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RESIDUAL ACTIVITY OF AT-PLANTING APPLICATIONS OF IMIDACLOPRID AND THIAMETHOXAM TO CONTROL COLORADO POTATO BEETLE, *LEPTINOTARSA DECEMLINEATA* (SAY), ADULTS

presented by

Eduardo Espitia-Malagón

has been accepted towards fulfillment
of the requirements for the

M.S. degree in Entomology

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By

Eduardo Espitia-Malagón

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ABSTRACT

RESIDUAL ACTIVITY OF IMIDACLOPRID AND THIAMETHOXAM TO CONTROL COLORADO POTATO BEETLE, *LEPTINOTARSA DECEMLINEATA* (SAY), ADULTS

By

Eduardo Espitia-Malagón

This research was aimed to study the effect of soil type (organic vs. mineral), application method (in-furrow vs. seed-treatment), water level (normal vs. excessive), active ingredient (imidacloprid vs. thiamethoxam), and rate (full vs. reduced) on the residual activity of at-planting treatments to control Colorado potato beetles. Treated potatoes were grown in the field, in 2002 and 2003, at two locations differing in soil type, and in greenhouse in 2004. Feeding bioassays were conducted on a weekly basis until affected beetles proportion was 20% or lower. Field studies showed: sustained high efficacy of both active ingredients until d 40 in mineral soils, and d 40 or less in organic soils; a steep efficacy drop after that date; reduced imidacloprid activity due to resistant strain; low evidence of cross resistance, late effect of resistant strain on thiamethoxam in mineral soils and sustained effect in organic soils; no effect with thiamethoxam activity at lower rate although imidacloprid activity was hampered; most factors responded in organic soils. For greenhouse, when total affected beetles recorded, the following was observed: sustained efficacy curve until d 80 even in organic soil; low effect of excess water (partly affecting thiamethoxam activity). When only dead beetles registered, efficacy was exhibited by thiamethoxam and; there was a strong effect of organic soil. Special attention should be paid under commercial conditions to potential hampering effects of all factors studied here.

To the memory of two of my most

loved ones and never-ending

source of inspiration:

Ana María Malagón

Guillermo Rondón Carvajal

May God have them at His side

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CHAPTER 1:

INTRODUCTION

Potato production

Potatoes, *Solanum tuberosum* L., have a long history as a cultivated food plant. This crop was introduced from its center of origin in South America, to different areas worldwide (Hijmans and Spooner 2001). Potato is a major food source and is fourth following wheat, maize and rice as the most produced crops in the world (Sattaur 1989). Potatoes are an important part of a typical American diet, with a consumption rate of 64 kilograms per person per year, exceeded only by wheat flour (Anon. 2003a). The United States is fourth in the world in potato production (Anon. 2004d) with annual yields of 21.0 million metric tons from about 445,000 ha planted. In 2001, the U.S. potato crop provided $ 2.5 billion in income for farmers, representing 15% of all annual income in the vegetable sector (Anon. 2003a). Michigan potato production corresponds to an annual average of 18,600 ha harvested (Anon. 2003b), yielding 629,495 metric tons in 2002 (Anon. 2003c) and 662,245 metric tons in 2003 (Anon. 2004a). In 2002 Michigan produced 3 % of the total U.S. potato crop (Anon. 2003c) and ranked 10th in the nation in potato production. Potato production adds almost $93 million to the state's economy each year and it is one of the state's top 10 crops (Anon. 2004a).

Colorado potato beetle

The Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae), is the most devastating defoliator of potatoes worldwide (Radcliffe 1982, Jacques 1988, Hare 1990, Ferro and Boiteau 1993) and is the most important arthropod pest of potatoes in North America. It is present throughout the U.S. except California, but it is most economically important in the northern regions of the midwestern and eastern U.S. (Ferro and Boiteau 1993). Two factors have established Colorado potato beetle as a key pest of potatoes: 1) its highly voracious feeding habits and 2) its ability to rapidly develop insecticide resistance. If uncontrolled, Colorado potato beetle can completely defoliate a potato field prior to tuber initiation (Hare 1990). By the early 1990s, Colorado potato beetle had developed resistance to virtually all the insecticides used to control it (Bishop and Grafius 1996).

The Colorado potato beetle probably originated in Mexico (Hare 1990, Casagrande 1987) and from there it invaded potato production areas in North America and, later on, in Eurasia. Potato crop damage was first reported in 1859 in Nebraska, apparently after a host shift to potatoes (Jacques 1988, Casagrande 1987). Populations in southern Texas, Arizona and Mexico still do not accept potatoes as a host (Hare 1990). The pest rapidly spread in the U.S. after shifting to the cultivated potato as a host plant and reached the U.S. East Coast from Connecticut to Virginia by 1874. It entered Canada in 1870 (Ferro and Boiteau 1993) and all of eastern Canada was infested by 1878 (Capinera

2001). It gradually spread southward, reaching Florida and Louisiana by 1900 (Capinera 2001). It was first introduced into Europe in 1875 (Jacques 1988), became established in Germany in 1914 (Jacques 1988), in France in 1921 (Webber and Ferro 1994), in Eastern Europe in the mid to late 1950s (Radcliffe 1982). It is also found throughout the former Soviet Union (Jacques, 1998). The Colorado potato beetle was reported in Iran in 1984 (Anon. 2004b) and reached China in 1992 (Xie and Zhang 2002, Jacques 1998).

The Colorado potato beetle's life cycle on potatoes is completed in as little as 3 wks under optimal conditions (Ferro and Boiteau 1993). Optimal temperature range for development is 25 – 33 °C, with a threshold of 10 °C (Hare 1990). Overwintered adults emerge in mid to late spring, generally at the time potato plants are emerging. In continuous potato cropping systems, the beetles may overwinter directly in the field (Caprio and Grafius 1993); however, they may also arrive from adjacent cultivated fields or distant wooded areas (Ferro and Boiteau 1993). After about 1 wk of feeding, mating occurs and females lay eggs in clusters of 25 – 40 on the underside of the foliage (Ferro and Boiteau 1993). Eggs are anchored to the leaf surface with a small amount of yellowish adhesive (Capinera 2001). Each female can lay up to 450 eggs, which hatch in 3 -10 d depending on temperature (Ferro and Boiteau 1993). All the eggs in a cluster hatch more or less simultaneously (Hare 1990); the newborn larvae consume the egg cases and after 1-2 d disperse and start feeding on foliage (Ferro and Boiteau 1993).

Larvae go through four instars. The first instars are bright red with black heads, pronotums and legs and older instars are light orange with black markings (Mahr et al. 1995). At the end of each stadium, from first to fourth, larvae will reach average lengths of 2.6, 5.3, 8.5 and 15 mm, respectively (Capinera 2001). Leaf consumption by larvae is estimated at 35 - 45 cm^2 (Capinera 2001). The first three instars cause minimal damage to the foliage, while the fourth instar is responsible for 77% of foliar consumption by the larvae (Hare 1990, Ferro and Boiteau 1993). The duration of the larval stage can be as short as 8 -11 d at 30 °C or as long as 31-35 d at 13 °C (Ferro and Boiteau 1993). Mature larvae stop feeding, fall to the ground, and burrow to a depth of 5 – 15 cm to pupate (Hartcourt 1971, Mahr et al. 1995).

Colorado potato beetle pupae are oval shaped and orange, about 9.2 mm long and 4.4 mm wide. After about 6 d pupae transform into adults, which spend their first 3 – 4 d in the soil before digging to the surface. Total time spent in the soil by the mature larva, the pupa and the newly emerged adult varies from 8.8 to 22.3 d at 28 to 15 °C, respectively (Capinera 2001).

Adults of this generation are referred to as "summer adults", and their emergence in the midwestern U.S begins in July. These beetles feed more than the overwintered adults and can do extensive foliar damage in a very short time if populations are high. Foliar consumption by adults is 7-10 cm^2 per day (Capinera 2001). They start laying eggs approximately 1 wk after emerging from the soil. At the end of the season, beetles burrow into the soil for overwintering at depths of 8 to 13 cm (Hare 1990). Colorado potato beetle larvae and adults feed on stem tissue and even on exposed tubers if all foliage has been consumed (Capinera 2001).

In the midwestern U.S., Colorado potato beetle populations complete one to three generations in a season, although the climate in the northernmost portion of its range usually allows only a single generation per year. In Maryland and Virginia three generations per year may occur, but many second generation adults go into diapause rather than laying eggs for a third generation (Capinera 2001). In the lower peninsula of Michigan one or two (rarely three) generations occur each season.

Males and females mate more than once and with different partners: at least three mating events are required to fill the female's spermatheca completely and multiple mating may enhance fertility (Boiteau 1988, Alyokhin and Ferro 1999). In studies of sperm utilization the importance of the second male in fathering offspring has been reported as 32-53% (Boiteau 1988) and 72% with a range of 16.7 – 100% (Alyokhin and Ferro 1999).

Colorado potato beetles disperse by walking, flight, and human-assisted transport. Flight is of variable importance among populations and can be of three types: short-range or trivial flight, long-range or migratory flight and the diapause flight (Hare 1990). Each one of these serves different purposes. Short range flight enhances mating and food resource use; migratory flight enables the insect to find new areas when searching for food; and diapause flight allows beetles to locate tall vegetation and wooded areas contiguous to the crops, which are then used as diapausing sites (Weber and Ferro 1994). Flight capability increases the area of dispersion of Colorado potato beetles up to several km (Radcliffe 1982, Weber and Ferro 1994).

Host plant range

The Colorado potato beetle is an oligophagous insect that feeds exclusively on Solanaceae, and primarily on about 20 species in the genus *Solanum* (Radcliffe 1982, Hare 1990). Different adaptation degrees to host in the United States are reflected in differential beetle body weight as observed in beetles that have fed on potatoes compared with those feeding on less suitable hosts (Hsiao 1978). The major factors determining the host plant range of this insect are: 1) feeding behavior, related to the insect's response to plant compounds, 2) host plant location, and 3) host geographical distribution (Hare 1990). In most of North America, in addition to potatoes, Colorado potato beetle feeds on its original hosts: buffalo bur (*Solanum rostratum* Dunal), silver-leafed nightshade (*Solanum elaeagnifolium* Cav.) and narrow-leaved solanum (*Solanum angustifolium* Mill.). Most-preferred hosts also include climbing nightshade (*Solanum dulcamara* L.) *and* the cultivated eggplant (*Solanum melongena* L.) (Hare 1990). Horsenettle (*Solanum carolinense* L.), *Solanum triquetrum* Cav., *Physalis* spp., and tomato (*Lycopersicon esculentum* Mill.) are also reported as important hosts (Jacques 1988).

Preference towards these hosts varies geographically. In north eastern U.S. and central states potato is the main host, in southern states (Colorado, New Mexico, Oklahoma, Texas) buffalo bur is the main wild host, followed by silver-leafed nightshade. This pattern may vary: in Arizona the predominant host is silver-leafed nightshade and horsenettle is an important host in central and southern states. In Mexico, it is found mainly on buffalo bur and narrow-leaved solanum. In Michigan, an important alternative host is horsenettle (Mena-Cobarrubias et al. 1996). A host in Central America (Honduras) is lanceleaf nightshade (*Solanum lanceolatum* Cav.), on which the insect completes two generations per year at that latitude (Cañas et al. 2002).

The diversity in host range as the insect colonized new areas represents a broadening of range rather than shifts from previous to new hosts (Hare 1990), but populations in the U.S. have evolved to prefer potato as a host (Hsiao 1978). Alkaloids and glycoalkaloides are the crucial semiochemicals determining host suitability. Due to the insect's ability to adjust to locally abundant hosts, its host range continues to expand within the Solanaceae (Hare 1990, Capinera 2001).

Diapause

Aestival and reproductive (hibernal) diapause occurs in Colorado potato beetle (Tauber et al. 1988). Univoltine traits and changes in food are related to aestival diapause. Late summer adults and second-generation adults that emerge in mid-August or later, delay development of the reproductive system and their flight muscles are underdeveloped (Ferro and Boiteau 1993). When the beetles are ready to overwinter, they fall to the ground or search for overwintering sites and then enter diapause in the soil. The most important factor that induces diapause is photoperiod; Colorado potato beetle is a long day insect with a critical photoperiod of ca. 15 h (De Wilde & de Loof, cited by Voss et al.1988). In addition to photoperiod, cool temperature and poor food quality also induce diapause (Hare 1990). These factors together trigger the physiological preparation for cold winter conditions. At higher temperatures, sensitivity to photoperiod is reduced; thus, southern populations generally require shorter photoperiod as a diapausing stimulus. Physiological condition of potato foliage also affects diapause induction; senescing foliage can induce diapause and reduce beetle reproductive functions (Hare 1990). Less suitable hosts also increase the incidence of aestival diapause (Hare 1983). If a combination of the above factors is in place, substantial variation in the proportion of beetles entering diapause occurs within generations and within populations (Hare 1990).

Management

Crop defoliation by Colorado potato beetle may occur even before tuber formation, leading to total yield loss if no control measure is taken (Hare 1990). Susceptibility to defoliation varies with cultivar and growth stage, but in general, plants just beginning to bloom and to form and fill tubers are most susceptible to yield reduction by Colorado potato beetle (Ferro and Boiteau 1993). Therefore, Colorado potato beetle management programs emphasize the need for early and midseason pest suppression with higher beetle densities tolerated later in the season.

Integrated management practices are recommended for Colorado potato beetle control. These practices include: monitoring, judicious use of synthetic insecticides, and cultural, physical and, to a limited extent, biological control. Throughout the history of Colorado potato beetle control, many insecticides have been used heavily, resulting in severe insecticide resistance problems (Casagrande 1987).

The backbone for any management program is monitoring. Through monitoring, Colorado potato beetle population levels can be estimated in the field, thence monitoring constitutes the main tool for decision-making regarding insecticide use. Both pest density and damage can be quantified.

A key element for Colorado potato beetle management is the understanding the pest's life cycle and to know the factors that affect it, especially temperature. Changes in temperature induce variation in stage length. Predicting pest development is based on quantification of degree-days (DD). In the Midwestern U.S., a base of 10 ºC is commonly used (Mahr et al. 1995).

Monitoring consists of weekly scouting of five adjacent plants at 5 - 40 sites throughout the field. Total plants sampled in a field would be 50 – 200, covering transects of 90 m that intersects 50-100 rows (Capinera 2001, Mahr et al. 1995, Ferro and Boiteau 1993). Scouting should start at plant emergence, examining leaf undersides for adults and egg masses. Initial infestation is often detected on field edges. Detected egg masses sites should be flagged and the hatch rate monitored. In the following weeks, scouting for larvae is important to detect young larvae which are more sensitive to insecticides. Head width is the instar-identifying character. Average head widths are 0.5, 1, 1.5, and 2 mm for first to fourth instars, respectively. Fourth instars need to be controlled before they drop to the ground for pupation. Summer adults can be detected by scouting as well. However, they can cause severe foliar injury and are more difficult to control than the overwintered adults. Newly emerged (less than 2 wks old) summer beetles have clear hind wings compared with the smoky orange wings with bright orange veins of overwintered adults or older summer adults (Mahr et al. 1995, Ferro and Boiteau 1993).

Crop rotation is a cultural practice that reduces and delays beetle infestations in the spring. Some beetles may overwinter for a year or more (Biever and Chauvin 1990, Peferoen et al. 1981, Ushatinskaya 1978 cited by Webber and Ferro 1994) or arrive from other fields after diapause (Weber and Ferro 1994). Early or very late potato varieties are recommended to reduce the effect of second generation beetles (Weber and Ferro 1994) although late varieties are not recommended where three insect generations occur (Ferro and Boiteau 1993). Trap crops can also be used; they consist of early-planted potatoes (for trapping overwintered adults), or late-planted potatoes (for trapping summer adults at the end of the season). Beetles concentrated on either type of trap crop can be confined and controlled by physical or chemical methods (Weber and Ferro 1994).

Several physical control techniques are effective on Colorado potato beetle but their feasibility is variable, therefore many of them have not been used commercially. Plastic lined trenches placed along the row sides to capture beetles seeking overwintering sites have shown efficiency of 95% (Misener et al. 1993). Disturbing overwintering sites significantly reduces adult survival (Milner et al. 1992). Use of steam has shown to inflict up to 50% mortality on beetles in field plots (Pelletier et al. 1998). Flaming is suitable for early-season control of adults on plants, especially for plants < 3 – 4 cm tall. Flames must be directed to the plant at an angle at the base of the plants, adjusting flame, distance and tractor speed to avoid damage to the plants. Mortality of beetles can reach more than 80% (Lague et al. 1999). Flaming may reduce egg hatch by 30% (Kuepper 2003) and it is comparable in cost to insecticide application. This method can also be used at the end of the season to kill both vines and beetles. Crop vacuums, which remove up to 70% of small larvae and adults, have also been used. The removal rate is variable and has been reported to be 34% - 48% of adults, 9 – 42 % of small larvae, and 29 – 50% of large larvae. Many larvae drop to the soil and are not picked up by the equipment (Hodik et al. 1999, Boiteau et al. 1992). The high cost of vacuuming makes its use limited. Combination of tactics, such as enhancing the value of crop rotation by means of early trap-cropping combined with diapause disruption may provide the greatest benefits from cultural control (Weber and Ferro 1994).

No Colorado potato beetle control program relies heavily on biological control. However, several biocontrol agents have been studied and their use may be more important in the future. Predators, parasitoids and entomopathogenic microorganisms have been identified as natural enemies, but none has been shown to consistently suppress beetle populations (Capinera 2001, Hare 1990, Mahr et al1995). *Predators:* The spined soldier bug (*Podisus maculiventris* Say); and, the two spotted stinkbug, (*Perillus bioculatus* Fabr.), are effective for egg and larval control, they can reach 80% control when an appropriate ratio predator:prey is established (Capinera 2001). The pink spotted lady beetle (*Coleomegilla maculata* De Geer) feeds extensively on Colorado potato beetle eggs and early instars (Ferro 1994). Carabids are reported as predators on Colorado potato beetle. *Lebia grandis* Hentz is the best known. Adults consume ggs and small larvae, and *Lebia* larvae parasitize prepupal stages. It appears to be one of the most promising endemic natural enemies (Groden 1989).

Parasitoids: The tachinid flies, *Myiopharus doryphorae* (Riley) and *M. aberrans* (Townsend), are larval-pupal parasitoids. *M. doryphorae* has been reported to kill about 50% of the season's last beetle generation (Casagrande 1987). The South America-originated wasp *Edovum puttleri* Grissell is a parasitoid of Colorado potato beetle eggs but its limitations for overwintering and adult food sources prevent use in commercial situations. Nevertheless, differences in its parasitism rate found at low temperatures show a potential for future use in temperate regions (Acosta and O'Neil 1999).

Neither predators nor parasitoids are practical for high levels of biological control under commercial conditions: multiple releases of *E. puttleri* and the spined soldier bug, despite effective control, are not feasible for large scale use due to high costs (Tipping et al. 1999).

Microorganisms: The entomopathogenic fungus, *Beauveria bassiana* Bals., is the most important of the identified fungi with activity on Colorado potato beetle. Factors related to *B. bassiana* deployment techniques and synergistic effects with imidacloprid, antifeedants and *Bacillus thuringiensis* var. *tenebrionis* have been studied and show compatibility and enhanced activity of the fungus (Fernandez et al. 2001, Furlong and Groden 2001, Furlong and Groden 2003). No adverse effects on biodiversity from *B. bassiana* applications have been found (Lacey et al.1999) but incompatibility with the fungicides chlorothalonil, mancozeb, maneb, thiophanate-methyl, metalaxyl+mancozeb and zineb has been reported (Jaros et al. 1999, Todorova et al. 1998). In contrast, the use of *B. t. tenebrionis,* in foliar sprays is recommended and effective to control early instars under commercial conditions and reduce resistance build-up to chemical insecticides (Mahr et al. 1995). *B. t. tenebrionis* can be used for larval control at 150 DD as an early spray or after suppressing beetle populations by other means (Mahr et al. 1995).

Chemical control and insecticide resistance

Because the Colorado potato beetle has developed resistance to most insecticides used for its control; only the novel neonicotinoid insecticides exhibit satisfactory control in areas where insecticide resistance to other chemical groups is widespread. Two neonicotinoids, imidacloprid and thiamethoxam, are registered in the United States for use against Colorado potato beetle and currently constitute the best tools available for the control of this pest. In 1995, imidacloprid was the first neonicotinoid to be registered for use in Colorado potato beetle control; thiamethoxam was registered in 2001. Other neonicotinoids are currently under evaluation. For Colorado potato beetle control imidacloprid and thiamethoxam are recommended as at-planting applications

(Boiteau et al 1997) and, to a lesser extent, foliar sprays. Due to their systemic and residual activity, a single at-planting application of neonicotinoids may eliminate the necessity for additional chemical control of beetles during the season; Boiteau et al. (1997) reported lasting control from a soil application at a rate of 0.03 g/m of imidacloprid.

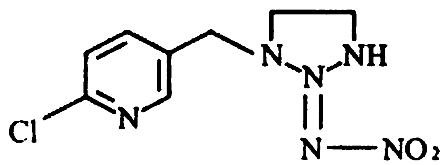
Neonicotinoid insecticides and control

Neonicotinoids have been the fastest growing chemical class of insecticides world-wide in terms of volumes produced and use. This success has been favored by characteristics like broad-spectrum activity, low application rates, excellent uptake and translocation in plants, a new mode of action, and a favorable safety profile (Maienfisch et al. 2001). Broad commercial use of these insecticides has increased in recent years world-wide for control of sucking and chewing insect pests.

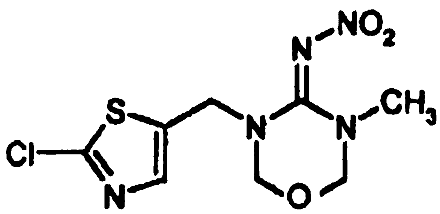
Of the three sub-classes of neonicotinoids known up to date, imidacloprid (Figure 1) belongs to the chloronicotinyls and thiamethoxam (Figure 1) to the thianicotinyls. The first neonicotinoid under commercial use, imidacloprid, was introduced in Japan and Europe in 1990 and first registered in the U.S. in 1992. Currently, it may be the most widely used insecticide globally (Ware, 2000).

Insecticide Resistance

The Colorado potato beetle ranks fourth among insects with the most reports of insecticide resistance worldwide (Anon. 2004c). Historically,



Imidacloprid



Thiamethoxam

Figure 1. Imidacloprid and Thiamethoxam structure

populations of Colorado potato beetle have developed resistance to every compound used for control (Bishop and Grafius 1996). However, chemical control remains a necessary component for Colorado potato beetle management program. In the 1900s, differences in rate effectiveness of Paris green (copper acetoarsenite), the earliest product used for chemical control of Colorado potato beetle, provided the first historic signal of insecticide resistance. Colorado potato beetle was the target of the development of arsenical insecticides and prompted the first large-scale use of insecticide in an agricultural crop for its control (Capinera 2001, Hare 1990). With the use of these insecticides in the 1940s the impact of the Colorado potato beetle was temporarily reduced (Radcliffe 1982).

Signs of resistance to arsenicals appeared in Long Island, New York, just before the introduction of DDT, the first chlorinated insecticide (Bishop and Grafius 1996). Resistance to different organochlorine insecticides was widespread by the late1950s and early 60s (Bishop and Grafius 1996). At that time, organophosphate and carbamate insecticides began to be used for Colorado potato beetle control. After great initial control, these products followed the fate of organochlorine insecticides, inducing pest resistance. Resistance to azinphosmethyl, oxamyl, phosmet and other organophosphates and carbamates became widespread in the northeastern U.S. by the 1980s and was confirmed in other regions of the U.S. during that decade. Resistance to pyrethroids, registered in the late 1970s and 1980s, evolved extremely rapidly. Before the mid-1980s, pyrethroids did not provide control of Colorado potato beetle populations in the northeastern U.S. In the 1990s, Colorado potato beetle resistance to all available insecticides was so prevalent that virtually no insecticide was effective for controlling the pest in most production areas of the eastern U.S. Based on this history Colorado potato beetle can be expected to develop resistance to any new insecticide deployed against it (Bishop and Grafius 1996).

Resistance has already been reported for imidacloprid in the United States including field populations from Long Island NY (Olson et al. 2000, Zhao et al. 2000), and Delaware (Bishop et al. 2003). Populations selected in the laboratory show high levels of potential resistance: a NY strain has developed more than 100-fold resistance (Bishop et al. 2003) and a Michigan strain has developed, more than 100-fold resistance (Bishop et al. 2003). In Canada, no resistance to imidacloprid had been found by 2002 (Brent and Gavloski 2002) but susceptibility level decreased in some populations during the period 1998 – 2001 (Hilton et al. 2002). No imidacloprid resistance has been found in Poland (Wegorek 2002).

Low levels of resistance (<20x) to thiamethoxam also occur in Colorado potato beetle populations from Long Island, NY (Bishop et al. 2003), Delaware and a Massachusetts strain that was 9-fold resistant to thiamethoxam (Bishop et al. 2003, Grafius et al. 2004). Since thiamethoxam had not been used at any of these sites, this low level resistance is likely due to cross resistance betweenthiamethoxam and imidacloprid. Since imidacloprid and thiamethoxam are chemically related (Figure 1), the chance for cross-resistance is high. Unlike most neonicotinoids, which bind on nicotinic-acetylcholine receptors, thiamethoxam has low affinity to these receptors (Nauen et al. 2003). However, thiamethoxam's metabolite, clothianidin, acts with high affinity on the imidacloprid binding site like other neonicotinoids, making thiamethoxam not an option for insecticide rotation with imidacloprid (Nauen et al. 2003). Cross-resistance between the two insecticides is less than 1:1 but is high enough to suggest that alternating between the two insecticides will not be an effective resistance management tactic (Grafius et al. 2004).

Insecticide Resistance Management

Intensive use of insecticides for control of Colorado potato beetle places great selection pressure on pest populations. With high resistance levels occurring in many areas, choosing the appropriate insecticide is very important. Techniques must be adopted wisely, when using chemical control, to reduce the risk of resistance build-up. Insecticide rotation is very important; insecticides from the same chemical group should not be used on more than one Colorado potato beetle generation in the same season. Since the chance of cross-resistance between imidacloprid and thiamethoxam is high, rotation must be done with other products. Crop rotation is the most effective option to reduce insecticide selection pressure, because other selection factors would take place like migration in search for food.

Maintaining susceptible populations in refugia can lower resistance build-up. With most resistance management programs relaying on the refugia/high-dose strategy (Alyokhin and Ferro 1999), the sperm mix resulting from the polygamous habits, enhances the occurrence of heterozygotes in the offspring coming from the mating between resistant beetles from the main crop area and susceptible beetles from the refugia. However the chance for matings taking place among resistant mates is high, because Colorado potato beetles often mate with conspecifics emerging close to the pupation site (Gould 1998 cited by Alyokhin and Ferro 1999). Early-planted refugia and the slow growth and development of resistant larvae may enhance the cross mating of susceptible and resistance populations (Alyokhin and Ferro 1999).

Criteria used to choose an insecticide include: degree of beetle resistance to the insecticide, stage of the pest to be controlled, and population density. Most insecticides are not effective on eggs, whereas larvae are easily controlled. When resistance is present or suspected, sprays to control larvae should start when 15 – 30 % of egg masses have hatched. Other products may be used for additional sprays, especially *Bacillus thuringiensis*, as described above.

Factors affecting soil-applied neonicotinoids

Soil characteristics

Because imidacloprid and thiamethoxam are recommended for at-planting applications for Colorado potato beetle control, the nature and characteristics of the soil are an important influence on compound activity. Soil (topography, physical conditions) interacting with water, can affect insecticide movement and half-life because the organic matter content and physical and chemical characteristics interact with the insecticide's intrinsic characteristics (chemical and biological) (Krohn and Hellpointner 2002).

The available amount of insecticide in the soil after application may vary since insecticides can interact with soil characteristics. In aerobic soil metabolism studies, the 1st order half-life of imidacloprid averaged 156 d (Krohn and Hellpointner 2002). Field dissipation studies conducted in the U.S. under crop conditions show a half-life for imidacloprid of 7 - 146 d. In studies in Europe a half-life range of 83 to 124 d has been reported (Krohn and Hellpointner 2002). Ground cover is one determining factor for half-life of imidacloprid (48 d for soil covered with grass vs. 190 d without cover) (Scholz and Spiteller 1992) in greenhouse studies. In field studies, on bare soil in Europe, the half-life averaged 174 d (Krohn and Hellpointner 2002). Dissipation studies in soil indicate that during its lifetime, imidacloprid remains in the upper 30 cm of the soil and is only partially translocated into soil beyond that depth (Krohn and Hellpointner 2002). Active imidacloprid sorption in the soil, especially by organic carbon, has been proposed to be the cause of the low mobility of the compound in the soil despite its high water solubility. However, in aqueous solutions the persistence of imidacloprid is also affected by pH (higher half life of the insecticide under alkaline compared with acidic conditions), and type of formulation (powder formulations have higher half life than liquids) (Sarkar et al. 1999). Organic soils may reduce insecticide activity compared with mineral soils because imidacloprid binds to the organic fraction of the soil (Krohn and Hellpointner 2002). A higher potential for binding in organic soils is supported by the finding that humic acids have higher sorption capacities than Ca-clay minerals (Liu et al. 2002). Also the differential effect of organic matter aging on half-life has been confirmed. Aged cow manure induced longer persistence of imidacloprid in soils than recently-applied manure (Anon. 2006). Many studies indicate that imidacloprid does not accumulate in the soil over the long term (Krohn and Hellpointner 2002); however, half lives of more than 365 d have been reported in the U.S. in crop situations (Anon. 2001)

Thiamethoxam has been studied mostly in laboratory conditions. In laboratory soils, thiamethoxam degrades at moderate to low rates. Its half-life ranges from 34 to 75 d under favorable growing conditions but may increase by a factor of three under unfavorable growing conditions (Maienfisch et al. 2001). Also in soil, thiamethoxam's half life is reported to be 101 d; photolysis in soil: 47 -54 d and in water 3 – 4 d (Anon. 2003d). Sensitivity studies for pH show that thiamethoxam is very stable at pH 5 and pH 7, but labile at pH 9 (Maienfisch et al. 2001).

Water

Reduction in activity of imidacloprid and thiamethoxam by excessive rainfall or irrigation is a concern because potatoes are generally grown on sandy loam or loamy sand soils. Water solubility for imidacloprid, 0.5 g/l at 20°C and pH 7, is considered moderate and places the compound as a hydrophilic substance and medium in mobility in soil (Krohn and Hellpointner 2002). Water solubility for thiamethoxam is 4.1 g/l and is ranked as medium to highly mobile in soil (Anon. 2003d). These products could potentially move through sensitive soil types including porous, gravelly, or cobble soils, depending on rain fall and irrigation practices (Jenkins 1994). Insecticide leaching also depends on the formulation components (Gupta et al. 2002) and pH of aqueous solution (Sarkar et al. 2001). Local soil compactness and water flow may extend leaching beyond the root zone (upper 30 cm) and could lead to a reduction in residual activity of these products (Gupta et al. 2002).

The effect of water flow in soil on neonicotinoid performance has been demonstrated by the differential performance of imidacloprid depending on the specific irrigation system. Enhanced whitefly control in poinsettias was reported for imidacloprid when used in sub-irrigated pots (van Iersel et al. 2001). A combination of application method (in irrigation water) and irrigation method (water layer on bench) improved plant insecticide uptake, providing uniform control throughout the growing season and improving whitefly control in poinsettias compared to other irrigation systems..

Application method

For at-planting applications with imidacloprid and thiamethoxam in potatoes, two methods are used: seed treatment and in-furrow application. Potato seed pieces can be treated with insecticide prior to placement in the furrow, this method is recommended for some soil pests. Genesis ® (imidacloprid, Bayer CropScience) and Cruiser ® (thiamethoxam, Syngenta Crop Protection Inc.) are the commercial formulations designed for this method of application. These compounds can also be used for in-furrow application as Admire ® 2F (imidacloprid, Bayer CropScience) or Platinum ® (thiamethoxam, Syngenta Crop Protection Inc.). For this method no extra step is required prior to planting.

Each of the two methods uses low amounts of pesticide and places the compound in the required site, close to the plant. However these formulations might interact with the other factors related to the application. When insecticides are applied at-planting, as neonicotinoids are recommended for Colorado potato beetle control, the effects of application method on product performance are still unclear. Although seed-treatment is reported to promote more insecticide uptake by plant tissue (Rouchaud et al. 1994), because the insecticide is applied in close proximity to the developing plant, the advantages or lack of advantages of this method in potatoes are unknown and it is important to compare this method with in-furrow application method.

Insecticide uptake

The physiology of neonicotinoid uptake has been studied extensively for imidacloprid – the first released and the best known of these insecticides. Fewer reports are available for thiamethoxam. Once applied to the soil, imidacloprid may have several routes of metabolism. Among its soil metabolites imidacloprid urea is the most abundant hydrolytic metabolite.

Water solubility is a determining factor for insecticide movement in soil, and ultimately insecticide availability to the plant. Imidacloprid is transported acropetally in the xylem. After application, the rate of insecticide concentration in the plant declines as a function of time. The decline of imidacloprid concentration in sugar beet leaves after seed treatment, decreases from 15.2 µg/g (fresh weight), on day 21, to 0.5 µg/g on day 97, and by day 97, 4.5% of the parent compound remains and 44.5% is in the form of absorbed metabolites (Westwood et al. 1998). By the end of the season, imidacloprid was no longer present in sugar beet (Rouchaud 1994) and only traces, considered safe for human consumption, were found in eggplant, cabbage and mustard at harvest (Mukherjee and Gopal 2000).

Movement of imidacloprid and thiamethoxam in the soil is a concern, especially given their high levels of water solubility. Although several studies report no leaching of imidacloprid under normal crop situations (Rouchaud et al. 1994, Rouchaud et al. 1996, Leib and Jarret 2003), there is potential for leaching especially under high rainfall shortly after the application, or through formation of stable, soluble organic fraction-pesticide interactions in solution (Gonzalez-Pradas et al. 2002). Further studies should address whether this potential leaching, related to excessive water, produces reduction in insecticide performance, especially for high soluble compounds.

It is also unknown how the combined effects of insecticide and application method affect beetle mortality when plants are grown in mineral vs. organic soils. A potential reduction in insecticide uptake will significantly affect mortality of susceptible beetles. If beetles are tolerant to imidacloprid, mortality from imidacloprid is expected to decline according to the degree of tolerance. Due to the chemical similarities between imidacloprid and thiamethoxam (Nauen et al. 2003) and the recent reports of resistance in Colorado potato beetles to thiamethoxam (Grafius et al. 2004), cross resistance of imidacloprid resistant strains to thiamethoxam, is likely.

The combined effects of application method and environmental factors could result in shorter half-lives of the compounds, higher degradation rates, and leaching or run-off losses. These changes could, in turn, lead to lower insecticide uptake by the plant tissue. A reduction of the insecticides' residual activity after at-planting application could select for beetles emerging late from overwintering or moderately resistant beetles, making control no longer effective and eventually requiring additional insecticide applications.

Insecticide efficacy may be impaired by: 1) environmental factors such as rainfall and soil type; 2) agronomic factors such as application method, and 3) ecological factors, such as insecticide resistance level of the pest populations. Consequently, it is necessary to determine whether and to what extent those environmental and agronomic factors affect insecticide performance and eventually modify application techniques accordingly to maintain insecticide performance and minimize potential for resistance build up.

Research Objectives

General objective

Determine whether and to what extent environmental (rainfall, soil type), ecological (pest population susceptibility to insecticide), and agronomic (application method) factors affect the efficacy and residual activity of the neonicotinoids, imidacloprid and thiamethoxam, when applied at planting for Colorado potato beetle control. I hypothesize that those factors affect the insecticides' performance for pest control in this system and that there will be significant interaction between factors.

Specific objectives for field studies

1) Compare the efficacy of the neonicotinoids imidacloprid and thiamethoxam, applied in the field at two rate levels, for Colorado potato beetles control.
2) Determine whether in-furrow application vs. seed treatment influences the efficacy and residual activity of these neonicotinoids
3) Determine the response of two strains of Colorado potato beetle, differing in susceptibility to imidacloprid, to the combined effects of insecticide, application method, and application rate. Since the two insecticides are from the same chemical class, cross-resistance is expected.
4) Identify any interactions and /or additive effects between the above.
5) Compare the responses in (3) at two sites that differ mainly in soil type (mineral vs. organic). The very high organic matter content of organic soils may hamper the efficacy and residual activity of these insecticides, compared with activity in mineral soils.

Specific objectives for greenhouse studies

1) Determine the effect of soil type (mineral soil versus organic soil) on beetle mortality from the two insecticides.

2) Determine under greenhouse conditions whether in-furrow application or seed treatment affects the efficacy and residual activity of these neonicotinoids.

3) Determine the effect of excessive irrigation at planting on the residual activity of imidacloprid and thiamethoxam.

4) Examine possible interactions between: soil type, application method, and insecticide, and their effect on beetle mortality.

CHAPTER 2:

THE EFFECT OF APPLICATION METHOD AND RATE ON THE RESIDUAL ACTIVITY OF THIAMETHOXAM AND IMIDACLOPRID FOR THE CONTROL OF THE COLORADO POTATO BEETLE, *Leptinotarsa decemlineata (SAY)* IN THE FIELD

INTRODUCTION

The Colorado potato beetle, *Leptinotarsa decemlineata* (Say), is the most important insect pest of potatoes in the midwestern United States (Foster and Flood 1995) and is the most devastating defoliator of potatoes worldwide (Hare 1990, Casagrande 1987). Larvae and adults can cause complete defoliation and total yield loss may occur if control measures are not taken. Potato plants in the bloom stage or at early tuber formation are especially vulnerable to beetle attack (Hare 1990). Consequently, control programs for this pest are aimed to minimize the effect of insect damage during early and mid season and rely extensively on synthetic insecticide use.

Currently, the two most widely used insecticides to control this pest are the neonicotinoids imidacloprid and thiamethoxam. These insecticides constitute the best control tools available where Colorado potato beetle shows resistance to other major insecticide groups. Imidacloprid was registered for use in Colorado potato beetle control in the United States in 1995 and thiamethoxam was registered in 2001.

These two systemic insecticides belong to the neonicotinoid chemical class: a relatively new group of compounds that act as nicotinic acetylcholine receptor agonists (Bi et al. 2002). Neonicotinoids can be applied by different methods, they have systemic residual action, require low application rates (Maienfisch et al. 2001), and are highly effective on many pests (Bi et al. 2002). Insecticide residual activity refers to the biological activity of the insecticide over a period of time after a single treatment application. It is governed by factors related to the nature of the product used, the sensitivity of the target, and the application conditions.

In the northeastern U.S., applications of thiamethoxam or imidacloprid are recommended at planting for control of Colorado potato beetle. A single application per season is desired because this can 1) lower the costs of control and 2) reduce the potential for insecticide resistance. However, the residual activity of these two chemicals when applied at planting depends on factors such as: soil characteristics, beetle resistance, application method and rate used. Understanding the effect of these factors on imidacloprid and thiamethoxam performance (in terms of residual activity and efficacy), and how they interact is crucial for optimal pest control.

Of the two insecticides, more research has been done on the use and fate of application of imidacloprid than thiamethoxam. The biological activity of imidacloprid varies depending on the soil´s agronomic conditions and the physical and chemical characteristics of the soil. Most field dissipation studies on crops show half-lives of imidacloprid of around 80 to 110 d (Krohn and

Hellpointner 2002) and up to more than 1 yr (Cox 2001). Longer half lives are reported in laboratory studies (Krohn and Hellpointner 2002). Vegetation is a major variable responsible for determining the product's half-life; longer persistence is observed on bare soils than when vegetation is present (Scholz and Spiteller 1992). No leaching of imidacloprid has been reported in terrestrial dissipation studies (Rouchaud et al. 1994, Rouchaud et al. 1996, Krohn and Hellpointner 2002, Leib and Jarret 2003) and the product has not been found to accumulate in the soil (Krohn and Hellpointner 2002). The persistence of imidacloprid in water solutions is affected by pH and formulation type (Sarkar et al.1999). The organic content of the soil, which may enhance compound binding, reduces the insecticide's activity (Krohn and Hellpointner 2002).

Thiamethoxam degrades at moderate to low rates in laboratory soils, with a half life of 34 to 75 d under favorable cropping conditions and longer under unfavorable cropping conditions (Maienfisch et al. 2001). Anon. (2003d) reported half life in soils in the laboratory of 101 d.

Imidacloprid and thiamethoxam for Colorado potato beetle control can be applied as a seed treatment or by application in furrow (Bird et al. 2005). For both application methods the insecticide is in proximity to the plant, but different insecticide placement with respect to the developing roots may result in insecticide uptake variation. The active ingredient placement and the effect of the insecticide formulation components may alter the efficacy of these insecticides.

Seed treatment is reported to promote more insecticide uptake by plant tissue because the insecticide is said to be applied in intimate proximity to the developing plant (Rouchaud et al. 1994). The advantages of this method in potatoes are however unknown and the effect of application method, on control of Colorado potato beetle, is still unclear. It is important to compare the effect of seed treatment methods with the more convenient in-furrow application methods. It is also important to know the effect that organic matter may have on insecticide performance in mineral vs. organic soils.

Imidacloprid and thiamethoxam currently provide acceptable control levels for Colorado potato beetle; however, resistance to both insecticides has been reported in the eastern United States. Resistance to imidacloprid in field populations of Colorado potato beetle has been reported in the United States in: Long Island, NY (Olson et al 2000, Zhao et al 2000), Massachusetts and Delaware (Bishop et al. 2003). In Michigan, a strain of Colorado potato beetle, selected in the laboratory is more than 100-fold resistant to imidacloprid indicating a high potential for resistance to develop in that state (Bishop et al. 2003). Similarly, increased tolerance to thiamethoxam already occurs in Long Island, NY, Delaware (Bishop et al. 2003), and Massachusetts (Grafius et al. 2004) apparently due to cross resistance with imidacloprid. Cross resistance is expected because imidacloprid and thiamethoxam belong to the same chemical class. Although cross-resistance between the two insecticides is not 1:1, it is high enough to suggest that alternating between the two insecticides may not be an effective resistance management tactic (Nauen et al. 2003, Grafius et al. 2004).

As indicated above, many factors may play a roll in determining the availability of the insecticide for plant uptake from application time throughout the time protection is expected to occur. The combined effect of these factors could result in faster decrease of the insecticide concentration in the soil. Insecticide uptake by the plant could be reduced and consequently, protection from the pest. Therefore, when the neonicotinoids thiamethoxam and imidacloprid are applied at planting in the field for control of Colorado potato beetle, resistance, application method, soil site conditions, and insecticide rate can affect the efficacy and the residual activity of the compound either directly or through interactions between those factors.

Objectives

The objectives of this research were to:

1. Determine the residual activity of the neonicotinoids imidacloprid and thiamethoxam, applied in the field at two rates, for control of Colorado potato beetle adults.
2. Determine whether in-furrow application vs. seed treatment influences the efficacy and residual activity of these neonicotinoids.
3. Determine the response of two strains of Colorado potato beetle, differing in susceptibility to imidacloprid, to the combined effects of insecticide, application method and rate.

4. Identify any interactions and /or additive effects between the above.
5. Compare the responses in (3) at two sites that differing mainly in soil type (mineral vs. organic). The very high organic matter content of organic soils may hamper the efficacy and residual activity of these insecticides, as compared with activity in mineral soils.

MATERIALS AND METHODS

The efficacy and residual activity of neonicotinoid insecticides on Colorado potato beetle adults were assessed in 2002 and 2003 through no-choice feeding bioassays conducted in the laboratory, using potato foliage collected from field plots at two experimental sites.

Field planting

Field research was conducted at the Michigan State University (MSU) Montcalm Potato Research Farm, Montcalm Co, MI, and the MSU Muck Soils Research Farm, Clinton Co, MI. These two sites were chosen to compare insecticide performance in mineral soil (Montcalm Farm) vs. organic soils (Muck Soils Farm). Soil at the Montcalm Farm is a McBride Sandy Loam, with < 5% organic matter and pH of 6.9. Soil at the Muck Soils Farm is a Houghton Muck containing 75 % organic matter with a pH between 6.2 and 6.9.

Two imidacloprid insecticide formulations were used: Admire® 2F (Bayer CropScience), and Genesis® (Gustafson) (Table 1). Two thiamethoxam insecticide formulations were used: Platinum® 2SC (Syngenta Crop Protection) and Cruiser 5FS (Syngenta Crop Protection). Admire and Platinum were applied in furrow and Genesis and Cruiser were applied as seed treatments. For seed treatment, the insecticide was applied to the potato seed pieces just before planting, spraying them with a mist spray bottle at the proper treatment rate (Table 1 and 2). In-furrow applications were made immediately after planting, but before covering the seed pieces with soil, using a single nozzle, hand held boom (30 gpa, 35 psi).

The four insecticides were applied at a single rate in 2002 (Table 1). In 2003, two insecticide rates were applied (Table 2): The maximum labeled rate (Bird et al. 2005) and a lower rate. In 2002 plots were planted on May 14 at the Montcalm Farm and on May 21 at the Muck Soils Farm using cv. Snowden at both sites. In 2003, planting was done on May 15 at the Montcalm Farm and on May 23 on the Muck Soils Farm, using cv. Atlantic at both sites. Rainfall was supplemented with irrigation as needed to provide adequate soil moisture conditions. Plots were 8 m long by 3 rows wide, with 0.8 m between rows and 30 cm between plants, and were arranged in a randomized complete block design with four replications. The crops were grown using standard commercial practices for irrigation, fertilization, herbicide and fungicides treatments.

Bioassays

Three weeks after planting (about 1 wk after plant emergence), foliage collection and bioassays were initiated. Four leaves from the youngest, fully

Table 1. Field treatments with neonicotinoid insecticides, made to mineral (Montcalm Farm) and organic (Muck Soils Farm) soils for control of Colorado potato beetle in 2002

Product	Active ingredient	Application method	Active ingredient Rate
Admire® 2F	Imidacloprid	In-furrow	252.0 g/ha
Genesis®	Imidacloprid	Seed treatment	277.1 g/ha*
Platinum® 2SC	Thiamethoxam	In-furrow	153.4 g/ha
Cruiser® 5FC	Thiamethoxam	Seed treatment	173.0 g/ha**
Control	----	Untreated	----

*0.090 g a.i. /kg seed at 75g/ seed piece

**0.056 g a.i. /kg seed at 75g/ seed piece

Table 2. Field treatments with neonicotinoid insecticides, made to mineral (Montcalm Farm) and organic (Muck Soils Farm) soils for control of Colorado potato beetle in 2003

Product	Active ingredient	Application method	Active ingredient High Rate	Active ingredient Low Rate
Admire® 2F	Imidacloprid	In-furrow	336 g/ha	218.0 g/ha
Genesis®	Imidacloprid	Seed treatment	370 g/ha*	231.1 g/ha*
Platinum® 2SC	Thiamethoxam	In-furrow	134 g/ha	109.0 g/ha
Cruiser® 5FC	Thiamethoxam	Seed treatment	150 g/ha**	127.0 g/ha**
Control	----	Untreated		----

*0.090 g a.i. /kg seed at 75g/ seed piece

**0.056 g a.i. /kg seed at 75g/ seed piece expanded leaves were collected, each one from a randomly selected plant in the center row of each plot. Leaves were transported to the laboratory with ice packs and kept in the refrigerator overnight. Bioassays were started the next day. In 2002, the leaves from all four plots of a given treatment were mixed and five leaves per treatment were randomly selected to use in the bioassays. In 2003, the foliage from each plot was kept separately. Leaves were placed in polystyrene Petri dishes (150mm diam. X 15mm) with the petiole inserted in a vial containing water; five beetles were then placed in the dish. The dishes were randomly arranged and the bioassays conducted at 22 – 25 °C. Food was replaced with freshly collected foliage on day 3. Treatments were evaluated on day seven by examining each beetle and classifying it as walking, poisoned or dead.

"Walking" beetles showed normal, coordinated, forward walking; "poisoned" beetles were unable to walk forward one body length; and "dead" beetles were non-responsive when probed with forceps and often exhibited dark coloration, shrunken abdomens or signs of decomposition. A combined category of dead and poisoned beetles was counted as "affected beetles" and used for analysis purposes later on. During treatment evaluation, Petri dish labels were covered to avoid bias in assessment. The bioassays were run every 2 wk in 2002 (June 4 through July 30) and weekly in 2003 (June 11 through July 25), until insecticide effectiveness had declined and no significant mortality occurred in most of the treatments

Beetle strains

Susceptible and imidacloprid-tolerant strains of Colorado potato beetle were used in the feeding bioassays. The susceptible beetles were either field-collected or reared in the laboratory. For bioassays conducted in 2002, beetles were collected from the Michigan State University (MSU) Montcalm Research Farm, Montcalm County, MI. For the 2003 bioassays, susceptible beetles were reared in the laboratory from egg masses purchased from the Phillip Alampi Beneficial Insect Rearing Laboratory, New Jersey Department of Agriculture.

The imidacloprid-tolerant strain was used in 2003 only and it was originally collected near Jamesport, Long Island, NY in 1999. This strain had been reared for over 12 generations in the laboratory without selection. At the time of the experiments, these beetles had an LD50 of 0.340 µg/beetle, ca. 9.4 fold tolerant to imidacloprid compared with New Jersey beetles, and also ca. 2.9 fold more tolerant to thiamethoxam than susceptible beetles with an LD50 of 0.096 µg/beetle (Grafius et al. 2004). Adults of both strains were kept at 22 - 25°C in the laboratory and fed daily with untreated, greenhouse potato foliage for 5 d after emergence from pupation. Afterwards they were transferred to growth chambers at 11°C, fed on untreated foliage and used in bioassays within 1 – 2 wks.

Data analysis

The efficacy of the treatments was the response variable and was evaluated based on the proportion of affected (dead and poisoned) beetles.

In 2002, the experimental design was a completely randomized design with five replications per treatment. In 2003, the experimental design was a randomized 4complete block design. Since the data did not have a normal distribution and the response variable was categorical, a generalized linear model was used to compare factors. Proc Genmod in SAS™ (software for Windows™, Release 8.02 TS Level 02M0) was used. Then a two-way, type 3 analysis was run using the Poisson distribution. This analysis was run for each bioassay of the experiment. An analysis was also done with all dates combined to find the significance of the factors studied over the season. In these analyses the significance of main effects ('days after planting', 'active ingredient' and 'application method'), and the interactions between them were tested. Each individual bioassay had non treated controls and affected beetle numbers in these controls were very low or zero, so the conditions of bioassays were assumed to be the same throughout the season so an overall analysis can be conducted.

To analyze the treatment residual activity, three treatment groups were considered here: 1) treatments with percentage affected > 50% throughout the experiment, 2) treatments with percentages affected always < 50% and, 3) those treatments with initial percentages affected > 50% and final percentages < 50%. Comparisons of residual activity in the latest group, was done by using Probit Analyses (Proc Probit, in the same SAS ® software mentioned above). For this purpose data were corrected for the proportion of affected beetles found in the untreated controls using Abott´s formula (Abbott 1925). The time after planting during which percentage affected was > 50% (median lethal time, LT_{50}) was determined. Also the times for efficacy of 95% (LT_{95}) and 5% (LT_5) were calculated and used to plot probability curves using the software Origin ®. From the percentage of affected beetles obtained at each evaluation time, the curves of time vs. affected beetles were constructed. After Proc Probit was run, the general analysis was discussed including the first two groups.

In 2003, the experimental design was a randomized complete block design with four replications (=blocks). The same categorical data analysis conducted for 2002 data was conducted for 2003 and then the Genmod procedure was used. The model aimed to find the effect of the tested factors ('days after planting', 'active ingredient', 'rate', 'application method' and 'strain'), and the interactions among them.

The analysis was done without correcting for control mortality, because the Poisson distribution in the model does not accept fractional numbers. Special caution is given in the discussion to those cases where mortality of untreated beetles was > 15%. Probit analyses were conducted to explore the residual activity of the treatments as was done for year 2002 data.

For the graphic representation of the results I used the proportion of affected beetles corrected for the affected beetles in the untreated controls using Abbott's formula (Abbott 1925). The graphic representation of results for year 2003, were grouped by beetle strain and by rate. Comparisons of a "best case scenario " of full dose and susceptible strain, to the combination of cases of low dose and resistant strain, are made (Figure 4, as example).

RESULTS AND DISCUSSION

Mineral soils site, year 2002

Weekly results. On day 28 after planting, only one treatment (imidacloprid as a seed treatment) affected less than 90% of the beetles, and affected significantly fewer beetles (LSMeans $P < 0.05$) than the other treatments. In general, thiamethoxam treatments affected significantly more beetles than imidacloprid ones and the in-furrow treatments resulted in more affected beetles than the seed treatments (Figure 2a, Table 3 for significant effect of the factor 'Application method'). Imidacloprid applied in-furrow affected significantly more beetles than imidacloprid applied as a seed treatment; however, both thiamethoxam treatments resulted in the same percentage of affected beetles (See significant interaction 'Application method (seed treatment vs. in-furrow) * active ingredient' (imidacloprid vs. thiamethoxam) in Table 3).

On day 42 after planting, two weeks later, thiamethoxam treatments affected significantly more beetles than imidacloprid (Figure 2a, Table 4). A higher percentage of affected beetles by the in-furrow treatments compared with the seed treatments may have been of biological importance since the P value was very close to significance for this date ($P=0.0507$). No significant effect for the interaction 'active ingredient * application method' was observed on this date. Thiamethoxam in-furrow (Platinum) continued to show high efficacy (ca. 90%

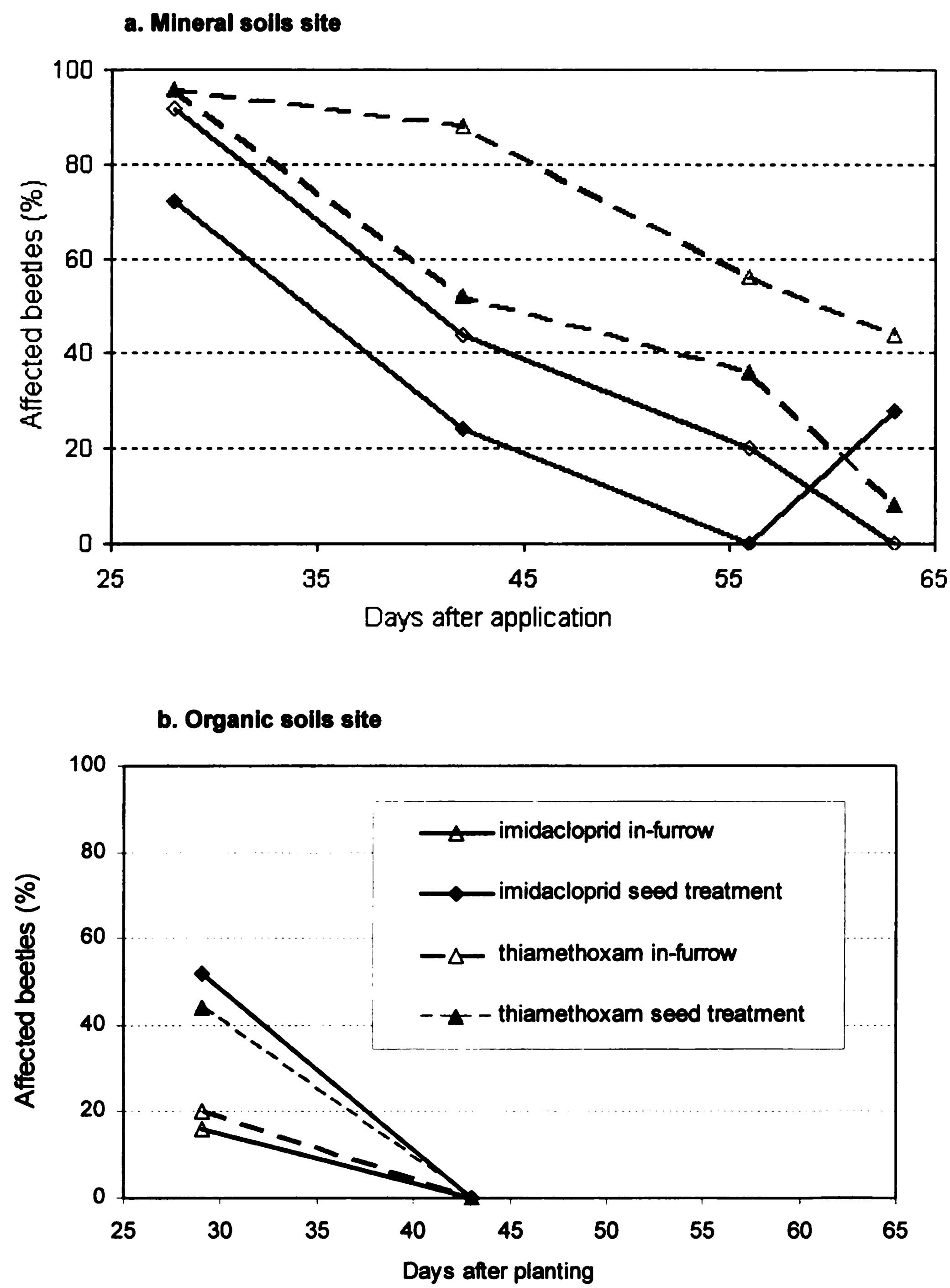


Figure 2. Percent of Colorado potato beetles affected by neonicotinoid insecticides-treated foliage from two experimental sites in 2002

Table 3. Main effects and interactions analyses on day 28 after planting for Colorado potato beetles affected in bioassays by foliage from plants treated with neonicotinoids at the mineral soils site in 2002 (linear regression statistics for Type 3 Analysis)

Factor	Numerator Degrees of freedom	Denominator Degrees of freedom	F Value	P Value	Chi-Square	P Value
active ingredient	1	16	10.36	0.0054	10.36	**0.0013**
application method	1	16	5.70	0.0297	5.70	**0.0170**
active ingredient * application method	1	16	5.70	0.0297	5.70	**0.0170**

Table 4. Main effects and interactions on day 42 after planting for Colorado potato beetles affected in bioassays by foliage from plants treated with neonicotinoids at the mineral soils site in 2002 (linear regression statistics for Type 3 Analysis)

Factor	Numerator Degrees of freedom	Denominator Degrees of freedom	F Value	P Value	Chi-Square	P Value
active ingredient	1	16	6.57	0.0208	6.57	**0.0104**
application method	1	16	3.82	0.0684	3.82	0.0507
application method * active ingredient	1	16	0.02	0.8990	0.02	0.8974

affected beetles). This was significantly higher than percentage affected beetles in the other treatments, which declined from the previous date and were around 20 - 50% (Figure.2a)

On day 56 after planting, again 'Active ingredient' and 'application method' significantly affected efficacy (Table 5). Thiamethoxam was significantly more effective than thiamethoxam and the in-furrow treatments resulted in significantly more affected beetles than the seed treatments. The interaction between these two factors was significant (Table 5) showing a differential effect of application method on active ingredient efficacy: imidacloprid as a seed treatment resulted in no affected beetles on this date while thiamethoxam applied in furrow affected 90% of the beetles (Figure 2a) significantly more than were affected by the two imidacloprid formulations.

On day 63, active ingredient and application methods factor were not significant (Table 6). All treatments affected less than 50% of beetles. Thiamethoxam in furrow affected significantly more beetles than thiamethoxam as a seed treatment ($X^2_{(1)} = 5.48$, $P = 0.02$). Imidacloprid as a seed treatment affected more beetles than during the previous week for the same treatment, but efficacy was $< 30\%$. For the rest of the treatments a general decline in affected beetles was observed (Figure 2a).

Treatment residual activity. In addition to the weekly analyses, treatment comparisons overall in the season showed the following: thiamethoxam applied in furrow exhibited the longest residual activity (Figure 3). Under the Montcalm Research Farm conditions, the median lethal time (LT_{50})

Table 5. Main effects and interactions on day 56 after planting for Colorado potato beetles affected in bioassays by foliage from plants treated with neonicotinoids at the mineral soils site in 2002 (linear regression statistics for Type 3 Analysis)

Factor	Numerator Degrees of freedom	Denominator Degrees of freedom	F Value	P Value	Chi-Square	P Value
active ingredient	1	16	25.73	0.0001	25.73	**<.0001**
application method	1	16	11.87	0.0033	11.87	**0.0006**
application method * active ingredient	1	16	6.21	0.0240	6.21	**0.0127**

Table 6. Main effects and interactions on day 63 after planting for Colorado potato beetles affected in bioassays by foliage from plants treated with neonicotinoids at the mineral soils site in 2002 (linear regression statistics for Type 3 Analysis)

Factor	Numerator Degrees of freedom	Denominator Degrees of freedom	F Value	P Value	Chi-Square	P Value
active ingredient	1	16	3.47	0.0809	3.47	0.0625
application method	1	16	1.68	0.2130	1.68	0.1946
application method * active ingredient	1	16	17.69	0.0007	17.69	***<.0001***

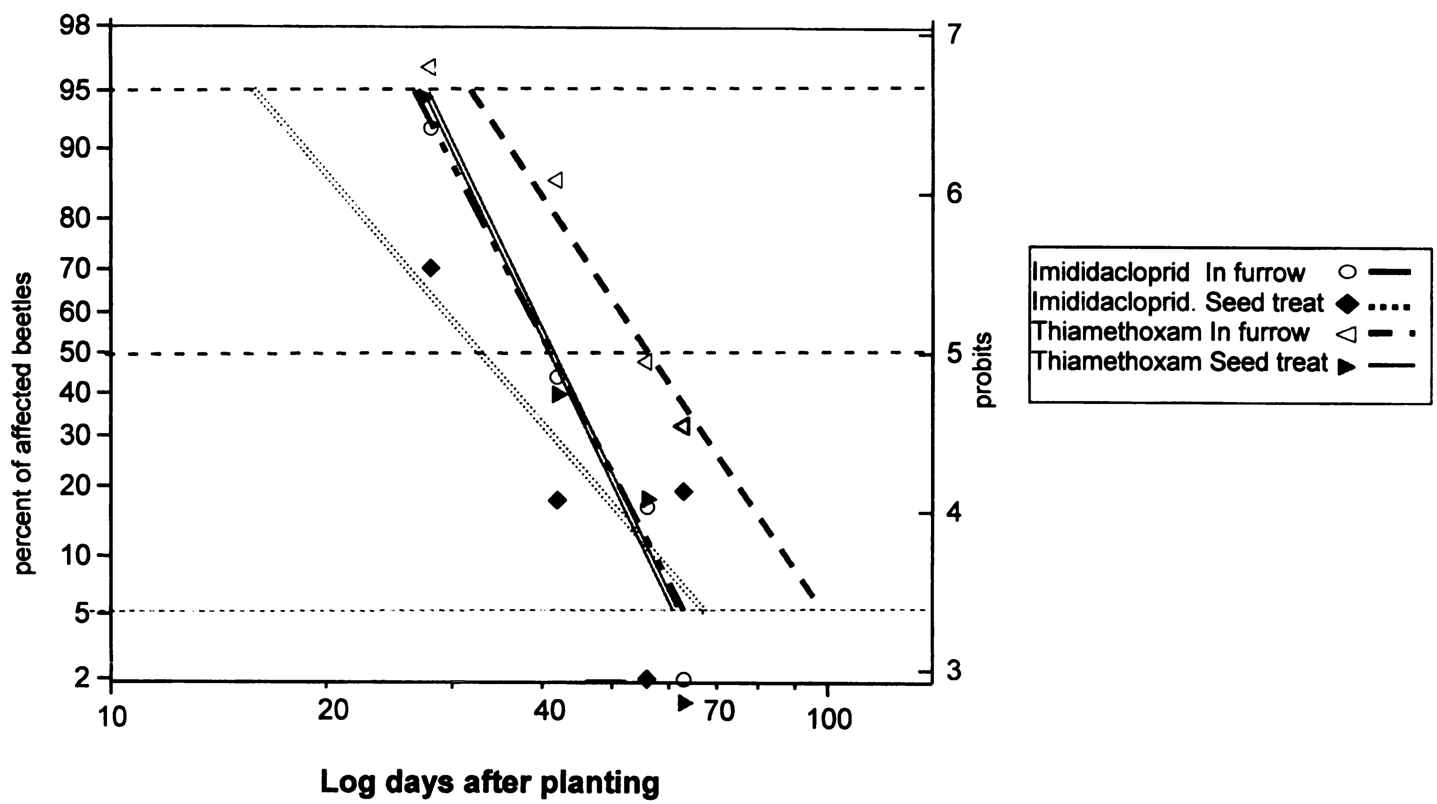


Figure 3. Log *days after planting* probit lines for affected Colorado potato beetles when fed with neonicotinoid-treated foliage from the mineral soil site in 2002

for this treatment was the longest of the four (Table 8), and significantly longer than for thiamethoxam applied as seed treatment or imidacloprid applied in furrow (Table 7). The percentage of affected beetles in of this treatment was also the highest for most individual dates and > 40% affected beetles on the last date of evaluation. The treatment with the lowest residual activity was imidacloprid applied as seed treatment. In addition, the projected probability curve in Figure 3 places this product far behind the residual activity of the other three. A final increment in the efficacy for this treatment (Figure 2a) raises questions about what could caused the plant to resume uptake. The higher efficacy of in-furrow applied treatments could be explained by the fact that more insecticide was readily available for uptake by potato roots.

With this application method, the insecticide is more evenly located along the furrow where many roots can come in contact with the product. For in-furrow imidacloprid residual activity experiments Boiteau et al. (1997) found protection to the crop from adults and larvae attack for about 50 - 54 days at similar rates, in contrast with the finding here that protection by imidacloprid against adults may not be that long.

Overall season results at the mineral soils site in 2002. Thiamethoxam products affected more beetles than imidacloprid products (Table 8), although 56 d after planting both active ingredients performed equally well. Imidacloprid seemed to have less residual activity than thiamethoxam as shown by the lower proportion of affected beetles throughout the season, and by the significant interaction 'active ingredient * time after application' ('days after

Table 7. Probit analyses for the evaluation of neonicotinoid insecticides in the control of Colorado potato beetle for the mineral soils site 2002

Treatment (active ingredient., application method)	**LT_{50} (days)**	**LT_{50} grouping**	**Fiducial limit 0.95**	**Slope ± SE**	**Pearson X^2/df=2 (P value)**
Imidacloprid in-furrow	40.60	b	36.76 - 44.10	-8.95 ± 1.42	2.19 (0.33)
Imidacloprid seed treatment	32.48	N/A	*	-5.24 ±2.59	11.38 (0.003)
Thiamethoxam in-furrow	55.44	a	50.28 - 63.15	-6.82 ±1.44	1.35 (0.51)
Thiamethoxam seed-treatment	40.90	b	37.10 - 44.32	-9.44 ±1.50	3.92 (0.14)

N/A, not available
* no fiducial limit available due to high variability

LT_{50} values followed by the same letter are not statistically significant due to overlapping fiducial limits

Table 8. Main effects and interactions analyses for Colorado potato beetles affected in bioassays by foliage from plants treated with neonicotinoids at the mineral soils site in 2002 (linear regression statistics for Type 3 Analysis)

Factor	Numerator Degrees of freedom	Denominator Degrees of freedom	F Value	P Value	Chi-Square	P Value
Days after planting	3	64	41.94	***<..0001***	125.82	<.0001
active ingredient	1	64	28.78	***<..0001***	28.78	<.0001
days after planting * active ingredient	3	64	6.97	***0.0004***	20.92	0.0001
application method	1	64	0.29	0.5908	0.29	0.5889
days after planting * application method	3	64	4.70	***0.0050***	14.11	0.0028
active ingredient * application method	1	64	0.15	0.6976	0.15	0.6963
Days after planting * active ingredient *application method	3	64	10.67	***<.0001***	32.01	<.0001

planting', Table 3). This interaction showed an earlier reduction in efficacy (lower residual activity) for imidacloprid over time with the initial high levels of affected beetles (ca. 90%) declining rapidly for imidacloprid.

The difference in percentage of affected beetles for the two application methods were evident after day 30 for thiamethoxam, while for imidacloprid that difference was evident from day 30. This suggested that the lower initial efficacy shown by imidacloprid as a seed treatment could be due to the application method interacting with active ingredient. The two application methods also affected residual activity as shown by the significant interaction 'application method * days after planting' (Table 3); the seed treatments resulted in shorter residual activity than did the in-furrow treatments. This effect could be explained by the allocation of insecticide in a larger area on the ground (in-furrow treatments) when the insecticide is applied along the furrow vs. the localized. seed treatment. As the plant grows and roots expand, more treated area is found and more insecticide uptake may occur. The best treatment, for a longer median lethal time was thiamethoxam applied in furrow (Table 7), with almost 15 d more effect for affected beetles.

Organic soils site, year 2002

For the organic soils site, on day 28 after planting, imidacloprid and thiamethoxam affected only 40%-50% of the beetles and no-significant differences between the insecticides were found (Table 9). Seed treatments affected significantly more beetles than in-furrow treatments (Figure 2b,

Table 9. Main effects and interactions on day 28 after planting for Colorado potato beetles affected in bioassays by foliage from plants treated with neonicotinoids in the organic soil site in 2002 (linear regression statistics for Type 3 Analysis)

Factor	Numerator Degrees of freedom	Denominator Degrees of freedom	F Value	P Value	Chi-Square	P Value
active ingredient	1	16	0.01	0.9356	0.01	0.9345
application method	1	16	10.33	***0.0054***	10.33	0.0013
active ingredient* application method	1	16	0.36	0.5566	0.36	0.5482

Table 9). By day 43, all the treatments had no affected beetles.

Because all percentage affected beetles were < 50%. No median lethal time analyses were conducted. There was a markedly low residual activity of insecticides under organic soil conditions with zero efficacies by day 43 after planting. Even on day 28 < 50% of the beetles were affected. This effect could be associated with the very high content of organic matter in the soil (75 -80%), supporting previous research showing a shorter half life of these products when organic matter is present (Krohn and Hellpointner 2002).

The effect of the organic soil in depressing insecticide efficacy was apparent both in the initial number of affected beetles and in the residual activity of the product. The negative effect on insecticide performance by the high organic matter could be reduced by the application as seed treatments because the insecticide is more in contact with the tuber than with the soil phase compared with the in-furrow treatment. This is shown by the higher efficacies of the seed applied products over the in-furrow treatments. This also corroborates the advantages of seed treatments in organic soils reported by other authors (Rouchaud et al. 1994).

Mineral soils site, Year 2003

Weekly results. On day 26 after planting, percentage affected beetles in the susceptible strain (80 - 100%) was generally higher than in the resistant strain (40 -90%) (Figure 4, Table 10). Seed treatments were generally more effective (Table 10, for significance of 'Application method') than in-furrow applications. There was a significant difference in percentage of affected

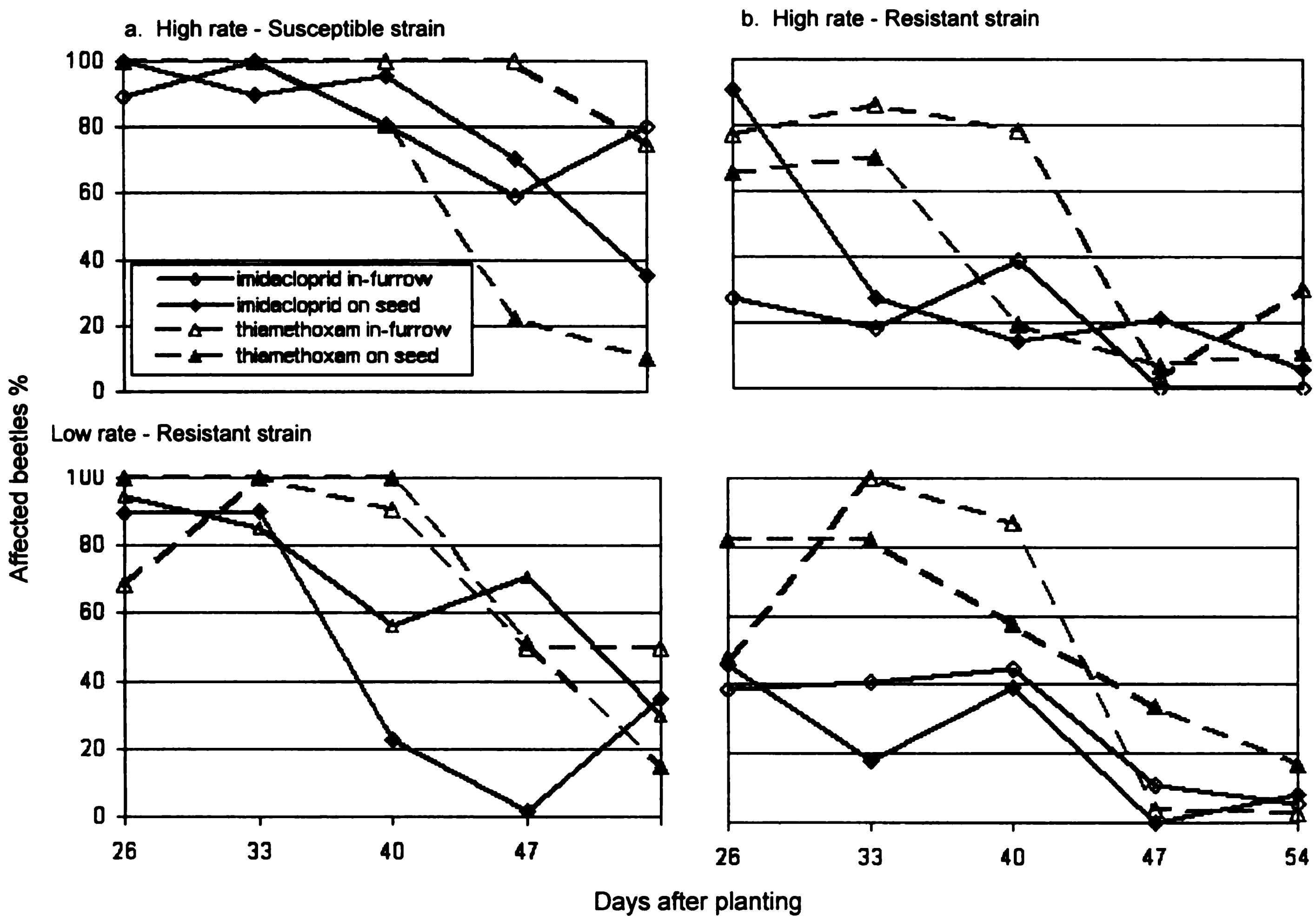


Figure 4. Percent of Colorado potato beetles affected by neonicotinoid insecticide-treated foliage from the mineral soils experimental site in 2003

Table 10. Main effects and interactions analyses for Colorado potato beetles affected in bioassays by foliage from plants treated with neonicotinoids in the mineral soil site on day 26 after planting in 2003 (LR Statistics for Type 3 Analysis)

Factor	Numerator Degrees of freedom	Denominator Degrees of freedom	F Value	P Value	Chi-Square	P Value
block	3	45	0.46	0.7139	1.37	0.7126
active ingredient	1	45	2.72	0.1059	2.72	0.0989
application method	1	45	6.94	***0.0115***	6.94	0.0084
rate	1	45	1.78	0.1887	1.78	0.1820
beetle strain	1	45	29.06	***<.0001***	29.06	<.0001
active ingredient* application method	1	45	0.74	0.3955	0.74	0.3910
active ingredient * rate	1	45	0.02	0.8895	0.02	0.8889
application method * rate	1	45	<0.01	0.9920	0.00	0.9920
active ingredient * beetle strain	1	45	3.43	0.0706	3.43	0.0641
application method * beetle strain	1	45	2.88	0.0968	2.88	0.0899
rate * beetle strain	1	45	0.16	0.6873	0.16	0.6854
active ingredient * application method * rate	1	45	7.44	***0.0090***	7.44	0.0064
active ingredient * application method * beetle strain	1	45	2.40	0.1281	2.40	0.1211
active ingredient * rate* beetle strain	1	45	0.30	0.5851	0.30	0.5824
application method * rate * beetle strain	1	45	0.18	0.6695	0.18	0.6675
active ingredient * application method * rate * beetle strain	1	45	2.37	0.1305	2.37	0.1235

beetles between the two thiamethoxam treatments for the susceptible strain at the low rate (Figure 4–d). This difference could be associated to potato plant development: at the time of evaluation, the plants were very small and the insecticide in seed treatments may have been more readily available for uptake. Neither 'rate' nor the 'active ingredient' alone was a significant factor. Both active ingredients showed high efficacy on this date for the susceptible strain (Figure 4 a,c), but efficacy for the resistant strain was variable (Figure 4 b,c). This interaction 'active ingredient * beetle strain' was close to the significance level of P = 0.05 (P = 0.064, Table 10). Also on this date, a significant effect of the three-factor interaction: 'active ingredient by application method by rate' was observed. The percentage of affected beetles in the untreated control for the susceptible beetle strain was 21% on this date and the reason of this high mortality in the control is unknown. However, the percentage of beetles affected by the treatments in this strain was very high (most cases more than 90%, only one case with 70%). A significant difference between the two application methods of imidacloprid at the high rate on the resistant beetle strain (X^2=11.39, P value <0.01) (Figure 4 c) was observed, the seed treatment affected 90 % of the beetles compared with <30% affected in the in-furrow imidacloprid treatments. On day 33 after planting, both active ingredients at either application method had high levels of efficacy when used at the high rate on the susceptible beetle strain. Under other conditions (low rate, resistant strain or both) thiamethoxam was more efficient than imidacloprid (Figure 4) as indicated by the significance of the factor 'active ingredient' (Table 11). The two strains responded differently

Table 11. Main effects and interactions analyses for Colorado potato beetles affected in bioassays by foliage from plants treated with neonicotinoids at the mineral soils site on 33 days after planting in 2003 (linear regression statistics for Type 3 Analysis

Factor	Numerator Degrees of freedom	Denominator Degrees of freedom	F Value	P Value	Chi-Square	P Value
block	1	45	0.66	0.5788	1.99	0.5743
active ingredient	1	45	22.21	***<.0001***	22.21	<.0001
application method	1	45	0.53	0.4699	0.53	0.4661
rate	1	45	0.23	0.6350	0.23	0.6327
beetle strain	1	45	29.56	***<.0001***	29.56	<.0001
active ingredient * application method	1	45	0.00	0.9976	0.00	0.9976
active ingredient * rate	1	45	0.00	0.9465	0.00	0.9462
application method * rate	1	45	0.64	0.4272	0.64	0.4230
active ingredient * beetle strain	1	45	16.76	***0.0002***	16.76	<.0001
application method * beetle strain	1	45	0.43	0.5164	0.43	0.5131
rate * beetle strain	1	45	0.53	0.4725	0.53	0.4687
active ingredient * application method * rate	1	45	0.73	0.3976	0.73	0.3930
active ingredient * application method * beetle strain	1	45	0.01	0.9432	0.01	0.9428
active ingredient * rate * beetle strain	1	45	0.03	0.8588	0.03	0.8580
application method * rate * beetle strain	1	45	1.10	0.2994	1.10	0.2938
active ingredient * application method * rate * beetle strain	1	45	1.22	0.2759	1.22	0.2700

('beetle strain' significant, Table 11); there were high numbers of affected beetles (85% or more, Figure 4 a,c) in the susceptible straincompared with a variable response (20 and 100%) in the resistant strain (Figure 4 b,d). The difference between the two active ingredients in the susceptible strain (Figure 4 a,c) was not significant (P = 0.5998), but it was significant (P < .0001) in the resistant strain; thiamethoxam treatments were more efficient than imidacloprid treatments at both rates (Figure 4 a,c). This variable response is confirmed by the significant interaction 'beetle strain * active ingredient' (Table 11) and could be due to direct effect of the imidacloprid-resistant strain that reduced the efficacy of that insecticide.

The treatment 'in-furrow thiamethoxam' applied at lower rate affected more beetles on day 33 (LSMeans P = 0.0457) than the week before (Figure 4 c,d). This could be due to increase in insecticide uptake of the young growing plant (plants were 15 to 20 cm tall on this date), which could be enough to counteract an early lower insecticide availability due to the low rate.

On day 40 after planting, there were significant differences between thiamethoxam and imidacloprid ('active ingredient' significance, Table 12). At the high rate and with the susceptible strain, all insecticides showed high efficacy levels (80% or more). For other conditions (low rate and/or resistant strain) most thiamethoxam treatments showed higher efficacy than imidacloprid treatments (Figure 4). On this date also, a decline in number of affected beetles was observed for imidacloprid when used at the low rate for the susceptible beetle strain (Figure 4 c) and for all thiamethoxam treatments for the resistant strain.

Table 12. Main effects and interactions analyses for Colorado potato beetles affected in bioassays by foliage from plants treated with neonicotinoids at the mineral soils site on 40 days after planting in 2003 (linear regression statistics for Type 3 Analysis

Factor	Numerator Degrees of freedom	Denominator Degrees of freedom	F Value	P Value	Chi-Square	P Value
block	3	45	0.60	0.6207	1.79	0.6173
active ingredient	1	45	9.14	***0.0041***	9.14	0.0025
application method	1	45	8.52	0.0055	8.52	0.0035
rate	1	45	0.33	0.5674	0.33	0.5645
beetle strain	1	45	15.19	***0.0003***	15.19	<.0001
active ingredient * application method	1	45	0.05	0.8213	0.05	0.8203
active ingredient * rate	1	45	2.17	0.1473	2.17	0.1403
application method * rate	1	45	0.99	0.3240	0.99	0.3186
active ingredient * beetle strain	1	45	0.01	0.9197	0.01	0.9193
application method * beetle strain	1	45	3.17	0.0817	3.17	0.0750
rate * beetle strain	1	45	9.53	***0.0035***	9.53	0.0020
active ingredient * application method * rate	1	45	1.03	0.3151	1.03	0.3096
active ingredient * application method * beetle strain	1	45	0.99	0.3259	0.99	0.3206
active ingredient * rate * beetle strain	1	45	1.64	0.2067	1.64	0.2002
application method * rate * beetle strain	1	45	3.87	0.0554	3.87	0.0492
active ingredient * application method * rate * beetle strain	1	45	0.70	0.4088	0.70	0.4044

The factor ‘application method’ was significant (Table 12): the seed treatments probably did not affect as many beetles as the in-furrow treatments. The factor ‘beetle strain’ was also significant (Table 12). The interaction ‘rate * beetle strain’ was also significant (Table 12); at the high rates, more beetles were affected in the susceptible strain than in the resistant strain; but at the low rate, the range in response was similar for both beetle strains.

On day 47 after planting, thiamethoxam applied in-furrow still had 100% affected beetles at the high rate in the susceptible strain. Most of the other thiamethoxam treatments had a steep decline in the percentage of affected beetles, especially with low rate and resistant strain. Most imidacloprid treatments resulted in a lower percentage of affected beetles on this date than on the previous evaluation date. The decline in thiamethoxam efficacy led to the two active ingredients to perform equally overall on this date. The resistant beetle strain had lower percentage of affected beetles than the susceptible strain. Only the main factor, ‘beetle strain’ showed a significant effect (Table 13). In the resistant strain no difference was observed between application methods, while in the susceptible strain the in furrow applications produced more affected beetles than the seed treatments (interaction ‘application method * beetle strain’, Table 13). The interaction ‘rate * beetle strain’ was also significant (Table 13) meaning that while the high rates produced more affected beetles compared to the low rate in the susceptible strain; there was no difference in affected beetles between rates for the resistant strain, mainly because all treatment effects had lowered to near zero at this date for the resistant strain.

Table 13. Main effects and interactions analyses for Colorado potato beetles affected in bioassays by foliage from plants treated with neonicotinoids at the mineral soils site on 47 days after planting (linear regression statistics for Type 3 Analysis)

Factor	Numerator Degrees of freedom	Denominator Degrees of freedom	F Value	P Value	Chi-Square	P Value
block	3	45	0.09	0.9639	0.28	0.9643
active ingredient	1	45	0.85	0.3625	0.85	0.3576
application method	1	45	0.34	0.5646	0.34	0.5617
rate	1	45	0.00	0.9792	0.00	0.9791
beetle strain	1	45	7.46	***0.0090***	7.46	0.0063
active ingredient * application method	1	45	0.01	0.9049	0.01	0.9044
active ingredient * rate	1	45	2.14	0.1506	2.14	0.1436
application method * rate	1	45	1.08	0.3036	1.08	0.2980
active ingredient * beetle strain	1	45	0.13	0.7198	0.13	0.7181
application method * beetle strain	1	45	7.11	***0.0106***	7.11	0.0076
rate * beetle strain	1	45	5.51	***0.0233***	5.51	0.0189
active ingredient * application method * rate	1	45	9.98	***0.0028***	9.98	0.0016
active ingredient * application method * beetle strain	1	45	1.42	0.2399	1.42	0.2336
active ingredient * rate * beetle strain	1	45	0.47	0.4967	0.47	0.4932
application method * rate * beetle strain	1	45	0.17	0.6811	0.17	0.6791
active ingredient * application method * rate* beetle strain	1	45	0.08	0.7747	0.08	0.7733

On day 54 after planting, significant differences were observed only between the two beetle strains (Table 14). More affected beetles were observed in the susceptible strain (20 to 80% affected) compared to the resistant strain (<20% affected (Figure 4).

Season Results. At the rates used in this experiment from the mineral soils, generally more beetles were affected by thiamethoxam than imidacloprid. This tendency was more consistent for the resistant strain (Figure 4). This showed that the specific active ingredient significantly determined efficacy in mineral soils ($P < 0.0001$ in Table15). As expected, compared with susceptible beetles, imidacloprid-resistant beetles were generally less affected by imidacloprid (significance of 'beetle strain' $P < 0.0001$, Table 10). The two factors ('beetle strain' and 'active ingredient') interacted in the following fashion: while most of the season both active ingredients had similar efficacy for the susceptible strain, the efficacy of imidacloprid was significantly lower than efficacy of thiamethoxam in most cases for the resistant strain. The numbers of affected beetles declined during the season, showed by 'Days after planting' significantly influencing efficacy. These results suggest little cross resistance of the imidacloprid-resistant strain towards thiamethoxam (as indicated by resistance ratios of 9.4 fold for imidacloprid vs. 2.9 fold for thiamethoxam. However the day-by-day analysis showed significant differences at day 47 between the thiamethoxam treatments on susceptible and resistant strains (from 100% affected beetles to ca. 5%). The active ingredient overall efficacy varied

Table 14. Main effects and interactions analyses for Colorado potato beetles affected in bioassays by foliage from plants treated with neonicotinoids at the mineral soils site on 54 days after planting in 2003 (linear regression statistics for Type 3 Analysis)

Factor	Numerator Degrees of freedom	Denominator Degrees of freedom	F Value	P Value	Chi-Square	P Value
block	3	45	0.94	0.4276	2.83	0.4185
active ingredient	1	45	0.86	0.3581	0.86	0.3531
application method	1	45	0.01	0.9208	0.01	0.9203
rate	1	45	0.06	0.8051	0.06	0.8040
beetle strain	1	45	14.22	***0.0005***	14.22	0.0002
active ingredient *application method	1	45	2.37	0.1310	2.37	0.1240
active ingredient * rate	1	45	1.28	0.2638	1.28	0.2578
application method* rate	1	45	0.00	0.9905	0.00	0.9904
active ingredient * beetle strain	1	45	3.83	0.0566	3.83	0.0503
application method* beetle strain	1	45	5.76	***0.0206***	5.76	0.0164
rate * beetle strain	1	45	0.54	0.4643	0.54	0.4605
active ingredient *application method* rate	1	45	2.49	0.1213	2.49	0.1142
active ingredient *application method* beetle strain	1	45	0.02	0.8755	0.02	0.8748
active ingredient * rate * beetle strain	1	45	3.16	0.0824	3.16	0.0756
application method* rate * beetle strain	1	45	0.64	0.4274	0.64	0.4232
active ingredient *application method* rate * beetle strain	1	45	2.85	0.0981	2.85	0.0912

Table 15. Main effects and interactions analyses for Colorado potato beetles affected in bioassays by foliage from plants treated with neonicotinoids at the mineral soils site in 2003 (linear regression statistics for Type 3 Analysis)

Factor	Numerator Degrees of freedom	Denominator Degrees of freedom	F Value	P Value	Chi-Square	P Value
block	3	237	1.00	0.3928	3.00	0.3909
rate	1	237	0.18	0.6754	0.18	0.6750
days after planting	4	237	39.96	***<.0001***	159.82	<.0001
active ingredient	1	237	14.74	***0.0002***	14.74	0.0001
application method	1	237	0.01	0.9254	0.01	0.9253
beetle strain	1	237	53.34	***<.0001***	53.34	<.0001
days after planting*active ingredient	4	237	1.65	0.1633	6.59	0.1594
days after planting*application method	4	237	2.91	***0.0222***	11.65	0.0201
active ingredient*application method	1	237	2.67	0.1034	2.67	0.1021
days after planting*rate	4	237	0.40	0.8105	1.59	0.8107
active ingredient*rate	1	237	0.01	0.9168	0.01	0.9167
application method *rate	1	237	0.26	0.6074	0.26	0.6069
days after planting*beetle strain	4	237	2.15	0.0754	8.60	0.0719
active ingredient*beetle strain	1	237	8.24	***0.0045***	8.24	0.0041
application method *beetle strain	1	237	9.87	***0.0019***	9.87	0.0017
rate*beetle strain	1	237	9.23	***0.0026***	9.23	0.0024
active ingredient* application method *rate	1	237	18.55	***<.0001***	18.55	<.0001

Table 15 (cont'd)

Factor	Numerator Degrees of freedom	Denominator Degrees of freedom	F Value	P Value	Chi-Square	P Value
days after planting*active ingredient*beetle strain	4	237	2.91	***0.0223***	11.65	0.0202
days after planting*rate*beetle strain	4	237	2.66	***0.0333***	10.65	0.0308
days after planting*am*beetle strain	4	237	5.47	***0.0003***	21.87	0.0002
active ingredient*am*beetle strain	1	237	1.89	0.1708	1.89	0.1695
days after planting*active ingredient*application method	4	237	0.78	0.5368	3.13	0.5355
days after planting*active ingredient*rate	4	237	1.59	0.1766	6.38	0.1728
days after planting*a application method * rate	4	237	0.76	0.5550	3.02	0.5539
days after planting*active ingredient* application method *beetle strain	4	237	0.39	0.8163	1.56	0.8165
active ingredient*rate*beetle strain	1	237	5.13	***0.0245***	5.13	0.0236
application method *rate*beetle strain	1	237	0.19	0.6599	0.19	0.6595
days after planting*active ingredient* application method *rate	4	237	2.45	***0.0472***	9.78	0.0442
days after planting*active ingredient* rate * beetle strain	4	237	1.40	0.2348	5.60	0.2313
days after planting* application method * rate*beetle strain	4	237	1.51	0.2009	6.03	0.1972
active ingredient* application method * rate*beetle strain	1	237	2.57	0.1104	2.57	0.1090
days after planting*active ingredient* application method *rate*beetle strain	4	237	1.49	0.2071	5.94	0.2034

depending on the beetle strain and in the case of imidacloprid applied in-furrow some efficacy may be lost due to the resistance strain.

No significant interactions between 'active ingredient * days after planting' were observed and both active ingredients exhibited the same declining pattern in efficacy throughout the experiment (Figure 4). A significant interaction 'rate * beetle strain' was also observed and this interaction may have been expressed especially beyond 33 days after planting: the high rates resulted in more affected susceptible than resistant beetles, while there was no difference between beetle strains at the lower rates. Since the level of resistance is directly related to the amount of insecticide the insect encounters, this interaction may be a direct response of the different resistant level of the beetle strains used. These first-order (two factors) interactions varied through the duration of the experiment, resulting in second-order interactions with 'days after planting'. Other interactions of second and third order were also observed (Table 15).

Treatment residual activity. Three treatment combinations ('imidacloprid - in-furrow - high rate - susceptible strain', 'thiamethoxam – in-furrow – high rate – susceptible strain' and 'thiamethoxam - in furrow – low rate – susceptible strain') affected more than 50% of the beetles throughout the season (Table 16). These treatments are assumed to be at the top of treatment performances when comparing the treatment residual activities, since their efficacy was higher than the rest of the treatments throughout the season. However, because percentage affected was always >50%, they were not included in the median lethal time analysis. Three other treatments ('imidacloprid–in furrow–high rate–resistant

Table 16. Probit analysis for the evaluation of neonicotinoid insecticides, at two rates and for two beetle strains in the control of Colorado potato beetle, Montcalm 2003

Treatment (active ingredient, application method, rate, beetle strain)	**LT_{50} (days)**	**LT_{50} grouping**	**Fiducial limit 0.95**	**Slope ± St Err**	**Pearson X^2/df=2 (P value)**
Imidacloprid, in furrow, high rate, susceptible	***				
Imidacloprid, seed, high, susceptible	51.95	a	47.78 - 60.68	-9.17 ± 2.05	5.88 (0.12)
Thiamethoxam, in furrow, high, susceptible	***				
Thiamethoxam, seed, high, susceptible	44.01	bc	41.97 - 46.07	-19.14 ±3.36	3.05 (0.22)
Imidacloprid, in furrow, low, susceptible	46.48	abc	42.12 - 54.52	-6.16 ±1.35	4.13 (0.25)
Imidacloprid, seed, low, susceptible	38.00	?	*	-7.94 ±4.02	27.93 (<.01)
Thiamethoxam, in furrow, low, susceptible	***				
Thiamethoxam, seed, low, susceptible	47.51	ab	45.52 - 49.51	-7.25 ±1.30	3.22 (0.36)

Table 16 (cont'd)

Treatment (active ingredient, application method, rate, beetle strain)	LT_{50} (days)	LT_{50} grouping	Fiducial limit 0.95	Slope ± St Err	Pearson X^2/df=2 (P value)
Imidacloprid, in furrow, high, resistant	**				
Imidacloprid, seed, high, resistant	31.33	d	21.41 - 37.15	-11.43 ±2.78	6.34 (0.10)
Thiamethoxam, in furrow, high, resistant	45.54	N/A	*	-3.62 ±2.04	9.70 (0.02)
Thiamethoxam, seed, high, resistant	31.96	N/A	*	-4.79 ±2.28	12.44 (<0.01)
Imidacloprid, in furrow, low, resistant	**				
Imidacloprid, seed, low, resistant	**				
Thiamethoxam, in furrow, low, resistant	35.83	N/A	*	-7.93 ±2.66	14.44 (<0.01)
Thiamethoxam, seed, low, resistant	39.19	cd	35.98 - 42.92	-7.25 ±1.30	5.00 (0.17)

* No fiducial limits available due to high variability
** Data below 50% mortality all season long
***Data above 50% mortality all season long
N/A not available

Values of LT_{50} followed by the same letter are not significantly different, due to-overlapping confidence intervals strain', 'imidacloprid-in furrow–low rate–resistant strain' and 'imidacloprid–seed treatment–low rate–resistant strain') affected less than 50% of the beetles throughout the season. These treatments were assumed to be the poorest treatments (in terms of treatment performance) for the season because they affected beetles in a lower percentage than the rest of the treatments throughout the season. Again, because percentage affected was always < 50%, they were not included in the median lethal time analysis.

Treatments applied to the seed usually had a steep decline in percentage affected beetles, especially notable for the thiamethoxam treatments even though they had an initial high percentage of affected beetles (Figure 5). Treatments with the susceptible beetle strain ranked as 'top treatments' or resulted in significantly higher (LT_{50}) median residual activity than treatments using the resistant beetle strain. Even though the significance of factor 'rate' was variable, the high rate had one treatment combination with imidacloprid as one of the most effective treatments. There was no effect on residual activity of the lower rate and there was no clear effect for the application method.

The LT_{50} levels for imidacloprid products are within the reported ranges for half life of the product (Figure 5, Table 15). Our results gave LT_{50} values of about 30 to 50 d and the dissipation studies show a range from 7 to 150d (Krohn and Hellpointner 2002). While some of the factors discussed above my influence the insecticide presence in the soil, the biological activity investigated is also dependant of the insecticide uptake by the plants.

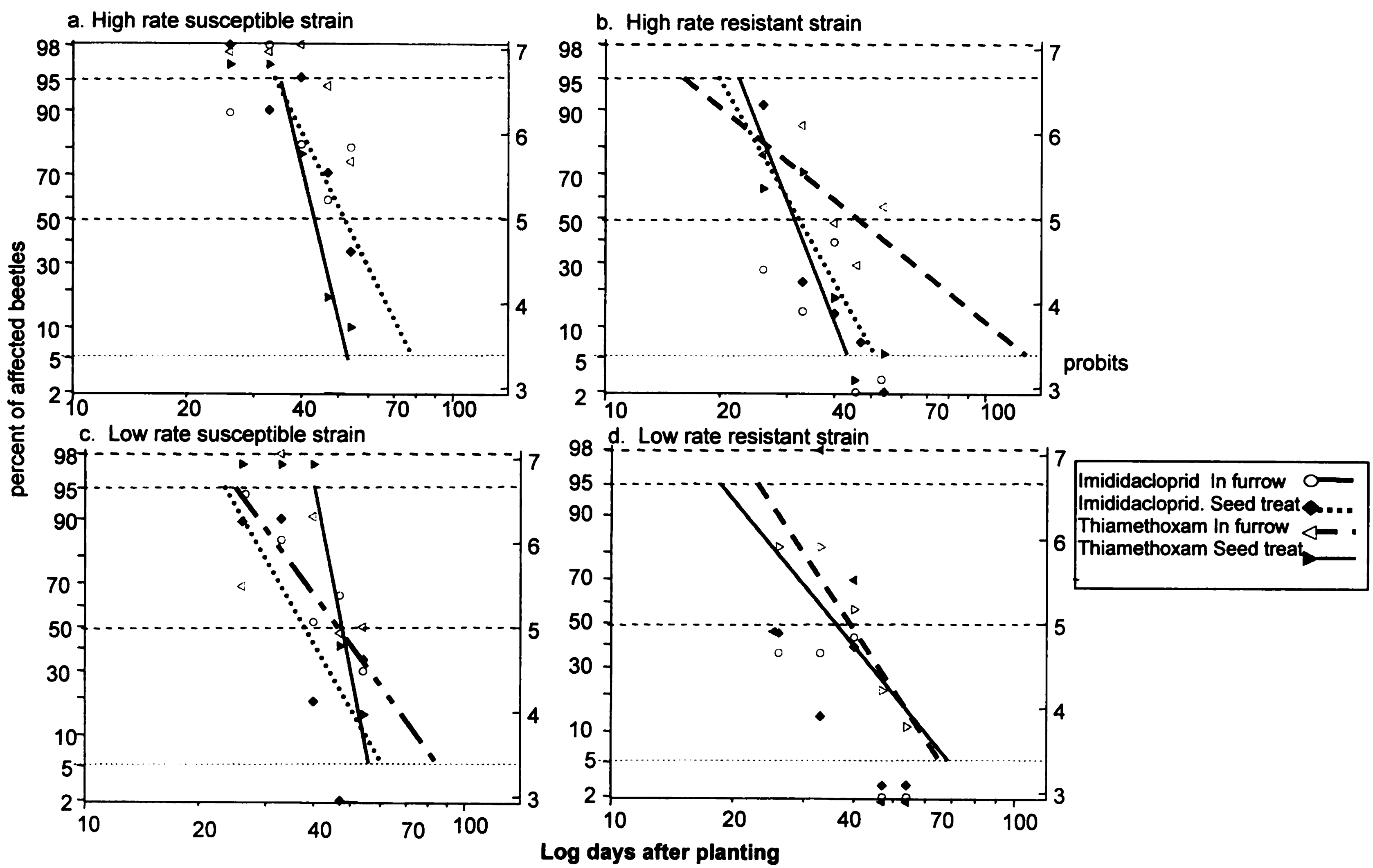


Figure 5. Log *days after planting* probit lines for affected Colorado potato beetles when fed with neonicotinoid-treated foliage from the mineral soil site in 2003. Treatment combinations are not included if percentage of affected beetles is always >50% or <50% throughout the season

Overall season results at the mineral soils site in 2003. At about day 40 after planting, the imidacloprid products declined in the 'high rate – susceptible strain' and were not as effective as thiamethoxam (Figure 4). At about the same date, thiamethoxam, when applied either at a low rate or to the resistant strain, declined to an efficacy level similar to imidacloprid.

In the mineral soils of this site, there were no differences in performance by the two active ingredients at a high rate and for the susceptible strain. When other factors are included (resistant strain and lower rate), there was higher efficacy for thiamethoxam than imidacloprid at about 40 days after planting and after day 47 both active ingredients had similar effects. No overall significance between rates used in this experiment was found for the mineral soils site. These results agree with results shown by Boiteau et al. (1997) who tested different rates of Admire ® (imidacloprid, in-furrow) for control of Colorado potato beetle with no major significance between 222 and 333 g A.I./ha. Rates lower than that may reduce the efficacy of the application, according to their results.

The decline in the residual activity for imidacloprid in furrow found in this study, along with the effect of resistant strains on such decline, confirmed previous findings about the residual activity of imidacloprid. Bishop et al. (2001) found a decline in affected beetles from 49 to 70 days after planting from 78% to 70% of affected beetles. The response in intermediate and resistant beetle strains was not higher than 40% in efficacy with a declining pattern. Boiteau et al. (1997) reported crop protection (from adults and larvae of the Colorado potato beetle) with in-furrow applied imidacloprid of 36-46 d and 71 d in two different seasons for susceptible field populations. Although results in this study showed efficacy of both neonicotinoids against adults until around day 40 after application, projecting these results to the field the treatments under mineral soils could show longer residual activity for field protection than the residual activity observed for affected adults because the crop is also attacked by the more insecticide-sensitive larvae.

The low rate did not have a major effect on the overall efficacy and residual activity of the insecticides, thus the lower rates could provide appropriate protection to the crop. However, some impact of the lower rate may have occurred in: 1) reducing the residual activity of imidacloprid when applied at the low rate; 2) hindering the efficacy of imidacloprid as a seed treatment on resistant beetles on day 26 after planting (Figure 4b) and, 3) reducing the residual activity of in-furrow thiamethoxam on susceptible beetles (Figure 4 a,c).

The apparent greater sensitivity of imidacloprid to the adverse effects of lower rate and insecticide resistance complies with the nature of the resistance of the strain I used (imidacloprid resistant) and with the fraction of the rate used for each compound (imidacloprid's low rate.= 0.64 high rate; thiamethoxam's low rate= 0.86 high rate). Then, reductions in efficacy, about day 40 after planting, or early drop in residual activity would be expected if low rates are used or imidacloprid resistance is present.

Seed treatments may have higher efficacy than in-furrow applications early in the season but that difference shifted 1 - 2 wks later or disappeared.

Low levels of resistance to imidacloprid on beetles (as the 9.4-fold for the strain treated here) severely hampered the performance of imidacloprid formulations and also could cause reduction of thiamethoxam products by day 40 – 47 after planting (Figure 4 b). The reduced efficacy due to the resistant strain was significant throughout the season for imidacloprid and important for thiamethoxam on day 40 after planting. The effect of some level of cross resistance made the efficacy of thiamethoxam decline by day 47 to levels lower than 20% affected beetles.

Despite of the random selection of plants in the field from the untreated check, I could have obtained selected foliage from these plots because over time the remaining foliage was only that not consumed by beetles. However the low mortality levels observed in the control made me assume that any change in that foliage was of no weight when comparing the effect of the insecticide treatments.

Organic soils site, year 2003

Weekly results. On day 26 after planting, the factor 'active ingredient' and its interaction with 'beetle strain' (Table 17) was significant. The susceptible beetles were significantly more affected (80 - 100% affected), than resistant beetles (2 – 80% affected, Figure 6) ('beetle strain' Table 17). Thiamethoxam treatments had significantly (LSMeans, $X^2_{(1)}$=13.53, P < 0.01) more affected beetles than imidacloprid treatments for the resistant strain, while both active ingredients showed equally high percentages (> 80% affected beetles) in the susceptible strain. Seed treatments resulted in higher percentage of affected beetles than the in-furrow applications (LSMeans $X^2_{(1)}$=6.44, P = 0.01).

Table 17. Main effects and interactions analyses for Colorado potato beetles affected in bioassays by foliage from plants treated with neonicotinoids at the organic soils site on day 26 after application in 2003 (linear regression statistics for Type 3 Analysis)

Factor	Numerator Degrees of freedom	Denominator Degrees of freedom	F Value	P Value	Chi-Square	P Value
block	3	45	0.53	0.6628	1.60	0.6604
active ingredient	1	45	17.73	***0.0001***	17.73	<.0001
application method	1	45	7.74	***0.0079***	7.74	0.0054
rate	1	45	1.75	0.1923	1.75	0.1856
beetle strain	1	45	60.07	***<.0001***	60.07	<.0001
active ingredient * application method	1	45	1.43	0.2379	1.43	0.2316
active ingredient * rate	1	45	0.19	0.6639	0.19	0.6618
application method * rate	1	45	0.52	0.4762	0.52	0.4724
active ingredient * beetle strain	1	45	20.45	***<.0001***	20.45	<.0001
application method * beetle strain	1	45	6.93	0.0116	6.93	0.0085
rate * beetle strain	1	45	1.71	0.1972	1.71	0.1905
active ingredient * application method * rate	1	45	0.13	0.7209	0.13	0.7192
active ingredient * application method * beetle strain	1	45	1.79	0.1882	1.79	0.1815
active ingredient * rate * beetle strain	1	45	0.47	0.4948	0.47	0.4912
application method * rate * beetle strain	1	45	0.12	0.7317	0.12	0.7301
active ingredient * application method * rate * beetle strain	1	45	0.54	0.4673	0.54	0.4635

For beetles fed with imidacloprid-treated foliage, strain was not a significant factor (Figure 6, Table 17). However the two beetle strains responded differently to thiamethoxam (more affected beetles in the susceptible strain than in the resistant strain, LSMeans $X^2_{(1)}$= 6.63, P =0.01). The interaction 'active ingredient * beetle strain' was significant on day 26 (Table 17).

For the resistant beetle strain only, seed treatments performed better than in-furrow applications (LSMeans X2 (1)= 7.26, P = 0.0071). There was no difference in efficacy between application methods for susceptible beetles. The interaction 'application method * beetle strain' was significant (Table 17). Seed treatments may reduce the effect of resistance early in the season by promoting more rapid insecticide uptake. The advantages of seed treatment due to the more availability of insecticide close to the emerging plant have been reported (Rouchaud et al. 1994) and may be playing a key role here in providing the roots with more available insecticide. Also, the insecticide is concentrated on the seed and there is less contact with the soil and less potential for binding. On day 33 after planting, 'active ingredient' and 'beetle strain' were the only factors that significantly affected efficacy (Table 18). Thiamethoxam at the rates used resulted in significantly more affected beetles than did imidacloprid ($X^2_{(1)}$ = 5.12, P = 0.02). Fewer affected beetles were found for the resistant beetle strain compared with the susceptible beetles (Figure 6).

By day 40 after planting, a steep decline in treatments efficacy for 'high dose – susceptible strain' conditions had occurred and all percentages of

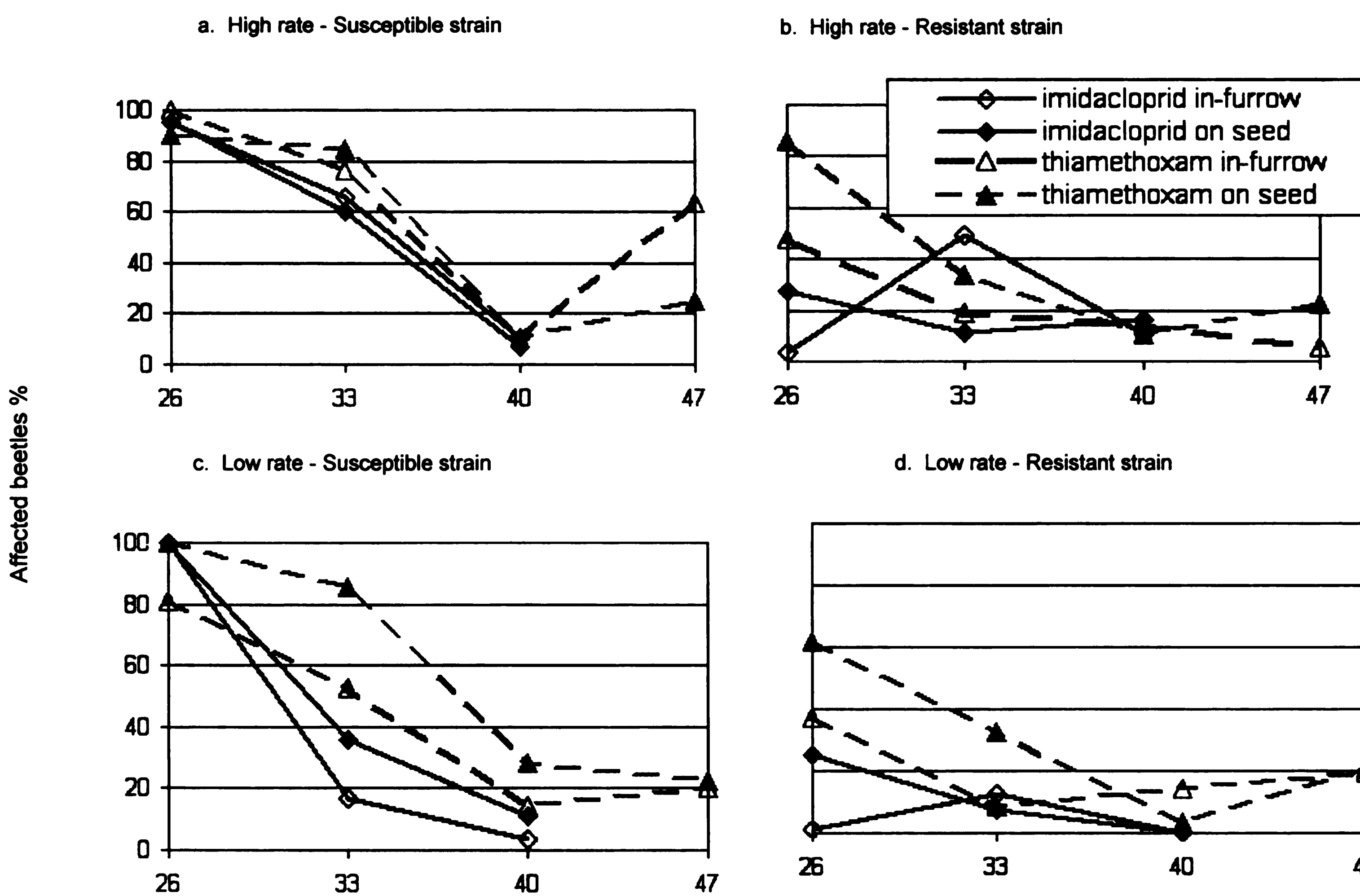


Figure 6. Percent of Colorado potato beetles affected by neonicotinoid insecticide-treated foliage from the organic soils experimental site in 2003

Table 18. Main effects and interactions analyses for Colorado potato beetles affected in bioassays by foliage from plants treated with neonicotinoids at the organic soils site on day 33 after application in 2003 (linear regression statistics for Type 3 Analysis)

Factor	Numerator Degrees of freedom	Denominator Degrees of freedom	F Value	P Value	Chi-Square	P Value
block	3	45	0.17	0.9142	0.52	0.9148
active ingredient	1	45	5.26	0.0266	5.26	0.0218
application method	1	45	0.59	0.4448	0.59	0.4408
rate	1	45	3.05	0.0876	3.05	0.0807
beetle strain	1	45	4.00	0.0515	4.00	0.0454
active ingredient *application method	1	45	0.36	0.5490	0.36	0.5459
active ingredient *rate	1	45	0.73	0.3969	0.73	0.3924
application method*rate	1	45	1.55	0.2200	1.55	0.2135
active ingredient *beetle strain	1	45	1.30	0.2601	1.30	0.2541
application method*beetle strain	1	45	0.87	0.3553	0.87	0.3503
rate*beetle strain	1	45	0.05	0.8277	0.05	0.8267
active ingredient *application method*rate	1	45	0.21	0.6523	0.21	0.6501
active ingredient *application method*beetle strain	1	45	1.35	0.2515	1.35	0.2453
active ingredient *rate*beetle strain	1	45	0.21	0.6527	0.21	0.6505
application method*rate*beetle strain	1	45	0.20	0.6603	0.20	0.6582
active ingredient *application method*rate*beetle strain	1	45	0.30	0.5850	0.30	0.5822

affected beetles were then at ca. 15% (Figure 6). The factor 'active ingredient' was significant (Table 19), significantly more beetles were affected by thiamethoxam than by imidacloprid (X2(1)= 5.99, P = 0.02). 'Beetle strain' was a significant factor as well (Table 19).

On day 47 after planting, the imidacloprid treatments were not included because <10% of the beetles had been affected the previous week. Only the factors: 'application method', 'rate' and 'beetle strain' were tested. Significantly more susceptible beetles were affected than resistant beetles (Table 20), but the individual treatment combinations did not show significant differences.

Season results. All the variables studied had significant effects during the season at the organic soils site (Table 21). For 'active ingredient', thiamethoxam showed significantly higher numbers of affected beetles than did imidacloprid (Figure 6). 'Beetle strain' also significantly affected the efficacy of the treatments and was probably the most important factor influencing differences among treatments (Table 21). Seed treatments generally resulted in significantly higher numbers of affected beetles than in-furrow treatments.

Finally, the two rates used influenced also produced significant differences (Table 21); the reduced rate resulted in fewer affected beetles than the high rate. This significance must be due to an additive effect date after date since significant differences were not seen for any individual date. The factor 'days after planting' was significant; all treatments had a declining efficacy through time as expected. The interaction of 'days after planting * beetle strain' was significant (Table 21). The main effect of this interaction was observed in the lower initial

Table 19. Main effects and interactions analyses for Colorado potato beetles affected in bioassays by foliage from plants treated with neonicotinoids at the organic soils site on day 40 after application in 2003 (linear regression statistics For Type 3 Analysis

Factor	Numerator Degrees of freedom	Denominator Degrees of freedom	F Value	P Value	Chi-Square	P Value
block	3	45	0.53	0.6611	1.60	0.6587
active ingredient	1	45	5.99	***0.0183***	5.99	0.0144
application method	1	45	0.60	0.4422	0.60	0.4382
rate	1	45	1.72	0.1964	1.72	0.1898
beetle strain	1	45	4.85	0.0328	4.85	0.0277
active ingredient *application method	1	45	0.10	0.7516	0.10	0.7501
active ingredient *rate	1	45	3.18	0.0812	3.18	0.0744
application method*rate	1	45	0.29	0.5898	0.29	0.5871
active ingredient *beetle strain	1	45	0.19	0.6628	0.19	0.6607
application method*beetle strain	1	45	0.61	0.4379	0.61	0.4338
rate*beetle strain	1	45	1.16	0.2880	1.16	0.2823
active ingredient *application method*rate	1	45	0.30	0.5846	0.30	0.5819
active ingredient *application method*beetle strain	1	45	0.10	0.7572	0.10	0.7557
active ingredient *rate*beetle strain	1	45	0.36	0.5490	0.36	0.5460
application method*rate*beetle strain	1	45	1.03	0.3155	1.03	0.3101
active ingredient *application method*rate*beetle strain	1	45	0.01	0.9373	0.01	0.9370

Table 20. Main effects and interactions analyses for Colorado potato beetles affected in bioassays by foliage from plants treated with neonicotinoids at the organic soils site on day 47 after application in 2003 (linear regression statistics For Type 3 Analysis)

Factor	Numerator Degrees of freedom	Denominator Degrees of freedom	F Value	P Value	Chi-Square	P Value
block	3	21	0.23	0.8760	0.68	0.8771
active ingredient	0	21	.	.	0.00	.
application method	1	21	0.12	0.7335	0.12	0.7300
rate	1	21	0.01	0.9313	0.01	0.9305
beetle strain	1	21	5.45	0.0296	5.45	0.0196
active ingredient *application method	0	21	.	.	0.00	.
active ingredient *rate	0	21	.	.	0.00	.
application method*rate	1	21	0.04	0.8412	0.04	0.8392
active ingredient *beetle strain	0	21	.	.	0.00	.
application method*beetle strain	1	21	2.18	0.1545	2.18	0.1397
active ingredient *application method*rate	0	21	.	.	0.00	.
active ingredient *application method*beetle strain	0	21	.	.	0.00	.
rate*beetle strain	1	21	3.02	0.0967	3.02	0.0821
active ingredient *rate*beetle strain	0	21	.	.	0.00	.
application method*rate*beetle strain	1	21	2.04	0.1680	2.04	0.1533
active ingredient *application method*rate*beetle strain	0	21	.	.	0.00	.

Table 21. Main effects and interactions analyses for Colorado potato beetles affected in bioassays by foliage from plants treated with neonicotinoids at the organic soils site overall season 2003 (linear regression statistics for Type 3 Analysis)

Factor	Numerator Degrees of freedom	Denominator Degrees of freedom	F Value	P Value	Chi-Square	P Value
block	3	165	0.39	0.7597	1.17	0.7596
days after planting	3	165	21.02	***<.0001***	63.06	<.0001
active ingredient	1	165	24.06	***<.0001***	24.06	<.0001
application method	1	165	4.31	***0.0394***	4.31	0.0379
rate	1	165	4.64	***0.0326***	4.64	0.0312
beetle strain	1	165	39.46	***<.0001***	39.46	<.0001
days after planting * active ingredient	2	165	0.54	0.5824	1.08	0.5814
days after planting * application method	3	165	0.74	0.5271	2.23	0.5254
active ingredient * application method	1	165	0.21	0.6504	0.21	0.6498
days after planting *rate	3	165	0.11	0.9552	0.32	0.9553
active ingredient *rate	1	165	3.08	0.0813	3.08	0.0794
application method *rate	1	165	1.79	0.1833	1.79	0.1815
days after planting * beetle strain	3	165	4.32	***0.0058***	12.95	0.0048
active ingredient * beetle strain	1	165	3.10	0.0799	3.10	0.0781
application method * beetle strain	1	165	0.96	0.3294	0.96	0.3279

Table 21 (cont'd)

Factor	Numerator Degrees of freedom	Denominator Degrees of freedom	F Value	P Value	Chi-Square	P Value
rate* beetle strain	1	165	0.02	0.8911	0.02	0.8909
days after planting * active ingredient * application method	2	165	0.95	0.3873	1.91	0.3852
days after planting * active ingredient *rate	2	165	0.37	0.6926	0.74	0.6920
days after planting * application method *rate	3	165	0.11	0.9523	0.34	0.9524
active ingredient * application method *rate	1	165	0.60	0.4403	0.60	0.4392
application method *rate* beetle strain	1	165	0.50	0.4792	0.50	0.4782
days after planting * active ingredient * beetle strain	2	165	8.63	0.0003	17.26	0.0002
days after planting * application method * beetle strain	3	165	3.06	*0.0299*	9.18	0.0270
active ingredient * application method * beetle strain	1	165	0.02	0.8770	0.02	0.8768
days after planting *rate* beetle strain	3	165	1.58	0.1973	4.73	0.1930
active ingredient *rate* beetle strain	1	165	0.20	0.6562	0.20	0.6556
days after planting * active ingredient * application method *rate	2	165	0.01	0.9907	0.02	0.9907
days after planting *active ingredient * application method * beetle strain	2	165	1.91	0.1511	3.82	0.1479
days after planting * active ingredient *rate* beetle strain	2	165	0.48	0.6206	0.96	0.6197
days after planting * application method *rate* beetle strain	3	165	0.75	0.5217	2.26	0.5201
active ingredient * application method *rate* beetle strain	1	165	0.45	0.5031	0.45	0.5021
days after planting * active ingredient * application method *rate* beetle strain	2	165	0.14	0.8730	0.27	0.8729

efficacy of several treatments against resistant beetles, especially those with imidacloprid. The residual activity was also influenced by the interaction 'active ingredient * beetle strain' as shown by the significant second-order interaction of 'days after planting * active ingredient' with 'beetle strain'. The interaction 'application method * beetle strain' varied through time, interacting significantly with 'days after application' and affecting the residual activity (Table 21).

The conditions of lower rate and resistant strain (or both) induced a decrease in efficacy of the products compared with the efficacy levels observed for high rate and susceptible strain.

High percentages of affected beetles were found on some treatments with the susceptible beetle strain, but those percentages declined quickly, principally at the lower rate for imidacloprid. With the resistant beetle strain only thiamethoxam as seed treatment (Cruiser) resulted in >60% affected beetles and then only on the first sample date (Figure 6).

The interaction 'active ingredient * beetle strain' for the whole season was not significant ($X^2_{(1)}$=3.1, P = 0.08), suggesting that both beetle strains responded similarly to the two insecticides and a that certain level of cross resistance in the resistant strain to thiamethoxam may be present. However on the first date, this interaction is significant, and then almost all efficacies decline rapidly. The similar performance of thiamethoxam and imidacloprid on the resistant strain could be due to overall ineffectiveness of the insecticides, rather than cross resistance to thiamethoxam in the imidacloprid-resistant strain.

The effective control afforded by imidacloprid and thiamethoxam in organic soils declined sooner than most of the reported half life ranges reported for the two active ingredients in soils. While the half life of imidacloprid is reported as 80 - 100 d, (Krohn and Hellpointner 2002), thiamethoxam half life values are reported as 34 to 75 d (Maienfisch et al. 2001) or 101 d (Anon. 2003d). The half life of imidacloprid is usually longer than the biological activity in plants, but the drastic declines in residual activity and efficacy of the different treatments in organic soils in this study suggest that the half life of these insecticides may be reduced as well.

Treatment residual activity Five treatment combinations (all with the resistant beetle strain) were not included in the Probit analysis because percentage of affected beetles was always < 50% (Figure 6). These treatment combinations were the least effective treatments where residual activity was severely depressed. From those included in the analysis, four Probit analyses gave no fiducial limits of comparison because of high variability (Table 22). Two of these treatment combinations were: 'thiamethoxam – in furrow – high rate – susceptible strain' and 'thiamethoxam – susceptible – high rate – seed treatment had a final increase in efficacy, thus increasing variability in the statistical analysis. For ′imidacloprid - in furrow – susceptible strain – low rate' and 'imidacloprid – seed treatment – low rate – susceptible strain′ a steep decline was seen just at the second date of study, increasing variability as well. LT50 for the treatment combination 'thiamethoxam – in furrow – high rate –susceptible strain' was the highest of all, but no fiducial limits were given, again because of a

Table 22. Probit analysis for the evaluation of neonicotinoid insecticides, at two rates and for two beetle strains in the control of Colorado potato beetle, in the organic soils site 2003

Treatment (active ingredient, application method, rate, beetle strain)	**LT_{50} (days)**	**LT_{50} grouping**	**Fiducial limit 0.95**	**Slope ± SE**	**Pearson X^2/df=2 (P value)**
Imidacloprid, in furrow, high, susceptible	31.60	b	30.00 - 33.21	-21.20 ±4.25	0.53 (0.47)
Imidacloprid, seed, high, susceptible	32.84	ab	31.19 - 34.60	-20.10 ±4.06	5.88 (0.12)
Thiamethoxam, in furrow, high, susceptible	57.70	***	*	-2.41 ±2.46	1.63 (0.20)
Thiamethoxam, seed, high, susceptible	36.38	***	*	-9.43 ±4.11	11.11 (<0.01)
Imidacloprid, in furrow, low, susceptible	31.81	N/A	*	-82.05 ±472796	****
Imidacloprid, seed, low, susceptible	29.38	N/A	*	-11.18 ±6.09	****
Thiamethoxam, in furrow, low, susceptible	29.38	ab	31.03 - 39.81	-5.79 ±1.47	0.67 (0.72)
Thiamethoxam, seed, low, susceptible	39.00	a	36.66 - 41.53	-12.93 ±2.34	4.31 (0.12)

Table 22 (cont'd)

Treatment (active ingredient, application method, rate, beetle strain)	LT_{90} (days)	LT_{50} grouping	Fiducial limit 0.95	Slope ± SE	Pearson X^2/df=2 (P value)
Imidacloprid, in furrow, high, resistant	**	**			
Imidacloprid, seed, high, resistant	**	**			
Thiamethoxam, in furrow, high, resistant	18.34	N/A	*	-2.07 ±2.57	6.16 (0.05)
Thiamethoxam, seed, high, resistant	30.98	N/A	*	-9.53 ±4.58	13.03 (<0.01)
Imidacloprid, in furrow, low, resistant	**	**			
Imidacloprid, seed, low, resistant	**	**			
Thiamethoxam, in furrow, low, resistant	**	**			
Thiamethoxam, seed, low, resistant	25.90	N/A		-1.79 ±2.48	6.19.00 (0.05)

* No fiducial limits available due to high variability
** Data below 50% mortality all season long
*** Data above 50% mortality all season long
**** No degrees of freedom to calculate goodness of fit
N/A Not available

Values of LT_{50} followed by the same letter are not significantly different due to overlapping confidence intervals large increase in percentage affected beetles on the last sample date. Most treatments were similar and the only significant difference detected was between 'thiamethoxam-seed treatment-low rate-susceptible strain' and 'imidacloprid-in furrow-high rate-susceptible strain' (Table 22, Figure 7). No differences can be detected between the thiamethoxam treatments of the susceptible strain at the low rate and the resistant strain at the high rate. No signs of cross resistance were observed.

Discussion Organic soils site 2003. For the first two evaluation dates (26 and 33 days after planting), consistently more beetles were affected for the susceptible strain than for the resistant strain (Figure 6). Differences in performance between imidacloprid and thiamethoxam have been found for other situations (Nault et al 2004). There were relatively few beetles and only minor defoliation in the field, so it is unlikely that beetles fed differentially on foliage with low toxin levels and left only foliage of unusually high toxicity for my samples

Overall analysis 2002 and 2003

For the two seasons, there was a decline of total affected susceptible beetles through time for all treatment combinations at both locations and this decline occurred much sooner on the muck soils site than in the mineral soils site (Figure 2, and 4, 6). This observation supports previous work that observed that organic soils reduce efficacy and residual activity of neonicotinoids due to binding of the insecticide to organic fraction in the soil (Liu et al. 2002, Krohn and

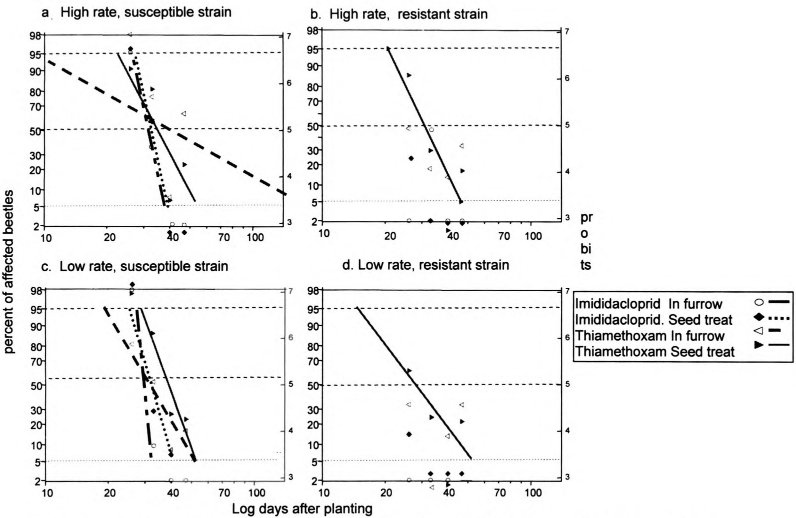


Figure 7 . Log *days after planting* probit lines for affected Colorado potato beetles when fed with neonicotinoid-treated foliage from the organic soil site in 2003. Treatment combinations are not included if percentage of affected beetles is always > 50% or <50% throughout the season.

Hellpointner 2002).

A decline in efficacy from highly, medium, low or non-existent to levels near zero is expected to occur within 25 to 40 days after planting in organic soils similar to those in the Muck Soils Farm. The organic soils also may emphasize the expression of cross resistance late in the season. This may be the result of reduced effective rate and less amount of insecticide taken up by the plant.

The effect of application method on efficacy and residual activity varied between the two sites. More beetles were affected by the in-furrow application in the mineral soils site than in the organic soils site in 2002, low effect of the application method in efficacy was observed for those soils in 2003. In the organic soils there was a more marked effect of application method, with the seed treatment affecting more beetles than in-furrow applications. This relation was found on the first date of assessment during both years. Seed treatment applications may improve insecticide performance on organic soils; probably by reducing the direct contact of more applied insecticide with the organic fraction of the soil, compared with the in-furrow application.

The insecticide performance with on resistant beetles or at the lower rate was relatively poorer for imidacloprid than for thiamethoxam, showing that imidacloprid products were less efficient under the detrimental factors tested in this study. It should also be noted that the resistant beetles had been selected for resistance to imidacloprid and showed only a low cross resistance to thiamethoxam. The percentage of affected beetles was initially high in most cases but declined more rapidly as the season progressed in the adverse conditions.

Thiamethoxam applied in-furrow, in 2002, and thiamethoxam whith either application method, in 2003, had the higher percentage of affected beetles with susceptible beetles in the mineral soils.

For the type of evaluation that was done here, laboratory bioassays, the products based on thiamethoxam produced more affected beetles. This result could be the effect of the retarded effect of thiamethoxam, which has to be converted into clothianidin to be active (Nauen et al. 2003). This delay may allow in the insect to consume more treated foliage before becoming intoxicated, ultimately producing more ingestion of the toxin and probably more insecticide in the insect's body than when foliage is treated with imidacloprid. This does not mean necessarily that thiamethoxam is more effective for control than imidacloprid, especially because in the field more factors interact to produce protection. Also, much of the crop protection the field involves control of larvae, which are more sensitive to imidacloprid than adults (Zhao et al.2000)

The median lethal time and effective control afforded by imidacloprid in organic soils was shorter than previous reports of residual activity of imidacloprid (Bishop et al. 2001, Boiteau et al. 1997). In mineral soils imidacloprid gave residual control comparable to those reported in that study. The efficacy of imidacloprid and thiamethoxam lasted longer in mineral soils than in organic soils probably because the insecticides bind to the organic matter of muck soils (Krohn and Hellpointner 2002).

The differences in the insecticide rates tested in 2003 did not have major effects on the efficacy of the insecticides or their residual activity. Both rates used were within labeled rates. In general efficacy was not affected by the lower rate but residual activity was reduced in mineral soils for susceptible beetles. In a two-year study, Boiteau et al (1997), found some differences in efficacy between rates of imidacloprid applied in furrow. In 1993, they assessed the residual activity of the insecticide by rating defoliation produced by adults and larvae of the Colorado potato beetle. The rates of 111 and 222 g [A.I.]/ha provided protection for 44 - 47 days after planting. Longer protection was observed at 333 g [A.I.]/ha (62 - 65 days) in 1994, less residual activity was observed at 111 g [A.I.]/ha compared with 222 g [A.I.]/ha or 333 g [A.I.]/ha. In the present study, differences in rates tested in 2003 did not have major effects on length of control. Only for imidacloprid as seed treatment on susceptible beetles around day 40 -47, and on resistant beetles at the beginning of the season, were differences in efficacy due to the different rates observed.

The differences in efficacy between resistant and susceptible beetles were in general statistically significant; with the resistant strain the efficacy of imidacloprid was generally lower than the efficacy of thiamethoxam. This is surprising because the resistance level to imidacloprid in the resistant beetle strain was ca. 9.4-fold which is considered a low level of resistance or tolerance. This study shows that even that low level can make a difference in beetle response to imidacloprid at field rates. This response may be magnified if more factors (insect plant interactions, environmental factors, etc) are involved. Low levels of resistance may be enough to produce a lower performance of insecticides especially if organic content of the soil is high.

At the beginning of the 2003 season, there were ca. 50% affected beetles for thiamethoxam in furrow in mineral soil at a low rate, and higher efficacy was attained the next week. This suggests that slower insecticide uptake could occur early during plant growth. One factor could contribute to this: the in-furrow application may delay insecticide uptake until the young plants have attained appropriate root development to reach the treated area in the soil. This in turn may result in low initial concentrations in the foliage that the insect consume. Those concentrations may not be enough to achieve high levels of control. Why this happened with thiamethoxam applied in furrow but not to imidacloprid remains unclear.

Conclusions

Imidacloprid and thiamethoxam at the rates used provided good levels of control of Colorado potato beetle adults when tested on the susceptible strain in mineral soil, beyond 30 days after planting; high efficacy continued until day 40 in mineral soils, for both products.

Colorado potato beetle control provided by each insecticide was much higher in mineral soil site than organic soil site.

The detrimental effect of organic soils on efficacy and residual activity of neonicotinoid insecticides may further result in lower efficacies if resistance to imidacloprid, is encountered in Colorado potato beetles.

Thiamethoxam applied in-furrow at a full rate on mineral soils was the most consistent treatment in efficacy and had the longest residual effect. High efficacies (more than 95% affected beetles) were observed up to at least day 47 after planting. This is very important for controlling beetles arriving late due to late emergence from diapause or migration from distant overwintering sites. It provided high control levels of control in 2003 under the optimal conditions and even at the lower rate.

Low levels of resistance (<10 fold) in Colorado potato beetle affect control by neonicotinoids and even lower levels of cross resistance of imidacloprid-resistant beetles to thiamethoxam (<3 fold) may influence the residual activity of the product.

CHAPTER 3:

THE EFFECT OF SOIL TYPE, EXCESS WATER, AND APPLICATION METHOD ON THE RESIDUAL ACTIVITY OF NEONICOTINOIDS FOR THE CONTROL OF COLORADO POTATO BEETLE *LEPTINOTARSA DECEMLINEATA (SAY)* UNDER GREENHOUSE CONDITIONS

INTRODUCTION

The Colorado potato beetle, *Leptinotarsa decemlineata* (Say), is the most important insect pest of potatoes in the northeastern and midwestern United States (Mahr et al. 1995, Ferro and Boiteau 1993). Worldwide, it is the most devastating potato defoliator (Hare 1990, Capinera 2001). Larvae and adults can cause complete defoliation and total yield loss may occur if control measures are not taken. Potato plants in the critical stages of bloom and early tuber formation are especially vulnerable to beetle attacks (Hare 1990). Control programs for this pest aim to minimize the effect of the insect during the early and mid season. Historically control has relied extensively on synthetic insecticides (Casagrande 1987).

Imidacloprid and thiamethoxam are currently the two most commonly used insecticides to control this pest in the northeastern and midwestern U.S. Imidacloprid was first registered in 1995 and thiamethoxam was registered in 2001 for Colorado potato beetle control in the United States. These insecticides are the products of choice especially in areas where there is Colorado potato beetle resistance to other major insecticide groups. Both insecticides are neonicotinoids, a relatively new group of compounds that act as nicotinic acetylcholine receptor agonists (Bi et al. 2002). Neonicotinoids can be applied through different methods, have systemic action, require low use rates, and are highly effective (Maienfisch et al. 2001). Thiamethoxam and imidacloprid applications are recommended at planting for Colorado potato beetle control (Bird et al. 2005). For both products a single application is desirable during the season because it can 1) lower the costs of control and 2) reduce the pest population potential for resistance build up. However, the efficacy and residual effect of these two chemicals when applied at planting may vary depending on factors like application method, rainfall or irrigation, and soil type.

For instance, the availability of soil-applied imidacloprid may vary through the season depending on cropping conditions, soil characteristics, and water flow. Under diverse cropping conditions imidacloprid half-life ranges differs greatly, average values are ca. 80 to 110 d, and can be up to 174 d in bare soils (Krohn and Hellpointner 2002). Scholz and Spiteller (1992) demonstrated the effect of vegetation in half life of imidacloprid: longer half lives are found on bare soil whereas shorter half lives are found in soil covered with grass. Imidacloprid remains mainly in the upper 30 cm of the soil during its lifetime and is only partially translocated into deeper soil (Krohn and Hellpointner 2002). In aqueous solution, acidic pH and liquid formulations produce a shorter half-life compared to alkaline pH and powder formulations of imidacloprid (Sarkar et al. 1999). Organic soils may additionally reduce insecticidal activity because the compound binds to the organic fraction of the soil (Krohn and Hellpointner 2002); humic acids have higher sorption capacity than Ca-clay minerals (Liu et al. 2002). Ultimately, imidacloprid does not accumulate in the soil (Krohn and Hellpointner 2002) although some studies have reported persistence of the compound in the soil for more than 365 d (Anon. 2001).

Water solubility is a determining factor for insecticide movement in the soil and also for insecticide availability to the plant. The activity of imidacloprid and thiamethoxam may be reduced by excessive rainfall or irrigation especially because potatoes are generally grown on sandy loam or loamy sand soils. Imidacloprid is a hydrophilic substance with water solubility values of 0.5 g/l at 20 °C at pH 7 (Krohn and Hellpointner 2002). Thiamethoxam is a hydrophilic substance as well, its water solubility is 4.1 g/l (Anon. 2003d).

Both Imidacloprid and thiamethoxam could easily move through sensitive soil types such as porous, gravelly, or cobble soils, depending on irrigation practices and rainfall (Jenkins 1994). Insecticide leaching depends also on the formulation components (Gupta et al. 2002) and pH of aqueous solution (Sarkar et al. 2001). Local soil compactness and water flow may extend compound leaching beyond the root zone (upper 30 cm) and could lead to a reduction in residual activity of these products (Gupta et al. 2002).

Imidacloprid is transported acropetally in the xylem of the plant and degrades almost completely depending on plant species and time after application (Buchholz and Nauen 2002). The rate of insecticide uptake by the plant declines after application as a function of time. For example, in sugar beet, concentration in foliage declined from 15.2 µg/g (fresh weight) on day 21 to 0.5

µg/g on day 97 (Westwood et al. 1998). By the end of the season little or no residues was present in sugar beet (Rouchaud 1994), eggplant, cabbage, or mustard (Mukherjee and Gopal 2000).

Thiamethoxam degrades at moderate to slow rates in laboratory soils, and its half-life ranges from 34 to 75 d under favorable crop conditions, but may increase by a factor of three under unfavorable crop conditions (Maienfisch, et al. 2001). pH sensitivity studies show that thiamethoxam is very stable at pH 5 and pH 7, but labile at pH 9 (Maienfisch et al. 2001).

The commercial formulations of imidacloprid and thiamethoxam available for Colorado potato beetle control are recommended as a range of rates and usually the highest rates are recommended for soil containing high levels of organic matter (Bird et al. 2005).

For at-planting applications, two methods are used: 1) seed treatment, or 2) in-furrow application. For seed treatment, potato seed pieces are treated with an insecticide solution prior to in-furrow placement. Genesis ® (imidacloprid) at a rate of 6.2–9.4 g active ingredient [a.i.]/100 kg seed, and Cruiser ® 5FS (thiamethoxam) at 3– 6 g a.i./100 kg seed are the recommended seed-treatment formulations (Bird et al. 2005). Seed treatments are advantageous in that the pesticide is placed in the required site and treatment is done prior to planting and can be combined with fungicides treatment.

For in-furrow application, the seeds are placed in the furrow and the insecticide is sprayed into the furrow before closing. For this application method Admire® 2F (imidacloprid) is recommended at application rates of 269 - 389 g [a.i.]/ha in furrow and Platinum ® (thiamethoxam) is recommended at a rate of 84 – 134 g [a.i.]/ ha (Bird et al. 2005). In-furrow application is convenient and does not require an extra step in the planting process.

Active ingredient placement, related to the plant site and root development, and probable effect of insecticide formulation components may produce a varying response in the efficacy of the insecticides. Although seed-treatment may promote more insecticide uptake by the plant, because the insecticide is applied in intimate proximity to the developing plant (Rouchaud et al. 1994), the advantages of this method in potatoes are unknown, especially since potato "seed" is actually whole or cut tubers. The effect of application method on the efficacy of Colorado potato beetle control is still unclear. It is important to compare seed treatment with the more convenient in-furrow application method, and to also study the effect that organic matter may have on performance in mineral vs. organic soils.

Movement of insecticides in the soil is a constant concern, especially with the relatively high water solubility values of imidacloprid and thiamethoxam compared to other insecticides. Although several studies report no leaching of imidacloprid under normal crop situations (Rouchaud et al. 1994, Rouchaud et al. 1996, Leib and Jarret 2003), there may be a potential for leaching especially under high rainfall events shortly after application, or through formation of stable, soluble organic fraction-pesticide interactions in solution (Gonzalez-Pradas et al. 2002). Water solubility of thiamethoxam (4.1 g/l at 25 °C) is higher than solubility of imidacloprid (0.5 g/l at 20 °C), thus increasing the leaching potential. Studies should address whether the leaching potential, related to excessive rainfall or irrigation, results in reduction of insecticide performance, especially for soluble compounds like imidacloprid and thiamethoxam.

It is also unknown how the combined effects of insecticide and application method affect beetle mortality when plants are grown in mineral vs. organic soils. A potential reduction in insecticide availability could significantly impact the mortality of Colorado potato beetle. If imidacloprid tolerant beetles are tested, then the mortality caused by imidacloprid treatments is expected to have a corresponding decline according to the degree of tolerance. Since imidacloprid and thiamethoxam are chemically related, the chance for cross-resistance is high. The thiamethoxam's metabolite, clothianidin, acts with high affinity on imidacloprid sites like other neonicotinoids, making thiamethoxam a poor option for insecticide rotation and resistance management (Nauen et al. 2003).

The effect of application method on the compound's length of activity is unclear, as is the effect of high rainfall or irrigation levels. How this response interacts with mineral vs. organic soils and against susceptible and resistant beetles is also unknown. Therefore, knowing the effect these factors have on insecticide performance and how they interact is crucial for chemical control optimization.

The objectives of this research were to:

Determine the effect of soil type (mineral soils versus organic soils) on beetle mortality from the two insecticides under greenhouse conditions.

Determine under greenhouse conditions whether in-furrow application or seed treatment increases the efficacy and residual activity of these neonicotinoids.

Determine the effect of excessive rainfall or irrigation at planting on the residual activity of imidacloprid and thiamethoxam.

Examine possible interactions between: soil type, application method, and insecticide and their effect on beetle mortality.

MATERIALS AND METHODS

The efficacy and residual activity of imidacloprid and thiamethoxam-based insecticides on Colorado potato beetle adults were assessed through no-choice feeding bioassays conducted in the laboratory, using treated foliage from greenhouse plants that had received an at-planting insecticide treatment.

Beetle strain

A susceptible strain of Colorado potato beetle was used in the study. Adults were purchased from the Phillip Alampi Beneficial Insect Rearing Laboratory of the New Jersey Department of Agriculture. Upon arrival, beetles were 3-5 d post emergence from pupation. They were kept for 5 d at room temperature (ca. 17°C) and fed daily on untreated greenhouse potato foliage, then transferred to growth chamber at 10°C, with only one initial food supply, and used in the study within 1 - 2 wks of arrival. The day prior to the bioassay, the beetles were taken out of the 10° C growth chamber and kept in the laboratory with no food to ensure that all were active and hungry.

Plants

Potato plants cv. Atlantic were grown under greenhouse conditions. Seed was manually cut and seed piece weight was 84.7 g ± 1.4 (SE). Two soil types were used: mineral soil from the Michigan State University (MSU) Montcalm Potato Research Farm, Montcalm Co, MI; and organic soil from the MSU Muck Soils Research Farm, Clinton Co, MI.

The Montcalm Farm has a typical mineral soil, used for potato production in Mid-Michigan, a McBride Sandy Loam, with pH of 6.7 < 5% organic matter. Fraction components for this soil are: sand 80%, silt 9% and clay 11% (Cottrell, 2003).

The MSU Muck Farm has a highly organic soil commonly used for vegetable crops, a Houghton Muck containing > 75% organic matter and a pH between 6.2 and 6.9 (Cottrell 2003).

Both soil types were collected from a depth of 0 – 25 cm and were transported in pots to the greenhouse. The pots used were cylindrical plastic pots 22 cm tall and 23 cm diameter, drilled with additional holes for drainage.

Watering

Two water levels were tested: 1) normal watering throughout plant growth and 2) excessive watering immediately after planting, followed by normal watering thereafter. Normal water corresponded to the irrigation provided with a sprinkler attached to a hose. Applied water amounts are shown in Figure 8. For

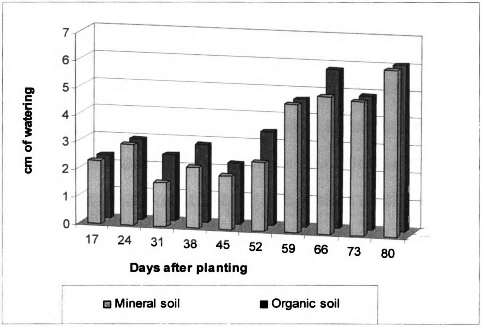


Figure 8. Accumulative weekly amounts of water applied on two soil types for potato development for the evaluation of neonicotinoid insecticides in greenhouse

the excessive watering, excess water was applied once, the day after planting, simulating the occurrence of a 7 cm, 24 h-long rainfall event that occurs in Michigan about every 5 yrs according to regional historic records (Andresen, MSU Department of Geography, Pers. Comm.). The corresponding amount of water for each pot was calculated for the hypothetical rainfall event. This amount of water was applied with a sprinkler in five waterings throughout the day.

Preliminary tests indicated that the potted soil did not have appropriate drainage conditions for excessive watering, especially with mineral soil. Therefore, the bottoms of all pots were lined with a 2.5 cm-thick bed of pea gravel and a plastic mesh (1 mm) for gravel retention. Additionally, the mineral soil was mixed with three parts volume: volume of sand (Play Sand ®). All pots were filled with soil to within 3 cm from the top. To achieve uniform water conditions in all the pots, they were saturated at field capacity 2 wks prior to planting. After the excess water treatment was applied, measurements of water leach-out were taken. An aluminum pan was placed under each pot and the water in the pans was measured. Average leach-out was 646.2 ml (±34.7 SE) for organic soil pots and 777.1 ml (±33.2 SE) for mineral soil pots in the excessive water treatments. No leach occurred in the normal water treatments. For the remainder of the experiment water was applied every day or every other day, cumulative weekly amount are reported in Figure 8. This water was applied with the sprinkler attached to a hose and the flow was constant and the time of watering controlled to ensure equal amounts each time. The differences in amount of water in some weeks are related to greenhouse temperatures and soil type requirement.

Insecticides

The insecticides used were Admire 2F®, and Genesis® (imidacloprid), Platinum 2SC® and Cruiser 5FS® (thiamethoxam). Insecticide application methods were: seed treatment (Admire and Platinum) and in-furrow (Genesis and Cruiser). For the seed treatments, the insecticide was applied to the cut seed at the recommended rate just before planting; and the in-furrow application was made immediately after planting and before seed furrow covering.

Treatments resulted from the combination of: active ingredient, application method, water level, and soil type (Table 23). Untreated controls for each soil type were also planted. Pots were arranged in the greenhouse in a randomized complete block design with five replications for each treatment and each experimental unit consisted of three plants to provide enough foliage for 2 x weekly collections. Blocking was set to account for gradients in physical conditions in the greenhouse (natural light, air flow, location of greenhouse entrance).

The potatoes were watered daily or every 2 d and fertilized weekly with 20-20-20 fertilizer (0.33 g/pot). The photo period was set at 15:9 h light: darkness and temperatures were 25 - 30°C.

Bioassays

For laboratory bioassays, starting the third week after planting, one leaf from the most developed and robust plant from each experimental unit (3 plants) was collected. In subsequent weeks plants were selected randomly from each experimental unit and the newest fully developed leaf was taken. After collection, the leaves were transported to the laboratory in a cooler and were used the same day in the bioassays.

Each leaf was placed in a Petri dish with the petiole in a vial with water; five beetles were placed in the dish. Food was replaced on day 3 with freshly collected foliage from the same experimental unit. The dishes were labeled with the corresponding treatment and replication, randomly arranged on a table in the laboratory, and kept at 20 – 25 ºC. The effect of the treatments on the beetles was registered after 7 d by recording walking, poisoned, and dead beetles. "Walking" were those active beetles that showed coordinated, forward walking; "poisoned" were those beetles unable to walk forward one body length; and "dead" beetles were unresponsive to probing with forceps and exhibited dark coloration, shrunken abdomens or signs of decomposition. Foliage consumption was estimated by a visual scale from 0 to 10 (from no consumption to totally consumed leaf). During treatment evaluation, Petri dish labels were covered to avoid bias. The bioassays were run weekly from 24 d after planting until 80 d after planting, when plants began to senesce.

Table 23. Greenhouse treatments with neonicotinoid insecticides, for control of the Colorado potato beetle.

Product	Active ingredient	Application method	Soil Type	Water level
Admire	imidacloprid	in-furrow	mineral	normal
Admire	imidacloprid	in-furrow	mineral	excessive
Admire	imidacloprid	in-furrow	organic	normal
Admire	imidacloprid	in-furrow	organic	excessive
Genesis	imidacloprid	seed treatment	mineral	normal
Genesis	imidacloprid	seed treatment	mineral	excessive
Genesis	imidacloprid	seed treatment	organic	normal
Genesis	imidacloprid	seed treatment	organic	excessive
Platinum	thiamethoxam	in-furrow	mineral	normal
Platinum	thiamethoxam	in-furrow	mineral	excessive
Platinum	thiamethoxam	in-furrow	organic	normal
Platinum	thiamethoxam	in-furrow	organic	excessive
Cruiser	thiamethoxam	seed treatment	mineral	normal
Cruiser	thiamethoxam	seed treatment	mineral	excessive
Cruiser	thiamethoxam	seed treatment	organic	normal
Cruiser	thiamethoxam	seed treatment	organic	excessive
Untreated	-	-	mineral	normal
Untreated	-	-	organic	normal

Recovery from intoxication

Each week, after evaluation on day 7, the treated foliage and dead beetles were removed and the walking and poisoned beetles were kept with untreated foliage under laboratory conditions to observe recovery of the poisoned beetles, 3 and 7 d later. Recovery from intoxication after lengthy periods sometimes occurs in the laboratory with neonicotinoids (Zhao et al. 2000) but its significance in the field is unknown. Harsh environmental conditions and predators or scavengers may not allow long periods of intoxication in the field. Recovery was recorded on day 10 and day 14 (from the initial the bioassay set up) as a new assessment in the categories walking, poisoned or dead beetles as used before.

Data processing and statistical analysis

The collected data of the experiments was computed as percentage of beetles from the total used per Petri dish. Two response variables were taken directly from data recorded in the experiment: the percentage of dead beetles and the percentage affected beetles (dead plus poisoned beetles). Mortality and affected beetles in the controls was zero or near zero on all dates so the treatment data were not corrected for control mortality.

For either response variable the following data analysis was done:

Since the data exhibited a Binomial distribution and because the response variables were categorical, the data were analyzed as categorical. Proc Genmod in the SAS System® (SAS Release 8.02 Level 02M0) and the Poisson distribution was used to describe the data distribution. Type 3 analyses were conducted to detect the significance of the main factors and interactions using the X^2 statistic. An analysis for the whole study was conducted to test the total effect of the variables studied. Then, individual analyses by each date were conducted. Least Square Means were also calculated to analyze differences between treatments.

Due to the many interactions this study involves, the main comparisons were grouped, contrasting a "best case scenario" of mineral soil and normal water level, compared to the treatment combinations with excessive water and/or organic soil. Graphic results are shown in split plots separating them by soil type and water level (Figure 9 -13).

The analysis of the treatment residual activity was assisted by Probit analyses, which were conducted when mortality of affected beetles included a range above and below 50% as time progressed. Parameters for this analysis (Median lethal time LT50 with confidential limits and slope) were estimated to compare the overall efficacy of the treatments.

Data for beetle recovery were grouped by treatment and are preliminarily presented here in a plot of treatments recovery vs. days after application. This intends to show general tendencies of this variable. Because initial numbers of poisoned beetles varied so widely (0 to 25 per treatment), statistical comparison of percent recovery would be of questionable value. For example 100% recovery could be 1 out of 1 initially poisoned or 25 out of 25 initially poisoned.

RESULTS AND DISCUSSION

Evaluation for total affected beetles

Weekly results. On day 24 after planting, all the treatments resulted in 60 -100% affected beetles (Figure 9). Efficacies in the mineral soils were in the range 90 -100% affected beetles while efficacies in the organic soils were more variable within a range 60 – 100% affected beetles. The mineral soil treatments resulted in significantly higher percentage of affected beetles than organic soil treatments (Table 24). 'Water level' also showed statistical differences (Table 24) in affected beetles with higher percentages for the 'normal water' vs. 'excess water'. This shows an initial reduction in percentage of affected beetles due to 'organic soil' and 'excess water'. On day 31 after planting, 'soil type' was the only main factor that caused significant effects in percentage affected beetles (Table 25); the mineral soil resulted in higher percentages of affected beetles than did the organic soil. Seed treatment affected more beetles than in-furrow applications for thiamethoxam, while imidacloprid affected more beetles with in-furrow applications than it did as seed treatments. This was evident in the significant interaction 'active ingredient * application method' (Table 25). And this interaction was more evident in the organic soil than in mineral soil, resulting in a significant second-order interaction 'application method * active ingredient * soil type' (Table 25).

There was no effect of 'water level' on day 31. Significant differences (LSMeans test P<5%) were only observed between the most effective treatments (near 100%) in the mineral soil and the two treatments with lowest efficacy, thiamethoxam in-furrow and imidacloprid, seed-treatment at the excessive water level in organic soils (Figure 9).

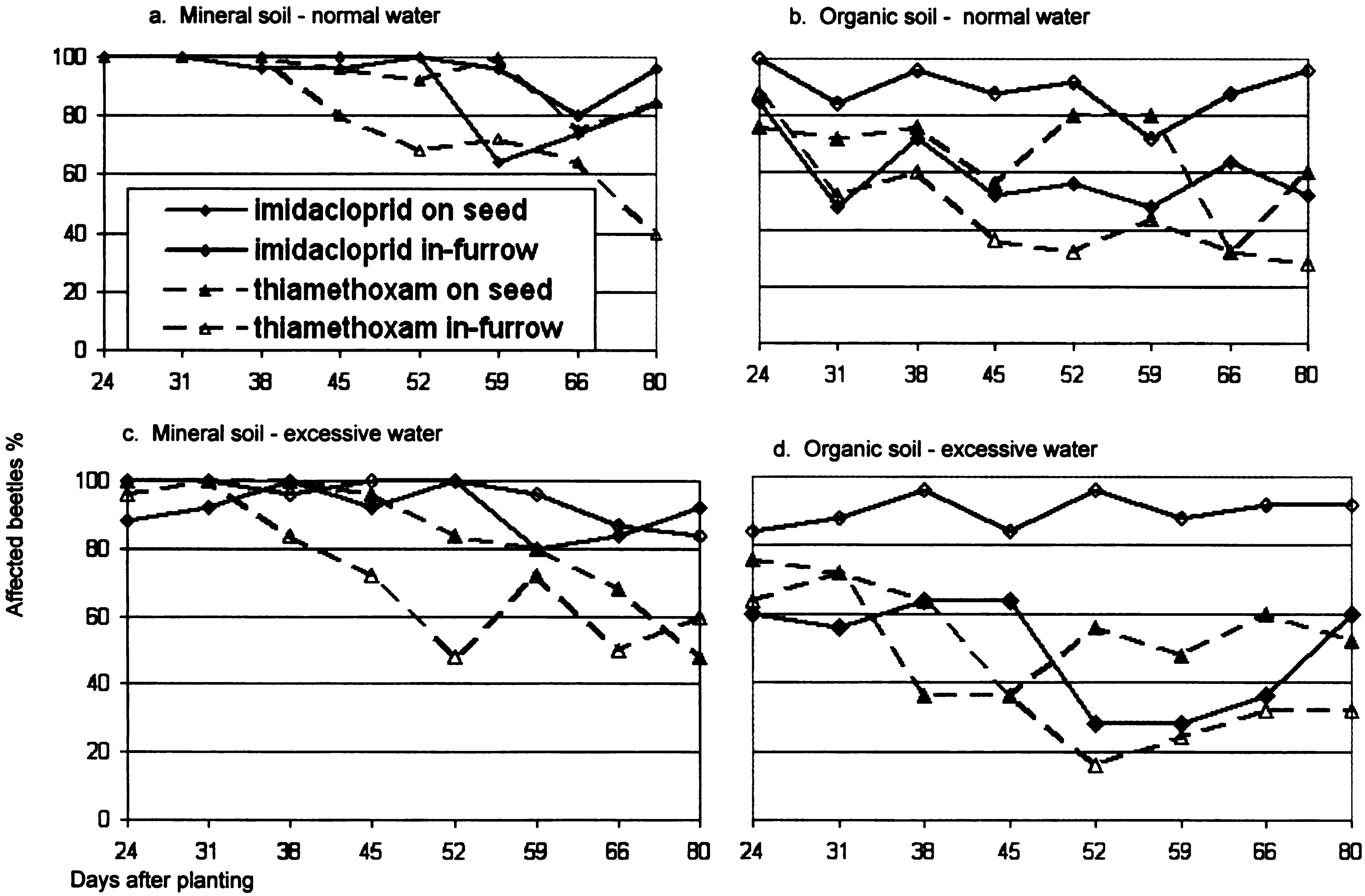


Figure 9. Percentage affected beetles by neonicotinoid insecticides from foliage treated in greenhouse under differing conditions of soil, watering, and application method

Table 24. Effect of neonicotinoid insecticides on percentage affected beetles under differing conditions of soil, water level, application method and active ingredient, on day 24 after application (linear regression statistics For Type 3 Analysis)

Factor	Numerator Degrees of freedom	Denominator Degrees of freedom	F Value	Pr > F	Chi-Square	Pr > ChiSq
block	4	56	0.30	0.8748	1.21	0.8762
soil	1	56	13.84	***0.0005***	13.84	0.0002
water level	1	56	4.47	***0.0390***	4.47	0.0346
active ingredient	1	56	0.16	0.6902	0.16	0.6886
application method	1	56	1.52	0.2235	1.52	0.2184
soil type*water level	1	56	1.85	0.1787	1.85	0.1733
soil type*active ingredient	1	56	0.51	0.4770	0.51	0.4740
soil type*application method	1	56	0.59	0.4463	0.59	0.4431
water level*active ingredient	1	56	0.29	0.5938	0.29	0.5916
water level*application method	1	56	0.01	0.9121	0.01	0.9117
active ingredient*application method	1	56	2.06	0.1571	2.06	0.1515
soil type*water level*active ingredient	1	56	0.08	0.7788	0.08	0.7778
soil type*water level*application method	1	56	0.27	0.6050	0.27	0.6030
soil type*active ingredient*application method	1	56	0.61	0.4394	0.61	0.4361
water level*active ingredient*application method	1	56	1.70	0.1980	1.70	0.1926
soil type*water level*active ingredient*application method	1	56	0.51	0.4792	0.51	0.4762

Table 25. Effect of neonicotinoid insecticides on percentage affected beetles under differing conditions of soil, water level, application method and active ingredient, on day 31 after planting (linear regression statistics For Type 3 Analysis)

Factor	Numerator Degrees of freedom	Denominator Degrees of freedom	F Value	Pr > F	Chi-Square	Pr > ChiSq
block	4	59	0.47	0.7549	1.89	0.7552
soil	1	59	28.80	***<.0001***	28.80	<.0001
water level	1	59	0.54	0.4641	0.54	0.4611
active ingredient	1	59	0.01	0.9246	0.01	0.9243
application method	1	59	1.68	0.1998	1.68	0.1947
soil type*water level	1	59	1.06	0.3082	1.06	0.3040
soil type*active ingredient	1	59	0.03	0.8627	0.03	0.8621
soil type*application method	1	59	1.01	0.3187	1.01	0.3146
water level*active ingredient	1	59	0.12	0.7324	0.12	0.7311
water level*application method	1	59	0.26	0.6116	0.26	0.6097
active ingredient*application method	1	59	5.67	***0.0205***	5.67	0.0172
soil type*water level*active ingredient	1	59	0.01	0.9406	0.01	0.9403
soil type*water level*application method	1	59	0.05	0.8266	0.05	0.8258
soil type*active ingredient*application method	1	59	4.46	0.0388	4.46	0.0346
water level*active ingredient*application method	1	59	0.35	0.5562	0.35	0.5540
soil type*water level*active ingredient* application method	1	59	0.74	0.3929	0.74	0.3894

On day 38 after planting the main factor effects of 'soil type' and 'active ingredient' significantly affected percentage affected beetles and 'water level' did not (Table 26). The percentage of affected beetles was consistently high for the mineral soil; more variability and fewer affected beetles were observed in the organic soil treatments (Figure 9). Imidacloprid products performed better than thiamethoxam. The interaction 'soil type * active ingredient' was significant as well (Table 26), this interaction shows that imidacloprid affected more beetles in organic soil than thiamethoxam did, but in the mineral soil, both active ingredients performed equally well (80 – 100% affected beetles). In the organic soils, imidacloprid applied in furrow affected high percentages of beetles at both water levels that significantly higher than results for other treatments (LSMeans $P < 0.05$) in the excess water conditions (Figure 9).

On day 45 after planting, the factors 'soil type' and 'active ingredient' continued to be statistically significant (Table 27). Imidacloprid products affected more beetles than did thiamethoxam products. Most treatments in mineral soils were close to the 100% efficacy while the organic soils induced much variability and the percentage of affected beetles was reduced in several treatments. The interaction 'active ingredient * application method' was also significant: while the imidacloprid in-furrow application resulted in more affected beetles than the seed treatment; the thiamethoxam seed treatment performed better than in-furrow application. All the treatments in the optimal soil and water conditions continued to affect > 75% of beetles. In the organic soil high variability between treatments was observed, but imidacloprid in-furrow continued to affect significantly more

Table 26. Effect of neonicotinoid insecticides on percentage affected beetles under differing conditions of soil, water level, application method and active ingredient, on day 38 after planting (linear regression statistics For Type 3 Analysis)

Factor	Numerator Degrees of freedom	Denominator Degrees of freedom	F Value	Pr > F	Chi-Square	Pr > ChiSq
block	4	60	0.62	0.6494	2.48	0.6476
soil	1	60	24.86	***<.0001***	24.86	<.0001
water level	1	60	2.85	0.0966	2.85	0.0914
active ingredient	1	60	6.68	***0.0122***	6.68	0.0098
application method	1	60	2.20	0.1428	2.20	0.1376
soil type*water level	1	60	1.17	0.2830	1.17	0.2787
soil type*active ingredient	1	60	5.11	***0.0274***	5.11	0.0238
water level*active ingredient	1	60	1.64	0.2057	1.64	0.2008
soil type*application method	1	60	4.37	***0.0408***	4.37	0.0366
water level*application method	1	60	1.36	0.2484	1.36	0.2438
active ingredient*application method	1	60	0.83	0.3648	0.83	0.3611
soil type*water level*application method	1	60	4.22	***0.0443***	4.22	0.0399
soil type*water level*active ingredient	1	60	0.46	0.5021	0.46	0.4995
soil type*active ingredient*application method	1	60	0.10	0.7570	0.10	0.7559
water level*active ingredient*application method	1	60	1.08	0.3024	1.08	0.2982
soil type*water level*active ingredient* application method	1	60	1.85	0.1784	1.85	0.1733

Table 27. Effect of neonicotinoid insecticides on percentage affected beetles under differing conditions of soil, water level, application method and active ingredient, on day 45 after planting (linear regression statistics For Type 3 Analysis)

Factor	Numerator Degrees of freedom	Denominator Degrees of freedom	F Value	Pr > F	Chi-Square	Pr > ChiSq
block	4	60	0.34	0.8507	1.36	0.8519
soil	1	60	30.54	***<.0001***	30.54	<.0001
water level	1	60	0.29	0.5912	0.29	0.5892
active ingredient	1	60	12.27	0.0009	12.27	0.0005
application method	1	60	0.00	0.9895	0.00	0.9895
soil type*water level	1	60	0.03	0.8678	0.03	0.8672
soil type*active ingredient	1	60	4.82	***0.0319***	4.82	0.0281
water level*active ingredient	1	60	0.70	0.4047	0.70	0.4014
soil type*application method	1	60	0.78	0.3802	0.78	0.3766
water level*application method	1	60	0.02	0.8756	0.02	0.8751
active ingredient*application method	1	60	5.37	***0.0239***	5.37	0.0205
soil type*water level*active ingredient	1	60	0.46	0.4987	0.46	0.4961
soil type*water level*application method	1	60	0.10	0.7535	0.10	0.7524
soil type*active ingredient*application method	1	60	0.66	0.4190	0.66	0.4157
water level*active ingredient*application method	1	60	0.48	0.4925	0.48	0.4898
soil type*water level*active ingredient*application method	1	60	1.13	0.2916	1.13	0.2873

beetles (statistical significance for LSMeans, $P < 0.05$) than other treatments in that soil.

On day 52 after planting, the factors 'soil type' and 'active ingredient' were again significant (Table 28). More beetles were affected in the mineral soils than in the organic soils and generally more beetles were affected in treatments with imidacloprid compared to treatments with thiamethoxam. 'Water level' was significant, after 4 wks of not showing significant effects; this may be the result of reduced amount of insecticide shortening residual activity. The interaction 'insecticide * application method' was also significant; again, imidacloprid generally was more effective when applied in-furrow, and thiamethoxam performed better as a seed treatment. Within the organic soils the factor 'water level' was not significant statistically but some biological effect may still exist ($X2(1) = 3.76$, $P = 0.0525$). The best-case scenario treatments (mineral soil and normal water) remained at the top with no differences between them. The effect of thiamethoxam in furrow continued to decline and its difference efficacy compared with the top treatments was significantly lower under excess water treatments (LSMeans $P < 0.05$). Under excessive water conditions on mineral soils thiamethoxam in-furrow was significantly less effective ($X2(1) = 4.41$, $P =0.0358$) than the other treatments. In the organic soils all treatments but imidacloprid in-furrow, affected less than 60% of beetles and percentage affected beetles continued a slight, steady decline especially in the excessive water treatments. Thiamethoxam in-furrow was statistically significant less effective

Table 28. Effect of neonicotinoid insecticides on percentage affected beetles under differing conditions of soil, water level, application method and active ingredient, on day 52 after planting (linear regression statistics For Type 3 Analysis)

Source	Numerator Degrees of freedom	Denominator Degrees of freedom	F Value	Pr > F	Chi-Square	Pr > ChiSq
block	4	60	0.90	0.4711	3.59	0.4642
soil	1	60	30.51	***<.0001***	30.51	<.0001
water level	1	60	6.59	***0.0128***	6.59	0.0102
active ingredient	1	60	16.14	***0.0002***	16.14	<.0001
application method	1	60	2.27	0.1373	2.27	0.1321
soil type*water level	1	60	2.46	0.1223	2.46	0.1171
soil type*active ingredient	1	60	0.40	0.5292	0.40	0.5268
water level*active ingredient	1	60	0.85	0.3595	0.85	0.3558
active ingredient*application method	1	60	36.27	***<.0001***	36.27	<.0001
soil type*application method	1	60	0.28	0.5978	0.28	0.5959
water level*application method	1	60	0.00	0.9654	0.00	0.9652
soil type*water level*active ingredient	1	60	0.00	0.9535	0.00	0.9533
soil type*water level*application method	1	60	0.82	0.3690	0.82	0.3654
soil type*active ingredient*application method	1	60	14.81	***0.0003***	14.81	0.0001
water level*active ingredient*application method	1	60	2.82	0.0981	2.82	0.0929
soil type*water level*active ingredient*application method	1	60	1.27	0.2646	1.27	0.2601

(X2(1) = 8.99, P = 0.0027) than thiamethoxam as a seed treatment. In both soil types this active ingredient was affected by excessive water. (Figure 9 c,d)
On day 59 after planting, only the factor 'soil type' was significant with more beetles were affected in mineral soil treatments than in organic soil treatments (Table 29). Also significant was the interaction 'application method * active ingredient'. Imidacloprid was more effective when applied in furrow than when applied as a seed treatment, and thiamethoxam was more effective as seed treatment. This interaction varied with 'soil type' when a second-order interaction 'soil type * active ingredient * application method' was also significant. The decline of affected beetles for thiamethoxam in-furrow in mineral soils stabilized at ca. 70%. In-furrow imidacloprid continued to be the most effective insecticide at both water levels in the organic soil. For the optimal conditions a decline in percentage affected beetles for imidacloprid as seed treatment was observed.

On day 66 after planting, the main factors 'insecticide' and 'soil type' had significantly affected percentage affected beetles (Table 30). Imidacloprid treatments generally affected more beetles than thiamethoxam treatments. The interaction 'insecticide * application method' continued to be significant (Table 30); imidacloprid affected more beetles when applied in furrow and thiamethoxam affected more beetles when applied as seed treatment. 'Water level' was not significant. In-furrow imidacloprid continued to be the most effective in both soil types, and resulted in > 80% of affected beetles. However, for first time in the study, this percentage was not close to the 100% and a slight decline was observed. The interaction 'soil type * water level * active ingredient'

Table 29. Effect of neonicotinoid insecticides on percentage affected beetles under differing conditions of soil, water level, application method and active ingredient, on day 59 after planting (linear regression statistics For Type 3 Analysis)

Source	Numerator Degrees of freedom	Denominator Degrees of freedom	F Value	Pr > F	Chi-Square	Pr > ChiSq
block	4	60	0.72	0.5802	2.89	0.5766
soil	1	60	19.12	***<.0001***	19.12	<.0001
water level	1	60	2.31	0.1338	2.31	0.1286
active ingredient	1	60	0.95	0.3334	0.95	0.3294
application method	1	60	0.24	0.6291	0.24	0.6274
soil type*water level	1	60	2.59	0.1130	2.59	0.1078
soil type*active ingredient	1	60	0.37	0.5432	0.37	0.5409
water level*active ingredient	1	60	1.60	0.2101	1.60	0.2052
soil type*application method	1	60	0.00	0.9449	0.00	0.9447
water level*application method	1	60	0.42	0.5216	0.42	0.5191
active ingredient*application method	1	60	17.38	***<.0001***	17.38	<.0001
soil type*water level*application method	1	60	0.54	0.4671	0.54	0.4642
soil type*water level*active ingredient	1	60	0.16	0.6915	0.16	0.6901
soil type*active ingredient*application method	1	60	4.02	0.0494	4.02	0.0448
water level*active ingredient*application method	1	60	0.21	0.6476	0.21	0.6459
soil type*water level*active ingredient*application method	1	60	1.77	0.1882	1.77	0.1831

Table 30. Effect of neonicotinoid insecticides on percentage affected beetles under differing conditions of soil, water level, application method and active ingredient, on day 66 after planting (linear regression statistics For Type 3 Analysis)

Source	Numerator Degrees of freedom	Denominator Degrees of freedom	F Value	Pr > F	Chi-Square	Pr > ChiSq
block	4	60	0.46	0.7678	1.82	0.7683
soil	1	60	9.17	***0.0036***	9.17	0.0025
water level	1	60	0.00	0.9634	0.00	0.9633
active ingredient	1	60	12.28	***0.0009***	12.28	0.0005
application method	1	60	0.09	0.7710	0.09	0.7700
soil type*water level	1	60	0.07	0.7944	0.07	0.7935
soil type*active ingredient	1	60	2.25	0.1386	2.25	0.1334
water level*active ingredient	1	60	0.45	0.5036	0.45	0.5011
soil type*application method	1	60	1.20	0.2783	1.20	0.2739
water level*application method	1	60	0.07	0.7882	0.07	0.7873
active ingredient*application method	1	60	7.42	***0.0084***	7.42	0.0065
soil type*water level*application method	1	60	0.06	0.8029	0.06	0.8020
soil type*water level*active ingredient	1	60	3.59	0.0631	3.59	0.0583
soil type*active ingredient*application method	1	60	2.12	0.1507	2.12	0.1455
water level*active ingredient*application method	1	60	2.66	0.1081	2.66	0.1029
soil type*water level*active ingredient*application method	1	60	1.28	0.2627	1.28	0.2582

was also significant.

On day 80, 'soil type' continued to have a significant effect, with generally more affected beetles on mineral soils (Table 31). 'Active ingredient' was highly significant in favor of imidacloprid treatments over thiamethoxam. Also significant was the interaction 'active ingredient * application method'. Again imidacloprid was more effective with the in-furrow application and thiamethoxam was more effective with the seed treatment, but this relation was more evident in the organic soil than in the mineral soil. In-furrow imidacloprid continued to affect > 80%or the beetles for both soil types and both water levels. Thiamethoxam in furrow affected < 40% of the beetles in all the conditions.

Overall results The factor 'soil type' was significant throughout the experiment (Table 32), showing the differential effect of the tested soil types on the insecticides' effectiveness (Figure 9). Under normal water conditions percentages of affected beetles were generally 70 – 100% throughout the experiment in mineral soils and 40 – 90% in organic soils (Figure 9). The factor 'active ingredient' was significant; imidacloprid generally affected more beetles than thiamethoxam at the application rates used. Overall the excessive water resulted in significantly reduced percentages of affected beetles (Table 32). 'Days after planting' was significant, showing the expected decline in percentage affected beetles, however the decline was not steep as expected based on the results from the field chapter 2. No overall effect of 'application method' on percentage affected beetles was detected.

Table 31. Effect of neonicotinoid insecticides on percentage affected beetles under differing conditions of soil, water level, application method and active ingredient, on day 80 after planting (linear regression statistics For Type 3 Analysis)

Factor	Numerator Degrees of freedom	Denominator Degrees of freedom	F Value	Pr > F	Chi -Square	Pr > ChiSq
block	4	60	0.52	0.7196	2.09	0.7193
soil	1	60	4.47	***0.0387***	4.47	0.0345
water level	1	60	0.02	0.8796	0.02	0.8791
active ingredient	1	60	17.46	***<.0001***	17.46	<.0001
application method	1	60	0.53	0.4681	0.53	0.4653
soil type*water level	1	60	0.11	0.7397	0.11	0.7385
active ingredient*application method	1	60	8.55	***0.0049***	8.55	0.0035
soil type*active ingredient	1	60	0.22	0.6388	0.22	0.6370
water level*active ingredient	1	60	0.07	0.7898	0.07	0.7889
soil type*application method	1	60	0.10	0.7537	0.10	0.7526
water level*application method	1	60	0.77	0.3835	0.77	0.3800
soil type*water level*active ingredient	1	60	0.00	0.9619	0.00	0.9617
soil type*water level*application method	1	60	0.48	0.4896	0.48	0.4869
soil type*active ingredient*application method	1	60	2.84	0.0971	2.84	0.0919
water level*active ingredient*application method	1	60	2.89	0.0941	2.89	0.0890
soil type*water level*active ingredient*application method	1	60	0.60	0.4427	0.60	0.4396

Table 32. Effect of neonicotinoid insecticides on percentage affected beetles under differing conditions of soil, water level, application method and active ingredient (linear regression statistics For Type 3 Analysis)

Factor	Numerator Degrees of freedom	Denominator Degrees of freedom	F Value	Pr > F	Chi -Square	Pr > ChiSq
block	4	503	1.03	0.3928	4.11	0.3917
days after planting	7	503	10.69	***<.0001***	74.82	<.0001
soil	1	503	143.93	***<.0001***	143.93	<.0001
water level	1	503	6.99	***0.0084***	6.99	0.0082
active ingredient	1	503	55.12	***<.0001***	55.12	<.0001
application method	1	503	0.24	0.6265	0.24	0.6263
days after planting*soil level	7	503	1.69	0.1100	11.80	0.1073
days after planting*water level	7	503	1.13	0.3431	7.90	0.3411
days after planting*active ingredient	7	503	4.34	***0.0001***	30.41	<.0001
days after planting*application method	7	503	0.93	0.4849	6.49	0.4839
soil type*water level	1	503	1.92	0.1660	1.92	0.1653
soil type*active ingredient	1	503	8.96	***0.0029***	8.96	0.0028
soil type*application method	1	503	4.82	***0.0286***	4.82	0.0282
water level*active ingredient	1	503	1.40	0.2367	1.40	0.2362
water level*application method	1	503	1.13	0.2879	1.13	0.2873
active ingredient*application method	1	503	74.93	***<.0001***	74.93	<.0001
days after planting*soil level*water level	7	503	1.00	0.4293	7.01	0.4279

Table 32 (cont'd)

Factor	Numerator Degrees of freedom	Denominator Degrees of freedom	F Value	Pr > F	Chi -Square	Pr > ChiSq
days after planting*soil level*active ingredient	7	503	0.64	0.7238	4.47	0.7241
days after planting*soil level*application method	7	503	0.25	0.9707	1.78	0.9710
days after planting*water level*active ingredient	7	503	0.66	0.7032	4.64	0.7034
days after planting*active ingredient*application method	7	503	3.86	***0.0004***	27.05	0.0003
days after planting*water level*application method	7	503	0.27	0.9649	1.90	0.9652
water level*active ingredient*application method	1	503	0.10	0.7574	0.10	0.7572
soil type*water level*application method	1	503	1.13	0.2882	1.13	0.2877
soil type*active ingredient*application method	1	503	23.74	***<.0001***	23.74	<.0001
soil type*water level*active ingredient	1	503	0.11	0.7403	0.11	0.7402
days after planting*soil level*water level*active ingredient	7	503	0.79	0.5962	5.53	0.5959
days after planting*soil level*water level*application method	7	503	0.65	0.7166	4.53	0.7168
days after planting*soil level*active ingredient*application method	7	503	1.49	0.1681	10.43	0.1653
days after planting*water level*active ingredient*application method	7	503	1.90	0.0679	13.28	0.0655
soil type*water level*active ingredient*application method	1	503	1.01	0.3156	1.01	0.3151
days after planting*soil level*water level*active ingredient *application method	7	503	1.31	0.2450	9.15	0.2424

The significant effect of the interaction 'days after planting * active ingredient' (Table 32) showed that the dynamics for both active ingredients did not follow the same trend. A significant interaction 'soil type * active ingredient' (Table 32) showed that the soil affected each active ingredient to a different extent: while in the mineral soil the imidacloprid products generally caused higher percentages of affected beetles; in the organic soils there was no consistent difference between the two active ingredients

The interaction 'active ingredient * application method' was significant (Table 32) showing a differential effect of the application methods tested in this work for each active ingredient. Imidacloprid affected more beetles when applied in furrow compared with the imidacloprid seed treatment, but thiamethoxam affected more beetles when applied as a seed treatment. This last interaction varied during the experiment resulting in a second order interaction 'days after planting * active ingredient * application method'. The interaction 'soil type * active ingredient * application method' was also significant (Table 32)

Some treatments were less sensitive than others to 'water level' or 'soil type': imidacloprid applied in furrow appeared to be unaffected by the excess water or by the organic soil (Figure 9). The persistence of imidacloprid, as has been reported in leaching studies (and probably enhanced by the application method or the formulation of this commercial product) may be an important factor for this effect. In contrast, thiamethoxam applied to seed or in furrow seemed to be more responsive to water and organic soil.

In the mineral soil treatments, a high initial level of affected beetles was observed in all treatments. After 3 wks effectiveness of thiamethoxam in furrow started to decline, and effects were accentuated by the excess water (Figure 9). The imidacloprid products were apparently non responsive to the water excess.

In the organic soil treatments, imidacloprid, applied in furrow caused the highest percentage of affected beetles. Thiamethoxam applied in furrow for both soils and water levels showed a steady decline all along the experimental period, this decline was also followed by thiamethoxam as seed treatment.

Foliage consumption

Overall, beetles in all treatments consumed little foliage compared to beetles that were fed untreated foliage (Figure 10) and consumption was inversely proportional to percentage of affected beetles, as expected. For the normal water level treatments consumption was less than 20% throughout the experiment, with a slight increase on the last date. For the excess water, consumption in the imidacloprid seed treatment appeared to be consistently around 30%. Although the percentage of affected beetles remained high for the same treatments (Figure 9, c). Higher foliage consumption was observed in the organic soil which agrees with the lower percentage affected beetles from treatments in this soil. High foliage consumption for thiamethoxam in furrow agrees with the low efficacy of that treatment under those conditions. In general excessive water may have had some effect in increasing foliage consumption for both soil types, for imidacloprid as seed treatment and in-furrow thiamethoxam.

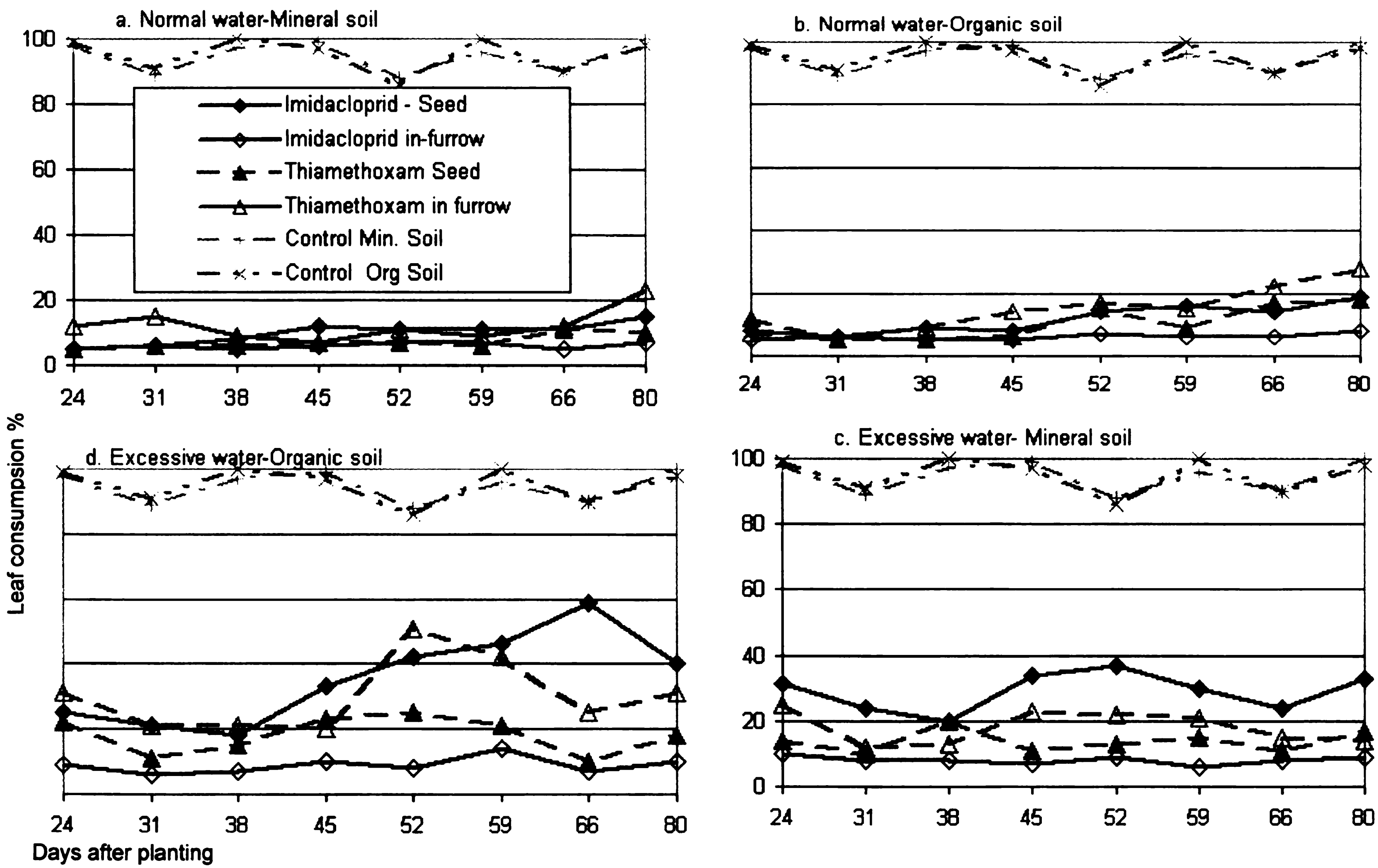


Figure 10. Percentage consumption of neonicotinoid-treated foliage in greenhouse under differing conditions of soil, watering, and application method, by Colorado potato beetles

This response agrees with the percentage affected beetles for these treatments (Figure 9).

Recovery of poisoned beetles

Recovery was observed for poisoned beetles in treatments in organic soils than in mineral soils throughout the study. High levels of recovery (75 - 100%) were registered for imidacloprid as seed treatment in the organic soil throughout the experiment (Figure 11). The organic soil seemed to have increases in recovery sooner than the other conditions. Most of the changes from low to high recovery were seen after day 38 after planting in nearly all cases.

These observations suggest that the residual activity that results in beetle poisoning induces weaker symptoms as time passes and makes the beetles recover sooner. Also, under greenhouse conditions, even though plants seem to maintain insecticide uptake throughout the season, the efficacy of the insecticide is lower as time passes. Zhao et al. (2000) reported more recovery of Colorado potato beetles from topical doses that caused intermediate mortality than from high doses.

Recovery from intoxication has also been observed in beetles treated topically with neonicotinoids (Zhao et al. 2000). However, much more recovery was observed in my study than in Zhao et al. (up to 100% of poisoned beetles recovered in this study). The higher levels of recovery are probably the result of the feeding type assay. Beetles did not receive a measured topical dose but ate only until they became intoxicated and likely did not receive high doses of

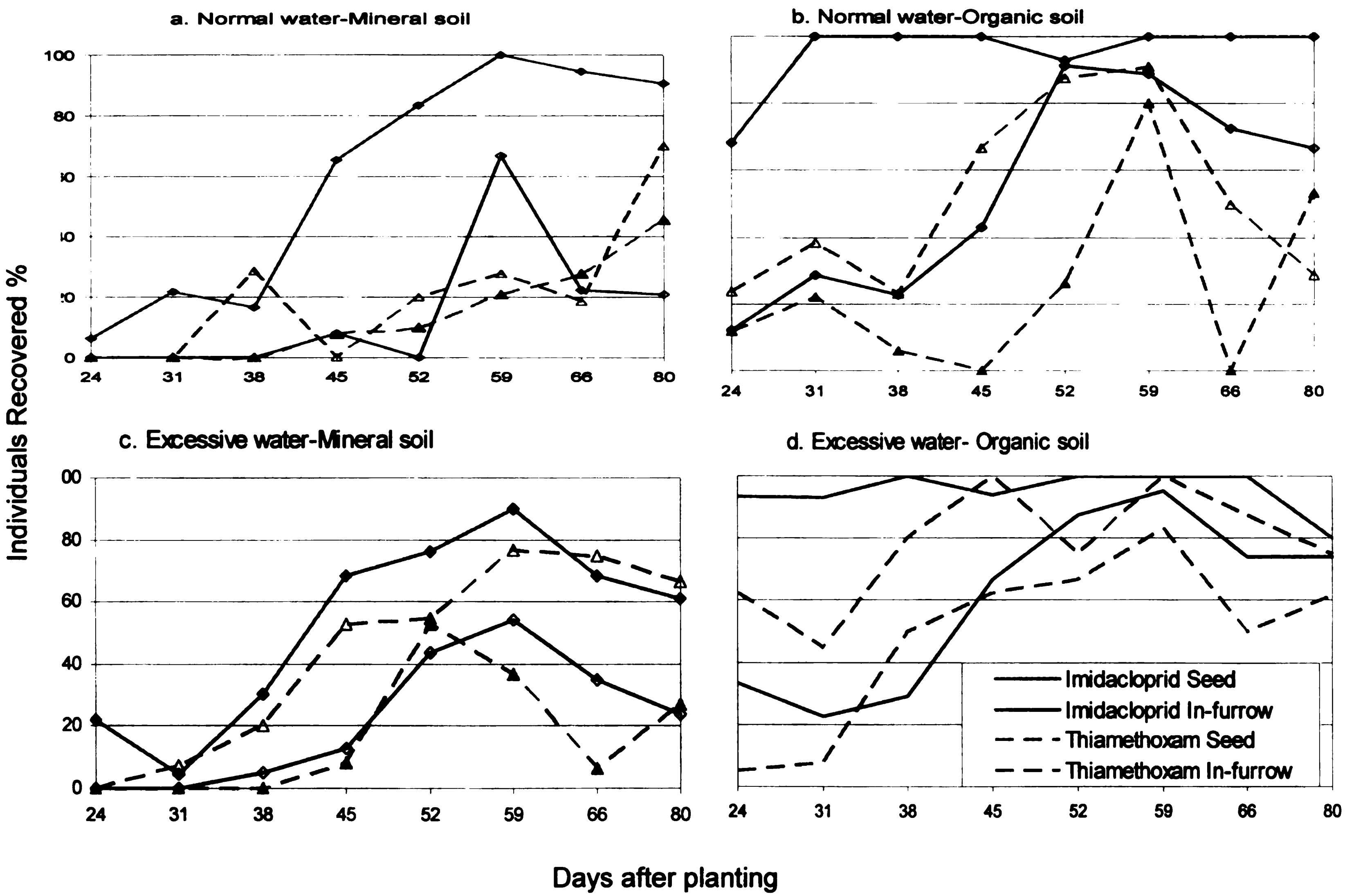


Figure 11. Percentage recovery of adult Colorado potato beetle on day 14 after being poisoned by nicotinoid-treated foliage from greenhouse under differing conditions of soil, watering, and application method

insecticide except in the most potent treatments. The significance for field situations of this recovery 7 d or more after intoxication is not known. It is questionable whether poisoned beetles would survive adverse environmental conditions in the field, predators, etc. for 7 to 14 d in the field and recover to become active pests.

Discussion of 'total affected beetles' variable

'Water level' probably had a delayed effect that was only statistically significant on day 52 and more easily seen for thiamethoxam treatments. After that date, that effect stabilized. Thiamethoxam applied in-furrow may have been more sensitive to the excessive water due to components in the formulation adding to the more water solubility of the active ingredient compared with imidacloprid. In addition, due to differences in product allocation in the in-furrow treatment (along a band as opposed to the more concentrated seed treatment), insecticide exposure to the water flow could have been higher.

For the potted plants in the greenhouse, the organic soil did not have the clear effect that was seen in field studies (Chapter 2). These results however, may

be contrasted with recovery information where the lasting effect of poisoning is tested. Although the recovery information gathered here is preliminary, some general tendencies probably occurred. The medium efficacy levels of imidacloprid seed treatments in soils were followed by high levels of recovery (ca. 90%) in almost the entire study. In contrast, in-furrow treatments, that had high percentage of affected beetles, did not show high recovery levels at the beginning of the season and they reached high recovery values only after day 45 after planting (Figure 11 a, b, c).

Imidacloprid in-furrow had a high efficacy in organic soil and was not affected by the water level at either soil type, as opposed to imidacloprid as seed treatment that was affected in the organic soils but not in the mineral soil by the water level. This was due probably to the lower solubility level of imidacloprid. Surprisingly the organic soil did not affect imidacloprid in furrow, as it did with the other treatments.

For the mineral soils the recovery values are low at the beginning of the study for all treatments (Figure 11 a, c). This corresponds to the high efficacy levels obtained at that time.

At the beginning of the season 'soil type' seems to be a main source of variation with higher recovery in organic soil treatments but that pattern fades in the last weeks of study (Figure 11). The product performances tend to stabilize for this response variable. Except that imidacloprid in-furrow and both thiamethoxam treatments in mineral soil shoed low recovery during most of the experiment.

Dead beetles (no movement, darkened color, sunken abdomen)

Mortality from neonicotinoids is slower than with other insecticides, despite intoxication occurring rapidly. Zhao el al. (2000) reported initial knock down in 2.4 h but no beetles died in the first day after treatment. In my study even on day 7, fewer beetles were dead than were rated as affected (poisoned or dead) in most treatments (Figure 9 vs. Figure 12). Dead plus affected beetles is probably the more accurate category for measuring overall treatment impact early in the course of this study. However, the high percentages of recovery late in the study, 7 – 14 days after treatment, in some treatments, confuses the pictures. As mentioned in the recovery section, whether recovery 7 – 14 d after treatments occurs under field conditions is unknown.

Weekly results. On day 24 after planting, the factor 'soil type' was the only main factor significantly affecting percentage mortality (Table 33). 'Water level' did not have a significant effect and this could be associated with small plants and short roots in the young plants that were tested on this date because all of them could reach the insecticide. Neither 'active ingredient' nor 'application method' had a significant effect on this date. Within the mineral soil, only 'active ingredient' was highly significant ($X^2_{(1)}$= 17.76, P <0.0001); thiamethoxam products caused higher mortality than imidacloprid, in all cases. Only thiamethoxam as seed treatment on mineral soil at both water levels was above 60% mortality. These insecticide treatments caused significantly higher mortality (LSMeans P < 0.05) than the other treatments on mineral soils or organic soil treatments. The other treatments in mineral soils reached low to medium mortality levels. In the organic soil no mortality was higher than 10%.

On day 31 after planting, 'soil type' was the only factor significantly affecting percentage mortality (Table 34). Within the mineral soils only, 'active ingredient' was highly significant ($X^2_{(1)}$= 51.22, p <.0001). 'Water level' was not

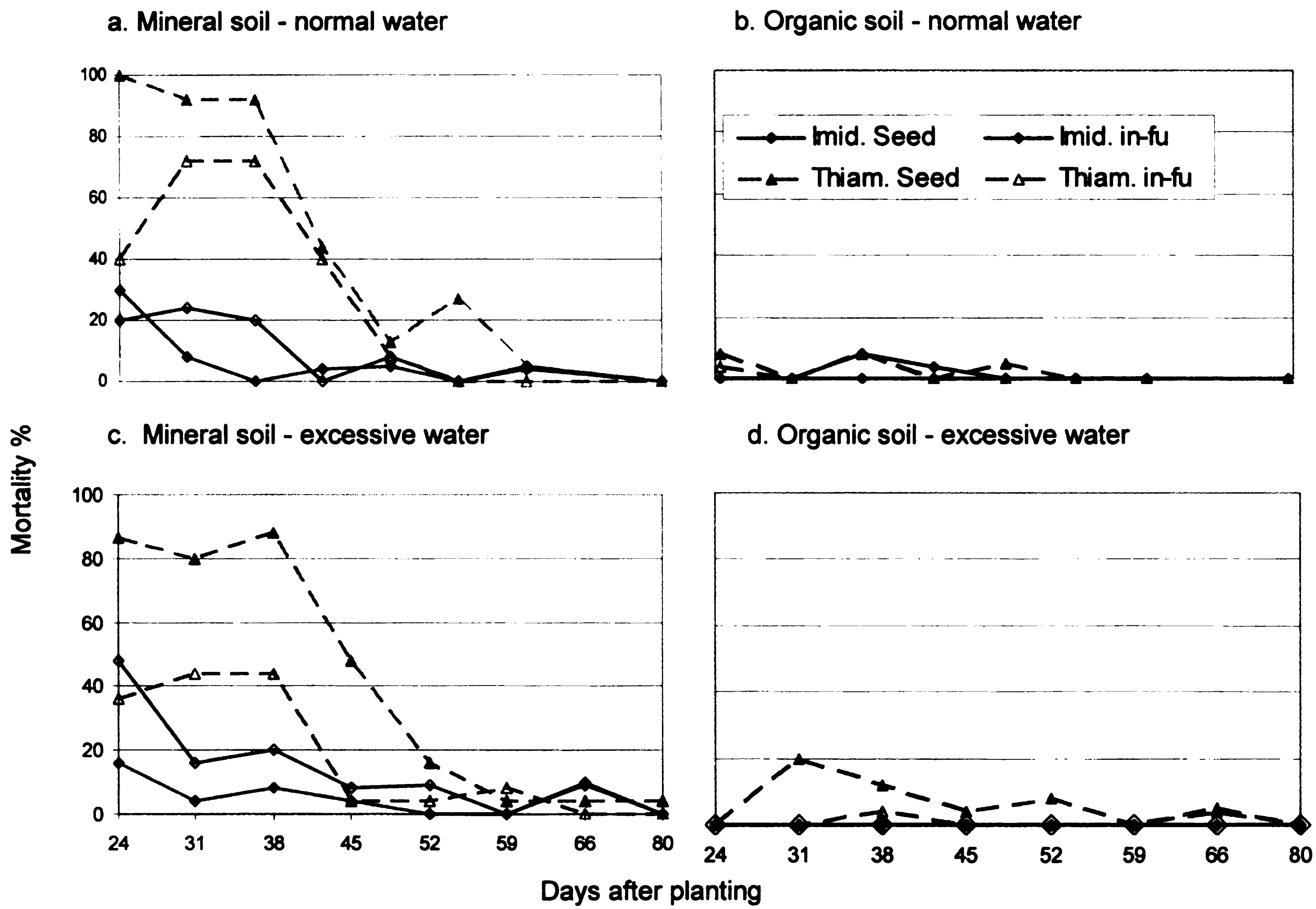


Figure 12. Percentage beetle mortality by neonicotinoid insecticides from foliage treated in greenhouse under differing conditions of soil, watering, and application method

Table 33. Effect of neonicotinoid insecticides on mortality percentage of beetles under differing conditions of soil, water level, application method and active ingredient, on day 24 after planting under greenhouse (linear regression statistics For Type 3 Analysis)

Source Factor	Numerator Degrees of freedom	Denominator Degrees of freedom	F Value	Pr > F	Chi-Square	Pr > ChiSq
block	4	56	2.12	0.0897	8.50	0.0749
soil	1	56	145.81	*<.0001*	145.81	<.0001
water level	1	56	0.58	0.4491	0.58	0.4459
active ingredient	1	56	1.14	0.2894	1.14	0.2848
application method	1	56	0.04	0.8441	0.04	0.8434
soil type*water level	1	56	0.55	0.4629	0.55	0.4598
soil type*active ingredient	1	56	0.19	0.6686	0.19	0.6669
water level*active ingredient	1	56	0.71	0.4046	0.71	0.4010
soil type*application method	1	56	0.00	0.9699	0.00	0.9698
water level*application method	1	56	0.08	0.7848	0.08	0.7838
active ingredient*application method	1	56	0.13	0.7227	0.13	0.7214
soil type*water level*active ingredient	1	56	0.52	0.4759	0.52	0.4729
soil type*water level*application method	1	56	0.01	0.9069	0.01	0.9065
soil type*active ingredient*application method	1	56	0.04	0.8415	0.04	0.8408
water level*active ingredient*application method	1	56	0.01	0.9349	0.01	0.9346
soil type*water level*active ingredient*application method	1	56	0.06	0.8116	0.06	0.8108

Table 34 Effect of neonicotinoid insecticides on mortality percentage of beetles under differing conditions of soil, water level, application method and active ingredient, on day 31 after planting under greenhouse (linear regression statistics For Type 3 Analysis)

Source	Numerator Degrees of freedom	Denominator Degrees of freedom	F Value	Pr > F	Chi-Square	Pr > ChiSq
block	4	59	1.62	0.1806	6.49	0.1654
soil	1	59	69.70	***<.0001***	69.70	<.0001
water level	1	59	0.12	0.7352	0.12	0.7340
active ingredient	1	59	1.48	0.2288	1.48	0.2240
application method	1	59	0.12	0.7264	0.12	0.7251
soil type*water level	1	59	0.50	0.4844	0.50	0.4816
soil type*active ingredient	1	59	0.11	0.7427	0.11	0.7415
water level*active ingredient	1	59	0.33	0.5668	0.33	0.5646
active ingredient*application method	1	59	0.74	0.3925	0.74	0.3890
soil type*application method	1	59	0.48	0.4909	0.48	0.4882
water level*application method	1	59	0.29	0.5925	0.29	0.5904
soil type*water level*active ingredient	1	59	0.23	0.6347	0.23	0.6330
soil type*water level*application method	1	59	0.27	0.6076	0.27	0.6057
soil type*active ingredient*application method	1	59	0.02	0.8790	0.02	0.8784
water level*active ingredient*application method	1	59	0.35	0.5536	0.35	0.5513
soil type*water level*active ingredient*application method	1	59	0.21	0.6493	0.21	0.6476

significant within the mineral soils. Thiamethoxam as a seed treatment continued to be the top treatment in mineral soils at both water levels (Figure 12 a, b). At normal water and in mineral soils, thiamethoxam caused the same mortality with either application method. The impact of water excess on the percentage mortality was not significant ($x^2_{(1)}$= 3.20, P = 0.0736). On this date three treatments caused > 70% mortality. Imidacloprid products fell below 20% mortality. In the organic soil, mortality in all treatments was near zero.

On day 38 after planting 'active ingredient' was the only factor that significantly affected percentage mortality, as was also shown by the LR Analysis (Table 35). However soil type was also significant using LSMeans ($x^2_{(1)}$= 5.71, p 0.02). The same three treatments continued to cause > 70% mortality. Thiamethoxam treatments caused similar levels of mortality to results of the previous weeks and were again statistically significantly higher than imidacloprid insecticides, all on mineral soil treatments.

On day 45 after planting soil type significantly affected percentage mortality (Table 36). There was also a 'block' effect observed on this date. Although 'active ingredient' did not significantly affect mortality in this analysis, the differences between the two active ingredients within the mineral soil was significant ($x^2_{(1)}$=7.39 p 0.0066) and thiamethoxam products continued to show better performance than imidacloprid Thiamethoxam products on the mineral soil started to reduce the obtained mortality levels. All of the thiamethoxam treatments decreased in mortality. Thiamethoxam as seed treatments was now around 40% at both water levels and thiamethoxam as in-furrow (Platinum)

Table 35 Effect of neonicotinoid insecticides on mortality percentage of beetles under differing conditions of soil, water level, application method and active ingredient, on day 38 after planting under greenhouse (linear regression statistics For Type 3 Analysis)

Source	Numerator Degrees of freedom	Denominator Degrees of freedom	F Value	Pr > F	Chi-Square	Pr > ChiSq
block	4	60	1.16	0.3362	4.65	0.3249
soil	1	60	2.31	0.1338	2.31	0.1285
water level	1	60	0.01	0.9163	0.01	0.9160
active ingredient	1	60	29.99	*<.0001*	29.99	<.0001
application method	1	60	0.72	0.3993	0.72	0.3959
soil type*water level	1	60	0.71	0.4025	0.71	0.3991
soil type*active ingredient	1	60	0.00	0.9714	0.00	0.9712
water level*active ingredient	1	60	0.01	0.9159	0.01	0.9156
soil type*application method	1	60	0.06	0.8027	0.06	0.8018
water level*application method	1	60	0.97	0.3287	0.97	0.3248
active ingredient*application method	1	60	1.30	0.2583	1.30	0.2538
soil type*water level*application method	1	60	0.01	0.9341	0.01	0.9338
soil type*water level*active ingredient	1	60	0.79	0.3783	0.79	0.3748
soil type*active ingredient*application method	1	60	0.05	0.8281	0.05	0.8273
water level*active ingredient*application method	1	60	0.53	0.4693	0.53	0.4665
soil type*water level*active ingredient*application method	1	60	0.01	0.9345	0.01	0.9342

Table 36 Effect of neonicotinoid insecticides on mortality percentage of beetles under differing conditions of soil, water level, application method and active ingredient, on day 45 after planting under greenhouse (linear regression statistics For Type 3 Analysis)

Factor	Numerator Degrees of freedom	Denominator Degrees of freedom	F Value	Pr > F	Chi-Square	Pr > ChiSq
block	4	60	2.41	0.0587	9.65	0.0467
soil	1	60	3.82	0.0553	3.82	0.0506
water level	1	60	0.03	0.8597	0.03	0.8591
active ingredient	1	60	1.00	0.3213	1.00	0.3173
application method	1	60	0.31	0.5788	0.31	0.5767
soil type*water level	1	60	0.03	0.8597	0.03	0.8591
soil type*active ingredient	1	60	1.00	0.3213	1.00	0.3173
water level*active ingredient	1	60	0.00	0.9826	0.00	0.9825
soil type*application method	1	60	0.31	0.5788	0.31	0.5767
water level*application method	1	60	0.29	0.5929	0.29	0.5909
active ingredient*application method	1	60	0.49	0.4877	0.49	0.4850
soil type*water level*active ingredient	1	60	1.55	0.2174	1.55	0.2126
soil type*water level*application method	1	60	0.71	0.4012	0.71	0.3978
soil type*active ingredient*application method	1	60	0.47	0.4961	0.47	0.4934
water level*active ingredient*application method	1	60	0.46	0.5000	0.46	0.4974
soil type*water level*active ingredient*application method	1	60	0.46	0.5000	0.46	0.4974

is 40% at normal water and less than 10% at excessive water and this differences are statistically significant ($X^2_{(1)}$ = 9.48, P = 0.0021). Thiamethoxam treatments on medium level of efficacy kept significant differences (LSMeans P < 0.05) with the bottom-controlling treatments. None of the treatments in the organic soil show more than 10% mortality.

On day 52 after planting no significant effects were observed on this date (Table 37). All treatments dropped to around < 20% mortality or less. No significant treatments effects were detected for this date.

On day 59 after planting no significant treatment effects were observed (Table 38) and mortality was < 10% in all treatments but thiamethoxam as seed treatment in the mineral soil. The higher level of mortality of this treatment was significant.

On day 66 and day 80 all treatments showed mortality levels near or equal to zero. No analysis was conducted.

Overall analysis for the 'dead beetles' variable

Overall, the soil type significantly affected percentage mortality (Table 39). The detrimental effect of the organic soil produced marked differences in mortality in contrast to the lower (but still significant) impact on percentage affected beetles. The factor 'active ingredient' was also significant; thiamethoxam products caused more mortality during the time span the insecticides were active for mortality. In contrast, imidacloprid often caused more "affected beetles" (poisoned plus dead) no other main factor effect or interactions were significant

Table 37 Effect of neonicotinoid insecticides on mortality percentage of beetles under differing conditions of soil, water level, application method and active ingredient, day 52 after planting under greenhouse (linear regression statistics For Type 3 Analysis)

Source	Numerator Degrees of freedom	Denominator Degrees of freedom	F Value	Pr > F	Chi-Square	Pr > ChiSq
block	4	60	0.69	0.6013	2.76	0.5984
soil	1	60	2.95	0.0909	2.95	0.0858
water level	1	60	0.10	0.7549	0.10	0.7538
active ingredient	1	60	1.43	0.2365	1.43	0.2318
application method	1	60	0.22	0.6433	0.22	0.6416
soil type*water level	1	60	0.21	0.6459	0.21	0.6442
soil type*active ingredient	1	60	0.05	0.8238	0.05	0.8231
water level*active ingredient	1	60	0.14	0.7067	0.14	0.7053
soil type*application method	1	60	1.03	0.3140	1.03	0.3099
water level*application method	1	60	0.03	0.8722	0.03	0.8717
active ingredient*application method	1	60	1.68	0.1997	1.68	0.1947
soil type*water level*active ingredient	1	60	0.05	0.8208	0.05	0.8201
soil type*water level*application method	1	60	0.10	0.7549	0.10	0.7538
soil type*active ingredient*application method	1	60	0.00	0.9441	0.00	0.9438
water level*active ingredient*application method	1	60	0.27	0.6022	0.27	0.6002
soil type*water level*active ingredient*application method	1	60	0.14	0.7067	0.14	0.7053

Table 38 Effect of neonicotinoid insecticides on mortality percentage of beetles under differing conditions of soil, water level, application method and active ingredient, day 59 after planting under greenhouse (linear regression statistics For Type 3 Analysis)

Factor	Numerator Degrees of freedom	Denominator Degrees of freedom	F Value	Pr > F	Chi-Square	Pr > ChiSq
blk	4	60	0.72	0.5802	2.89	0.5766
soil type	1	60	19.12	***<.0001***	19.12	<.0001
water level	1	60	2.31	0.1338	2.31	0.1286
active ingredient	1	60	0.95	0.3334	0.95	0.3294
application method	1	60	0.24	0.6291	0.24	0.6274
soil type*water level	1	60	2.59	0.1130	2.59	0.1078
soil type*active ingredient	1	60	0.37	0.5432	0.37	0.5409
water level*active ingredient	1	60	1.60	0.2101	1.60	0.2052
soil type*application method	1	60	0.00	0.9449	0.00	0.9447
water level*application method	1	60	0.42	0.5216	0.42	0.5191
active ingredient*application method	1	60	17.38	***<.0001***	17.38	<.0001
soil type*water level*active ingredient	1	60	0.16	0.6915	0.16	0.6901
soil type*water level*application method	1	60	0.54	0.4671	0.54	0.4642
soil type*active ingredient*application method	1	60	4.02	0.0494	4.02	0.0448
water level*active ingredient*application method	1	60	0.21	0.6476	0.21	0.6459
soil type*water level*active ingredient*application method	1	60	1.77	0.1882	1.77	0.1831

Table 39. Effect of neonicotinoid insecticides on mortality percentage of beetles under differing conditions of soil, water level, application method and active ingredient (linear Regression Statistics For Type 3 Analysis)

Source	Numerator Degrees of freedom	Denominator Degrees of freedom	F Value	Pr > F	Chi-Square	Pr > ChiSq
block	4	503	4.49	***0.0014***	17.98	0.0012
days after planting	7	503	3.27	***0.0021***	22.88	0.0018
soil	1	503	28.15	***<.0001***	28.15	<.0001
water level	1	503	0.07	0.7903	0.07	0.7902
active ingredient	1	503	9.05	***0.0028***	9.05	0.0026
application method	1	503	0.66	0.4185	0.66	0.4182
days after planting*soil level	7	503	1.79	0.0873	12.52	0.0847
days after planting*water level	7	503	0.26	0.9674	1.85	0.9677
soil type*water level	1	503	0.05	0.8308	0.05	0.8307
days after planting*active ingredient	7	503	0.95	0.4699	6.63	0.4687
soil type*active ingredient	1	503	0.10	0.7538	0.10	0.7536
water level*active ingredient	1	503	0.17	0.6761	0.17	0.6759
days after planting*application method	7	503	0.30	0.9546	2.09	0.9549
soil type*application method	1	503	0.00	0.9676	0.00	0.9676
water level*application method	1	503	0.25	0.6157	0.25	0.6155
active ingredient*application method	1	503	4.75	***0.0298***	4.75	0.0294
days after planting*soil level*water level	7	503	0.44	0.8747	3.10	0.8753
days after planting*soil level*active ingredient	7	503	0.70	0.6764	4.87	0.6764

Table 39. (cont'd)

Source	Numerator Degrees of freedom	Denominator Degrees of freedom	F Value	Pr > F	Chi-Square	Pr > ChiSq
days after planting*water level*active ingredient	7	503	0.25	0.9705	1.78	0.9708
soil type*water level*active ingredient	1	503	0.55	0.4596	0.55	0.4592
days after planting*soil level*application method	7	503	0.43	0.8828	3.02	0.8834
days after planting*water level*application method	7	503	0.34	0.9375	2.35	0.9379
soil type*water level*application method	1	503	0.59	0.4434	0.59	0.4431
days after planting*active ingredient*application method	7	503	0.21	0.9829	1.48	0.9831
soil type*active ingredient*application method	1	503	0.13	0.7174	0.13	0.7173
water level*active ingredient*application method	1	503	0.12	0.7310	0.12	0.7308
days after planting*soil level*water level*active ingredient	7	503	0.60	0.7558	4.20	0.7562
days after planting*soil level*water level*application method	7	503	0.14	0.9948	0.99	0.9949
days after planting*soil level*water level*application method	7	503	0.14	0.9948	0.99	0.9949
days after planting*soil level*active ingredient*application method	7	503	0.19	0.9865	1.36	0.9867
days after planting*water level*active ingredient*application method	7	503	0.34	0.9365	2.36	0.9370
soil type*water level*active ingredient*application method	1	503	0.03	0.8565	0.03	0.8564
days after planting*soil level*water level*active ingredient *application method	7	503	0.19	0.9870	1.35	0.9871

for the total analysis.

Highly significant differences occurred in percentage mortality between the two soil types. For the mineral soils, mortality in all insecticides declined to less than 20% by day 52. For the organic coil mortality was never > 20%The effect of soil tends to diminish after day 38 and then all treatments produced low mortality after that.

In thiamethoxam treatments mortality was significantly higher than in imidacloprid treatments until about day 52 as well. The excessive water may have a slight positive effect on these products on organic soil, perhaps compensating slightly for binding with the organic soil fraction. Due to the low mortality in the organic soils no LT50 could be calculated for this soil type. In the mineral soil LT50 only could be calculated for the thiamethoxam treatments that resulted in some mortality. Under these conditions the expected declining curve of mortality is only shown by the thiamethoxam treatments (Figure 13)

Treatment residual activity

The percentage of dead beetles in most treatments was < 50% and probit analysis could not be run. Only three treatments were included in this analysis, all of them for mineral soils with thiamethoxam as active ingredient. The treatments were: seed treatment and in-furrow application for the normal water and the seed treatment for the excessive water. The three treatments analyzed had similar Median Lethal Time LT50 values of 32.6 to 45.4 d (Table 40).

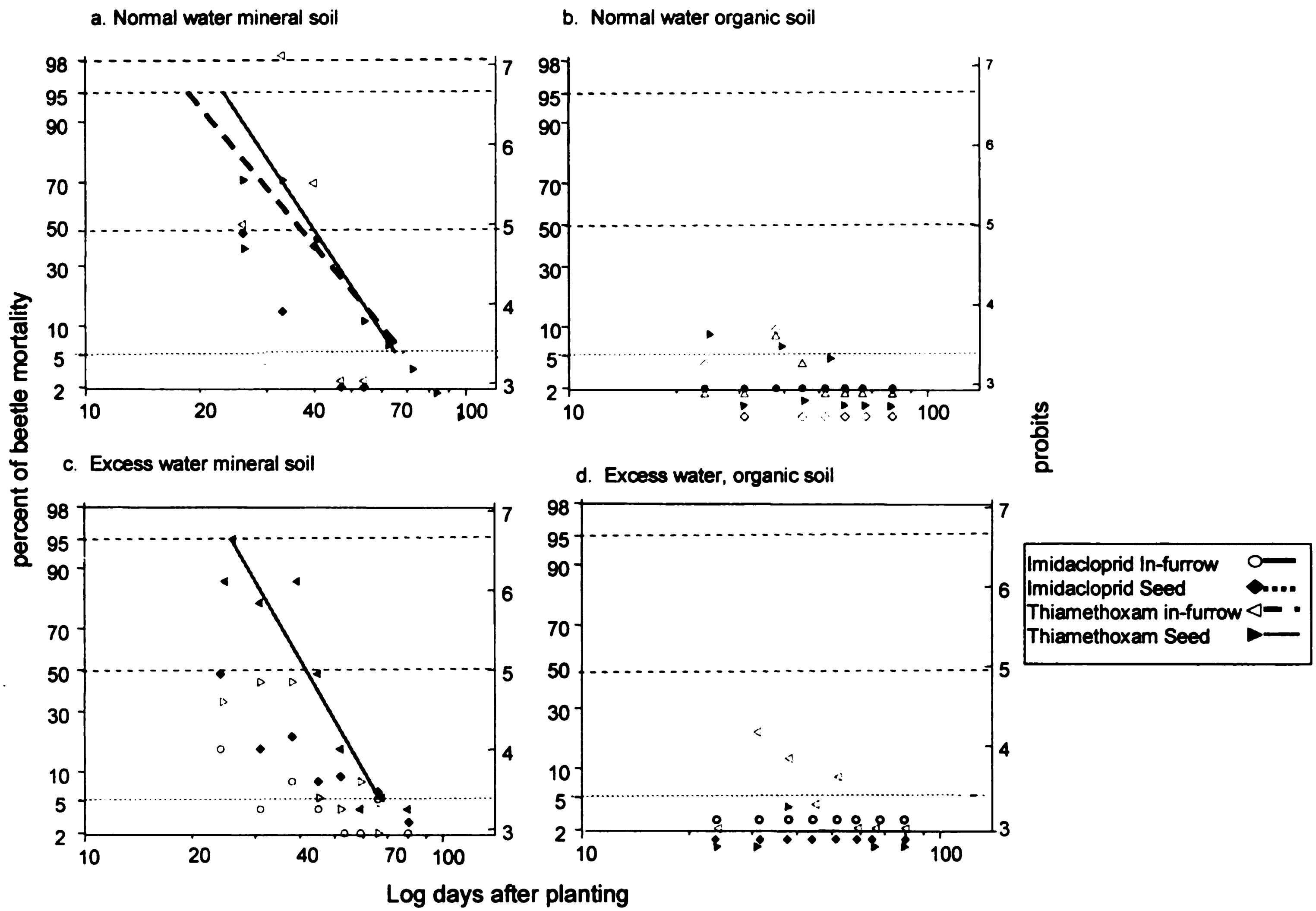


Figure 13. Log *days after planting* probit lines for mortality of Colorado potato beetles when fed with neonicotinoid-treated foliage from greenhouse under differing conditions of watering, soil, and application method

Table 40. Probit analysis for the evaluation of neonicotinoinds for control of the Colorado potato beetle under greenhouse conditions - Mineral soils only

Treatment (a.i., apl. meth., water level)	LT_{50} (days)	LT_{50} grouping	Fiducial limit 0.95	Slope ± SE	Pearson $X^2/df_{=2}$ (p value)
Imidacloprid, in furrow, normal water				*	
Imidacloprid, seed, normal water				*	
Thiamethoxam, in-furrow, normal water	32.55	a	1.09 - 46.17	-4.94 ± 1.90	5.88 (0.12)
Thiamethoxam, seed, normal water	45.37	a	40.14 - 50.36	-10.49 ± 1.90	13.09 (0.04)
Imidacloprid, in furrow, excessive water				*	
Imidacloprid, seed, excessive water				*	
Thiamethoxam, in furrow, excessive water				*	
Thiamethoxam, seed, excessive water	41.86	a	33.77 - 49.07	-7.25 ± 1.30	19.70 (0.003)

* Mortality levels below 50% throughout the experiment

Values of LT_{50} followed by the same letter are not significantly different, by not overlapping confidence intervals

General discussion

Imidacloprid as seed treatment showed medium to high percentages of affected beetles in treatment combinations, but its mortality levels were very low. This difference however, agrees with the higher foliage consumption by the end of the study period (compared with the rest of the treatments, Figure 10) and high recovery (Figure11).

Imidacloprid applied in furrow, also showed a high percentage of affected beetles (Figure 9) and resulted in low foliage consumption (Figure 10). Recovery of the beetles poisoned in this treatment was especially common in the second half of the study (Figure 11), although that recovery was not associated with foliage consumption. Even though in-furrow imidacloprid showed high recovery and low beetle mortality, the low foliage consumption is a positive effect of those treatments, which would give this insecticide eligibility as plant protection tool.

The effect of the excessive water used in this study was generally low, contrary to what I had expected given the high solubility values imidacloprid and thiamethoxam. These results however, support the findings that no effect of water-saturated soil on the persistence of imidacloprid has been reported (Indumathi et al. 2001). An initial small difference to the effect of water is rapidly balanced and only a late effect was observed in the in-furrow thiamethoxam treatments. The watering technique I used probably created conditions of water saturation but not the water flow dynamics that may occur in the soil when low and constant irrigations are used. Improvement of imidacloprid performance by means of irrigation system manipulation has been demonstrated for potted plants (van Iersel et al. 2001). However this benefit seems to be possible only under certain range of water content, way below the field capacity where more interaction insecticide-water flow is allowed improving insecticide uptake. This was not the case in this study due to the high requirements of potato plants under these greenhouse conditions (Figure 7) and the poor drainage conditions observed. Still water level may have some effect in reducing the numbers of affected beetles and shortening residual activity in sub-lethal (total affected beetles) but not for lethal effects (dead beetles).

Organic soils not only induced a lower performance on the insecticides, also makes the interactions with the soil factors more variable and less predictive.

The number of total affected beetles by imidacloprid in-furrow was not affected by the soil type in the greenhouse. This is contrary to what was observed in the field (Chapter 2). No clear explanation for this finding can be reported at this time; the rapid plant growth, the irrigation regime, and higher desorption of the active ingredient, may all could be involved in this response. For the potted plants the root system has more chance to use the applied insecticide, perhaps explaining this phenomenon. By the end of the experiment plant roots had entirely filled the pots, as can be seen in Figure 14.

The noticeable difference between the variables poisoned and dead beetles in the greenhouse experiments could be explained in part by the differences in symptoms that sub lethal and lethal neonicotinoid poisoning have.

Thus, for the experimental arrangement used, the pot probably generated favorable conditions for

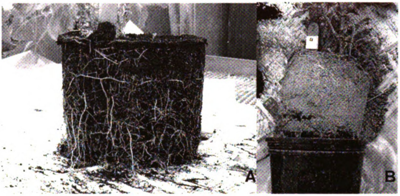


Figure 14. Root growth of potted potatoes grown under greenhouse conditions to study residual activity of neonicotinoid insecticides in mineral soil (A) and organic soil (B).

sustained insecticide availability at low concentration, producing sub-lethal effects. The insecticide that is taken by the potted plants could be not enough to kill or severally poisoned the beetles, but just to produce a knock-down effect, that did not last more than 7 d and recovery occurred during that time.

This difference between percentage affected and percentage dead beetles appeared to be larger for imidacloprid treatments, suggesting that imidacloprid has a broader range of concentration for the production of sub-lethal effects, than thiamethoxam.

The effect of application method could have been masked by the set up conditions used in the experiment. After planting, and with the continuous watering and confinement in the pot, the insecticides distribution in the pots could be equalized for the two application methods, this is supported by the fact that movement of neonicotinoids is reported to be active only in 20 – 30 cm upper levels of the soil (van Iersel et al. 2001, Krohn and Hellpointner 2002). Additionally the appropriate greenhouse conditions for the plants make them grow fast and rapidly place their roots in the available space.

All responses in affected beetles may have been influenced by the arrangement in which the plants were set up. More effect was seen in imidacloprid products than in thiamethoxam. The pots I used were 22 cm tall but the root system of this potato variety may go far beyond that. After removing plants form the pots it was observed that the root system had grown to much of the available spaces in the pot. As the plant grows, and due to the persistence of the neonicotinoids in the first 30 cm of the soil, the root system may be taken more insecticide that is desorbed through time in the pots. These conditions did not promote insecticide uptake to levels enough to cause more mortality on beetles.

Uptake and biological effect of neonicotinoids in potato seem to be very related with plant physiology and the environmental conditions that govern plant growth and development. An optimal uptake could be obtained if the root system is always in the zone were the insecticides are present in the soil.

Conclusions Greenhouse studies

A regime of excessive water in the way was applied here, did not have a detrimental effect for the residual activity of neonicotinoids.

Confined conditions for plant development, like potted plants provide them with a modified environment that affect neonicotinoid residual activity, presumably by affecting insecticide uptake.

Beetle true mortality is a variable that define better a differential effect of the two neonicotinoids studied.

A complement of the analysis for insecticide effect is the recovery that some treatments may be followed by.

CHAPTER 4:

OVERALL CONCLUSIONS

Imidacloprid and thiamethoxam are good options for control of Colorado potato beetle adults. Both products show ≥ 80% affected beetles or more under field conditions with loam sandy soil, which is the most common type of soil for potato production.

Although soil- or seed-applied neonicotinoid insecticides are a good strategy for control of the Colorado potato beetle, reduced activity may occur if factors like insecticide tolerance or high organic matter content are present.

Organic matter content is detrimental to the residual activity of neonicotinoid insecticides when used as at planting applications for Colorado potato beetle control. Seed treatments may reduce this detrimental effect compared to in-furrow applications.

When applied in organic soils such as the muck soils studied here, efficacy and residual activity of neonicotinoids was severely hampered. Under greenhouse experiments this detrimental effect was even more apparent on percentage beetle mortality than on percentage affected beetles.

Planting conditions, like those imposed under greenhouse, may alter the expected detrimental effect of organic soils on residual activity of neonicotinoid insecticides. Different patter of water flow, root growth would be associated with these changes. However, when lethal effects (dead beetles) were recorded the depressing effect of organic soils on these neonicotinoid insecticides efficacies was clearly observed.

Water regime at the levels tested in this study may have an impact in reduce the activity of thiamethoxam soil applied formulations. Excessive water regimes may reduce thiamethoxam efficacy. In this case seed treatments may be an alternative to limit that effect.

Both experiments showed that there is potential for a reduced efficacy in the middle of the season (about day 50 after application) for thiamethoxam applied in furrow (Platinum) and the water excess may be associated with that potential.

The low rate used in this work did not reduce product efficacy; it provided good performance and could be used under commercial conditions to reduce the amount of product applied thus reducing application costs.

No general conclusion about the effect of excess water can be drawn from this study; however these results indicated that excess water may be associated with reduced biological activity.

A steep decline in percent affected beetles under field conditions is expected by day 40-45 after application.

Imidacloprid products are more sensitive than thiamethoxam to adverse factors studied in this research.

Seed treatment applications may help circumvent the detrimental effect of organic soils. Effect of application method is variable in mineral soil, but in furrow applications tended to have longer residual activity.

Greenhouse conditions such as potted plants, have strong effect in the residual activity of neonicotinoid insecticides, making it difficult to apply results to field situations. Changes in the plant physiology may be involved in such effect.

CHAPTER 5:

RESEARCH PROJECTION

The hypothesis about the negative influence of organic soils on neonicotinoid activity was confirmed by this research. Soil type, related to organic matter content, strongly determines neonicotinoid residual activity when applied at planting for control of Colorado potato beetle. This phenomenon was observed between the two sites of the field studies and confirmed in the greenhouse studies.

Once the association of organic soil with a drastic reduction in insecticide efficacy is determined, the next step is to determine what level of organic matter is critical in reducing the efficacy of the products. Also important it is to determine if certain types of soil (from those found in commercial potato areas) are more sensitive to changes in this soil feature.

Feeding bioassays are a useful tool for determination of insecticide biological activity and residual activity of neonicotinoid insecticides on Colorado potato beetle under controlled conditions. This technique could be used in future studies that address similar questions to those approached here. Particularly, keeping turgid leaves, during the time each bioassay was conducted, was important for consistent results.

The set up used here, worked well when plants from the field were taken. However, for the plants kept in greenhouse, it may need some adjustments to more effectively reflect the conditions that plants encounter under field conditions. Some of the adjustments to the set ups use here could include:

bigger pots, non disturbed soil and a watering regime that better simulates the rainfall situation that a potato crop has in the field.

Confirming that the reduced application rate does not have important effect on affected beetles or dead beetles is important in terms of optimizing the management program. Reduced rates are expected to results in shorter residual activity. However, under the production conditions of mineral soil and susceptible beetle strains, the reduced rates may give results as good as the full rate.

The excessive irrigation simulated here was chosen as one of the extreme situations that can occur under field conditions. However this is not the only variable that can be encountered in the field and further evaluations are required where other regimes may be tested for their effect on neonicotinoid efficacy and potential leaching. More detailed research is recommended on the effects that excessive rainfall seasons may be causing for neonicotinoid programs of Colorado potato beetle control, testing a more precise level and frequency of rainfall event that may be detrimental for insecticide performance.

Future research on effects of irrigation and rainfall should be conducted in the field with undisturbed soil structure. Watering regimes that affects potato root growth in the soil profile should be considered as a variable.

Complementarily to what was seen here, overall length of effective control in the field is likely to be longer than reported here because larvae, which commonly occur early in the season, are much more susceptible to these products than adults.

The neonicotinoid insecticides evaluated here give effective control under the common conditions for potato growth. However detrimental factors like organic soil or a low level of resistance to imidacloprid may induce a reduction in efficacy. Longer residual activity is in general expected for soils with a sand fraction and mineral composition than in organic soils. Duration of protection found here and elsewhere shows that periods of 40 -50 days are acceptable as good treatments. Imidacloprid however has been found to have long lasting protection against aphids (3-4 months). This difference may be because the Colorado potato beetle consumes the entire foliage, with a lower concentration of insecticide than the phloem sap ingested by sucking insects.

Thiamethoxam had a more lethal effect in my experiments perhaps because this insecticide is metabolized to clothianidin before lethal action is reached. This could allow the insect to ingest more treated foliage before becoming intoxicated and consequently to reach lethal amounts of the insecticide.

Although these experiments depicted an approximate idea of the performance of the insecticide, when used in control, caution must be taken if the results are intended to be extrapolated to field conditions. This is true especially because of the artificial conditions that the feeding bioassays impose. Two factors are related to the conditions of the experiment: (1) the protection the beetles have inside the Petri dish with no other factor inflicting mortality, and (2) the continuous feeding stops that beetles experimented as a response to poisoning.

Conducting this research will give more sustained technology for use of these products with less chance for insecticide resistance occurrence. A judicious use of the insecticides in the appropriate rates according to the varying factors should be followed to avoid the occurrence of resistance.

To study the processes that govern water distribution in the soil, their relation with insecticide movement and their effect on insecticide uptake is crucial to better understand the effect of heavy rainfall regimes in potato crops.

The inferences obtained here may be better supported by absorption studies in plant tissue. Contrasting absorption curves, given by such studies, and the residual activity curves will explain better whether the factors we assume affect residual activity are really producing changes in insecticide content in plant tissue and then protecting plants from insect attack.

An approach to the effect in the field will also be supported by physiological studies on plants under field conditions to know how the insecticide is distributed in the parts of the plant. In this way the projection for the effect on larvae of the Colorado potato beetle will be better proposed. Rapid growth of the potato plant makes very important to find a rapid and consistent absorption of insecticide for the crop protection

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