# Movement and Spatial Costs of Resistance in the Colorado potato beetle, *Leptinotarsa decemlineata* (Say), Coleoptera: Chrysomelidae

By

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# Abstract

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The Colorado potato beetle, *Leptinotarsa decemlineata* is infamous for its' ability to develop resistance to insecticides and remains the most important insect defoliator of potatoes today. Long Island populations of the Colorado potato beetle have been at the forefront of developing resistance to every newly developed insecticide. Managing the evolution of resistance requires cultural as well as chemical means. Cultural efforts include field rotation, crop rotation, chemical rotation as well as refugia. Movement plays an integral part of both, the cultural schemes intended to thwart resistance evolution and the life history traits of the Colorado potato beetle.

The use of refuges and crop rotation are often promoted to supplement the use of chemical pesticides in an effort to control crop pests. Refuges are untreated areas adjacent to treated crops, where susceptible genes can survive. The efficacy of refuges depends on movement between treated and untreated areas. Differences in movement between resistant and susceptible beetles can play a big role in the success of the refuge or rotation plan. Crop rotation can reduce the amount of insecticide used through dosage levels or frequency of application and slows

insects' resistance evolution. Resistance to insecticides often has fitness costs associated with that resistance. I hypothesized that resistance to the insecticide Imidacloprid is correlated with reduced movement capability in Colorado potato beetles, *Leptinotarsa decemlineata* (Say) (henceforth potato beetles), the primary insect defoliator of potato plants.

I examined whether migratory ability or flight propensity have a cost of resistance to imidacloprid in Colorado potato beetles, *Leptinotarsa decemlineata* (Say) by examining $LD_{50}$'s of flying emergers and walking emergers in the spring. Imidacloprid is the most widely used and in some cases the only effective insecticide for Colorado potato beetle control and there is currently a wide range of variation in resistance. In the spring overwintering potato beetle adults halt diapause and emerge from overwintering sites. For the purposes of this work I will use the definition of diapause presented by Tauber et al (1986): "a neurohormonally mediated, dynamic state of minimal activity that occurs during a genetically determined stage(s) of metamorphosis, usually in response to environmental stimuli that precede unfavorable conditions." Diapause in the Colorado potato beetle begins before the harsh conditions set in (loss of host and cold temperatures). It is an important strategy employed by many temperate zone insects for overwintering. Upon emergence from the overwintering site they emigrate to colonize local and distant fields. Emergence from diapause therefore offers an opportunity to sample genetically diverse groups of beetles.

My results indicate that emerging flyers have a higher level of resistance than emerging walkers from overwintering sites. I also examined populations that were under intense selection pressure from one chemical, Spinosad, and largely isolated from other fields or populations. Spinosad is

produced by a soil dwelling bacterium called *Saccharopolyspora spinosa* and it kills by ingestion. Spinosad is currently the only approved chemical available to Organic farmers on Long Island. These results indicate complete failure of Spinosad on that population but less resistance on distant populations and less resistance on populations from conventionally managed fields, all in Suffolk County, Long Island. Additionally early spring colonists of rotated and ‘non-rotated’ fields were evaluated for resistance levels for 3 years. For two of the three years, colonists on long distance rotated fields had high $LD_{50}$. Assuming long distance colonization is more likely dependent on flight, this is consistent with my results that emerging flyers have a higher $LD_{50}$ than emerging walkers.

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# Evolution of Resistance and the Colorado potato beetle

## Introduction

Insecticide resistance offers a contemporary opportunity to observe evolution in action. Efforts to slow down the evolution of resistance can benefit the environment and growers alike. The primary method proposed to slow resistance evolution is the use of refuge crops and in potatoes, the primary methods used by growers are rotation of fields and rotation of treatments within fields. Refuge crops and crop rotation both depend critically on movement (or restriction of movement) for their success. The Colorado potato beetle was the reason for the first large scale use of insecticides on agricultural crops (Casagrande, 1987).

The evolution of resistance has long been observed as plants and herbivores, respectively, have engaged in developing chemical defenses and overcoming them (Georghiou, 1986). Crops and crop pests also engage in this battle under increasingly intense conditions. It is no surprise that this evolution of resistance has continued, if not escalated, with the advent of chemicals designed to kill these crop pests. While resistance was rare before the 1950's (Casagrande 1987; Baker et al, 2005) it is currently rare to find susceptible populations.

Many insecticides are broad-spectrum biocides and have exceeded their intended targets. They have selected for resistance in insects and mites, but also in every other organism from bacteria to mammals as illustrated in Figure 1-1 from Georghiou, 1986.

The same phenomenon was not observed for fungicides, antimalarials, nematocides, rodenticides or herbicides or other groups of xenobiotics. (Georghiou, 1986).

In 1938 there were 7 insect species resistant to DDT and by 1984 there were 447 species resistant to members of five principal groups of insecticides. These groups include DDT, cyclodienes, organophosphates, carbamates and pyrethroids. Almost all of these 447 species are of agricultural or veterinary significance. There are 17 species that can resist chemicals in all 5 compound groups including one of our most important pests, the Colorado potato beetle (Georghiou, 1986). With each introduction of new chemicals there is a danger of pests developing resistance more rapidly due to similar modes of action or similar metabolic pathways for detoxification. This is called cross-resistance and occurred when pyrethroids were introduced. The semi-recessive kdr gene played a major role in DDT resistance and was shown to provide some insects with resistance to pyrethroids. With DDT resistance so widespread, the 'shelf life' of pyrethroids was questionable. Adding to this complication is the fact that populations can develop more than one mechanism of resistance. The known mechanisms of resistance in Colorado potato beetles include decreased sensitivity at the target site, enhanced metabolism, reduced penetration of the insecticide and increased excretion (Alyokhin et al 2008)(Alyokhin et al 2009). However, none of the known mechanisms present in field populations exclude any other mechanism from evolving (Georghiou, 1986). All resistance mechanisms reported in insects have been documented in Colorado potato beetles and in the absence of selection pressure, insecticide resistant Colorado potato beetles can retain resistance mechanisms (Bishop and Grafius 1996).

Resistance does not evolve at the same rate for all organisms and resistance can differ geographically. During many years of DDT use in the corn belt of the U.S. there was no evidence of resistance in the corn borer but house flies developed DDT resistance. Insecticides that do not work in one region may offer control where selection pressure is less intense. One example is organophosphates that controlled the Colorado potato beetle in the Midwest but at the same time, were entirely ineffective on Long Island (Georghiou, 1986). Forgash (1981, 1984) reported on the greater propensity for developing resistance in the Colorado potato beetles from Long Island when compared with the mainland. When resistance is genetically entrenched and/or geographically widespread it places an additional financial burden on growers who typically respond with increased frequency and/or quantity (concentrations) of sprays.

The process of resistance development is an evolutionary one driven by survival of the fittest or survival of individuals with genes that enable them to survive exposure to the insecticide. Resistance initially appears as a mutation which increases in frequency through selection or even genetic drift. There are many factors influencing the evolution of resistance, some of which are not under our control. Obvious ones we can control and that affect selection pressure include the timing and dosage of insecticide applications, chemical rotation (when alternate insecticides are available), and rotating crops and fields. Dominance or recessiveness is a property of the mutation itself and can have a profound impact on the rate of resistance evolution. Immigration can alter evolutionary dynamics either by introducing resistance alleles (like mutation) or by swamping allelic frequencies of local populations. Immigration depends on the pattern and extent of movement which can be studied in the field and plays a vital role in the Colorado potato beetle life history, diapause, feeding and reproduction (Alyokhin et al 2008)(Alyokhin et al 2009).

The functional dominance or recessiveness of resistance cannot always be readily identified. Further, adjusting the dose of insecticide can alter the results. With some insecticides a large dose can kill all homozygous susceptible (SS) and most heterozygous (RS) insects. In this way the S allele is functionally dominant. Conversely using a small dose will kill all homozygous susceptible (SS) insects but leave most heterozygous (RS) forms alive, making the S allele functionally recessive. If resistance is recessive or can be made to behave in a recessive manner by application of a dose that kills heterozygotes (RS) as well as homozygous recessive (SS) individuals, then after insecticide use only a few homozygous resistant(SS) are left. (Taylor and Georghiou, 1979).

Resistance in the potato beetle has been shown to be incompletely dominant or incompletely recessive with one or more genes involved (Alyokhin et al 2008) (Alyokhin et al 2009)and is insecticide dependent. Confounding the problem is the case of cross resistance, where populations resistant to one insecticide have lower susceptibility to another insecticide. One example is the DDT resistant populations of potato beetle that rapidly developed resistance to pyrethroids upon their introduction (Ioannidis and Grafius 1988)(Harris and Svec 1981). For this reason rotation of ‘treatments’ is strongly recommended to slow down evolution of resistance and to reduce chances of promoting cross-resistance.

The insecticide used for this research is Imidacloprid which was introduced in 1995. This is a neonicotinoid insecticide with a new mode of action, offering great promise in control of the Colorado potato beetle on Long Island. Even with new insecticides there is a danger of cross resistance and/or multi-resistance. Discovering new chemicals with new modes of action or with superior control is increasingly difficult. In addition to that, each possible candidate must have a net positive effect in a cost-benefit analysis. If the estimates do not show a return on the cost of development, the possible chemical may not be pursued. Part of this estimate is based on the potential for the development of resistance. Therefore attempts to control pests must always include responsible management of the evolution of resistance. Resistance is predicted by evolutionary theory and can never be ignored or considered trivial. Rotation of fields, rotation of chemicals and rotation of crops will all play vital roles to extend the life of current and future insecticides. Efforts to manage the evolution of resistance are crucial worldwide and are not only important to contain agricultural costs or to minimize environmental contamination but they are also critical in the fight to control disease vectors such as *Anopheles* and the spread of malaria.

## The Life Cycle of *Leptinotarsa decemlineata* (Say)

The Colorado potato beetle, *Leptinotarsa decemlineata* (Say), remains the primary insect defoliator of potatoes in North America (Alyokhin et al, 2008). The Colorado potato beetle is in some ways, uniquely suited for researching the evolution of resistance and its' potential relationship to beetle movement. It has been credited with being responsible for the creation of today's insecticide industry (Gauthier et al, 1981). The Colorado potato beetles enter diapause in the fall and will overwinter in the soil as adults (Lashomb et al, 1984). The diapause sites are woodlots or hedgerows near fields. Resistance to insecticide in the Colorado potato beetle is a worldwide phenomenon (Boiteau 1988; Noronha 2001; Pourmirza 2005; Mota-Sanchez et al. 2006) and the Long Island populations have some unique characteristics making them even more suitable for studies in resistance and movement. The resistance profile is a summary of the chemicals and levels of chemicals to which a population is resistant. The resistance profile of the Long Island populations exceeds those from the mainland and may indicate the future resistance path for other populations (Georghiou 1986; Forgash 1981, 1984). The Long Island populations had high levels of resistance to most synthetic insecticides and there was a reported 16 fold increase in resistance to imidacloprid in the Long Island beetles, before wide spread commercial use (Olson et al 1996;Olson et al 2000; Zhao et al. 2000; Tomizawa and Casida 2003).

The Colorado potato beetle is readily raised and maintained in laboratory colonies and can be found in diapause sites in the field throughout the year, providing the ground is not frozen. The Colorado potato beetle can have one to four generations per year (Hare, 1990). In the fall short photoperiod induces diapause (De Kort, 1990) and most adults head toward tree lots or hedgerows to diapause (Weber and Ferro 1993; Boiteau et al, 2003) but some overwinter in the field (French et al. 1993). They are usually at a depth of 7.6cm – 12.7cm (Lashomb et al, 1984). In the spring these beetles emerge from the soil and disperse by walking and flying (Voss & Ferro, 1990a; Boiteau et al. 2003). These beetles are referred to as overwintered beetles. They feed immediately upon finding a suitable host. Shortly after emerging the females begin depositing eggs, usually 20-50 on the underside of leaves of the host potato plant, *Solanum tuberosum*. Adult beetles mate before and after diapause and mating is multiple and promiscuous (Tauber et al., 1988). Emerging females overwinter with viable sperm from fall matings and are able to deposit viable eggs in the spring without spring matings. However sperm precedence for spring matings was demonstrated by Baker et al (2005). It has been shown that starved overwintered females flew almost four times as much as fed overwintered females

(Ferro et al., 1991). This could imply that upon emerging, females are more likely to fly as they have not eaten since the fall. Female beetles can deposit as many as 600 eggs. Eggs laid by overwintered females begin to hatch within a week and the larvae begin feeding immediately on the potato foliage where they spend most of their time. They molt three times in 10-20 days and the fourth instars burrow into the soil to pupate. The pupa stage can take up to 15 days until they dig out of the soil and emerge as adults. Optimal conditions of 25-30$^{o}$C, long day photoperiod (>16h), and high quality food, can yield a generation from egg to adult in 21 days (Ferro et al., 1985). The first adults to emerge from these eggs deposited by overwintered females are called the first summer generation (Ferro et al, 1985). These first summer generation adults will take seven to nine days to develop flight muscles and their reproductive system. The first summer generation produces offspring which are called the 2nd summer generation (Ferro et al 1985). Adult beetles that emerge from pupation in late summer under short photoperiod conditions do not develop flight muscles or reproductive systems before entering diapause. Only adult Colorado potato beetles overwinter.

Exact timing of emergence differs with latitude, where those in warmer environments emerge sooner (Hurst 1975). A nine year study in the Netherlands had a variation for emergence of up to 71 days (Hurst 1975). In New Brunswick, NJ there was 50% emergence around May 15th, 1980 (135 Julian) which corresponded to roughly 80 Degree Days (DD) accumulated (Lashomb and Ng 1984). A DD is a measure for each degree above a base temperature. Degree days summarize the amount of heat available above a threshold temperature since a given day. Often the number of DD accumulated over a period of time is related to phenological events of insects (and plants). For Colorado potato beetles the threshold temperature is 50$^{o}$F or 10$^{o}$C. The average emergence in Massachusetts is early June (Voss and Ferro 1990b). There was no significant difference in the emergence of male and female beetles in Massachusetts and emergence commenced with as few as 50DD (Yang 1994).

Another factor that contributes to the high predisposition of resistance development in potato beetles is protection of offspring. Offspring losses are sheltered from complete collapse due to the spatial and temporal distribution of eggs by the Colorado potato beetle (Voss and Ferro 1990a). The migration, reproduction and diapause behavior of Colorado potato beetles allows them to mitigate the effects of catastrophic loss. The offspring benefit from this spatial and temporal distribution and reduce the likelihood of total annihilation due to insecticides or rotation events (Alyokhin and Ferro 1999b).

The potato beetle has some ‘bet-hedging’ strategies that contribute to its’ success. A bet-hedging strategy is one which will minimize loss in the face of uncertainty or change. Potato beetles were introduced into Europe in the last 80 years and research has demonstrated that they have had a change in photoperiod response. The capacity of the potato beetle to have this rapid shift in photoperiod response has benefitted the species and made it more adaptable to changing environments (Lehmann et al 2012). Walking or flying to colonize host plants, mating pre and post diapause, local and long distance field flight to deposit eggs and/or colonize new fields, are all examples of the ‘bet-hedging’ strategies that make the Colorado potato beetle such a formidable foe in the fight to control it. Some techniques have been developed that reduce spring colonization by 90% and more. These include flame throwers, plastic lined trenches and crop rotation schemes. While these have initial success it has been shown on Long Island that the population size recovers at the end of the first larval generation (Wright, 1984). Potato beetles are highly mobile, fecund and have repeatedly demonstrated their ability to evolve resistance to chemical insecticides. Populations with a larger reproductive potential can tolerate a higher intensity of selection. As a result potato beetles remain a major challenge today.

## Ecology and Movement

Colorado potato beetles are capable of movement by walking and flying and both of these can serve to enable colonization on host fields. Voss and Ferro (1990a & 1990b) described three types of flight, local, long distance and diapause flight. They describe local flight as occurring within the host habitat. The purpose could be distribution of eggs, to find a mate or to minimize host plant depletion. They observed overwintered beetles engaging in this flight one to two weeks after colonizing a field and summer generation beetles in this type of flight shortly before engaging in diapause flight.

They define long distance flight as one that removed the Colorado potato beetle from the habitat and it can be several kilometers. A Colorado potato beetle can fly more than 100km with high temperatures, clear skies and no wind (Hurst, 1969; Wiktelius, 1981).

Diapause flight brings the beetles from the host fields to untilled, unfarmed areas near the potato fields. These areas can be shrubs and hedgerows along the field edges or wooded lots. The beetles burrow into the soil just near the edges of these uncultivated areas in sometimes dense groups to overwinter. This strategy enables them to avoid the cold and the lack of host plants.

When the overwintered beetles emerge in the spring, they can walk or fly in search of host plants and these adults are called overwintered or post-diapause beetles. Dispersal patterns have been studied by Szendrei et al (2009) and it was shown that beetles moved preferentially into tilled plots when compared to their movement into plots of complex habitats. They strongly suggest incorporating dispersal patterns into management control plans (Szendrei et al 2009).

While it has been shown that unmated females fly more than mated ones for summer-generation beetles (Alyokhin & Ferro 1999a), it has also been shown that flight of post diapause beetles is not affected by their mating status (Ferro et al 1999). Sometimes, even in the presence of host plants these emerging overwintered beetles engage in long distance flight (Yang, 1994).

De Kort (1969) showed that first summer generation adults feed for seven to nine days to complete flight muscle development. However Ferro (1991) demonstrated that overwintered beetles were capable of flight on a flight mill on the first day after emerging. Second summer generation beetles move almost exclusively by walking (Voss et al., 1988). These beetles emerge in late summer and early fall when there is a short photoperiod, and hosts are less plentiful and senescing. Their flight muscles do not reach the same size as the summer generation that developed under long photoperiod conditions (De Kort 1969). These beetles that emerge under short photoperiod and senescing or no host, walk to the overwintering site to diapause.

In the spring flight muscles must regenerate and Stegwee (1964) confirmed this and demonstrated that complete regeneration of the flight muscles had occurred at the time of emergence from the soil. Upon emerging the beetles are on the move, some walking and some flying. On the other hand, De Kort (1969) also reported that flight muscles totally degenerate in diapausing beetles and they take seven to nine days to complete development after emerging from the soil. De Kort (1969) also reported that summer generation beetles also require seven to nine days to complete development of flight muscles after completing pupation and emerging as adults

Contrary to De Kort (1969), post diapause beetles have been shown to fly on a flight mill on the first day after emergence from diapause (Ferro et al. 1991) and flight on the first day after emergence is consistent with Stegwee's findings (1964). Furthermore, Yang (1994) demonstrated that flight muscle regeneration relied only on accumulated heat, whether or not the beetle stayed in the soil or fed. Post diapause beetles required ~150-200 DD (degree days) to complete muscle regeneration. The regeneration could be completed without food (Yang 1994). Yang (1994) demonstrated that 20% of the population had fully developed flight muscles while still in the soil and in 50% of the population spring emergence coincided with flight muscle regeneration. It has been demonstrated that some of the overwintering population can fly long distances and survive for up to 4 weeks without food (Ferro et al. 1991). I have observed overwintered adults emerging from the soil and engaging in flight. It is consistent with Stegwee, Yang, Ferro and my observations that emerging overwintered adults can generate flight muscles while still in the soil and without food.

The degeneration and regeneration of flight muscles in the Colorado potato beetle is controlled by Juvenile hormone (JH) (De Kort, 1969). Short days result in a decrease of JH, relocation of

Colorado potato beetle to diapause sites, the onset of burrowing and degeneration of flight muscles. In spring the lengthening photoperiod reverses these processes to increase in JH production, halt diapause, commence emergence from the soil and initiate regeneration of flight muscles and departure from the overwintering site (De Kort 1969; De Kort et al. 1982).

Flight in the potato beetle is powered by Proline and carbohydrates (Weeda et al., 1979). Levels of proline in Colorado potato beetle flying for nine minutes under long photoperiod conditions dropped rapidly while the alanine concentrations rose (Brouwers et al., 1979). Proline concentrations in overwintered beetles just emerging from the soil were 11.2 µg/µl. If these beetles remained unfed, this level remained constant to day 20 (Yang, 1994). Overwintered fed beetles had proline concentrations of 13.6 µg/µl while unfed beetles the concentration stayed at the same levels to day 20 (Yang, 1994). Emerging overwintered beetles could survive and fly without food but emerging summer generations that did not find food, died within ten days (Yang, 1994). Proline is an ideal energy substrate for Colorado potato beetle flight because of many positive characteristics. It produces a high yield of metabolic energy, is highly soluble allowing large stores of energy in solution in body fluids and it contains less nitrogen. The detoxification of nitrogen requires a cost of 1.5 mole of ATP per atom of Nitrogen (Bursell, 1981).

## History of potato production on Long Island and resistance

Potato production on Long Island was once an economic stronghold and the Long Island Vegetable Research Station was established at Riverhead 1922 (Brigden et al 2006). In 1946 Long Island potato production peaked at 62,000 acres planted (Spears 1968). The steadily declining farm acreage and production is due to many factors, but the resistance of Colorado potato beetles to all approved chemicals was the major one. Potato farmers were unable to control the pest and therefore unable to realize a profitable harvest. This led to a major sell off of farm acreage to developers (Brigden et al 2006). The lack of control of the Colorado potato beetle presented such a management challenge that in the 1970's the Long Island Vegetable Research Station expanded their applied research programs to include one solely focused on potatoes.

This sell-off of potato farms for development reduced the total number of fields available and thus reduced future options for field rotations. The nearness of ground water on Long Island adds another constraining factor. Residents and government agencies alike are wary of contaminating this ground water with chemicals. As a result, chemicals must be approved (labeled) for NY and specifically approved for use on Long Island. As if this were not enough, another pest, the Golden Nematode added additional regulations on Long Island (Golden Nematode Act of Congress 1948) and in 1994 a second race (Ro2) of the Golden Nematode was found on Long Island (Hays 1996). Upon passage of the 1948 Act, Levitt & Sons began buying Golden nematode infested potato farms for low cost housing (Levittown Historical Society. 2004).

The Colorado potato beetle not only made headlines for its' impressive abilities to develop resistance, but on Aug 31, 1877 it made the following headline in the Plattsburgh Sentinel: "Prof. Lintner of NYS Geological Hall is surprised to find COLORADO POTATO BEETLE at 4400 feet elevation on Mount Marcy". Mount Marcy is the highest peak in the Adirondack Mountains.

The Long Island populations have received particular notoriety for shortening the shelf life of insecticides. Forgash (1984) already noted the Colorado potato beetle "has weathered the onslaught of arsenicals, chlorinated hydrocarbons, organophosphorus compounds, carbamates and pyrethroids." At that time it was thought that there were 1 to 2 summer generations per year on Long Island, but see Appendix 1 (In 2008 and 2010 I cage reared 3 summer generations in a Long Island field under environmental conditions.) An increased number of generations per season can increase the chance for mutations and can also shorten the time required for evolution of resistance. The intense chemical selection and higher number of summer generations rapidly increase the evolution of resistance.

When an insecticide appears to be losing effectiveness the typical first response is increased frequency of treatments or increased dosage, or both. If this doesn't work then the next usual response is a new chemical, if available, which is usually more expensive. Both responses result in an increase in direct costs of pest control as well as increasing selection pressure for resistance.

The introduction of new chemicals has been enhanced by our understanding of basic biology, biochemistry and physiology of crop pests. However this pest's response to new insecticides is daunting as evidenced by the historical record presented in Gauthier et. Al, 1981 and Forgash 1984.

DDT was the first synthetic chemical used for selection in Colorado potato beetles on Long Island. Potato beetles on Long Island took seven years to develop resistance. The Long Island potato beetle population has required increasingly less time to develop resistance to subsequent chemicals. Rotenone is an insecticide from a plant derivative that had been used for more than a century until the introduction of DDT. On Long Island in 1984 it had to be combined with a synergist, piperonyl butoxide to provide control of the potato beetles. The fact that Rotenone alone could not provide control indicates that enzymes to detoxify rotenone were present in the Long Island populations.

The Colorado potato beetle originated in the southwest section of North America, Mexico where the host plants were buffalo bur, *Solanum rostratum* and *Solanum angustifolium*. Both of these host plants also have their origins in Central Mexico (Casagrande, 1987). We do not know when the Colorado potato beetle or *S. rostratum* spread to the United States. Today the Colorado potato beetle host plants are members of the Solanaceae family. In addition to potato, tomato, and eggplant, this family includes tobacco, deadly nightshade and jimson weed, to name a few. As a group they produce quite a range of toxic glycoalkaloids and the ability of the Colorado potato beetle to survive on plants in this family may predispose them to detoxifying or tolerating other poisonous chemicals (Ferro 1993).

The first report of Colorado potato beetle as a crop pest was on a potato field in Nebraska in 1859 (Casagrande, 1987) and this population that had adapted to potatoes as a host plant and reached the East coast in 1874, just 15 years later. Potato fields in the southeast and to the west were scarce and the spread of Colorado potato beetle to these areas was slower (Riley 1877). Damage by the Colorado potato beetle to potatoes was extensive causing many farmers to abandon the crop in the 1860's. A horse drawn beetle collector was invented in 1866 and Riley reported widespread use of Paris green against the Colorado potato beetle in 1873 (Casagrande, 1987). In 1871 recommendations were made to use Paris Green, (copper acetoarsenite) an arsenic, in an effort to control the Colorado potato beetle. 1876 found swarms of Colorado potato beetles infesting the concentrated potato fields of New York and New England. These occurred in such numbers that a New York Times article reported the beetles causing the NY Central Railroad to stop, until the beetles were swept from the tracks. (Casagrande, 1987). It seemed that the pest had been brought under control with the use of arsenic compounds such as Paris Green.

The switch to using DDT on Long Island was made in 1945 (Gauthier, 1981) and resistance was reported in 1952. The arsenates were also no longer effective against this pest and L.I. growers switched to dieldrin. The chart from Georghiou (1986) is a good chronology of the efforts on Long Island to control this crop pest, and their response. Development of resistance to every insecticide used against it has forced a change in the chemical every few years. (Forgash, 1984). The introduction of imidacloprid in 1995 has offered relief but resistance is once again rearing its' ugly head (Baker et al. 2007) and has become a popular focus for recent research (Alyokhin et al. 2008). Because of, or motivated by, the development of resistance as demonstrated in Georghiou (1986), non-chemical solutions were resurrected.

In 1985 several Long Island potato growers were again using beetle-collecting devices, albeit a tractor run vacuum this time. This was expensive due to fuel and labor as well as extremely time consuming to vacuum each field regularly. Maintenance of the equipment was also costly and time consuming. It was in response to not having a clear chemical control agent for the Colorado potato beetle. Neonicotinoid insecticides arrived on the scene in 1995. The novel mode of action in this class of insecticides offered much needed help to many potato growers who had run out of viable chemical controls. This class displays low mammalian toxicity and long residue time in the field. Because of these characteristics commercial growers readily embraced the new chemical, abandoning other chemical and mechanical means. In 2010 when resistance to an organic insecticide appeared on organic farms, the vacuum returned to Suffolk county, because all else had failed (This volume, 2010).

### Refuges

Refuges are the primary method advocated for managing resistance, though predominantly used on transgenics, where it is mandated by the EPA since 1999 (Bates *et al.* 2005, Gould 2000). Refuges provide an untreated area adjacent to the treated host crop. The refuge strategy rests on the principle that resistance is a heritable genetic trait and the refuge is to provide a safe haven for susceptible genes (Onstad & Gould, 1998).

Beetles on the refuge are not exposed to the insecticide, which, in theory, allows individual beetles with susceptible genes to survive and reproduce. Optimally the goal is to have resistant beetles mate with susceptible ones, which necessitates movement between the two areas. This is required because in the adjacent treated fields, only resistant larva can survive to adulthood.

Therefore, if resistant adult beetles mate within the treated field, there is a high probability of producing homozygous resistant offspring.  The strategy of a refuge is to reduce the selection pressure put on the susceptible population and allow the susceptible alleles to survive in individuals feeding on non-treated host plants.  Mating between resistant and susceptible adults will produce heterozygotes.   This can slow or limit the evolution of resistance (Tabashnik 1994).

Factors such as genetics, environment and random chance contribute to the variation between individuals in their phenotype or observable characteristics.  Heritability of a population is the proportion of observable variation among individuals due to genetics.  The refuge strategy strives to decrease the functional dominance of resistance (Gassman et al 2009, Gould 1998).  If the toxin kills heterozygotes (R/S) then resistance is functionally recessive.  This would slow the rate of spread of the R allele in the population.  Conversely if heterozygous (R/S) individuals survive and reproduce, the rate of spread of the R allele is increased substantially.

Refuge success requires that resistance is recessive or functionally recessive (kills heterozygotes).  As discussed earlier resistance alleles may be made to respond differently to different levels of insecticide.  If resistance is recessive or functionally recessive, then heterozygotes (R/S) in the treated fields will be killed by the insecticide, along with the homozygous susceptible (S/S) population.  When heterozygote individuals die this decreases frequency of the resistant allele (R), which can delay the evolution of resistance.  The levels of insecticide (dosage, frequency of applications) can determine if resistance behaves in a recessive manner.  At low levels of insecticide a heterozygote may be able to survive and reproduce, but would die at higher concentrations.  This is why resistance sometimes increased faster at lower doses (Roush and McKenzie 1987). Over time, failure of the refuge and saturation of the resistant phenotype is predicted by evolutionary theory (Gould 1998)

Another factor contributing to delay of the evolution of resistance is fitness costs. Fitness costs can be described as a trade off in which alleles conferring higher fitness in one environment reduce fitness in an alternative environment. For example, in the absence of the toxin the resistant individuals could be less fit, as when resistant individuals produce fewer offspring. Fitness costs are recessive when fitness in refuges is lower for homozygous resistant (R/R) than for homozygous susceptible (S/S) but does not differ between (R/S) and (S/S). Fitness costs are nonrecessive when fitness in refuges is lower for (R/S) than for (S/S).

Refuge success depends critically on the movement of individuals between treated and untreated fields, and more specifically on mating between these two populations. If refuges and treated areas are too isolated from each other, then susceptible insects will not mate with those on the treated areas, and the refuge effect will be lost. However if susceptible insects travel farther than resistant ones, refuge success might be enhanced by some degree of isolation from treated areas

## Fitness Costs of Resistance : Bt and Imidacloprid

Resistance is one characteristic that can have a negative association or potential trade-off to individuals, and is referred to as life history trade-offs (Zera and Harshman 2001). These trade-offs are also referred to as costs or fitness costs and a significant fitness cost associated with resistance has been demonstrated (Onstad and Gould 1998). In the absence of insecticides, homozygous resistant individuals are less fit than their susceptible counterparts, both homozygous and heterozygous (Onstad and Gould 1998). As discussed, refuges can offer a safe habitat for the susceptible genes to survive and thus slow the spread of resistant alleles. Fitness costs can also help delay resistance. It is fairly common that resistant genotypes have fitness costs shown to exist when the insecticide is absent (Crow 1957).

*Bacillus thuringiensis, (*henceforth *Bt*) is an insecticidal bacterium which occurs as a large number of subspecies. Some of the characteristics used to identify the subspecies of *Bt* include surface antigens, plasmid arrays and the scope of species affected by its insecticidal actions. *Bt* is a soil inhabiting gram positive sporulating bacterium which produces crystals. These crystals are composed of delta-endotoxins which are large proteins. The delta-endotoxins bind to specific receptor sites on the insect gut epithelium. This slowly leads to degradation of the gut lining ending in starvation. Insects will be killed several days after ingesting *Bt* products. *Transgenic* organisms are genetically altered by artificial introduction of DNA from another organism and the artificial gene sequence is referred to as transgenes. Plants with these transgenes are also called genetically modified (GM). The utilization of Bt genes transplanted into crops, provides insecticidal control against crop pests.

Fitness costs associated with resistance of Colorado potato beetle to *Bacillus thuringiensis* have been demonstrated. Trisyono and Whalen (1997) found that while eggs of resistant beetles were viable longer, resistant larval development was slower, resistant females produced 60% fewer eggs, had shorter oviposition periods and had fewer eggs per egg mass. Studies in the laboratory have shown that resistance to *B. thuringiensis* declined in the absence of selection (Tabashnik et al. 1991, Rahardja and Whalon 1995). Alyokhin and Ferro (1999b) demonstrated that Bt-susceptible male beetles copulated more than Bt-resistant males, susceptible female beetles produced more eggs and more larva than resistant females and twice as many Bt-susceptible beetles survived the overwinter diapause. Nonrecessive fitness costs occur in heterozygote offspring of resistant and homozygous dominant susceptible parents and favor reducing the resistant allele through selection against heterozygotes on the refuge. These nonrecessive costs can select more strongly against resistance, when fitness of the heterozygotes is less than the fitness of homozygous recessive individuals on the refuge. The critical factors are dominance of these fitness costs as well as dominance of resistance (Gassman et al 2009)

Specific fitness costs of resistance to Imidacloprid in the Colorado potato beetle have also been demonstrated. Alyokhin et al. (2006) report an increased mortality of imidacloprid resistant larva at the control (0) dose when compared with susceptible larva in the same study. All larvae were reared under the same conditions which suggest fitness disadvantages affiliated with imidacloprid resistance. Other life history trade-offs include fecundity and walking speed (Baker et al, 2007) and overwintering survival (Baker and Porter, 2008).

To manage the evolution of insecticide resistance we necessarily must understand the dynamics of the movements involved as well as the cost. The Colorado potato beetle has quickly and consistently developed resistance to almost every insecticide used on it (Casagrande 1987). Placing untreated fields at appropriate distances can have a great impact on this process (Baker et al, 2001). Over-wintered beetles use walking or long distance flight in colonizing new potato fields (Weber et al, 1995). It is suggested that longer distances will encourage recruitment by flight (Boiteau, 2001). The use of refuges has been employed with genetically modified crops and it has been shown that resistance evolved slower, but the mechanism for that has not been demonstrated. It is on this genetic nature of resistance that the premise of the refuge area was built (Onstad & Gould, 1998).

Costs of resistance have frequently, but not uniformly, been observed in a wide variety of traits (Reviewed in McKenzie 2001, Roush & McKenzie 1987). These costs explain why resistant organisms are rare in the absence of the pesticide. Pesticide resistance in the Colorado potato beetles was demonstrated to have high fitness costs to *B. thuringiensis* toxin (Alyokhin and Ferro. 1999b). When resistance has high fitness costs the removal of the toxin will give an evolutionary advantage to the susceptible population (Laxminarayan et al 2002). Resistance to imidacloprid in the Colorado potato beetles is costly in terms of fecundity, development time, maximum walking speed and overwintering survival (Baker et al. 2007).

## Crop Rotation and Movement

Crop rotation is a method used by farmers to reduce pest invasion, keep soils healthy and maintain healthy crops. Two or more crops are planted in different sections of a farm. In subsequent seasons they can switch sections in the farm or a section may be left unplanted for wild grasses to grow, which is called fallow. Pests in the switched or fallow section will not find their host plant and now must disperse to seek it out. This is how movement plays a critical role in the pest management technique of crop rotation. Moving fields at the right spatial distance can delay discovery by the crop pest. This can reduce crop damage, reduce the use of insecticides and increase yields.

Crop rotation has been advocated for controlling several potato pathogens including common scab, fusarium wilt, verticillium wilt, and early blight, as well as for controlling weeds and improving the soil. Both Wright (1984) and Lashomb & Ng (1984) demonstrated that rotation can reduce initial Colorado potato beetle populations in the fields.  Crop rotation is one of the oldest control strategies and is also highly recommended and used to manage insect populations, as well as to reduce resistance.  Other cultural controls to manage potato beetles include early or delayed planting, trap crops, mulches, plastic lined trenches and removal of overwintering sites but crop rotation remains the single most important cultural tool (Alyokhin et al 2008) (Alyokhin et al 2009).

Crop rotation can work by increasing the diversity (instability) of the pest environment and create discontinuity in the supply of host plants.  It can reduce or delay spring colonization by Colorado potato beetles emerging from diapause (Hough-Goldstein et al., 1996).  Sexson and Wyman (2005) demonstrated that crop rotations greater than 400m reduced spring infestations by 90%.  Rotation has been shown to delay the appearance of Colorado potato beetle larva (Lashomb & Ng, 1984) and reduce adult densities by as much as 70-90% compared to non-rotated fields (Wright, 1984). However, crop rotation works best for pests with a narrow host range, which are not highly agile or mobile, use annual or biennial hosts and are present before the crop.  Rotation alone many not work well for the Colorado potato beetles because they are highly mobile.(Weber et al., 1995).

## Crop Rotation and Resistance

Crop rotation can reduce resistance indirectly by requiring fewer applications of insecticide, which reduces the selection pressure (Roush et al., 1990).  Crop rotation could also directly reduce resistance if resistant insects do not disperse as far as susceptible ones.  Crop rotation and refuge crops can both benefit from decreased movement in resistant versus susceptible insects.

Considering the observed frequency of resistance costs and observed frequency of life history trade-offs with migration, this is a logical prediction. However, the one time the prediction was examined the results were equivocal (Roush et al., 1990).

As stated earlier many characteristics have fitness costs and involve tradeoffs, and insecticide resistance is such a characteristic (Zera and Harshman, 2001). Movement and migration have also frequently been shown to involve life history tradeoffs or costs (Rankin and Burchsted 1992) (Dingle, 1995) and are important characteristics in the life history of potato beetles. Migration has energy costs of the actual movement and can also increase predation susceptibility. Migrating colonists should have a suite of traits to be successful. They should migrate efficiently, reach reproductive maturity quickly and be fecund. Some insect lines selected for large wings (long migrators) have been shown to have higher early fecundity (Ranking and Burchsted, 1992).

The presence of an “oogenesis-flight syndrome” (Rankin and Burchsted, 1992) implies that reproduction and migration are mutually exclusive physiological states. Costs associated with the capability to fly include delayed reproduction, overall decreased fecundity, increased development time and a shortened life span. Rankin and Burchsted (1992) conclude that reproductive costs often exist in wing polymorphic species, but development and longevity costs are not always apparent, if existent. Migrants also run the risk of not finding suitable host habitat as well as potential reproductive costs caused by possible ovipositing delay(s).

Although intuitively the cost of flight is perceived to be high (Ranking and Burchsted, 1992), colonists using flight can escape bad conditions. Insects rely on movement to escape toxins, poor quality or no hosts, or adverse environmental conditions. The ability to fly even short distances to find hosts can often make up for the costs and risks (increased predation, failure to find hosts) of that flight. Rankin and Burchsted (1992) suggest that benefits of flight are often greater than the costs and laboratory experiments may not be designed to allow the colonists to reap the benefits of flight. One cost of resistance may involve migratory flight capability though it is rarely looked at and has rarely been observed (but see Foster et al. 1997). If movement has a resistance cost then rotation on the right spatial scale can reduce resistance and have far reaching impacts in agriculture.

### ***Resistance, movement and costs on Long Island potato beetles***

The potato beetles' host plants are members of the Solanaceae family which is known to produce toxic glycoalkaloids. Potato beetles have developed ways to deal with these toxins and this may predispose them to neutralizing new chemicals, such as insecticides. As stated earlier, several traits make the Colorado potato beetle and the populations found on Long Island in particular, an excellent model system for examining the effects of resistance on movement. The population on Long Island has received special notoriety because they develop resistance quickly against all chemicals and ahead of other populations (Forgash 1982,1984; Georghiou 1986; Olson et al 1996; Olson et al 2000; Zhao et al 2000; Tomizawa and Casida 2003).

As stated earlier movement is an important part of the Colorado potato beetle life cycle. If movement has fitness costs involved in resistance to imidacloprid, it could have implications for growers and methods used to slow resistance evolution. Most of the overwintering beetles are found in woodlots adjoining potato fields from the previous season (Weber and Ferro 1993), emerging adults from these overwintering spots can be separated into walkers and flyers. The induction of diapause and its termination can be predicted with some certainty and observed readily in the field. Diapause has been introduced under laboratory conditions (Yang 1994; Alyokhin and Ferro 1999b). Colorado potato beetles employ diverse bet-hedging strategies in its movements (Boiteau et al, 2003) so there is a wide range of movement that resistance can affect, much of it on the 10's and 100s of meters scale. It is these characteristics which make the Colorado potato beetle uniquely suited for measuring dispersal behavior and any fitness consequences.

Efforts to manage this challenging potato beetle population on Long Island, can have repercussions throughout all populations. As discussed it is not prudent to wait for new chemicals to emerge and extending the shelf life of chemicals through currently available means, such as the use of refuges as well as field and crop rotation, are goals we can strive for now.

Little is known about the movement of recently emerged overwintered beetles (De Kort, 1990; Boiteau et al, 2003). The Colorado potato beetle's emergence pattern has been described by Lashomb et al (1984) as Waldbauer's (1987) polymodal type A. This emergence pattern can be described as when all individuals that enter diapause in a given year, terminate diapause the following growing season and demonstrate a bimodal emergence curve. This pattern can increase survival when confronted with annual variations in environmental conditions. This results in two sympatric phenotypes, early and late emergers. They differ in the time of adult emergence from diapause. I observed bimodal peaks on Long Island in spring emergence and collected ‘early’ emergers and ‘late’ emergers. Both phenotypes were bioassayed with imidacloprid but I did not observe significant differences in $LD_{50}$ resistance levels for the two groups.

This research examines if movement or migratory ability has a fitness cost involved in resistance to imidacloprid in the potato beetle. This research on the movement/migratory capabilities and resistance of the Colorado potato beetle emerging from diapause provides insight into costs of resistance, spatial optimization of refuge crops and rotation and evolution of resistance. This is the first study on effects of resistance on movement, on the scale of agricultural landscapes (10’s, 100’s and 1000’s of meters). As stated earlier the observed frequency of resistance costs and tradeoffs involved with migration we had predicted that flying potato beetle migrants would be more susceptible to imidacloprid (Ranking and Burchsted 1992) (Dingle 1995). My results indicate that flyers had higher $LD_{50}$’s than walkers in all years and at all sites indicating that flight is not a cost of resistance.

The larger implication to sustainable potato production is that long distance rotation on Long Island may not provide the protection I had thought it would. However, rapid development on Long Island is causing a reduction in the number of available fields which limits the crop rotation options. Given the diminishing choices of available fields for crop rotation combined with my results that the most resistant beetles colonize as flyers, the distance factor may not be as important on Long Island.

The one previous study of migration and resistance in the potato beetle had equivocal results (Roush et al., 1990) The insecticides used came from the five major groups of organic synthetic compounds in use at the time. They calculated the mean of the percentage of mortalities for all five compounds to use as the standard of comparison. Since they pooled insecticide resistance over all major classes of insecticide for analysis, this may not be illustrative of resistance since resistance is not a singular trait and is dependent upon dose, insecticide and insect. Pooling the results for all chemicals used in the Roush study may have confounded the results.

## Objectives:

1. In the spring, overwintered adults emerge onto a barren landscape. They walk or fly in search of hosts and/or mates. Little is known about this movement of emergers in the spring and if there is a movement costs of resistance, then flyers would be less resistant to imidacloprid. My objective was to evaluate and compare resistance levels of overwintered emerging beetles that walked from the overwintering site, (Walkers), with overwintered emerging beetles that fly from the overwintering site (Flyers).

2. Organic growers in Long Island have a very restricted list of chemicals they can use. In practice, on Long Island, only one chemical, Spinosad, offered any level of control of the potato beetles. This study followed up on reported failure of Spinosad by organic growers. My objective was to compare Spinosad resistance levels of beetles captured on organic fields with beetles captured on traditionally managed fields.

3. Rotation is an important management technique to control crop pests. On Long Island, agricultural fields are being converted out of potato. This reduces and limits the available options for rotating potato fields. My objective was to evaluate and compare the resistance level to imidacloprid of colonizers on new potato fields that were long distances (>300m) from the prior year potato with resistance levels to imidacloprid of colonizers on potato fields that were short distances (<25m) from the prior year's potatoes.

## Overall Conclusions

In Chapter 2, my first study compared the $LD_{50}$ to imidacloprid of spring emerging beetles that flew from the overwintering grounds, with the $LD_{50}$ of spring emerging beetles that walked from the overwintering grounds. The $LD_{50's}$ of flyers, to imidacloprid, were higher at all sites in all years. Flyers had a higher resistance to imidacloprid at all sites in all years. This could have implications when planning field rotations. If flyers are more resistant then long distance field rotations may not be an important consideration. Flying beetles can move further in a shorter period of time. So if Flyers are more resistant than walkers, resistance may be enhanced by long distance field rotations as the colonizers on these fields will be mostly flyers.

In Chapter 3 I compared the resistance levels to Spinosad, of beetles captured on organically managed fields with beetles captured on traditionally managed fields. This field study on the use of a singular chemical, Spinosad, documents greatly increased resistance when compared with populations subjected to multiple chemicals. In this study organically managed fields had populations that were significantly more resistant to Spinosad. One factor that likely is involved is the strength of direct selection. These organic fields have been treated with one chemical, Spinosad. Organic fields in Suffolk County have a very short list of chemicals available to them that are approved in this area. Growers have increasingly relied on Spinosad as their results with other chemicals failed to control the potato beetles. Organic growers adopted spinosad earlier than traditional grower and apply it more frequently than conventional growers. However, this recent large increase in resistance to spinosad in Suffolk county Colorado potato beetles has not been match by an equal large increase in resistance to imidacloprid.

In chapter 4 I compared the LD50 of colonizing beetles on long distance rotated fields with short or no distance rotated fields.  Long distance rotated fields are those fields that are more than 300m from the closest prior year's potato fields.  Short distance rotated fields are those fields that are less than 25m from the closest prior year's potato fields.  Colonizing beetles on fields that were rotated long distances had higher resistance levels to imidacloprid.  The high precipitation in one year may have resulted in a more homogenous mixture of flying and walking colonists on all fields.  Beetles that fly can travel longer distances in shorter times.  Colonists of long distance rotated fields are not less tolerant of imidacloprid as predicted by general resistance-cost theory.  There may be a trend towards higher resistance in colonists of longer distance fields.

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# Resistance levels of spring emerging beetles: Flyers and Walkers

## *Abstract*

Insecticide resistance is associated with fitness costs in many insects and the Colorado potato beetle is no exception. Imidacloprid resistance in Colorado potato beetles has been shown to impact larval mortality, fecundity, mating and sprint speed and here I investigated if flight would be similarly affected. Overwintered adults were collected upon emergence from known overwintering sites and defined by their mode of exodus from these sites. Those captured while walking are classified walkers, those captured while flying are classified flyers. The results of this study suggest that flying spring emergers always have higher resistance levels to imidacloprid than their walking contemporaries. The importance of this dichotomy to the development of Integrated Pest Management plans for Long Island potato farms is examined.

## *Introduction*

The Colorado potato beetle remains the primary insect defoliator of potatoes in North America (Alyokhin et al. 2008). It also has an enormous reputation for developing resistance to every insecticide used against it (Forgash 1984). The populations on Long Island also have developed resistance to insecticides before other populations (Georgiou 1986; Forgash 1984). As a result Long Island growers have been battling this crop pest sometimes with no clear chemical control option. In 1995 a new class of insecticides was introduced, namely the neonicotinoids. Imidacloprid is a member of this class and its’ introduction came when Long Island growers had no effective chemical to use against the potato beetle.

The neonicotinoid class of insecticides is called neonicotinoids because of their similarity with and to nicotine.  Nicotine is found naturally in tobacco plants which the Colorado potato beetle can use as an alternate host.  The Insecticide Resistance Action Committee's formal designation is: "Nicotinic acetylcholine receptor (nAChR) agonists".  The primary excitatory neurotransmitter in insects' central nervous system is acetylcholine.  The action of the neonicotinoid group causes hyperexcitation by mimicking the agonist action of acetylcholine at nAChR sites.  [IRAC 2010].  A nicotinic agonist is a drug which enhances the action at the nAChR.  Examples include nicotine, acetylcholine and choline, among others.  Neonicotinoids have a high affinity for and act selectively on insect nAChR sites explaining at least partially the low mammalian toxicity characteristic which make this group so attractive.  Low mammalian toxicity and the fact that it offered better control at less cost on Long Island made it the overwhelming number one choice for chemical control.  The most widely used neonicotinoid on Long Island in potatoes is imidacloprid.

Upon halting diapause and emerging from the soil Yang (1994) found 20% had flight muscles fully regenerated.  This regeneration had happened while the Colorado potato beetle was still in the soil and is evidence that some beetles complete flight muscle regeneration prior to halting diapause and emerging from the soil.

Yang (1994) further demonstrated that post diapausing beetles require 150-200 GDD to regenerate flight muscles and only the accumulated heat matters .  It does not matter if they remain in the soil or emerge from the soil and it also does not matter for flight muscle regeneration if they have not fed. However Ferro (1991) showed that unfed beetles fly more often and farther.

Comparing the resistance of emerging potato beetles that walked away from the diapause site with that of beetles that flew from the diapause site could provide insight as to costs of resistance as it relates to movement, or migration at the time of emergence from diapause. If we could determine the right spatial scale for field rotation it could be a valuable tool in managing this crop pest and extending the shelf life of the one chemical that provides some control on Long Island. Said another way, if flyers are more susceptible then increasing distances between fields would lead to preferential colonization by susceptible beetles and they can be better controlled by imidacloprid. This could be part of an Integrated Pest Management (IPM) plan to reduce the quantity or frequency of imidacloprid applications. If early maturing potato plants were used in new potato fields that are a long distance from last year's potato then the plants might already be mature and producing the potato tubers by the time of colonization by potato beetles. These mature plants would not suffer as much of a decline in potato production as immature plants. And if the flying beetles were less resistant then the colonizing population could be better controlled by imidacloprid.

In general many costs of resistance to insecticides have been shown to exist. (Zera and Harshman 2001; Onstad and Gould 1998; Crow 1957). Fitness costs can be detrimental to resistant individuals in the absence of the insecticide (Roush and McKenzie 1987; Tabashnik et al. 1991; Rahardja and Whalon 1995; Alyokhin and Ferro 1999b; Carrière et al 2001a; 2001b; Laxminarayan and Simpson, 2002; Alyokhin et al. 2006; Baker et al. 2007). Initially I hypothesized that since evolved resistance might be associated with reduced metabolic abilities, then movement or migration in the form of flight would be more impacted than walking.

One tool developed to improve pest management is the concept of 'Growing Degree Days' (GDD). This has been utilized to predict different events in a pest's life cycle. Insect activity, growth and/or development are often temperature related. Variations in temperature between seasons and days can result in varied responses from the insects over the same time period from year to year. The easiest method to calculate GDD is to average the daily minimum temperature plus the maximum temperature and deduct the base temperature of the insect in question. Base temperature reflects the minimum temperature at which growth and/or development occurs (Boyd, 1976). The Colorado potato beetle base temperature used in agricultural calculations is $10^{o}$C ($50^{o}$F) (Yang1994). Although there are many factors involved with halting diapause, with the use of this simple tool, we can anticipate emergence from diapause and other important developments. This includes when the 150-200 GDD threshold has been reached which is required by post-diapause beetles to complete flight muscle regeneration.

This research focuses on this dichotomy of movement between the walkers and the flyers upon emergence. The majority of emerging beetles are found walking from the overwintering site, but an observable proportion does fly. The goal was to see if there was a movement cost of resistance between those that only walked from the overwintering site in search of hosts and those that took to flight from the overwintering site.

## *Materials and Methods:*

I identified two overwintering sites. These were both treed areas surrounded by a mat of weeds and grasses. At both sites, next to the grassy mat was a dirt field road and fields on the other side of that road. I collected the beetles on the road edge of the overwintering sites and on the roads bordering those sites. Those that were walking from the overwintering site were labeled 'walkers'. Those that were flying over this road, away from the overwintering site were labeled 'flyers'. Collections began before potato plants had emerged in the field. Collections stopped before any possibility of encountering summer generation adults. This confined the specimens to only overwintered adults.

These two study sites were conventional commercial potato-growing farms of Suffolk County, Long Island, NY. Samples were taken at the time of spring emergence from the overwintering sites during April, May and June of 2008, 2009 and 2010. All samples were taken prior to there being any possibility of a first summer generation. For this study preliminary (bi-weekly) field visits started in March and continued more intensively (daily) from mid-April through June.

Site A has a central woodlot, surrounded by approximately 94 hectares (232 acres) of fields. The central woodlot is approximately 1.85 hectares (4.57 acres). The fields surrounding this woodlot were continuously planted in potato. Beginning in 2008, a portion of one side near the overwintering site was rotated and planted with grain. All remaining fields surrounding the central overwintering site were planted with potatoes. When beetles emerge in the spring potato fields are as close as 3- 4 meters. These fields are treated with Imidacloprid in furrow at planting. The fields are scouted throughout the season to determine when and if additional treatment is necessary. The growers work very close with the extension agent on all aspects of pest management. This site must also manage for Golden Nematode. In 2010 they used Alias® brand of imidacloprid along with foliar sprays of Baythroid ® (Bayer).

At Site B the field closest to the overwintering site is approximately 12.8 hectares (31.7acres) and is at least 22.5 meters from the overwintering site. It is planted in potato in even numbered years (e.g. 2008, 2010) and rotated in grain in odd numbered years (2009). In even numbered years the beetles emerging from this overwintering site need only move 22 meters to find host plants however, they must cross an active railroad track to get there. In odd numbered years the beetles would have to travel to a different field to find host plants. The closest field in these odd numbered years is 158m to the west. The field management at this site requires this longer movement to find host plants every other year. Another difference at Site B is the woodlot, which is peripheral, not central. The fields near this woodlot are rotated with potatoes one year and grain the next. This field is treated with Imidacloprid in furrow at planting and weekly scouting is done throughout the season to determine when and if more treatments are necessary. This site never used Assail® or foliar spray Admire® and is not required to manage for Golden Nematode. Additional insecticides used when and if necessary include SpinTor® (Radiant®) and Cryocide®.

Drift fences were constructed and installed shortly after the fields were planted. These drift fences consisted of metal roofing installed upright with periodic coffee cans buried in the soil to the top of the can, to trap walkers. I emptied the traps at the start of each day to increase the likelihood of collecting new emergers. In addition to drift fences, I also employed trap plants at the edge of the overwintering site. These greenhouse grown untreated plants were placed on the ground to attract walkers, with full acknowledgment that some beetles could have flown to these plants.

A combination of harp traps, passive trap plants and active hand held traps were used to capture flyers. Harp traps of $1m^2$ (modeled after Boiteau 2000) were constructed and holes drilled every 1 cm. I threaded fishing line through each hole and then mounted these traps onto wooden legs, which were installed in the ground at the edges of the overwintering site. I alternated the fishing line between 2 pound test and 6 pound test. I coated rain gutters with Fluon® (trademark AGC Chemicals Americas, Inc) and installed them on the harp trap frame, underneath the fishing line. The Fluon® kept flyers in the rain gutter until collection. I coated the legs of the traps with Tanglefoot® (trademark Contech Enterprises) partway up both legs to eliminate any walkers from entering the rain gutters.

I designed a passive trap plant for the field. Potted potato plants were nailed on to a garden stake and these were installed at the edge of the overwintering sites. I grouped 4 to 5 of these trap plants together. Tanglefoot was applied partway up each stake to eliminate walkers from reaching the plant and any nearby weeds and debris were cleared. This reduced the risk of walkers accidentally getting onto the trap plants. Any Colorado potato beetle on the plant had to fly to get there. These passive trap plants were installed along the edges of the overwintering site while the fields were still barren. These passive trap plants were constructed and installed at both overwintering sites in 2009 and 2010.

Hand held badminton raquettes and nets were used for active capture of flyers in 2009 and 2010. I spotted flyers from quite a distance away and then chased them down and manually captured them in the net or raquette. I collected walkers and flyers on the same day, within the same time period to reduce day to day variability.

All specimens were fed *ad libitum* untreated potato leaves from greenhouse grown plants, for 2-3 days in 8”x4”x6” plastic cages. They were then treated one time with 1 of 8 concentrations of imidacloprid. I placed a 1-2 µl drop on their abdomen (2 µl were required for only the highest dose) and then placed the treated beetles into a new Petri dish with filter paper. The petri dishes were given daily cleanings and beetles had daily access to fresh water and untreated potato foliage for 7 consecutive days. On day 7 they were scored as Dead, Alive or Moribund.

Dead is the absence of any signs of life. Moribund meant there was some sign of movement but they were unable to right themselves. Moribund movements include trembling legs or antennae, mouthpart movements etc. Alive indicated that they could right themselves within 45 seconds, when flipped. For analysis moribund was pooled with dead.

The imidacloprid doses used for abdominal bioassay on adults are shown in Table 2-1.
For analysis I used Proc Probit, (2002-2003 by SAS Institute Inc., Cary, NC, U.S.A.) to determine the LD50 for each group. Each year I attempted to test at least 30 flyers and 30 walkers from each field at each dose.

Four models were used for *Akaike Information Criteria* (AIC) analysis with SAS. The first model was a normal distribution with the calculated response of the control dose Dose O or mortality at zero concentration. The second model was also a normal distribution but forced mortality to zero for the control dose. This model assumes that observed mortality at concentration 0 (control dose) is sampling error. Model 3 used a logistic distribution and calculated mortality for Dose 0 as above in Model 1. Model 4 was also a logistic distribution but forced mortality to zero for the control dose, again assuming that observed mortality at concentration 0 is sampling error.

Following Burnham and Anderson (2002) our models were contrasted with respect to underlying distribution (normal or logistic) and whether a control level (zero dose) effect was assumed or estimated. The model with the highest Akaike Information Criteria relative weight was used to estimate the LD50 and associated upper and lower 95% Confidence Limits.

Temperature and solar insolation play integral roles in the flight behavior of the Colorado potato beetle. The average temperatures for Riverhead, NY are as follows. (Riverhead Research Farm)

| Month: | March | April | May | June |
|---|---|---|---|---|
| Maximum | 9oC | 15$^{o}$C | 22$^{o}$C | 26$^{o}$C |
| Minimum | 0$^{o}$C | 4$^{o}$C | 10$^{o}$C | 15$^{o}$C |
| Mean | 5$^{o}$C | 10$^{o}$C | 16$^{o}$C | 21$^{o}$C |

Caprio and Grafius (1990) reported that in Michigan no beetles flew below 15$^{o}$C and all unfed beetles flew at 20$^{o}$C. The earliest observed flight during the three years of this study occurred in late April in 2010.

At the study site the number of days that the maximum temperature was at 20°C or above (and thus conducive to potato beetle flight) is as follows:

| **Year:** Month | **2008** | **2009** | **2010** |
|---|---|---|---|
| March | 0 | 0 | 4 |
| April | 7 | 5 | 15 |
| May | 18 | 15 | 25 |
| June | 29 | 24 | 30 |

The number of days in each month, below 15°C when no beetles were observed to fly are as follows:

| **Year:** Month | **2008** | **2009** | **2010** |
|---|---|---|---|
| March | 28 | 27 | 22 |
| April | 14 | 16 | 6 |
| May | 5 | 3 | 2 |
| June | 0 | 0 | 0 |

Growing Degree Days table below shows the date I first observed potato beetles emerging from the overwintering site and the accumulation of GDD.

| Year | Date of first emergence | GDD accumulated |
|---|---|---|
| 2008 | April 03, 2008 | 78 |
| 2009 | May 1, 2009 | 109 |
| 2010 | April 15, 2010 | 100 |

Spring emergence began was determined by my observation when making field visits and marks the date that I saw the first Colorado potato beetle emerged. It should be noted that 2009 was an extremely wet spring preventing plantings in some cases until mid-May and had a late first emergence date at the study site. Colorado potato beetles emerge between 50GDD and 250

GDD. There is no difference in emergence between the sexes (Ferro et al. 1991). In Massachusetts approximately 50% of the Colorado potato beetles emerge from diapause after 150 GDD (Yang 1994; Ferro et al 1991 – oviposition & flight behavior). However Lashomb reported 90GDD required for 50% emergence in New Jersey. (Lashomb et al 1984)

## *Results:*

Flying emergers were significantly more resistant than walkers in all years at all sites.

Table 2-2 has the sites for each year along with the sample sizes and $LD_{50}$ values. In all years at all sites, the sample size of walkers (n=226 to 426) was larger than the sample size of flyers (n=61 to 224). In 2008 Site A, I captured and dosed 426 walkers and 153 flyers. In 2009 Site A, I captured and dosed 254 walkers and 129 flyers. In 2010 field A, I captured and dosed 250 walkers and 224 flyers. In 2009 at Site B, I captured 380 walkers and 61 flyers. In 2010 at Site B, I captured 226 walkers and 198 flyers. The sample size of walkers was larger at all sites in all years because walkers were easier to capture.

Figure 2-1 is a graph comparing the values for walkers and flyers by year and by site. In all years at all sites the flyers had a higher $LD_{50}$ than the walkers. The $LD_{50}$ for flyers ranged from 5.7 to 7.56 µg/insect while The $LD_{50}$ for walkers ranged from .275 to 3.928 µg/insect.

Figure 2-3 is a graph of the LD50 for the Flyers and Walkers for the 3 years of this study. The error bars represent a 95% CI. In all 3 years at all sites Flyers had a higher LD50 to imidacloprid than walkers and lacked any overlap between the 95% CI and the LD50 estimates of the other group.

Statistics were calculated with Proc Probit (2002-2003 by SAS Institute Inc., Cary, NC U.S.A.), AIC and 4 models. Using model fitting – an information criterion by Akaike we fit data to a model and find the log likelihood of that model. Model 1 assumed a normal curve with the calculated mortality at dose 0. Model 2 assumed a normal curve with mortality at does 0 forced to be zero. Model 3 assumed a logistic curve with calculated mortality at dose 0. Model 4 assumed a logistic curve with mortality at dose 0 forced to zero.

## *Discussion:*

Late in the growing season senescing hosts or no hosts in conjunction with short photoperiod and lower temperatures signal adult Colorado potato beetles to begin diapause. Some have been shown to overwinter directly in the soil of the potato field (French et al. 1993). Most have been shown to overwinter as adults in the soil at the edge of a tree lot or hedgerows surrounding that year's potato field (Weber and Ferro 1994; Boiteau et al, 2003). The overwintering sites near tree lots provide an excellent opportunity for research on emerging potato beetles in the spring. In spring the overwintered adult Colorado potato beetles halt diapause and emerge from the soil. They burrow out of the soil leaving a hole behind them. I observed that upon emerging most potato beetles walk in search of suitable hosts but some fly. This flight can happen immediately upon emerging from the soil or the beetles engage in a short walk and then fly. I define the 'walkers' are those that are walking at the time of capture, from the overwintering site in search of suitable hosts. I define the 'flyers' are those that are engaged in flight at the time of capture, from the overwintering site. I did not differentiate between those that engaged in flight immediately upon emerging or engaged in a short walk before flight.

Flight muscles totally degenerate in diapausing Colorado potato beetles. Upon halting diapause, Colorado potato beetles must regenerate their flight muscles in the spring. Flight muscle degeneration is a common phenomenon in insects and can take place during adult, larval or pupal stages. The benefits can be an increase of available space for energy stores and/or to eliminate energy consumption by flight muscles. In the Colorado potato beetle flight muscle degeneration happens only after beetles disperse to diapause sites and coincides with the onset of diapause. This happens at the end of the season when there are senescing (poor quality) or no host plants available. Since Colorado potato beetles cannot feed at overwintering sites and thus cannot increase their energy stores, De Kort implies that at least in the Colorado potato beetle flight muscle degeneration is to reduce energy consumption. (De Kort 1969).

De Kort further demonstrated that flight muscle degeneration and regeneration are controlled by Juvenile hormone which is also one factor that regulates the termination of diapause in the Colorado potato beetle.

De Kort (1969) reported that flight muscles totally degenerate in diapausing beetles and they take seven to nine days to complete development <u>after</u> emerging from the soil. This is the same time requirement as summer generation beetles upon completing pupation and emerging as adults. Contrary to that, Stegwee (1964) reported that flight muscles do regenerate in the spring and he demonstrated that complete regeneration of the flight muscles had occurred at the time of emergence from the soil. Upon emerging the beetles are on the move, some walking and some also flying. Additionally post diapause beetles have been shown to fly on a flight mill on the first day after emergence from diapause (Ferro et al. 1991). Flight on the first day after emergence is consistent with Stegwee's findings (1964). Finally, Yang (1994) further demonstrated that flight muscle regeneration relied only on accumulated heat. Flight muscle regeneration was not dependent upon whether or not the beetle stayed in the soil or feeding. And regeneration could be completed without food (Yang 1994). This is of great significance as it means that beetles could remain in the soil while regenerating flight muscles. This would enable flight upon emergence, which is consistent with Ferro et al (1991) and Stegwee (1964) as well as supporting what I have observed in the field. This also affords the beetle an opportunity to avoid predation while developing flight muscles and if there are no host plants upon emerging, they could immediately take flight in search of hosts.

Little is known about the movement of recently emerged overwintered beetles (De Kort, 1990) (Boiteau et al, 2003). This study indicates that there is a trend of flyers having higher resistance than walkers at all sites for all years. One factor that I could not entirely separate is completely differentiating between flyers and walkers. Colorado potato beetles have some pre-flight behaviors that delay takeoff. This delay seems to be pretty widespread in the Long Island populations that I observed. They will climb a blade of grass or the stalk of a weed or a bamboo skewer that I've placed near the overwintering site, climbing towards the top. There is the chance that a beetle I captured while 'walking' and I labeled a 'walker' was actually a flyer who was conducting a pre-flight walk.

In prior year's potato fields where rye is the rotated grain crop in spring, the beetles will climb to the top of the rye, all the way to the seed head. I have observed them holding on in one spot for more than two hours. At some point, the conditions signal flight and multiple beetles take off, most usually flying in the same general direction. Temperature has been shown to have an effect on flight in Colorado potato beetles as well as solar insolation, wind and feeding status (Caprio and Grafius 1990; Ferro et al. 1991). These studies involved removing diapausing Colorado potato beetles from the soil and bringing them into the controlled laboratory environment. They demonstrated that spring unfed Colorado potato beetles are capable of long distance flights and a single female that mated pre-diapause represents a viable founding population in spring. Caprio and Grafius (1990) reported that no beetles flew below 15$^{o}$C and all unfed beetles flew at 20$^{o}$C. Sequential days at or above the 20$^{o}$C threshold begin to appear in the Long Island field sites on 4/17/2008; 4/17/2009 and 4/11/2010.

Emerging beetles enter a barren landscape as the field plants have not yet emerged, thus they are unfed. Both overwintering sites are surrounded by a mat of grass and then a field road (and railroad tracks in one case) before reaching the fields. It is on these grass mats and the adjacent field roads that I collect walkers and flyers.

The $LD_{50}$'s of flyers are higher for all sites and all years. This is not what I expected or predicted. I use the term long distance rotation to mean potatoes grown in year t+1 are greater than 300m from potatoes grown in year t. This could imply that long distance field rotations may not have great value in managing potato beetle damage; however this may not be as detrimental on Long Island as one would suspect. The results may reflect no cost of resistance and flight. They could also represent a flight benefit of resistance. The results could reflect sublethal effects of pesticide exposure. In these populations all are exposed to neonicotinoids and possibly only the most resistant are in good enough condition. Whether or not it's a benefit of resistance or a consequence of exposure, rotation of crops may favor resistance evolution if only the most resistant are flying to rotated fields. In Suffolk county, agricultural lands have been converted into development, vineyards and other uses out of agriculture. This limits the options growers have to rotate fields and long distance rotations are not generally a viable option on Long Island. The results of this study seem to indicate that long distance rotation is not an important pest control tool in Suffolk County.

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Figure Headings

Fig 2-1: Graph of Flyers vs. Walkers over 3 years from 2 different sites.

Figure 2-1: Graph of LD50 for Flyers and Walkers for 3 years.

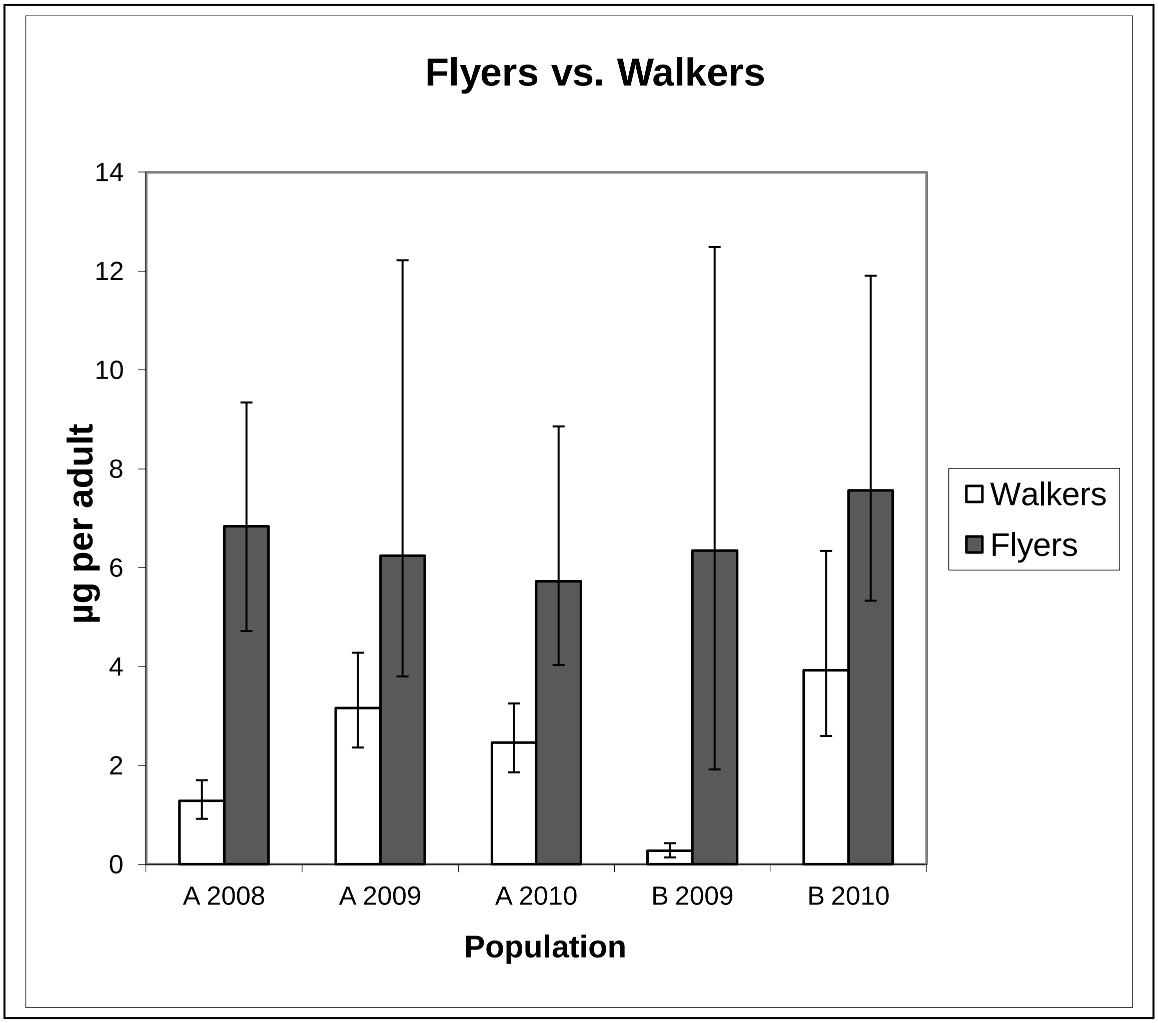

**Table 2-1 Imidacloprid doses for abdominal bioassay**

| Dose | [ ] g/ml |
|---|---|
| 1 | 0 |
| 2 | 3.125e ^$^{-4}$ |
| 3 | 6.25e ^-4 |
| 4 | 1.25e $^{\wedge -3}$ |
| 5 | 2.5e $^{\wedge -3}$ |
| 6 | 5e $^{\wedge -3}$ |
| 7 | 1e $^{\wedge -2}$ |
| 8 | 2e $^{\wedge -2}$ |

**Table 2-2: LD$_{50}$ Estimates to imidacloprid for all sites and years. Estimates are derived from the AIC model with the highest AIC weight.**

**Legend:**

**Aw = Site A walkers**

**Af = Site A flyers**

**Bw = Site B walkers**

**Bf = Site B flyers**

| Field | Year | *n*[a] | LD$_{50}$[b] | Lower[c] | Upper[c] | Model used [d] |
|---|---|---|---|---|---|---|
| Aw | 2008 | *426* | 1.2808 | .9187 | 1.699 | 2 |
| | 2009 | *254* | 3.1579 | 2.3619 | 4.2796 | 4 |
| | 2010 | *250* | 2.4618 | 1.8594 | 3.2532 | 2 |
| Af | 2008 | *153* | 6.8332 | 4.7171 | 9.3429 | 1 |
| | 2009 | *129* | 6.2354 | 3.8000 | 12.221 | 4 |
| | 2010 | *224* | 5.7265 | 4.0289 | 8.8585 | 4 |
| Bw | 2009 | *380* | .2753 | 0.138 | 0.4249 | 4 |
| | 2010 | *226* | 3.9282 | 2.5943 | 6.3393 | 4 |
| Bf | 2009 | *61* | 6.3482 | 1.9178 | 51.4902 | 4 |
| | 2010 | *198* | 7.5604 | 5.3315 | 11.9068 | 4 |
| | | | | | | |

[a] Number of adults tested

[b] LD$_{50}$s are in units of µg/insect

[c] 95% Fiducial limits

[d] AIC model used

Model 1 assumed a normal curve with the calculated mortality at dose 0.

Model 2 assumed a normal curve with mortality at does 0 forced to be zero.

Model 3 assumed a logistic curve with calculated mortality at dose 0.

Model 4 assumed a logistic curve with mortality at dose 0 forced to zero.

# Spinosad resistance on Long Island populations of the Colorado potato beetle

## *Abstract*

Spinsosad is a biologically based insecticide used by organic and conventional growers. While used by both organic and conventional growers, it is the sole approved insecticide for use on Long Island organic farms. In 2006 resistance to spinosad in seven conventionally managed field populations on the South Fork of Long Island and one susceptible laboratory population (control) was measured in 2nd instar larvae. All field populations were more resistant to spinosad than the control lab population and the maximum Resistance Ratio (RR) using $LD_{50}$ was 9.8 ($LD_{50}$=Lethal Dose that kills 50% of the population). Spinosad resistance was measured again in 2010 after reports of complete failure on organic potato fields. In 2010 the larval resistance ratios of commercial fields varied from 17.5 to 5750.3, a dramatic increase in spinosad tolerance. Further, the three most resistant fields were all organically managed meaning they could only use Spinosad, while the four less resistant fields were all conventionally managed using a variety of insecticides from different chemical classes. In this instance, strength of selection and number of applications appears to have been more potent factors than population size or selection with other chemicals for evolution of resistance to spinosad.

**Keywords:** Colorado potato beetle; *Leptinotarsa decemlineata;* imidacloprid; Spinosad; neonicotinoids; cross-resistance; Chrysomelidae

## *Introduction*

Effective management of insect pests via insecticides almost guarantees strong selection for resistance, and has predictably led to repeated cases of resistance evolution (Denholm et al. 2002, Alyokhin et al., 2008). While resistance to biologically-based toxins (e.g. azadiractin; Feng and Isman 1995, Spinosad; Sayyed *et al.* 2004, *Bt*; Tabashnik 1994, Tabashnik *et al.* 2008, Storer *et al.* 2010) and biocontrol agents (CpGV; Eberle and Jehl 2006) have been reported there is less attention given to insecticide resistance in organic agriculture (Zehnder *et al.* 2007). Most often direct comparisons of organically and conventionally managed or IPM (Integrated Pest Management) population resistance use organically managed fields as alternately-treated controls to look at synthetic chemical insecticides (Reuveny and Cohen 2004). Persistent use of a singular chemical insecticide increases selection pressure and selects for resistance. The absence of processes such as immigration and/or refugia dynamics combined with this lack of chemical rotation options can hasten the evolution of resistance.

Though the same ingredient is sometimes used in both conventionally managed and certified organic farms, several factors may differ in their effects on resistance. On conventionally managed fields, larger population sizes, possibly longer persistence of biologically-based insecticides, and previous selection with chemical insecticides with shared resistance mechanisms can all favor resistance evolution. Organically managed farms are more likely to use non-chemical control techniques. On the other hand, conventional management may offer more choices of ingredients, which can reduce reliance on, and selection by, any single ingredient. A lack of alternatives may lead to earlier adoption and more frequent use of biologically-based insecticides, which will lead to higher resistance evolution to those ingredients. Here we compare resistance in Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (henceforth Colorado potato beetle) , to a biologically based toxin, spinosad, on organically and conventionally managed fields in eastern New York (Suffolk County).

The Colorado potato beetle is the primary insect defoliator of potato (Weber and Ferro 1994) and is renowned for rapidly evolving resistance to every insecticide widely used in its control (Forgash 1985; Casagrande 1987; Ioannidis et al. 1991). Neonicotinoid insecticides (Tomizawa and Casida 2003) have been in wide use for just over a decade and are widely used against Colorado potato beetles in eastern NY. Despite increasing resistance (Olson et al, 2000; Alyokhin et al, 2006; Mota-Sanchez et al. 2006; Alyokhin et al. 2007), use of neonicotinoids has remained high, even in areas with higher levels of resistance, due to its in-furrow application, systemic expression and effectiveness against other insect pests; primarily aphids and leafhoppers. To avoid multiple treatments with neonicotinoids and to control beetle populations, many growers will choose an alternative to neonicotinoids later in the season.

In Long Island, NY, for example, most growers using conventional management apply in-furrow imidacloprid at planting, but later in the season apply a different insecticide usually with a different mode of action. This strategy has been highly effective (Alyokhin et al. 2006). Organic growers in Eastern NY are allowed to apply a total of 6oz of Entrust™. The rate is 1 to 2 oz per acre therefore growers can apply either 6 or 3 applications per year. The Cooperative Extension recommends only the high rate, due to the failing nature of the product (S. Menasha, pers. comm.) Conventional growers have more chemical options for insecticide use but limits apply as well. Conventional growers are limited to 32 fluid oz of Radiant applied to potato per acre per year. The rate of Radiant on potato is 6-8 fl Oz. Again the cooperative extension recommends only the high rate. Restrictions for Assail and Provado (another name for Alias) are as follows: Assail should not exceed 16 fl oz/acre for potato per year and no more than 4 applications per crop per season. The rate is 1.5-4fl. Oz. per acre. Provado: Growers should not exceed 16 fl. Oz/acre/crop/year and the rate is 3.8 fl oz/acre for potato.

Spintor™ is an insecticide product of Dow AgroSciences that contains spinosad as its active ingredient. Spintor™ has been approved for use on vegetables since 1999, but was not adopted widely on Long Island until the mid-2000's, and organic growers have been using spinosad since 2005.. Spinosad is a mixture of two compounds, spinosyns A and D, derived from fermentation of the actinomycete *Saccharopolyspora spinosa* (Kirst 2010). *S. spinosa* was isolated from soil samples collected in 1982 in the Caribbean (Mertz and Yao 1990) and identified as a potential insecticide from mosquito bioassays. Spinosyns are placed in Group 5 by Insecticide Resistance Action Committee (IRAC) (2010) and their formal designation is nicotinic acetylcholine receptor allosteric modulators. This group allosterically activates the Nicotinic acetylcholine receptor (nAChR) causing hyperexcitation of the nervous system. When allosteric activators bind to regulatory sites of enzymes, the shape of the active site changes so it can bind its substrate and catalyze production.

Imidacloprid is a neonicotinoid class insecticide and they are placed in IRAC main group 4. They are called neonicotinoids because of their similarity with and to nicotine. Nicotine is found naturally in tobacco plants which the Colorado potato beetle can use as an alternate host. The IRAC formal designation is nAChR agonists. The primary excitatory neurotransmitter in insects' central nervous system is acetylcholine. The action of this group causes hyperexcitation by mimicking the agonist action of acetylcholine at nAChR sites (IRAC 2010). A nicotinic agonist is a drug which enhances the action at the nAChR. Examples include nicotine, acetylcholine and choline, among others. These two insecticides, Spinosyns (spinosad) and neonicotinoids (imidacloprid), were placed in different Mode of Action classes but have a somewhat similar mode of action and cross resistance is a potential problem. Alyokhin et al. (2006) caution against relying solely on spinosad as an alternative to imidacloprid due to the similar modes of action.

Isolation or the absence of immigration has been shown to have adverse effects enhancing the evolution of resistance. In California house flies, the populations with the highest resistance levels were found inside the poultry houses. These houses were screened, presumably to exclude flies but once inside they were trapped there. The screens diminished immigrants which probably contributed to higher levels of resistance (Georghiou and Taylor, 1986). It can be argued that isolation is a factor with two of the organic fields as these populations are near each other and yet are more than 7 miles away from the closest agricultural field. These two populations have the highest resistance levels of all those tested. Quite often subjective grower assessment of poor insecticide performance is supported and confirmed by scientific studies (Boiteau et al 1987; Stewart et al 1997; Alyokhin et al 2007; Alyokhin et al 2008 ; Alyokhin et al 2009). This is such a case.

Spinosad resistance has been artificially selected for or observed in field or greenhouse populations in several species (Table 3-1). $LC_{50}$ values are the concentration of chemical that kills 50% of the population in a given time. $LC_{50}$ values are used when the dose is not controlled such as in a leaf dip or artificial diet experiment. The insects consume an unknown quantity but the concentration of the medium (leaf dip or diet) is known. Field resistance ratios of $LC_{50}$ vary from fully susceptible in several species to 20,600 in Diamondback moth, *Plutella xylostella* (Sayyed *et al*. 2004). Selection in the lab has yielded resistance ratios from 21.7 to 1068 (Table 1). The present study reports field resistance from several Eastern NY (Suffolk Co.) farms. Some farms were sampled both in 2006 and 2010, some only collected from in one of those years. In 2006 only conventionally managed fields were sampled. In 2010, enough organic and conventional farms were visited to allow comparison of the resistance ratios found in both management methods.

Resistance ratios are ubiquitous in entomology, toxicology and animal testing and are used to compare resistance among different populations or among different ingredients. Simply stated, the Resistance Ratio (RR) is the resistance of one population over the resistance of a reference population. The reference population can be a lab susceptible population, a field susceptible population or the population with the lowest resistance.

## *Materials and Methods*

### Field surveys

**2006:**

Eight fields were sampled in 2006 near Bridgehampton, NY, on the South Fork of Long Island. Six of the fields were treated with imidacloprid (Admire™ Pro, Bayer CropScience, Kansas City, MO) at planting, and two were 4-acre portions of larger imidacloprid-treated fields left untreated at planting. Initial egg collections of Colorado potato beetle were made in June before any spinosad applications. Only two fields had a second summer generation large enough to assay. Of these, one was untreated with imidacloprid but received two spinosad (SpinTor 2SC Naturalyte™, Dow AgroSciences, Indianapolis, IN) treatments (Field “5”), and one field was initially treated with imidacloprid at planting and treated with Cryolite (Kryocide™, Cerexagri, King of Prussial, PA) later in the season (Field “1”). An additional field (Field “8”) was identified by a grower in late July as being highly resistant to spinosad. In that field, adults were collected and reared in a 46 x 61 x 51 cm cage on untreated foliage. Enough eggs were collected to assay resistance to spinosad. A laboratory susceptible colony, originating from field-collected clutches in Maryland, was used as the baseline.

**2010:**

Three fields located on the eastern end of Long Island were identified as having populations that could not be controlled by Spinosyn in 2009. These fields are isolated and are more than 7 miles away from any other agricultural fields. One of these isolated fields has not been in potatoes for more than 25 years. These three fields are surrounded largely by estates and golf courses. All three fields are owned by one entity but half the acreage is rented out to a separate grower with slightly different field treatments. In 2009 growers reported a lack of control with spinosyn. The plants were destroyed and the fields plowed under when I arrived. As a result there were not enough beetles to do a bioassay analysis for 2009.

In 2010 I arrived prior to plant emergence in the field and installed passive trap plants. The trap plants were untreated and grown in a greenhouse. Three organic farms and four conventional farms were identified for collecting specimens. Two organic fields are located on the south fork and one organic field is located on the north fork. Three traditional fields are located on the north fork and one traditional field is located on the south fork. Sample sizes from organic and conventional fields are in Table 3.4

### Larval assays

Field clutches were collected and raised to second-instar larva in the lab. These second-instar larvae weighing 6-8.5 mg were assayed by direct topical application on the abdomen of a 1 µl drop of spinosad dissolved in HPLC-grade (0.995) acetone. Spinosad was extracted from SpinTor 2SC Naturalyte® (Dow AgroSciences LLC) by first diluting 1:9 using HPLC-grade (0.995) acetone, then vacuum filtered twice to remove remaining particulates. The extraction used to create all solutions used in this study was confirmed by a specific immunosorbent assay performed by Environmental Micro Analysis, INC, Woodland, CA to have 0.59 efficiency from the stated concentration of active ingredient in the product label. Up to 7 concentrations from $4.2\times10^{-3}$ to $1.35\times10^{-1}$ µg/larva in 2006, and up to 17 concentrations in 2010 of spinosad, from $4.2\times10^{-3}$ to 85 µg/larva, plus an acetone control, were used. Following application, larvae were placed on a potato leaf cutting and held at 25°C for 24 hours until scoring. Mortality was defined as failure to move a leg for 10 seconds after the larva was placed on its back.

### Adult assays

Emerging spring adults were collected from trap plants initially and then from trap and field plants once the field plants emerged. These beetles were placed in a plastic cage (8”x4”x6”) with untreated potato foliage and kept in a cooler for the day.

All specimens were fed untreated potato leaves from greenhouse grown plants, for 2-3 days. In all years each adult received one bioassay treatment. The bioassays were by direct topical application of a 1µl drop of spinosad dissolved in HPLC-grade (0.995) acetone on the abdomen. Spinosad was extracted from SpinTor® 2SC Naturalyte® as described above for larva. Four different concentrations of spinosad dissolved in acetone plus an acetone control was used.

Following bioassay the adults were placed in a petri dish with untreated potato foliage and fresh water. All petri dishes were cleaned daily and given fresh water and untreated potato foliage for 7 consecutive days. On day 7 they were scored as Dead, Moribund or Alive. Dead is the absence of any signs of life. Moribund is when there was some sign of movement but they were unable to right themselves in 60 seconds. Moribund movements include trembling legs or antennae, mouthpart movements, etc.

## *Analysis*

Dose-mortality curves were analyzed using a probit analysis (Le Ora Software 1987) to estimate the slope and the fiducial limits around the estimate of the dose necessary to achieve 50% mortality ($LD_{50}$). We attempted to assay at least 30 individuals at each dose. If fewer were available at the correct size, we included the $LD_{50}$ results only if the index of significance for potency estimation, $g$, was less than 0.7 at the 0.95 confidence level (Finney 1971). Larval resistance ratios were calculated at the $LD_{50}$ relative to that of the susceptible colony. Adult resistance ratios were calculated at the LD50 of the most susceptible field population. For Adult analysis the moribund were pooled with the dead. Analyses other than the Probit analyses were used to create figures and were carried out in JMP® 6-8 (2009).

## *Results*

The larval resistance of field populations and the susceptible laboratory colony from 2006 are presented in Table 3-2. Resistance ratios to spinosad ranged from 1.8 to 9.8 times that of the susceptible population. The field that was identified by a grower late in the summer as being highly resistant to spinosad had a resistance ratio more than twice that of the next most resistant field.

All commercial fields in 2010 had higher LD$_{50}$s than those sampled in 2006. Larval resistance of North and South fork organic and conventional fields in 2010 and laboratory control are presented in Table 3-3. The laboratory control strain, though of different origin than 2006, was almost identical in tolerance to the 2006 control line. The least resistant commercial field in 2010 had an LD$_{50}$ that is twice that of the most resistant field LD$_{50}$ in 2006.

Organically managed fields were significantly more resistant than conventionally managed fields (Figure 1; Wilcoxon; n=7, df=1, $\square^2$=4.5, P=0.03). Though there was not a large enough number of fields sampled in 2010 to test statistically, it appears that there was a geographical trend in resistance, with North Fork fields generally less resistant than South Fork fields (Figure 3-2). Adult resistance of field populations from 2010 are presented on Table 3-4. These results also show the highest LD$_{50s}$ were from populations of potato beetles in the three organic fields.

## *Discussion*

Resistance to spinosad increased consistently in Eastern Long Island from 2006 to 2010, with no overlap in field resistance ratios between the two sampling years. Because different locations were sampled between the 2 years, there might be spatial variability as well. Spinosad may no longer be effective in the south fork of eastern Long Island, and the observed increase in resistance suggests a high potential for resistance evolution in other locations, in organically managed as much or more than in conventional fields. The rate of resistance change from 2006 to 2010 is well within the range observed for resistance evolution in CPB to a wide range of chemical insecticides. For example, in Georgiou's (1986) review, the number of years to report of control failure to 14 different insecticides ranged from 0-60, with a median of two years, or 4-6 generations, on Long Island populations of CPB.

Resistance is a quantitative trait, and may progress either gradually or rapidly. Resistance evolution to spinosad has been much more rapid than to the neonicotinoids, which are the most commonly used insecticides by conventional growers for CPB control, and which have led to resistance ratios in the 10's for larvae, and the low 100's for adult assays as presented here. The levels of resistance observed here are the largest measurable with this ingredient using topical assays of the ingredient dissolved in acetone or water. The highest doses used in these trials left a residue on the larvae and led to a plateau in mortality, leading to large confidence intervals around $LD_{50}$ estimates.

Evolution of resistance to spinosad has been faster than to the most commonly used bacterially based toxin, *Bacillus thuringiensis* (Tabashnik 1994, Tabashnik *et al.* 2008, Moar *et al.* 2008). Larger population size and higher doses usually, but not always, favors higher resistance in field populations vs. laboratory selected populations, and this seems to be borne out for spinosad. While reported selection experiments increased resistance above that of less resistant populations, the four highest reported rates of resistance, including this study, were reported from field or commercial greenhouse populations.

In 2010, beetles from organically managed fields were significantly more tolerant of Spinosad than were samples from conventionally managed fields as shown in Figure 3-1. Organically and conventionally managed fields differed in several ways, however, making it impossible to distinguish among several potentially causal factors. Factors that generally favor resistance evolution include large population size under selection, number of generations selected, and strength of selection. Organic plantings are smaller than conventional plantings in Eastern NY, and the largest organically managed farm sampled planted 6 acres of potato, much less than the smallest conventional farms sampled. Larger population size should increase the chance that favorable mutations appear. Though organically managed fields are often maintained at a higher population density of CPB, the 1-2 orders of magnitude difference in planted area makes up for higher density to still have a larger overall population size in conventionally managed fields and farms. In this case population size is not correlated with resistance.

Strength of direct selection has likely been higher in organically managed fields. Organic fields have been treated with spinosad more frequently and for more years. Though Spintor 2 SC™ has been listed for conventionally managed potato since 1999, and Entrust™ has been listed only since 2003, organic growers adopted it earlier and can apply spinosad up to three times per season, as opposed to once or twice per season by conventional growers using spinosad as a supplement. Most conventional fields in eastern NY have been managed using systemic neonicotinoids at planting since 1996 (Olson et al. 2000). Spinosad was adopted at different times by different conventional growers as a mid-season complement to in-furrow neonicotinoids starting around 2005. The labeled rate per application is similar for both formulations, 20-40 gm/acre for Spintor™ and 22.7-45.4 gm per acre for Entrust™, and any differences in persistence of each formulation is not known. Spintor™ has been reported to cause mortality after 12 days of aging in the field (Liu *et al.* 1999), and persistence of Entrust™ has not been published.

Previous selection by chemical insecticides does not explain the difference in resistance between conventional and organic fields. Neonicotinoids have been the most commonly used insecticides in Eastern NY since the late 1990's. Both Spinosad (Kirst 2010; Orr et al. 2009; Scott 2008) and neonicotinoids (Tan *et al.* 2008) attack nAChR subunits, but at specific sites (Orr et al. 2009). Some positive cross resistance was observed in populations with low levels of resistance to spinosad (Mota Sanchez *et al.* 2005, Baker unpublished data), but the recent large increase in resistance to spinosad in Long Island CPB has not been accompanied by large increases in resistance to imidacloprid (unpublished data), which has been more gradually increasing in resistance over the last decade (Olson *et al.* 2000; Baker *et al.* 2007; Mota-Sanchez *et al.* 2005; Alyokhin *et al.* 2008).

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## Figure Headings

Figure 3-1: South Fork Spinosad Fields

Figure 3-2: South Fork Traditional Fields

Figure 3-3: South Fork All Fields

Figure 3-4: North Fork – All Fields

Figure 3-5: North Fork spinosad field

Figure 3-6: All fields used in this study

Figure 3-7: Larval resistance ratios relative to the laboratory susceptible strain of organically and conventionally managed fields in Eastern Long Island in 2010.

Figure 3-8: Larval resistance ratios relative to the laboratory susceptible strain of North and South fork fields on Eastern Long Island in 2010.

## Figure 3-1: South Fork Spinosad Fields

## Figure 3-2: South Fork Traditional Fields

**Figure 3-3: South Fork All Fields**

## Figure 3-4: North Fork – All Fields

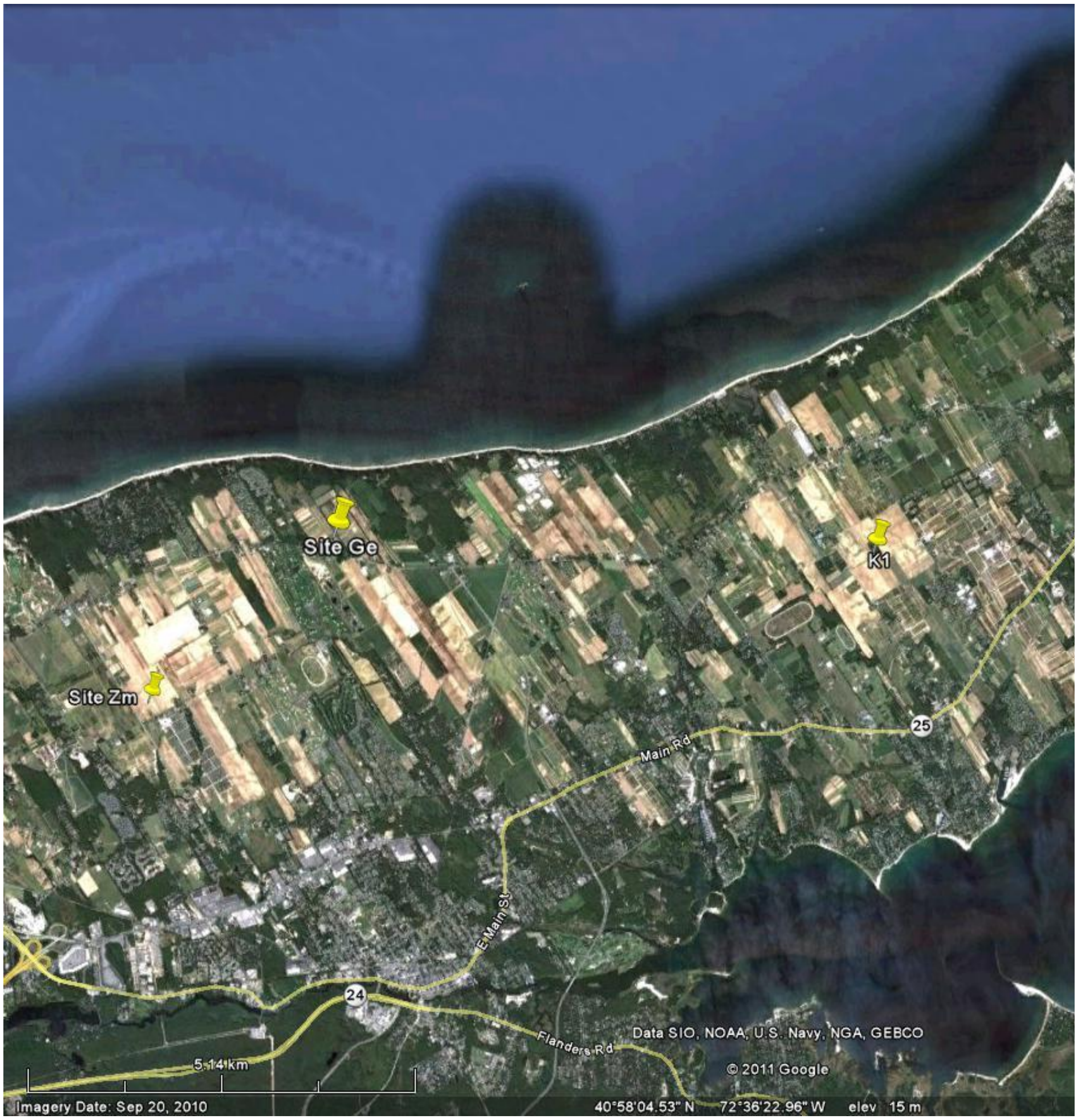


**Figure 3-5: North Fork spinosad field**

**Figure 3-6: All fields used in this study**

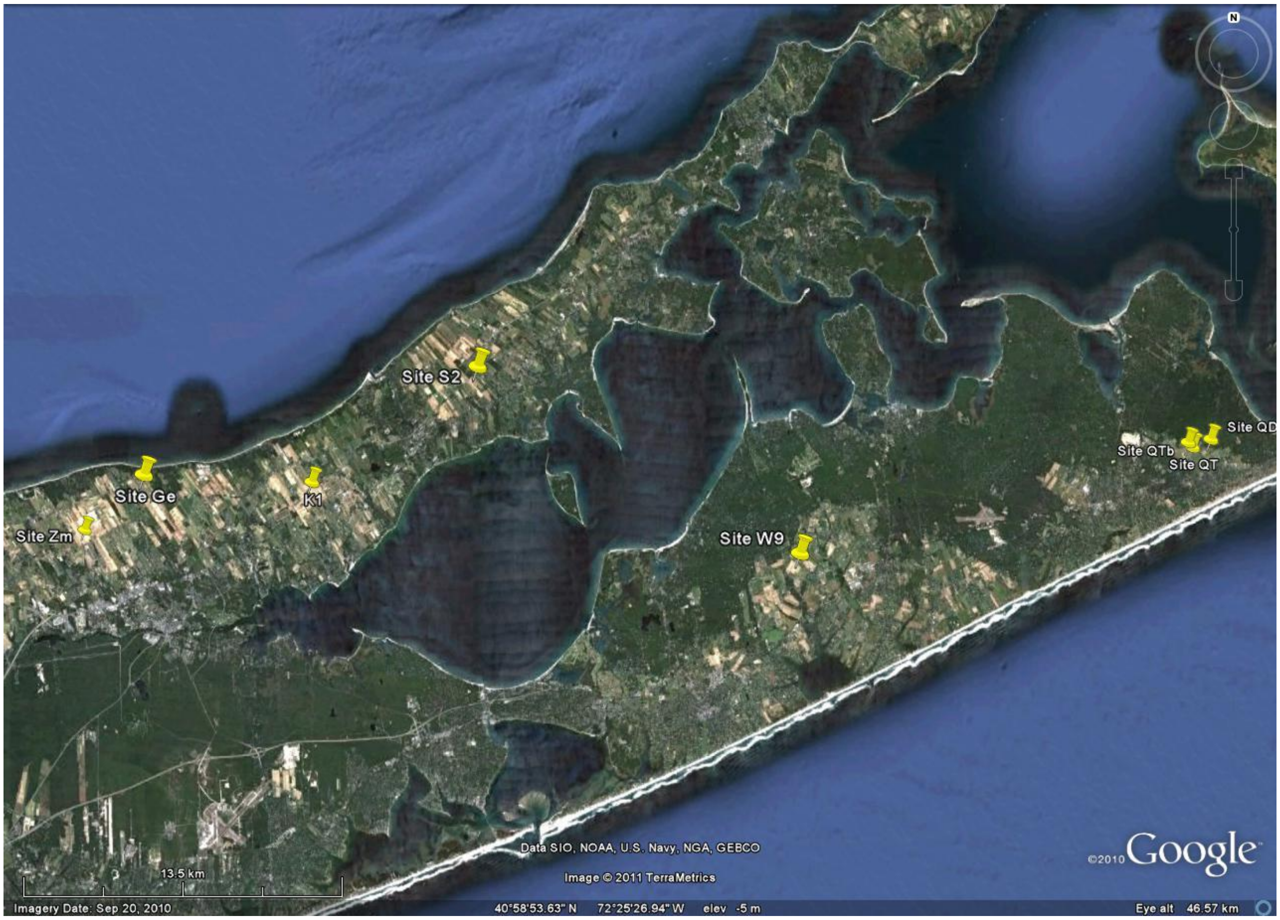


**Figure 3-7: Larval resistance ratios relative to the laboratory susceptible strain of organically and conventionally managed fields in Eastern Long Island in 2010.**

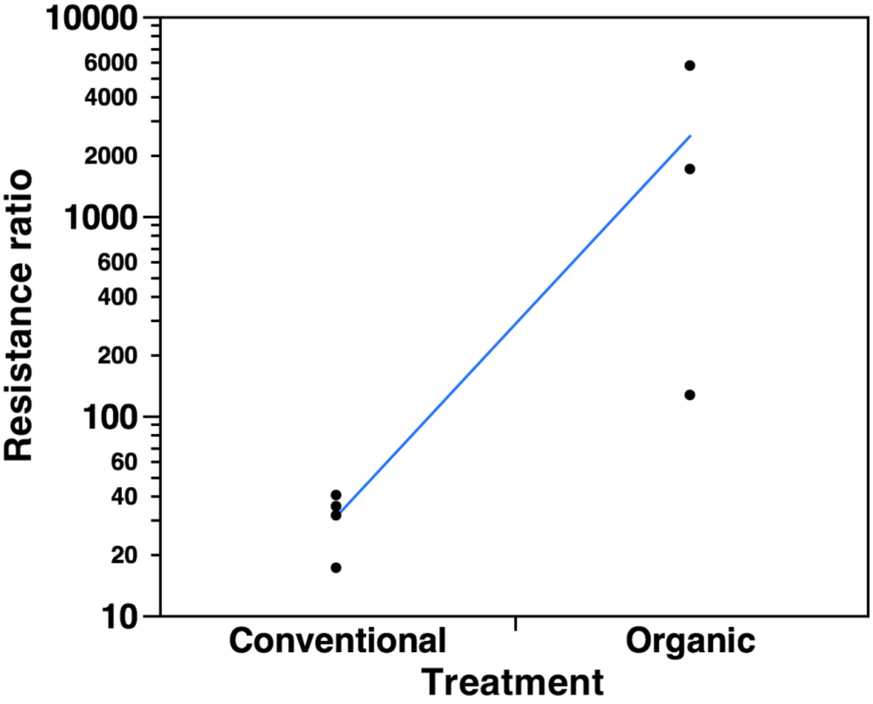
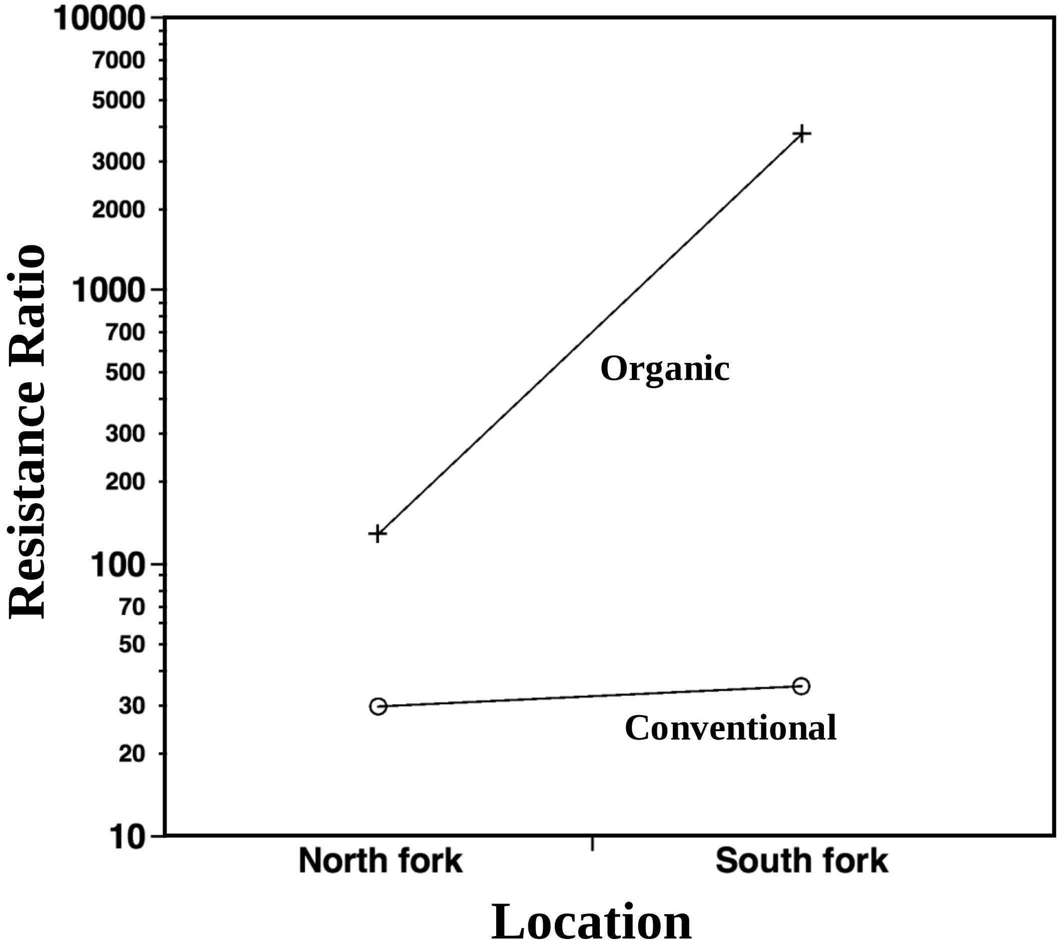


**Figure 3-8: Larval resistance ratios relative to the laboratory susceptible strain of North and South fork fields on Eastern Long Island in 2010.**



## Table 3-1. Reported spinosad resistance ratios from field and laboratory populations .

| Species | Population | Resistance Ratio | Source |
|---|---|---|---|
| Cotton bollworm, *Helicoverpa armigera* | Field | 6.5 | Wang *et al*. 2009 |
| Diamondbacked moth, *Plutella xylostella* | Field | 20,600 | Sayyed *et al*. 2004 |
| Olive fruit fly, *Bactrocera oleae* | Field | 4-13 | Kakani *et al*. 2010 |
| Whitefly, Bemisia tabaci | Field | 5.7-6.4 | Wang et al. 2010 |
| Leafminer, *Liriomyza trifolii* | Greenhouse | 188-1192 | Fergeson 2004 |
| Western flower thrips, *Frankliniella occidentalis* | Greenhouse | 13,500 | Bielza *et al.* 2007 |
| *Cotesia plutellae* | Laboratory Selected | 21.7 | Liu *et al.* 2007 |
| House fly, *Musca domestica* | Laboratory Selected | 247 | Shi *et al.* 2011 |
| House fly, *Musca domestica* | Laboratory Selected | 331[b] | Kaufman *et al.* 2010 |
| House fly, *Musca domestica* | Laboratory Selected | 150 | Shono and Scott 2003 |
| Oriental fruit fly, *Bactrocera dorsalis* | Laboratory Selected | 408 | Hsu and Feng 2006 |
| Tobacco budworm, *Heliothis virescens* | Laboratory Selected | 1068 topical, 317 diet | Young *et al*. 2003,<br>Roe *et al.* 2010 |

[a] $LD_{50}$ of a given selected, field, or greenhouse population / $LD_{50}$ of a susceptible laboratory line

## Table 3-2 Resistance of Long Island field populations and a susceptible laboratory population to spinosad in 2006.

| **Field** | **Treatment Prior to collection** | $n^a$ | **LD$_{50}$**$^b$ | **Lower**$^c$ | **Upper**$^c$ | **RR**$^d$ |
|---|---|---|---|---|---|---|
| 1 | Imidacloprid | 220 | 20.63 | 14.21 | 29.79 | 3.2 |
| 2nd Gen. | Imidacloprid | 253 | 19.73 | 13.03 | 31.65 | 3 |
| 2 | None | 73 | 27.22 | 11.68 | 73.9 | 3.1 |
| 3 | Imidacloprid | 98 | 18.57 | 8.2 | 35.78 | 4.3 |
| 4 | Imidacloprid | 366 | 11.58 | 6.67 | 16.07 | 2.9 |
| 5 | None | 482 | 17.58 | 10.96 | 27.64 | 1.8 |
| 2nd Gen. | Spinosad | 275 | 20.77 | 11.35 | 33.03 | 3.8 |
| 6 | Imidacloprid | 247 | 18.91 | 14.78 | 23.64 | 2.8 |
| 7 | Imidacloprid | 252 | 24.46 | 16.06 | 35.31 | 3.3 |
| 8 | Imidacloprid and Spinosad | 117 | 62.35 | 39.47 | 99.64 | 9.8 |
| Laboratory Susceptible | None | 243 | 6.39 | 4.78 | 8.22 | 1 |

[a] Number of 2nd instar larvae tested

[b] LD$_{50}$s are in units of ng/insect

[c] 95% fiducial limits

[d] LD$_{50}$ of a given field population / LD$_{50}$ of the susceptible laboratory line

## Table 3-3. 2010 Larval Spinosad resistance of L.I field populations and a susceptible lab population

| **Field** | **Insect management** | ***n*[a]** | **$LD_{50}$[b]** | **Lower[c]** | **Upper[c]** | **RR[d]** |
|---|---|---|---|---|---|---|
| Q | Organic | 569 | 12.16 | 6.73 | 27.50 | 1744.1 |
| B | Organic | 574 | 40.10 | 6.33 | 9259.36 | 5750.3 |
| G | Organic | 206 | 0.90 | 0.41 | 2.64 | 128.7 |
| M | Conventional | 158 | 0.12 | 0.07 | 0.52 | 17.5 |
| S | Conventional | 373 | 0.28 | 0.07 | 1.94 | 40.6 |
| K | Conventional | 158 | 0.22 | 0.07 | 0.55 | 32.1 |
| W9 | Conventional | 233 | 0.25 | 0.02 | 2.37 | 35.8 |
| Lab Susceptible | None | 217 | 0.0069 | 0.001 | 0.014 | 1.0 |

[a] Number of 2nd instar larvae tested

[b] $LD_{50}$s are in units of µg/larva

[c] 95% fiducial limits

[d] $LD_{50}$ of a given field population / $LD_{50}$ of the susceptible laboratory line

*Table 3-4:* **. Adult resistance of L.I field populations to spinosad in 2010.**

| **Field** | **Type** | **Location**[a] | **n**[b] | **LD50**[c] | **Lower**[d] | **Upper**[e] | **RR**[f] |
|---|---|---|---|---|---|---|---|
| B | Org | South | 167 | 26.2367 | 1233.43 | 3455.85 | 23.36 |
| Q | Org | South | 141 | 18.4222 | 1078.9 | 1798.9966 | 16.40 |
| G | Org | North | 165 | 17.1637 | 1697.9 | 20858 | 15.28 |
| K | Trad | North | 207 | 1.9168 | 3444.9 | 16306 | 1.71 |
| M | Trad | North | 193 | 1.7820 | 9364.2 | 9142100 | 1.59 |
| S | Trad | North | 174 | 1.1233 | 10700 | 5062100 | 1.00 |
| W9 | Trad | South | 130 | 3.3395 | 12108 | 16889000 | 2.97 |

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[a] North Fork or South Fork of Suffolk county

[b] Number of adults tested

[c] LD50 are in units of µg/insect

[d] 95% fiducial limits

[e] 95% fiducial limits

[f] LD50 of given field population / LD50 of most susceptible field population ( Field S)

# Colonizers on rotated and non-rotated fields: Imidacloprid resistance levels

## *Abstract*

Rotation is one of the oldest and most widely practiced management techniques to control crop pests and/or the evolution of resistance and remains an important tool for managing Colorado potato beetles. Colonizing Colorado potato beetles were captured on long distance rotated (>300m), and short-distance rotated fields (<25m), in Suffolk County, Long Island over 3 consecutive years. Long distance rotated fields are fields that are >300m from the closest potato fields of the prior year. Short distance rotated fields are fields that are <25m from the closest potato fields of the prior year. Each year they were bioassayed with imidacloprid. In two of three years of this study (2008 & 2010), colonizers on fields rotation >300m from prior year potatoes had higher $LD_{50}$. In 2009 colonizers on fields rotated <25m from prior year potatoes had the higher $LD_{50}$.
The remaining year, 2009, had mixed results with no clear trend. 2009 was also one of the coolest and wettest in Long Island. Benefits of long distance rotation were not readily apparent in $LD_{50}$s of colonizing beetles.

## *Introduction*

Controlling crop pests through Integrated Pest Management, or IPM, requires a multi-pronged approach. We cannot rely solely on the use of chemicals to control crop pest populations as this increases the likelihood of the evolution of resistance and this can result in a lack of control by the insecticide. Crop rotation is advocated for controlling many crop pests as well as controlling weeds and improving the soil. In the context of pest management, the key to success is the distance between the new planting of a crop and the previous year's planting of that crop and the movement of the pest between the two sites. It is important to stress that crops at both sites are rotated – from the host plant at the original site and to the host plant at the new site the following year. The strategy can work by increasing the instability of the pest environment and create discontinuity in the supply of host plants, forcing the pest to leave in search of host plants.

Crop rotation is recommended for controlling several potato pathogens such as early blight and common scab, among others. Crop rotation has been shown to reduce or delay the colonizing Colorado potato beetle populations (Wright 1984; Lashomb & NG 1984; Hough-Goldstein et al. 1996). Sexson and Wyman (2005) determined that rotation of crops more than 400m away from current host locations, reduced spring colonization by 90% and rotation has also been show to delay the appearance of Colorado potato beetle larva (Lashomb & Ng 1984). Crop rotation can indirectly reduce resistance by requiring fewer applications of insecticide which reduces selection pressure (Roush et al., 1990).

Managing the evolution of resistance is critical when dealing with the Colorado potato beetle. It is an extreme example of resistance to pesticides and has evolved significant resistance to all major classes of modern synthetic insecticides in at least some areas of eastern North America (Forgash 1984, 1985, Roush et al 1990). It is the most important insect defoliator of potatoes (Weber and Ferro 1994) and poses an annual challenge to potato growers. Potato growers in some areas of NY (Long Island) spend more than $400/acre/year for insecticide control of Colorado potato beetle while in Massachusetts it averaged $230/acre at the same time period (Roush et al. 1990). The number of available fields on which to rotate crops on Long Island is continually decreasing due to land development for other purposes, such as housing and vineyards. As a result, long distance rotation of fields may not always be possible on Long Island and may be a contributing factor to the Long Island Populations being at the forefront of developing resistance to insecticides.

Crop rotation offers control of weeds, plant diseases, nematodes and insect crop pests (Wright 1984, Hough-Goldstein and Whalen 1996) and crop rotation can reduce resistance indirectly by requiring fewer applications of insecticide, which reduces the selection pressure (Wright 1984, Roush et al 1990). Rotation has been shown to reduce adult densities by as much as 70-90% from non-rotated fields (Wright 1984). Early in the season rotated fields generally have fewer Colorado potato beetles (Lashomb and Ng, 1984, Wright 1984, Weisz et al 1994, Hough-Goldstein and Whalen 1996) however later in the season that benefit disappears (Wright 1984). Crop rotation works best for pests with a narrow host range and which are not highly mobile. Rotation alone may not be an effective management technique for the Colorado potato beetle as they are highly mobile (Weber et al. 1995). Roush (1990) posits that if the colonizers on rotated fields were less resistant then that would reduce the need for insecticide applications. That study asked if differential movement of resistant and susceptible Colorado potato beetles leads to a reduction of resistance in rotated fields, although they failed to detect an effect (Roush et al. 1990).

Initially I hypothesized that crop rotation could also directly reduce resistance if resistant insects do not fly as far as susceptible ones (but see this volume Chapter 2). Considering the observed frequency of resistance costs and observed frequency of life history trade-offs with migration, this is a logical prediction. However, the one time this prediction was examined the results were equivocal (Roush et al. 1990). They pooled insecticide resistance results for analysis, which may not be illustrative of resistance, since resistance is not a singular trait and is dependent upon dose, insecticide and insect. The Colorado potato beetle has quickly and consistently developed resistance to almost every insecticide used on it (Casagrande 1987).

To manage the evolution of insecticide resistance we necessarily must understand the dynamics of the movements involved as well as the costs. I define long distance rotated fields as those that are more than 300m from the prior year's potato. I define 'non-rotated' fields as fields that are rotated less than 25m from the prior year's potato crop. I test the resistance levels from spring emerging colonizers of long-distance rotated fields and short distance rotated fields. Placing untreated fields at appropriate distances can have a great impact on this process (Baker et al 2001.). Overwintered adult beetles emerge from the ground in spring and use walking or flight to colonize new potato fields (Weber et al, 1995). It is suggested that longer distances will encourage recruitment by flight (Boiteau 2000).

The use of refuges has been employed with transgenic crops and it has been shown that resistance evolved slower, but the mechanism for that has not been demonstrated. One cost of resistance may involve migratory flight capability though it is rarely looked at and has rarely been observed (but see Foster et al. 1997). If movement has a resistance cost then rotation on the right spatial scale can reduce resistance and have far reaching impacts in agriculture as well as the environment. If rotation occurs on a larger spatial scale than the average distance travelled by colonizing pests, then colonization of new fields should fail or take significantly more time.

There are very few studies linking resistance with movement. One exception is Foster et al (1997) that worked on within plant movement of peach-potato aphids. My study works on agricultural distances in use by commercial growers. Emerging beetles (overwintered adults) in the spring colonize fields by walking and/or flying to suitable hosts. If flight is a cost of resistance then flying colonizers would be less resistant than their walking counterparts and rotating host crops long distances each season will help delay resistance. These emerging overwintered adult beetles will be the only generation on the plants until the appearance of the first summer generation larva.

## *Materials and Methods*

Three traditional commercial farms with many fields and well documented rotation plans were identified and used to study differences in resistance based upon rotation status. These farms are designated as Farm T, Farm E and Farm I. While all fields undergo some manner of rotation, I defined a classification to differentiate between long-distance rotated fields and short distance rotated fields. If the fields had potatoes in the prior year or were within 25m of the prior year's potatoes, then that field was classified as "non-rotated". This often happens when fields are across a farm road from each other and they alternate potatoes back and forth between the two fields. Two farms, Farm T and I, are located on the north shore while Farm E is located on the south shore of Long Island. Historically the Colorado potato beetles emerge at least a week earlier on the north fork fields. For 2008 one pair of fields for each grower were studied for a total of 6 fields. Farm T had 1 rotated and 1 non-rotated field. Farm I had 1 rotated and 1 non-rotated field. Farm E had 1 rotated and 1 non-rotated field. Due to the trends of the 2008 data I increased the number of fields for 2009.

In 2009 there were 12 fields. Farm T had 2 rotated, 1 non-rotated fields. Farm I had 2 rotated and 2 non-rotated fields. Farm E had 3 rotated and 2 non-rotated fields.

In 2010 there were 6 fields. Farm T had 1 rotated and 1 non-rotated field. Farm I had 1 rotated and 1 non-rotated field. Farm E had 1 rotated and 1 non-rotated field.

Fields were scouted early in the season beginning in March of each year, to determine first arrival dates as well as the invasion edge of each field. I wanted to evaluate the overwintered generation after it emerged and colonized a potato field in the spring. In an attempt to capture newly arriving colonists, all field collection sites were cleared of all beetles upon arrival each day. A minimum of 1 hour after arrival I would start collecting beetles from the field plants along the invasion edge. These beetles were placed in a plastic cage (8”x4”x6”) with untreated potato foliage and kept in a cooler for the day.

All specimens were fed untreated potato leaves from greenhouse grown plants, for 2-3 days. In all years adults received one bioassay treatment. The bioassays were by direct topical application of a 1µl drop of imidacloprid dissolved in HPLC-grade (0.995) acetone on the abdomen, except for the highest dose which required 2 µl drops. I used 7 different concentrations of imidacloprid dissolved in acetone and the lowest dose was a control of pure acetone. The imidacloprid doses used for abdominal bioassay on adults are as follows:

| Dose | [ ] | g/ml |
|---|---|---|
| 1 | 0 | 0 |
| 2 | 3.125e ^$^{-4}$ | 0.03125 |
| 3 | 6.25e ^-4 | 0.0625 |
| 4 | 1.25e $^{\wedge-3}$ | 0.125 |
| 5 | 2.5e $^{\wedge-3}$ | 0.25 |
| 6 | 5e $^{\wedge-3}$ | 0.5 |
| 7 | 1e $^{\wedge-2}$ | 1 |
| 8 | 2e $^{\wedge-2}$ | 2 |

After administering the dose, the beetles were placed into a new Petri dish with filter paper. They were given access to fresh water and untreated potato leaves from plants grown in a greenhouse. All petri dishes were cleaned daily and replenished with fresh water and untreated potato leaves for 7 days. On day 7 they were scored as Dead, Alive or Moribund.

Dead is the absence of any signs of life. Alive indicated that they could right themselves within 45 seconds, when flipped. Moribund meant there was some sign of movement but they were more dead than alive. Moribund movements include trembling legs or antennae, mouthpart movements etc. For analysis the moribund were pooled with the dead. I used Proc Probit, (2002-2003 by SAS Institute Inc., Cary, NC, USA.) to determine the $LD_{50}$ for each field and I attempted to have at least 30 at each dose from each field for each year.

Four models were used for AIC analysis with SAS. The first model was a normal distribution with the calculated response of the control dose, Dose 0, or mortality at zero concentration. The second model was also a normal distribution but forced mortality to zero for the control dose. This model assumes that observed mortality at concentration 0 (control dose) is sampling error. Model 3 used a logistic distribution and calculated mortality for Dose 0. Model 4 was also a logistic distribution but forced mortality to zero for the control dose.

## *Results*

In 2008 and 2010 adult Colorado potato beetle emerged in sufficient numbers for collecting during the first week of May. In 2009 however, adult Colorado potato beetle did not emerge in sufficient numbers for collecting until the second week of May. Adults emerged on the north fork at least 1 week before any adults were spotted on the south fork in all three years.

To analyze the data all colonists of short distance rotations were pooled for each year and compared with all colonists of long distance rotations by year using SAS to calculate the $LD_{50}$ and 95% Confidence intervals. In 2008 (Fig 4-4) and 2010 (Fig 4-6) there was a higher $LD_{50}$ on colonists from the longer distance rotated fields. This would be consistent with the trend of flyers being more resistant than walkers (see Chapter 2 this volume) as flyers could travel faster and go further. However in 2009 (Fig 4-5)colonists on short distance rotated fields had a higher $LD_{50}$ than their long distance colonist's counterparts. In 2009 there was overlap between the 95% confidence intervals of short distance colonists and the LD50 of the Long distance colonists. Complete results for each individual field for all years are in Table 4-1..

## *Discussion*

There are several factors that could account for the inability to detect a consistent significant difference in resistance levels of colonizers on rotated and non-rotated fields. Date of first emergence and peak emergence differ between the north and south forks of Long Island. The north fork emergence dates are always earlier by about one week or more. Another factor is that each commercial farm has different management practices. Farm T rotates fields out of potato on alternate years. Farm I grows potato for 2 years and then fumigates the field and rotates out of potatoes for one year. Farm E also grows potatoes for 2 years and then rotates out for one year but does not fumigate the fields. Another differential is the weather from year to year. The 2009 season was among the wettest on record for the area. This did result in later emergence from diapause on both forks and could have played a major role on the behavior of the Colorado potato beetle. I noticed that there did not seem to be a clear peak in emergence activity in 2009. There was just a slow continual emergence day after day (pers. obs.)

The Colorado potato beetle is most active when it is sunny and warm. Warmth and no wind enhance flight activity. If the Colorado potato beetle was staying close to where it emerged and walking to colonize fields, this would change the demographics of the colonizers on rotated fields. If the weather was not conducive to Colorado potato beetle flight, then all emergers would walk, taking more time to travel shorter distances. The resistance profile would be a mixture of all emerging beetles and not a subset according to distance traveled. Potato beetles have emerged from diapause and engaged in long distance flight, even when suitable hosts were available (Ferro 1999). If these beetles have different resistance profiles then I could have detected that with colonizers of long distance rotated fields. If conditions were not appropriate for flight and the resistance profiles for these beetles were different, then they would be mixed in with those that emerged from diapause and did not engage in long distance flight and I would not be able to detect differences.

Despite the mixed results there are noteworthy trends. In both 2008 and 2010 the fields that were long distance rotated from prior year potatoes tended to have colonists with higher resistance levels to imidacloprid. The wet 2009 season may have resulted in a more homogenous mixture of flying and walking colonists on all fields. There are several possible mechanisms that could underlie the pattern. A positive relationship between resistance and rotation distance could reflect a lack of costs, movement benefit of resistance or greater overall health in the more resistant beetles. All beetles were exposed in all fields to insecticides the previous season and/or early in the current season. If there is a movement enhancement of resistance, or a reduction in flight capacity of the most susceptible due to insecticide exposure then crop rotation could accelerate resistance evolution.

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## Figure Headings

Figure 4-1: 2008 Rotated & Non-rotated Fields

Figure 4-2: 2009 Rotated & Non-rotated Fields

Figure 4-3: 2010 Rotated & Non-rotated Fields

Figure 4-4: Chart of LD50 for 2008 Fields

Figure 4-5: Chart of LD50 for 2009 Fields

Figure 4-6: Chart of LD50 for 2010 Fields

**Figure 4-1: 2008 Fields**

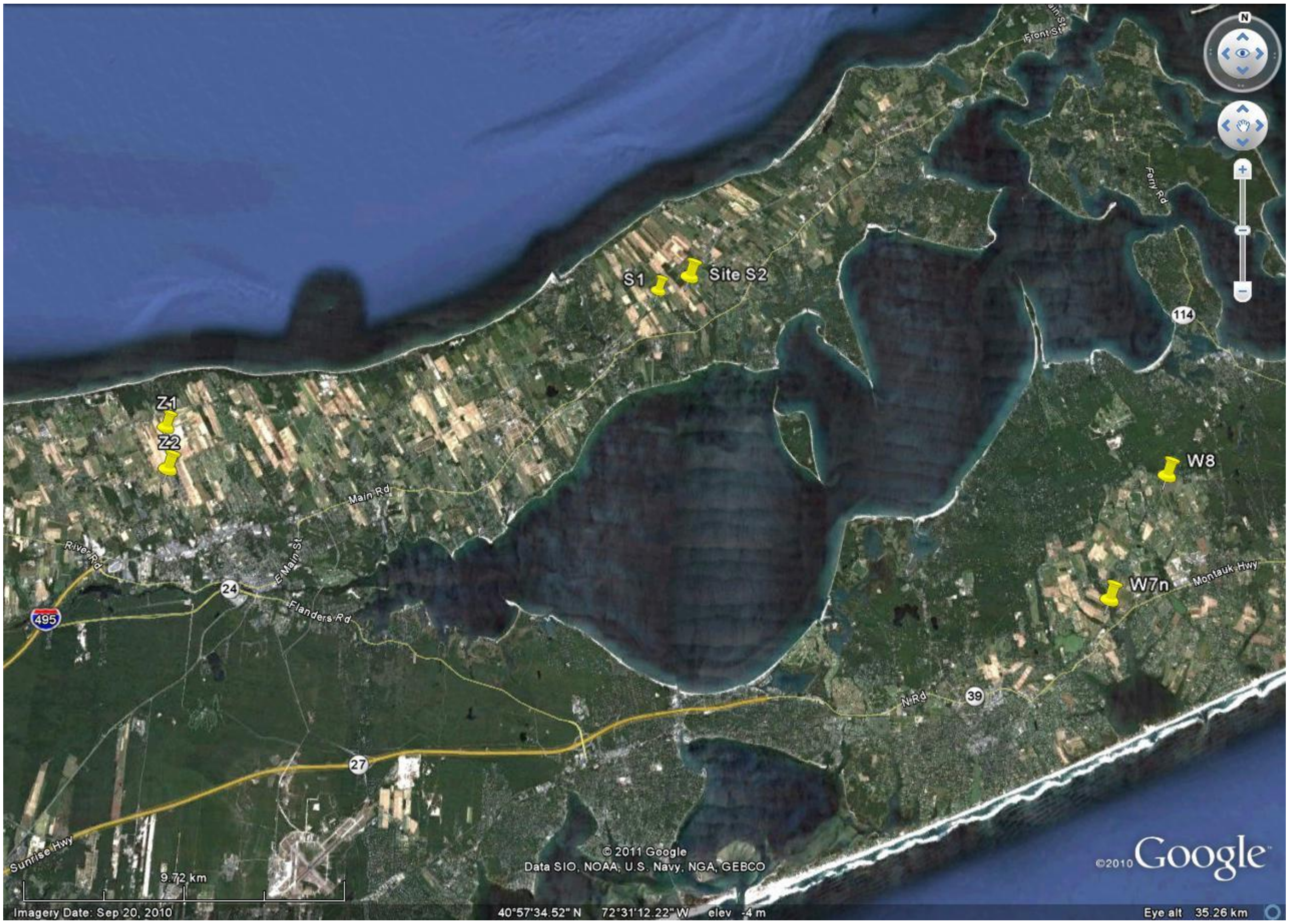
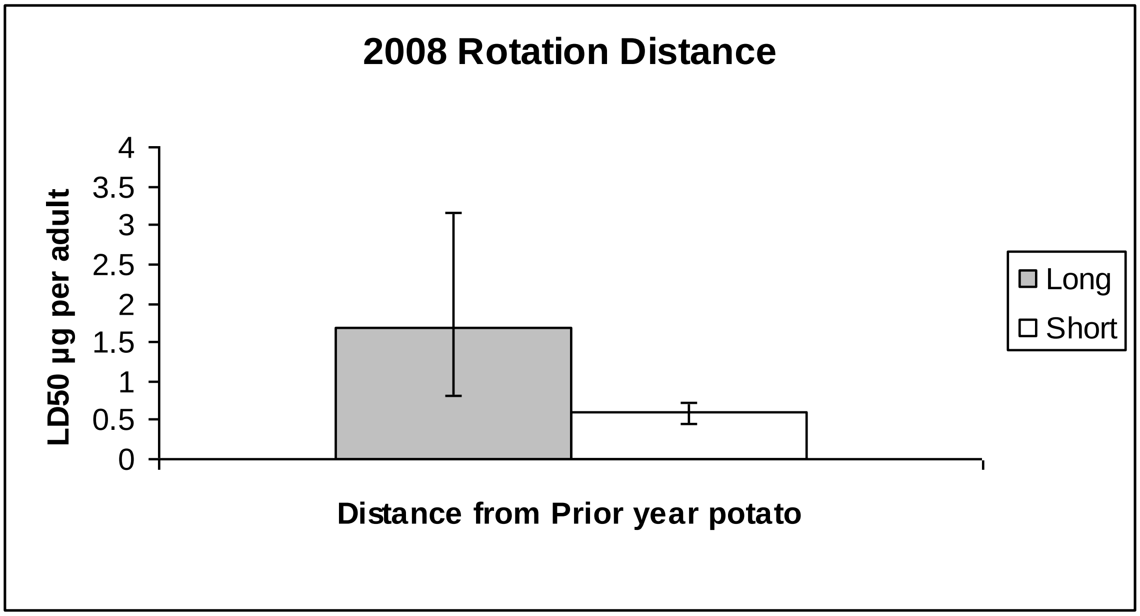


**Figure 4-2: 2009 Fields**

**Figure 4-3: 2010 Fields**

**Figure 4-4 Chart of $LD_{50}$ values for 2008 Fields**



| Type[1] | N[2] | $LD_{50}$ [3] | Lower[4] | Upper[4] |
|---|---|---|---|---|
| Long | 538 | 1.6694 | 0.812 | 3.145 |
| Short | 823 | 0.591 | 0.463 | 0.7232 |

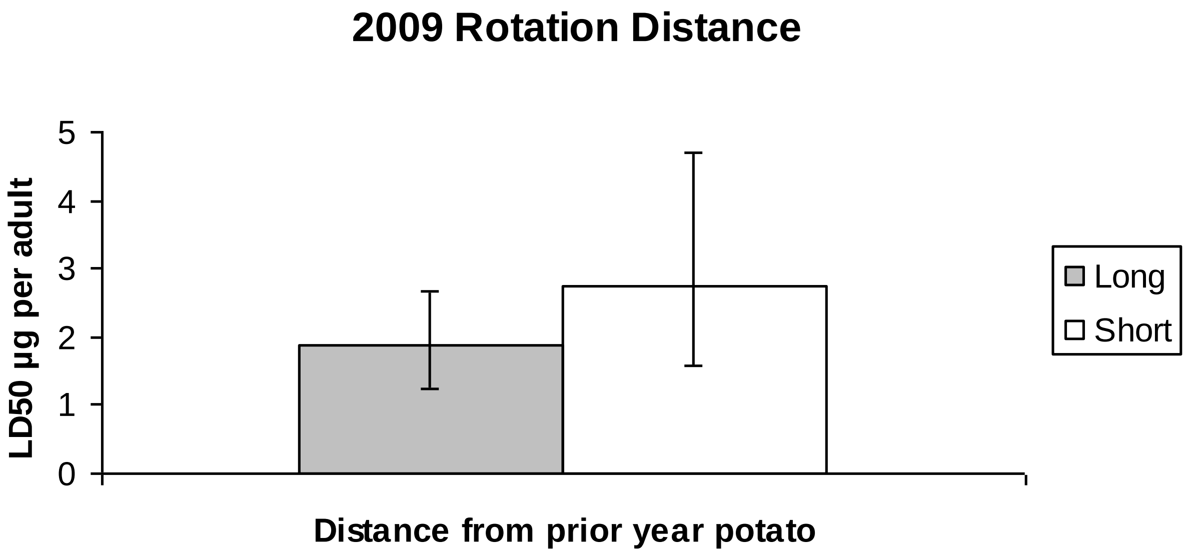
[1] Long = rotated > 300m from prior year potatoes. Short = rotated<25m from prior year potatoes

[2] Number of adults assayed

[3] LD50s are in units of µg/insect

[4] 95% fiducial limits

**Figure 4- 5:Chart of $LD_{50}$ values for 2009 Fields**



| Type[1] | N[2] | $LD_{50}$ [3] | Lower[4] | Upper[4] |
|---|---|---|---|---|
| Long | 2105 | 1.865 | 1.2515 | 2.6858 |
| Short | 1785 | 2.737 | 1.594 | 4.705 |

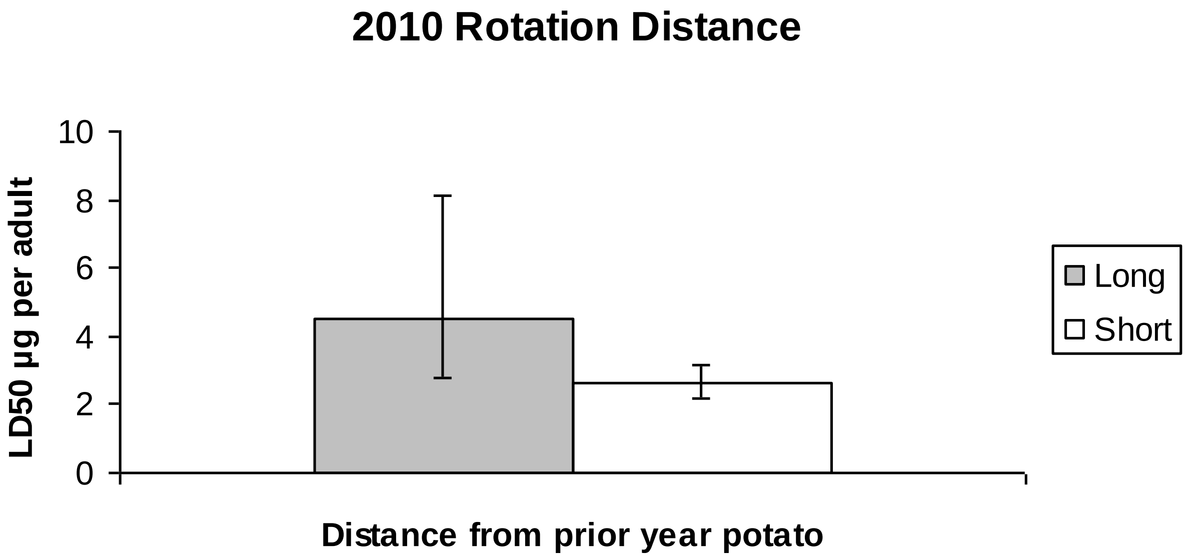
[1] Long = rotated > 300m from prior year potatoes. Short = rotated<25m from prior year potatoes

[2] Number of adults assayed

[3] LD50s are in units of µg/insect

[4] 95% fiducial limits

**Figure 4-6: Chart of $LD_{50}$ values for 2010 Fields**



| Type[1] | N[2] | $LD_{50}$ [3] | Lower[4] | Upper[4] |
|---|---|---|---|---|
| Long | 765 | 4.527 | 2.790 | 8.135 |
| Short | 810 | 2.634 | 2.195 | 3.174 |

[1] Long = rotated > 300m from prior year potatoes. Short = rotated<25m from prior year potatoes

[2] Number of adults assayed

[3] LD50s are in units of µg/insect

[4] 95% fiducial limits

## Table 4-1 Growing degree days

| | **North Fork (Jamesport)** | | | | **South Fork - Bridgehampton** | | |
|---|---|---|---|---|---|---|---|
| **DATE** | Temp | Temp | GDD | | Temp | Temp | GDD |
| 04/01/08 | 57 | 41 | 4 | | 56 | 48 | 3 |
| 04/15/08 | 60 | 33 | 19 | | 57 | 31 | 17 |
| 05/01/08 | 60 | 32 | 77 | | 58 | 30 | 66 |
| 06/01/08 | 84 | 62 | 323 | | 81 | 57 | 272 |
| 04/01/09 | 49 | 37 | 8 | | 46 | 32 | 2 |
| 04/15/09 | 56 | 43 | 16 | | 52 | 42 | 7 |
| 05/01/09 | 64 | 50 | 109 | | 58 | 50 | 90 |
| 06/01/09 | 65 | 43 | 408 | | 65 | 42 | 321 |
| 04/01/10 | 65 | 43 | 16 | | 61 | 43 | 6 |
| 04/15/10 | 72 | 40 | 100 | | 69 | 47 | 58 |
| 05/01/10 | 77 | 46 | 162 | | 73 | 47 | 100 |
| 06/01/10 | 80 | 64 | 574 | | 77 | 61 | 462 |

## Table 4-2 Imidacloprid resistance LD$_{50}$ values:

Adults Imidacloprid assays - rotated / non-rotated

| Field | Year | Type[g] | $N$[h] | LD$_{50}$[i] | Lower[j] | Upper |
|---|---|---|---|---|---|---|
| S1 | 2008 | r | 274 | 1.5992 | 0.6631 | 3.1686 |
| Z2 | 2008 | r | 115 | 1.2303 | 0.7325 | 1.948 |
| W8 | 2008 | r | 129 | 5.1474 | 3.201 | 6.6563 |
| S2 | 2008 | n | 261 | 0.9859 | 0.7295 | 1.2707 |
| Z1 | 2008 | n | 180 | 0.4708 | 0.2717 | 0.6747 |
| W7 | 2008 | n | 329 | 0.4269 | 0.236 | 0.6335 |
| S5 | 2009 | r | 326 | 2.4147 | 0.5243 | 6.4396 |
| S6 | 2009 | r | 379 | 1.7088 | 0.5344 | 4.4742 |
| Z5 | 2009 | r | 322 | 2.4611 | 1.9399 | 3.1072 |
| Z3 | 2009 | r | 279 | 4.9864 | 3.2294 | 8.5338 |
| W10 | 2009 | r | 332 | 1.3532 | 1.0429 | 1.7183 |
| W14 | 2009 | r | 259 | 1.5189 | 1.1067 | 2.0402 |
| W9 | 2009 | r | 220 | 0.9246 | 0.6346 | 1.257 |
| S4 | 2009 | n | 291 | 1.9696 | 0.4305 | 5.6178 |
| Z4 | 2009 | n | 462 | 1.6689 | 1.0109 | 2.5649 |
| Z2 | 2009 | n | 469 | 5.7727 | 4.4546 | 6.8922 |
| W13 | 2009 | n | 239 | 5.9042 | 3.0756 | 10.7217 |
| W8 | 2009 | n | 324 | 2.9187 | 1.4803 | 5.826 |
| S2 | 2010 | r | 226 | 3.9282 | 2.5943 | 6.3393 |
| Z6 | 2010 | r | 286 | 4.7662 | 3.0187 | 8.1778 |
| W7 | 2010 | r | 253 | 5.7805 | 4.1449 | 8.6593 |
| S3 | 2010 | n | 244 | 2.9043 | 1.4449 | 6.5216 |
| Z3 | 2010 | n | 252 | 5.2427 | 3.8924 | 7.3171 |
| W9 | 2010 | n | 314 | 1.4365 | 1.0824 | 1.8744 |

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[g] r =long distance rotation; n=short distance or no rotation

[h] Number of adults assayed

[i] LD$_{50}$s are in units of µg/insect

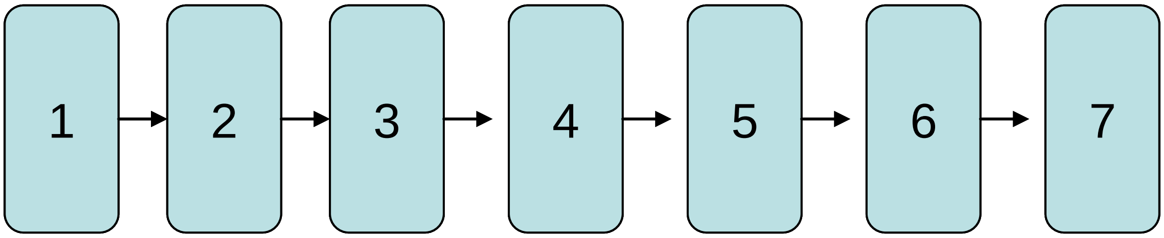
[j] 95% fiducial limits

# Appendix 1:

## *Quantifying Summer Generations on Long Island*

Several different geographical locations with significant commercial potato acreage have documented the number of summer generations of the Colorado potato beetle, I was unable to find anything for Long Island although it is often stated they are multivoltine on Long Island. Considering that Long Island beetles are on the forefront of resistance and somewhat infamous for their ability to develop resistance, the question begged: ‘How many summer generations?” The more generations per summer, the higher the chance for mutations (resistant alleles?) and the shorter the time required for evolution of resistance.

Illustration of cages used to quantify the number of summer generations



(NOTE: IN all cases, all the cages were left in the field under environmental conditions. All plants were started in a greenhouse and all were untreated.)

### Cage 1: Overwintered Adults

Beginning with the first week of emergence, usually in early to mid-May, I collected spring emerging adults from the overwintering site(s) and passive trap plants. The field plants had not yet emerged and at least in two years, planting had not been completed by the time of the first emergence. I placed ~25 adults in this cage in the field with an untreated potato plant. The beetles were fed liberally and checked daily for egg masses. Egg masses were removed daily and placed into cage #2.

Cage 2: 1st Summer Generation (1st S.G.) – Eggs and Larva

Egg masses placed in here represented the first summer generation (1st S.G.). Again they were fed liberally and as they completed pupation and emerged from the soil as adults, they were removed and placed into cage #3.

Cage 3: 1st S.G. adults

This population was also fed liberally and checked daily for egg masses. These egg masses represented the 2nd summer generation (2nd S.G.) and they were removed an placed into cage #4.

Cage 4: 2nd S.G. - Eggs and Larva

When these eggs hatched and development was completed, the emerging adults were placed into cage #5.

Cage 5: 2nd S.G. Adults

The cage was checked daily and egg masses removed. These represented the 3rd summer generation (3rd S.G.). These egg masses were placed into cage #6.

Cage 6: 3rd Summer generation – Eggs and Larva.

Again, upon completion of development the emerging adults were removed and placed into Cage #7.

## *Results and Discussion:*

2008: Traditional fields

Three summer generations were raised to adults however, the 3rd S.G. cage had only 2 adults in it when there was an accident. All cages had tire track marks and were destroyed August 12. Based on the size of the tire marks it appears to have been done by a four wheeler. Moral of the story – don't put all your eggs in one cage.

2009: Traditional fields

Two summer generations

This was one of the coldest and wettest seasons on record which appears to have contributed to limiting the number of generations to just two.

2010: Traditional fields North Fork (First emergers appeared May 1, 2010)

**1st S.G**. cage started May 14, 2010. Completed addition of 25 adults on May 21, 2010.

**2nd S.G**. 40 Adults emerged by June 24, 2010. First clutches collected June 29, 2010. First Adult July 27th.

**3rd G.G**. 37 Adults emerged by September 4th.

2010: Organic fields South Fork (First emergers appeared May 23, 2010)

**1st S.G**. Cage started May 23. Completed addition of 25 adults on June 1, 2010.

**2nd S.G**. 11 Adults emerged by July 7, 2010. First clutch July 11, 2010. First Adult August 4th.

**3rd S.G**. 3 Adults emerged by September 4th.

The experiment was concluded on September 4th, 2010 due to resource constraints and the impression that there would not be a 4th summer generation. Emergence on the south fork of Long Island is always later than emergence on the North Fork (Pers. Obs.). Overall the south fork organic population laid less clutches and took longer to develop. It should be noted that the south fork traditional population cage suffered a raccoon attack which resulted in incomplete data. The differences between the 2010 populations may be due to the geographic differences (North fork vs. South fork) or the field treatments (traditional vs. organic) or some combination of both.

In 2008 and 2010 there were three summer generations raised in field cages. In 2009 there were 2 summer generations raised in this manner. 2009 was one of the coolest and wettest seasons on record for Suffolk County. At least one grower commented that it was the first season he could remember that he did not have to irrigate at all.

It is reasonable to claim 3 summer generations in an average year based on conditions. Based on my observations in the fields over the past 3 years, I fully suspect 4 generations are possible.

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