application					
		2	4	6	8	10	12
Imidacloprid	0.30	58± 3	62 ± 3	90 ± 2*	71 ± 6*	85 ± 5*	83 ± 5*
Imidacloprid	0.60	29± 3	23 ± 6	93 ± 2*	94 ± 2*	94 ± 2*	94 ± 2*
Imidacloprid	0.90	100± 0	100 ± 0	100 ± 0*	100 ± 0*	100 ± 0*	100 ± 0*
F-Statistic		0.83	1.85	18.48	43.60	57.87	37.54
n		20	20	20	20	20	20
P-value		0.50	0.17	0.0001	0.0001	0.0001	0.0001

Means within columns followed by * are significantly different than the untreated (Tukey Test; P=0.05).

Table 15. Leaf defoliation (0-5) ± SE after *O. melanopus* larvae emerged from eggs applied to 4-leaf stage barley seed treated with imidacloprid under greenhouse conditions.

Treatment	Rate (gai/kg)	Days Post Egg Application							
		2	4	6	8	10	12	14	16
Imidacloprid	0.30	0.0± 0.0a	0.2± 0.1b	0.1± 0.1b	0.4± 0.1b	0.3± 0.2b	0.4 ±0.2b	0.4± 0.2b	0.3± 0.2b
Imidacloprid	0.60	0.0± 0.0a	0.1± 0.1b	0.1± 0.1b	0.2± 0.1b	0.2± 0.1b	0.1 ±0.1b	0.2± 0.1b	0.2± 0.1b
Imidacloprid	0.90	0.0± 0.0a	0.0± 0.0b	0.0± 0.0b	0.0± 0.0b	0.0± 0.0b	0.0 ±0.0b	0.0± 0.0b	0.0± 0.0b
Untreated		0.2± 0.1a	0.6± 0.2a	0.7± 0.1a	1.6± 0.3a	2.3± 0.2a	2.8± 0.2a	3.5± 0.3a	3.4± 0.2a
F-Statistic		2.67	5.79	11.71	18.79	48.56	67.05	62.84	78.69
n		20	20	20	20	20	20	20	20
P-value		0.08	0.007	0.0003	0.0001	0.0001	0.0001	0.0001	0.0001

Means within columns followed by similar letters are not significantly different (Tukey Test; P=0.05).

Table 16. Corrected mortality ± SE after *O. melanopus* larvae emerged from eggs applied to flag-leaf stage barley seed treated with imidacloprid under greenhouse conditions.

Treatment	Rate (gai/kg)	Days Post Egg Application					
		2	4	6	8	10	12
Imidacloprid	0.30	0± 6	0± 8	11± 9	0 ± 8	13 ± 11	22 ± 12
Imidacloprid	0.60	0± 5	71± 4*	42 ± 9	22 ± 6	21 ± 5	26± 3
Imidacloprid	0.90	100± 0	78± 7*	57± 10*	52 ± 10	52 ± 10	52 ± 10
F-Statistic		2.06	13.05	3.78	2.94	2.73	2.13
n		20	20	20	20	20	20
P-value		0.14	0.0001	0.03	0.06	0.08	0.13

Means within columns followed by * are significantly different than the untreated (Tukey Test; P=0.05).

Table 17. Leaf defoliation (0-5) ± SE after *O. melanopus* larvae emerged from eggs applied to flag-leaf stage barley seed treated with imidacloprid under greenhouse conditions.

Treatment	Rate (gai/kg)	Days Post Egg Application					
		2	4	6	8	10	12
Imidacloprid	0.30	0.8 ± 0.2a	2.0 ± 0.3a	2.6 ± 0.5a	3.2 ± 0.5a	3.4 ± 0.5a	3.6 ± 0.4a
Imidacloprid	0.60	0.6 ± 0.2a	0.8 ± 0.2bc	2.0 ± 0.3ab	2.5 ± 0.4a	3.0 ± 0.3a	3.4 ± 0.4a
Imidacloprid	0.90	0.0 ± 0.0a	0.4 ± 0.2c	1.0 ± 0.3b	2.0 ± 0.5a	2.3 ± 0.7a	2.6 ± 0.9a
Untreated		0.4 ± 0.2a	1.2 ± 0.2ab	2.2 ± 0.2ab	3.1 ± 0.3a	3.8 ± 0.6a	4.0 ± 0.4a
F-Statistic		2.92	7.78	3.71	1.57	1.44	1.08
n		20	20	20	20	20	20
P-value		0.07	0.002	0.03	0.23	0.27	0.38

Means within columns followed by similar letters are not significantly different (Tukey Test; P=0.05).

Table 18. Summary of repeated measures analysis of mortality, leaf defoliation, field densities, and damaged tillers after beetle larvae infested 2-leaf stage barley treated with foliar and seed treatments of imidacloprid, and foliar carbofuran near Huntley, MT.

Mortality	DF	F-Statistic	Pr>F
Trt	3	2.32	0.17
Time	1	1.00	0.35
Trt by Time	3	0.33	0.73
Leaf Defoliation			
Trt	3	0.93	0.48
Time	1	0.25	0.63
Trt by Time	3	1.39	0.33
Damaged Tillers[a]			
Trt	3	0.18	0.90
Time	1	18.37	0.0001*
Trt by Time	3	0.29	0.93
Field densities			
Trt	3	2.56	0.08
Time	1	12.39	0.0002*
Trt by Time	3	2.12	0.09

* Represents values significant at $P<0.05$.

[A] Represents data obtained from the natural infestation of beetle larvae in the area.

Table 19. Summary of repeated measures analysis of mortality, leaf defoliation, field densities, and damaged tillers after beetle larvae infested 4-leaf stage barley treated with foliar and seed treatments of imidacloprid, and foliar carbofuran near Huntley, MT.

Mortality	DF	F-Statistic	Pr>F
Trt	3	13.22	0.004*
Time	2	6.55	0.003*
Trt by Time	6	2.18	0.07
Leaf Defoliation			
Trt	3	22.03	0.001*
Time	2	10.62	0.0003*
Trt by Time	6	5.28	0.001*
Damaged Tillers[a]			
Trt	3	29.75	0.0001*
Time	2	27.09	0.0001*
Trt by Time	6	6.22	0.0006*
Field densities			
Trt	3	30.11	0.0001*
Time	2	1.43	0.26
Trt by Time	6	1.71	0.16

* Represents values significant at $P<0.05$.

[A] Represents data obtained from natural infestation of beetle larvae in plots.

Table 20. Corrected mortality ± SE after *O. melanopus* larvae emerged from eggs applied to 4-leaf stage barley treated with foliar and seed treatments of imidacloprid, and foliar carbofuran on plots near Huntley, MT.

Treatment	Rate (gai/ha)	Days Post Foliar Application[a]			
		Pretreatment	4	8	12
Imidacloprid	0.45 (kg)	53± 5	33 ± 13	30 ± 7	32 ± 8
Foliar Imidacloprid	51.50	34± 18	96 ± 3*	98 ± 1*	95 ± 1*
Carbofuran	210.50	60± 2	100 ± 0*	100 ± 0*	100 ± 0*
F-Statistic		2.21	7.23	5.08	6.12
n		12	12	12	12
P-value		0.18	0.02	0.04	0.02

Means within columns followed by * are significantly different than the untreated (Tukey Test; P=0.05).

[a] Foliar application occurring at the early tillering (growth stage 24, Zadok et al. 1974) of barley.

Table 21. Mean rating of leaf defoliation (0-5) ± SE after *O. melanopus* larvae emerged from eggs applied to 4-leaf stage barley treated with foliar and seed treatments of imidacloprid, and foliar carbofuran on plots near Huntley, MT.

Treatment	Rate (gai/ha)	Days Post Foliar Application[a]			
		Pretreatment	4	8	12
Imidacloprid	0.45 (kg)	0.5± 0.2a	0.8 ± 0.2b	2.5 ± 0.5a	2.5 ± 0.3a
Foliar Imidacloprid	51.50	0.8± 0.4a	0.2 ± 0.1c	0.2 ± 0.2b	0.2 ± 0.2b
Carbofuran	210.50	0.5± 0.0a	0.1 ± 0.1c	0.0 ± 0.0b	0.0 ± 0.0b
Untreated		1.1± 0.5a	1.4 ± 0.2a	2.8 ± 0.6a	2.2 ± 0.7a
F-Statistic		1.11	24.78	12.14	13.44
n		12	12	12	12
P-value		0.41	0.0009	0.005	0.004

Means within columns followed by similar letters are significantly different (Tukey Test; P=0.05).

[a] Foliar application occurring at the early tillering (growth stage 24, Zadoks et al. 1974) of barley.

Table 22. Mean surviving beetle larvae per m^2 ± SE, on barley treated with foliar and seed treatments of imidacloprid, and foliar carbofuran near Huntley, MT.

Treatment	Rate (gai/ha)	Days Post Foliar Application[a]			
		Pretreatment	4	8	12
Imidacloprid	0.45 (kg)	10.8 ± 6.3a	37.8 ± 18.0a	65.7 ± 4.5a	83.7 ± 11.7a
Foliar Imidacloprid	51.50	28.8 ± 14.4a	8.1 ± 4.5a	8.1 ± 1.8b	6.3 ± 3.6b
Carbofuran	210.50	11.7 ± 2.7a	2.7 ± 1.8a	4.5 ± 2.7b	6.3 ± 5.4b
Untreated		17.1 ± 9.9a	42.3 ± 11.7a	62.1 ± 14.4a	53.1 ± 3.6a
F-Statistic		0.70	2.67	17.09	34.39
n		12	12	12	12
P-value		0.58	0.14	0.005	0.0004

Means within columns followed by similar letters are significantly different (Tukey Test; P=0.05).

[a] Foliar application occurring at the early tillering (growth stage 24, Zadoks et al. 1974) of barley.

Table 23. Percent damaged tillers ± SE from natural infestation of *O. melanopus* larvae in barley treated with foliar and seed treatments of imidacloprid, and foliar carbofuran near Huntley, MT.

Treatment	Rate (gai/ha)	Days Post Foliar Application[a]			
		Pretreatment	4	8	12
Imidacloprid	0.45 (kg)	73 ± 2a	86 ± 3a	76 ± 2a	75 ± 1a
Foliar Imidacloprid	51.50	74 ± 3a	83 ± 6a	43 ± 2b	23 ± 8b
Carbofuran	210.50	77 ± 3a	73 ± 10a	38 ± 2b	26 ± 7b
Untreated		68 ± 8a	81 ± 1a	78 ± 2a	81 ± 10a
F-Statistic		0.75	0.80	86.83	19.86
n		12	12	12	12
P-value		0.56	0.53	0.0001	0.001

Means within columns followed by similar letters are significantly different (Tukey Test; P=0.05).

[a] Foliar application occurring at the early tillering (growth stage 24, Zadoks et al. 1974) of barley.

Discussion

Low mortality and high rate of oviposition were recorded after adult beetles were removed from 12 weeks of cold storage. Similar findings were found by Hoxie and Wellso (1983). They reported 30% adult cereal leaf beetle mortality when extracted from cold storage. Low rates of mortality may be due to starvation, fungi, and/or genetic factors. High rates of oviposition are consistent with results obtained from observations made by Connin et al. (1968), with each female depositing 150 - 200 eggs. The rearing procedures used in this study successfully produced an adequate number of eggs from a limited number of adults and can be followed for subsequent cereal leaf beetle studies.

Greenhouse Trials

Seed treated with imidacloprid at all rates caused a high level of mortality and protected plants from cereal leaf beetle larval feeding at the 2-leaf stage (Table 12 and Table 13). Larvae were observed feeding only briefly before expiring. As expected, rapid mortality caused low defoliation levels on imidacloprid treated plants, but this was not necessarily due to repellency. This mortality may have been due to the high toxicity of this chemical to larvae at the labeled rates on 2-leaf stage barley.

Mortality was decreased when larvae were applied at the later plant growth stages. At the 4-leaf stage, mortality was reduced in the 0.60 gai/kg and 0.30 gai/kg rates, to 94% and 83%, respectively (Table 14). Mortality became unacceptable at all rates when larvae were applied at the flag-leaf stage (Table 16). A loss of this seed treatment's toxicity as a plant matures has been observed in trials evaluating imidacloprid vs. various

species of aphids. Elbert et al. (1990) reported >90% mortality of *Schizaphis graminum* on seed treated wheat for up to 40 days post sowing (Elbert et al. 1990). After that time period mortality was reduced significantly. Altmann (1991) indicated *Aphis fabae* Scopoli mortality to be reduced from 6 - 10 weeks after emergence to 100% and 45%, respectively (Altmann 1991). Each study indicates a loss of toxicity approximately 6 weeks after emergence which agrees with results obtained from the flag-leaf stage of this experiment. As a seed treated plant grows beyond the younger stages, the degree of protection from insect pests is minimized (Altmann 1991).

All rates caused a high level of protection from damage up to the 4-leaf stage even though mortality was relatively low (Table 15 and Table 17). Perhaps this seed treatment has a repellent effect on cereal leaf beetle larvae. Schmidt (1989) and Zeller (1990) noticed a repellent effect that lasted over a week on many hymenopterans, *Apis mellifera*, when imidacloprid was applied as a foliar on flowering crops. Although applied directly on flowering crops, imidacloprid caused no reduction in pollination. Schmuck (1991) also observed carabid, *Poecilus cupreus*, populations being reduced by 15% with imidacloprid. This reduction was primarily due to the repellent effect of imidacloprid since there were no indications of treatment related mortality.

Field Trials

No differences in mortality, leaf defoliation, damaged tillers, and/or field densities were observed at the 2-leaf stage (Table 18). This was believed to be caused by a departure from normal temperatures and precipitation of - 4° C and 6.5 cm, respectively. Shade et al. (1970) indicated that water droplets, winds, and lower temperatures caused

significant mortality to cereal leaf beetle larvae. Temperature and precipitation did not become favorable to beetle survival until the early tillering stage of barley, resulting in increased mortality of eggs applied at the 2-leaf stage in this study (Table 22). Consequently, low rates of larval emergence at the 2-leaf stage are believed to be responsible for the lack of differences among treatments used in that trial.

A high rate of hatch did not occur until precipitation ended eight days after eggs were applied on 4-leaf stage barley. Carbofuran and foliar imidacloprid treatments resulted in high levels of mortality, providing an acceptable level of protection from damage (Table 20 and 21). Ruppel (1973) indicated foliar carbaryl and endosulfan treatments to cause > 90% mortality of cereal leaf beetle larvae when applied to oats. Comparable toxicities between carbofuran, carbaryl, endosulfan, and foliar imidacloprid confirm the effectiveness of foliar imidacloprid on cereal leaf beetles in barley. High beetle mortality by foliar imidacloprid was confirmed when the natural infestations within the plots were measured. Carbofuran and foliar imidacloprid treated plots contained a mean 6.3 larvae per m^2, compared to 53.0 per m^2 in the untreated (Table 22). These data suggests that foliar imidacloprid may be a promising alternative for managing cereal leaf beetle infestations.

Imidacloprid applied as a seed treatment, however, did not cause a high level of larval mortality or leaf repellency in either artificial or natural infestations at the early tillering stage of barley (Table 20, 21, 22 and 23). Previous studies have indicated this treatment as effective in reducing populations of aphids, whiteflies and/or thrips at similar concentrations (Elbert et al. 1990, Pawar et al. 1993, Sloderbeck et al. 1996). This

indicates that this treatment would not control cereal leaf beetle infestations at the early tillering stage of barley. This agrees with laboratory trials in this study which suggest toxicity to be reduced by dilution through plant tissues somewhere between the 4-leaf and flag-leaf stage of barley. It is reasonable to assume that mortality may be initially increased if emergence occurred earlier in the year, thereby infesting earlier staged barley. However, due to earlier studies indicating that larval emergence may occur over a 2 - 3 week period, and infestations commonly occurring past the 4-leaf stage (Blodgett and Tharp 1996, Webster and Smith 1979), this seed treatment may be of limited use for managing this pest.

CHAPTER 5

CONCLUSIONS

Grasshopper Studies

Carbofuran was far more effective than applications of imidacloprid in bioassay and/or field trials, which indicated the limited potential imidacloprid has when used alone to control grasshoppers.

The high levels of debilitated grasshoppers suggest the possibility of combining imidacloprid with other pathogens and/or chemicals to induce an acceptable level of mortality. Combining imidacloprid with *Beuvaria bassiana* or *Metarhizium anispliae* may provide adequate levels of control. These pathogens have raised German cockroach, *Blattella germanica,* and root weevil, *Diaprepes abbreviatus,* mortality to higher levels when used in combination with imidacloprid. High rates of debilitation may hinder a grasshoppers ability to thermoregulate or remove fungal conidia, in response to being infected by this fungi. Either combination may offer a direction for future research in the control of grasshoppers on cereals.

Cereal Leaf Beetle Studies

Laboratory trials indicate that the seed treatment of imidacloprid is highly efficacious towards cereal leaf beetle larvae, with high mortality maintained through the 4-leaf stage of barley. However, when larvae fed on treated barley after this stage (early tillering and

flag leaf stage), mortality decreased to low levels. In this study and others, larval emergence may occur well past the 4-leaf stage. As a result, this seed treatment offers limited potential for control of cereal leaf beetle infestations.

Foliar imidacloprid gave excellent protection from larvae throughout all field experiments. Mortality was > 90%, and was not significantly different from the carbofuran treated plots. Consequently, foliar imidacloprid may be another possible alternative when managing the cereal leaf beetle. Future research may concentrate on residual and efficacy tests of this chemical, which are some steps needed to initiate the labeling of this product for use.

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