Research Article

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Resistance to neonicotinoid insecticides in field populations of the Colorado potato beetle (Coleoptera: Chrysomelidae)

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Abstract

BACKGROUND: Neonicotinoid insecticides were first used commercially for Colorado potato beetle [*Leptinotarsa decemlineata* (Say), Coleoptera: Chrysomelidae] control in the United States in 1995, and since then have been critical for management of this pest. Field populations from the northeastern and midwestern United States were tested from 1998 to 2010 for susceptibility to imidacloprid and thiamethoxam using standard topical dose assays with adults.

RESULTS: From 1998 to 2001, imidacloprid resistance was present in only a few locations in the eastern United States. By 2003, imidacloprid resistance was common in the northeastern Unites States. In 2004, imidacloprid resistance in Colorado potato beetle was detected for the first time in the midwestern United States. In 2003, the first case of resistance to thiamethoxam was found in a population from Massachusetts. Neonicotinoid resistance in summer-generation adults was higher than in overwintered adults from the same locations. By 2009, 95% of the populations tested from the northeastern and midwestern United States had significantly higher LD_{50} values for imidacloprid than the susceptible population.

CONCLUSIONS: The increasing resistance to neonicotinoid insecticides raises concerns for the continued effective management of Colorado potato beetles in potatoes and highlights the need for more rigorous practice of integrated pest management methods.
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Keywords: *Leptinotarsa decemlineata*; insecticide resistance; resistance management; monitoring

1 INTRODUCTION

Resistance to pesticides is an increasing global problem in the management of numerous weeds, diseases and insects. A major difficulty is compiling data on resistance build-up in field populations, which requires regular, large-scale monitoring to determine the levels of resistance in the field and the geographic distribution of resistant populations. This kind of information is essential to make educated management decisions and forecast efficacy of a pesticide.

There are 553 species of arthropods known to be resistant to at least one pesticide, and the number of cases is increasing.[1,2] The Colorado potato beetle [*Leptinotarsa decemlineata* (Say)] presents one of the most notorious examples of insecticide resistance in agriculture; this insect first developed insecticide resistance in the 1940s[2] and is now reported to be resistant to most classes of synthetic insecticides.[1,3] As it is the most severe defoliator of potatoes worldwide,[4,5] and potatoes are the most important non-grain food crop in the world (http://www.potato2008.org/), serious concerns are well justified about the future of sustainable, effective and economical Colorado potato beetle control methods.

The two main neonicotinoid insecticides used in commercial potato production in the United States are imidacloprid and thiamethoxam. Imidacloprid was registered in 1995 and thiamethoxam in 2002 for Colorado potato beetle management on potatoes in the United States. These products appeared on the market at a time when most other registered insecticide classes were ineffective against this pest in the field. This clear need for an effective control measure led to a rapid and wide-scale adoption of neonicotinoids by commercial potato growers. According to a 2005 national survey, neonicotinoid insecticides were used on 60–70% of commercial potato acres in the United States (http://www.nass.usda.gov/). In a 2010 grower survey conducted in Michigan it was found that 80% of the Michigan commercial potato acreage was treated at planting with a neonicotinoid insecticide, and that the proportion of the total potato acreage treated with neonicotinoid insecticides had stayed approximately the same over the past 10 years.[6]

Unfortunately, as has been true for other classes of insecticides, Colorado potato beetles rapidly developed resistance to neonicotinoid insecticides. Tenfold differences in LC_{50} values (concentrations lethal to 50% of the population) were reported between susceptible larvae and larvae from Long Island, New York, populations in 1995–1998, using a diet incorporation bioassay.[7] High levels of resistance to imidacloprid in the field were first

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documented in 1997; LD_{50} values (doses lethal to 50% of the population) for Colorado potato beetle adults from Long Island, New York, were more than 100 times the LD_{50} values for susceptible beetles.[8] Adults were more resistant than larvae from this population, consistent with findings of other authors,[7] and resistance appeared to be inherited in a semi-recessive autosomal manner.[8]

The objective of this study was to survey Colorado potato beetle adult resistance to two neonicotinoid insecticides (imidacloprid and thiamethoxam) in the northeastern and midwestern United States from 1998 to 2010. LD_{50} values between two generations of Colorado potato beetles – overwintered and summer adults – were also compared.

2 METHODS

2.1 Colorado potato beetle collection

Colorado potato beetle adults were collected by cooperators from commercial potato fields and, in some cases, from university research farms from 1998 to 2010 (Table 1). For preliminary screens and full bioassays with both imidacloprid and thiamethoxam, 600 beetles were requested from each site, although fewer beetles were received from some sites. Collection sites were not randomly selected; often it was not possible to sample beetles from the same site in each year because of variable insect pressure between years and field rotation. Most often, collections were from fields with significant numbers of beetles because of poor crop rotation or control failures due to environmental factors, insecticide application problems or insecticide resistance. The exact age of the beetles was unknown, but adults were either overwintered or from the generation emerging in the field during mid-summer. The beetles were shipped in insulated and cooled containers by overnight express mail to the authors' laboratory. Upon arrival, beetles were placed in 9 × 12 × 4 cm deep plastic containers (25 beetles each), kept in the laboratory (22–25 °C, 16 : 8 h light : dark) and fed with greenhouse-grown potato foliage (cv. Snowden or Atlantic) for 1 week prior to the bioassays to allow recovery from any adverse effects of collection and transportation. If multiple populations arrived for testing at the same time, beetles were kept in a growth chamber at 10 °C (16 : 8 h light : dark) until assays could be conducted (usually <3 weeks). Beetles kept at 10 °C were moved to room temperature each week for 2–3 days and fed fresh foliage, and then returned to 10 °C. Beetles were kept at room temperature and fed for a minimum of 2 days before bioassays.

Table 1. The total number of Colorado potato beetle populations sampled in the survey by year

Year	Number of populations
1998	11
1999	11
2000	11
2001	7
2002	15
2003	9
2004	16
2005	19
2006	46
2007	54
2008	33
2009	26
2010	13

2.2 Susceptible strains

Susceptible Colorado potato beetles were collected in 1998 from potatoes grown on an organic farm in the Upper Peninsula of Michigan (Houghton County). These beetles were reared in the authors' laboratory on greenhouse-grown potato foliage, and new field-collected individuals were added to the colony annually until 2003. Starting in 2004, susceptible Colorado potato beetles were attained as needed from a colony maintained by the New Jersey Department of Agriculture (Phillip Alampi Beneficial Insect Rearing Laboratory, West Trenton, NJ). This strain had been in continuous culture since 1983 without exposure to insecticides, and it was used as a susceptible reference strain by Olson *et al*.[7] As this strain became unavailable in 2008, a colony was established from these insects in the authors' laboratory in 2008. It was continuously reared thereafter, and was used as the susceptible reference strain up to 2010.

2.3 Bioassays

Beetles from a site were randomly placed into petri dishes (150 × 15 mm; VWR, Radnor, PA) in groups of ten. Beetles were treated with 1 µL of insecticide + acetone solution per beetle, applied to the underside of the abdomen with a microapplicator (Hamilton Company, Reno, NV). A comparison group of beetles was treated with acetone alone for determination of control mortality. Beetles were kept at room temperature (22–25 °C) for 7 days after treatment and fed fresh greenhouse potato foliage daily. After 7 days, beetles were examined and classified as healthy (walking, normal antennal movement, etc.), poisoned (unable to walk one body length) or dead (no movement, abdomen sunken and darkened). Previous research[8] showed that significant recovery from neonicotinoid intoxication (beetles classified as poisoned) can occur within 3–5 days after treatment. This recovery after initial intoxication is especially common for resistant beetles.[8] Poisoned beetles rarely recover after 7 days,[8] so poisoned and dead beetles were combined for data analysis.

2.4 Dose range determination

Preliminary screens (5–15 beetles dose^{-1} at 3–6 doses) were used to help determine the optimum dose range for full bioassays. Once an appropriate dose range was determined, complete bioassays were conducted using 5–6 doses, giving from near zero to near 100% mortality (1–3 replications each of 15 beetles dose^{-1}, plus 1–3 replications receiving acetone only). Control mortality was usually zero, but, if mortality in the acetone-only controls was >20%, data for the entire replication were discarded.

2.5 Data analysis

Dose–mortality data were analysed using log dose–probit mortality regression (SAS Institute 1999), with treatment mortality corrected for mortality in the controls.[9] LD_{50} values and 95% confidence limits were calculated, and significant differences were determined on the basis of non-overlapping confidence limits.

A linear, cubic, quadratic and exponential model was fitted and compared to find the best-fit curve to describe the change in LD_{50} values over time (Proc Reg; SAS Institute, 2009). Owing to the non-linear relationship between LD_{50} values and time, non-linear regression with Marquardt's algorithm[10] was used to analyze the

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data. Efron's pseudo-R^2 values were used to assess the goodness of fit of the exponential model to the dataset. In calculation of Efron's R^2, the model residuals are squared, summed and divided by the total variability in the dependent variable, and this is equal to the squared correlation between the predicted and actual values. The value of Efron's pseudo-R^2 ranges from 0 to 1, with higher values indicating better model fit; *P*-values are not available in this case.

Slopes of the change in LD_{50} values over time for different groups of insects were compared with a mixed-model analysis of variance using year as a repeated variable. Prior to analysis of variance, LD_{50} values were log transformed to meet statistical assumptions.

3 RESULTS

3.1 Temporal change in neonicotinoid insecticide resistance

The exponential model provided the best description of the relationship between the LD_{50} values and time (Table 2). Nationally, LD_{50} values for both insecticides were exponentially increasing between 1998 and 2010 (Figs 1A and 2A). There was a statistically significant difference between the change in imidacloprid and thiamethoxam LD_{50} values, with a steeper increase in the case of imidacloprid compared with thiamethoxam (nationally – *t*-value 5.89; df = 1, 563; $P < 0.01$; in Michigan – *t*-value 4.18; df = 1, 228; $P < 0.01$). Similar to the national trend, in Michigan the LD_{50} values for both insecticides exponentially increased over the examined time period; this change, however, was significantly higher in the case of summer adults than in the case of winter adults (*t*-value 5.11; df = 1, 116; $P < 0.01$) (Figs 1B and C).

3.2 Geographic distribution of neonicotinoid-resistant populations

3.2.1 Imidacloprid

The first detection of a Colorado potato beetle population with an imidacloprid LD_{50} value significantly higher than the susceptible comparison was in New York in 1998 (2.83 µg beetle^{-1}) (Fig. 3A). By 2002 there was an increasing number of populations with significantly higher LD_{50} values than the susceptible strain. In 2002, the two highest values measured in the national survey were from a Delaware population (5.051 µg beetle^{-1}) and from New York (5.000 µg beetle^{-1}), which were >130 times the LD_{50} value compared with the New Jersey susceptible strain (0.036 µg beetle^{-1}). LD_{50} values between 1999 and 2004 for populations from the midwestern United States were low and generally not significantly different from LD_{50} values for the susceptible strains, while the number of populations and LD_{50} values from the East Coast continued to rise during this time period (Figs 3A and B).

The first record of resistance (defined as an LD_{50} value more than tenfold higher compared with the susceptible population) to imidacloprid from the midwestern United States was in 2004. In 2004, LD_{50} values for beetles from two sites in Michigan were 23 and 16 times the LD_{50} value for the susceptible strain (0.828 and 0.572 µg beetle^{-1} respectively, as opposed to 0.036 µg beetle^{-1}). These LD_{50} values were significantly higher than any found previously for Colorado potato beetle populations from the midwestern or western United States (unpublished data). LD_{50} values for these two groups of beetles overlapped and could represent a single interbreeding population, as the two collection sites were only 1 km apart. LD_{50} values for other populations from the midwestern United States in 2004 and 2005 were low and were generally not significantly different from those of the susceptible strain. Exceptions were three populations from Wisconsin: LD_{50} values were higher, but less than 3 times the LD_{50} value for the susceptible strain, within the range found for other field populations from the midwestern United States in previous years.

By 2005, approximately 50% of the tested populations in the national survey had significantly higher LD_{50} values than the susceptible comparison, and this value continued to increase to 95% by 2009–2010 (Fig. 3C). The highest value measured in the

Table 2. Model fit comparison to describe the change in LD_{50} values over time for Colorado potato beetle resistance to imidacloprid (Proc Reg; SAS Institute, 2009)

Model	*t*-value	*P*-value
Exponential	3.21	<0.01
Linear	−0.12	0.90
Quadratic	0.10	0.92
Cubic	−0.34	0.73

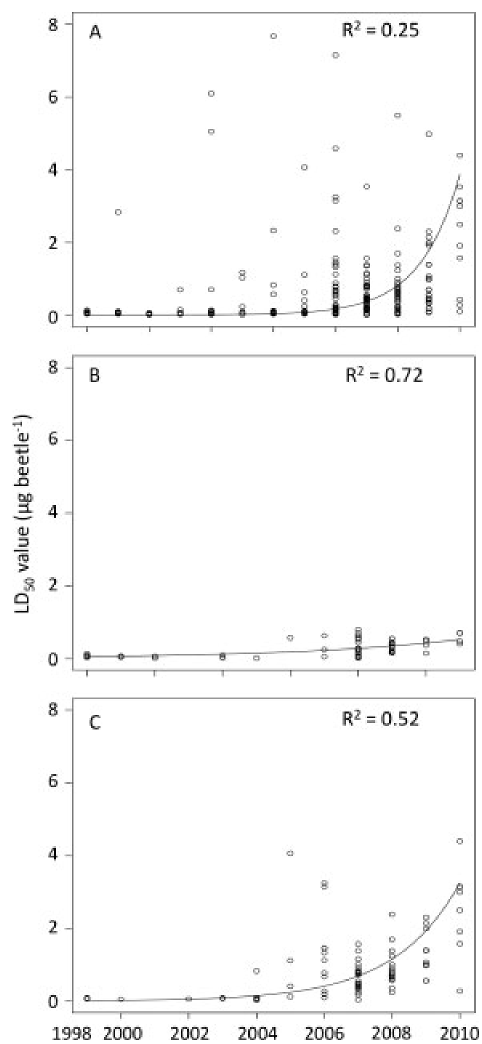

Figure 1. Change over time in LD_{50} values for Colorado potato beetle adults tested for imidacloprid resistance from (A) the national survey, (B) Michigan overwintered adults and (C) Michigan summer-generation adults. Circles represent the LD_{50} value of individual populations, and the line is an exponential model fit. Efron's R^2 values are displayed on each graph for the corresponding group of insects.

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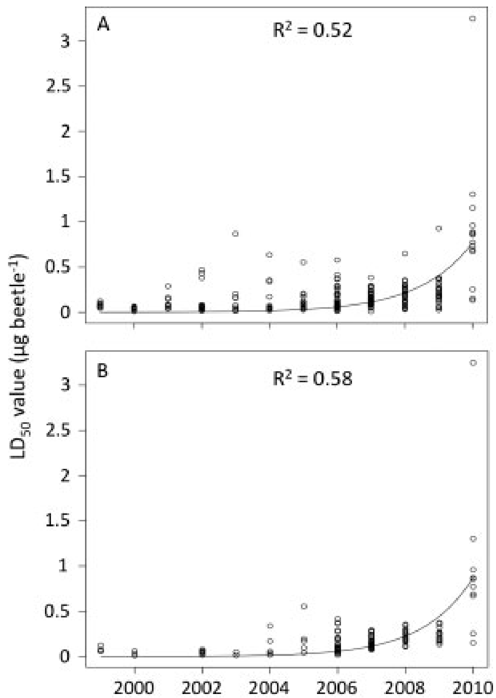

Figure 2. Change over time in LD_{50} values for Colorado potato beetle adults tested for thiamethoxam resistance from (A) the national survey and (B) Michigan overwintered adults and summer-generation adults. Circles represent the LD_{50} value of individual populations, and the line is an exponential model fit. Efron's R^2 values are displayed on each graph for the corresponding group of insects.

2010 national survey was 20.43 µg beetle^{-1} for a population from New York. In Michigan, the number of resistant populations continued to increase from 2004 to 2010 for both overwintered and summer-generation adults (Figs 3D to I). In 2010, approximately 50% of the tested populations had imidacloprid LD_{50} values more than 20 times the LD_{50} values of the susceptible strain (Fig. 3I). The highest LD_{50} value measured in Michigan was 12.73 µg beetle^{-1} in 2010.

3.2.2 *Thiamethoxam*

LD_{50} values for thiamethoxam were generally lower than for imidacloprid. The LD_{50} value measured for thiamethoxam for a Colorado potato beetle population from Massachusetts ($LD_{50} = 0.867$) in 2002 was 26 times higher than the LD_{50} value for the susceptible strain. This was one of the first reported cases of resistance to thiamethoxam.[3] In 2002–2003, LD_{50} values for thiamethoxam were still low in the midwestern United States, but started increasing thereafter. In Michigan, the first record of a thiamethoxam-resistant population was found in 2004. The LD_{50} values for two populations of beetles that were already resistant to imidacloprid were 5 and 10 times higher, respectively, than the LD_{50} value for the susceptible strain. By 2010, 80% of tested populations from the national survey had significantly higher thiamethoxam LD_{50} values compared with the susceptible strain.

4 DISCUSSION

New insecticides are increasingly difficult and costly to develop; therefore, it is crucial that the value of the currently used insecticides is not lost to agriculture through the development of insecticide resistance. The ability to understand the current status of insecticide resistance, along with the underlying genetics and the risk of resistance development, can significantly contribute to the development of sustainable pest management strategies. The present 12 year field monitoring fulfills a part of this need by describing the pattern of Colorado potato beetle insecticide resistance development over space and time. Although there are a large number of pesticide resistance cases recorded in the literature, the authors were not able to find any that document resistance development in the field over an extended time span and in a similar way to the present dataset.

Colorado potato beetle LD_{50} values increased exponentially for both imidacloprid and thiamethoxam, first in the eastern United States and, more recently, in the midwestern United States. The build-up of resistance over the 12 year period is not surprising given the history of Colorado potato beetle resistance to registered insecticides and the dependence on neonicotinoid insecticides since 1995. First records of resistant populations in the present sampling originated from the eastern United States and were detected in 1998, the year sampling began. Six years later, in 2004, neonicotinoid-resistant populations appeared in the midwestern United States. Reasons for the geographic pattern of resistance development are not obvious from the dataset, but it is likely that pesticide resistance management tools such as crop and insecticide rotation practices could be part of the explanation. On the East Coast in the United States, field size and availability for rotation may be more limited than in the midwestern and western United States, leading to smaller and more isolated populations, setting the stage for a faster rate of increase in LD_{50} values in this area. The gradual east-to-west increase in LD_{50} values over time is probably due to the natural rate of development of insecticide resistance under different management practices.

With an exponential change over time in LD_{50} values, the initial rate of increase is slow; therefore, management problems under field conditions early on in the process are less noticeable. According to the present data, the rate of change begins steeply to increase around 2007–2008, especially for summer-generation adults, and thus management problems are more likely to be first noticed in this group of insects (Figs 1 and 2). Summer-generation adults are significantly more resistant than overwintered adults. This could be due to the loss of fat reserves during the winter diapause, leading to reduced overall fitness in overwintered adults and/or because the summer adults encounter sublethal doses of the insecticide in the plant later in the season. Low pesticide rates may increase the rate of resistance development.[11] Encountering sublethal doses of insecticides is possible during the summer because neonicotinoids are most often applied at planting, and the toxic effect wanes over time. Regardless of the increasing LD_{50} values, the majority of Michigan growers are still able to control Colorado potato beetles with neonicotiniods.[6] The challenge is that there is currently no information available on which LD_{50} values correspond to insecticide failure in commercial production; therefore, the longevity of neonicotioinds in the field cannot be predicted. Understanding the genetic background of Colorado potato beetle insecticide resistance and predicting the longevity of commercially used insecticides are areas of high-priority research in light of the present results.

Changes in LD_{50} values for imidacloprid are occurring at a faster pace than for thiamethoxam, which likely is caused by a more widescale use of imidacloprid compared with thiamethoxam in most potato-growing areas[6] (http://www.nass.usda.gov/). Although

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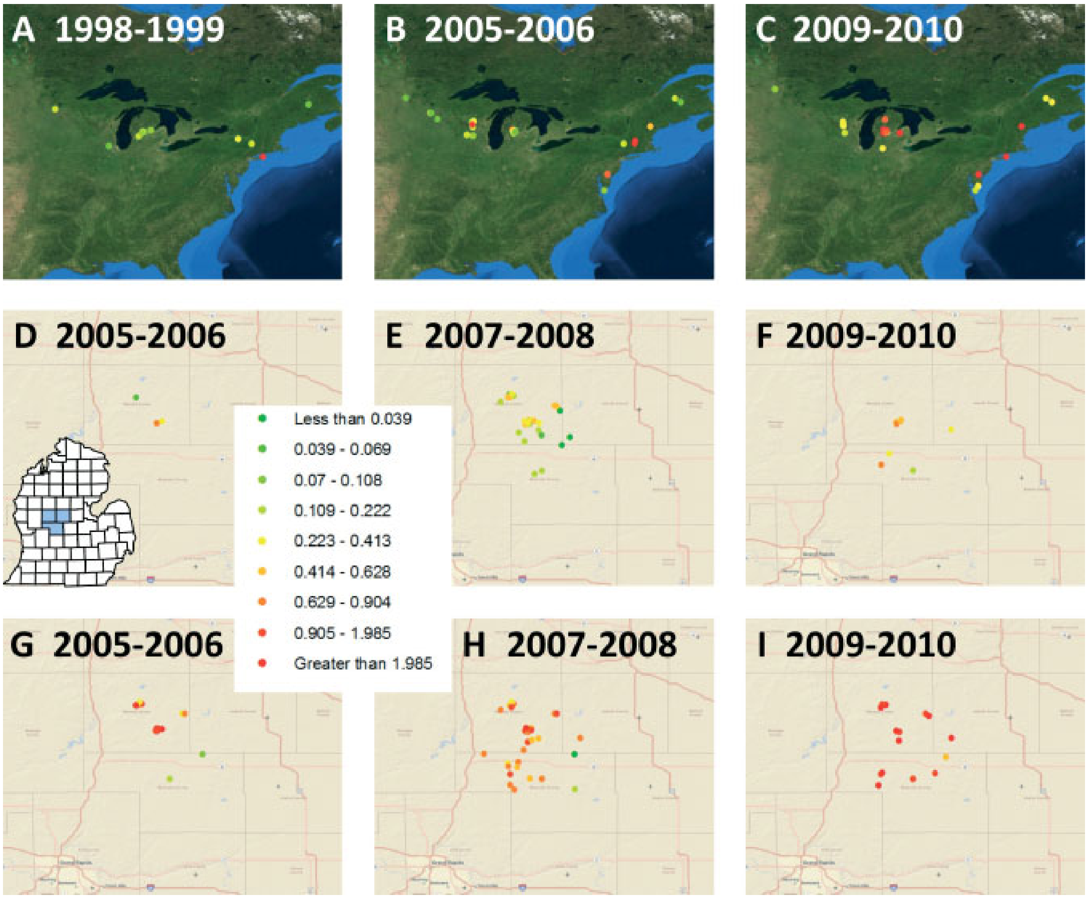

Figure 3. Geographic distribution of Colorado potato beetle adult imidacloprid resistance over time. The dots on the maps represent the source of the beetle populations tested, and the color of the dots indicates the level of resistance [see the insert with ranges of LD_{50} values (μg beetle^{-1}) corresponding to different colors]. Maps A to C display results of the national survey, panels D to F display LD_{50} values of populations from Michigan overwintered adults and panels G to I show results of summer-generation adults from Michigan. The area on maps D to I corresponds to the blue counties on the Michigan map insert on panel D. Samples were collected in the time period indicated on each panel.

cross-resistance is likely for the two neonicotinoids tested, the differences between the rate of LD_{50} value change over time indicates that the cross-resistance is not complete. Alternating imidacloprid with other neonicotinoid insecticides is not an effective insecticide resistance management strategy.[12] Resistance to thiamethoxam in Colorado potato beetle was among the first reported for any insect,[1,12] followed closely by the whitefly *Bemisia tabaci* being reported resistant to thiamethoxam.[13] Resistance to thiamethoxam and other neonicotinoids in Colorado potato beetles from Long Island, New York, is apparently the result of cross-resistance to imidacloprid, as thiamethoxam and the other neonicotinoids had never been used commercially in this area.

The US National Potato Council developed specific guidelines to discourage the repeated use of neonicotinoid insecticides in potatoes within a growing season and thus manage insecticide resistance (http://www.nationalpotatocouncil.org/). Insecticide class rotation is recommended as one of the resistance management strategies, and there are available options to follow the at-planting neonicotinoid insecticides with alternative classes of foliar insecticides applied later in the season. Effective management of resistance and preserving the effectiveness of insecticides for control of Colorado potato beetles will be critical to the future economics of potato production in the United States and worldwide.

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