

MONITORING RESISTANCE IN COLORADO POTATO BEETLE POPULATIONS TO IMIDACLOPRID AND OTHER NEONICOTINOIDS

Report of 2004 and 2005 Studies

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Continued monitoring was conducted in 2004 and 2005 to detect shifts in Colorado potato beetle susceptibility to imidacloprid. To examine possible cross-resistance relationships, bioassays also were performed on selected populations to estimate susceptibility to other neonicotinoid insecticides and to spinosad. Overwintered or summer beetles were collected from potato farms by Bayer field personnel and sent to the College Park laboratory, where susceptibility of each population was determined by exposing first instars to a potato-based agar diet containing a series of insecticide concentrations. Populations were selected partly on the basis of availability but many were also collected from farms reporting field control failures or reduced residual activity of imidacloprid. There were no changes in the protocol used in previous bioassay work, except that populations were reared on potted potato plants in small cages held in an environmentally-controlled macrocosm. Previous rearing was conducted outdoors in larger field cages over potato plants. This change resulted in greater time efficiency in colony maintenance, less mortality due to soil-borne fungal pathogens, and more egg production per female. Standard probit analysis (SAS Proc Probit) was used to model the concentration-mortality responses to estimate 50% lethal concentrations. Beetles from a New Jersey laboratory colony were used as a reference population to verify the repeatability of bioassay results compared to previous years.

Concentration-mortality responses to imidacloprid

Table 1 summarizes the bioassay results on 43 populations collected in 2004 from potato production areas in 13 states and 2 provinces. Imidacloprid susceptibility expressed as 50% lethal concentrations ranged from 0.18 to 11.7 ppm. Table 2 summarizes the concentration-mortality responses of 54 populations collected in 2005 from 12 states and 4 provinces. Of these, 32 populations were collected on separate potato farms, while the remaining populations were special collections for Dr. Andrei Alyokhin's insecticide efficacy plots at Fryeburg, ME. LC₅₀ levels for imidacloprid ranged from 0.28 to 17.6 ppm.

Bioassay responses relative to reported field efficacy

Figs. 1 and 2 show the range in susceptibility responses to imidacloprid for each year with respect to the field efficacy reported by the grower. The majority of populations with LC₅₀ values exceeding 4 ppm were collected from farms where growers reported either fair or poor performance of imidacloprid. The most resistant populations were collected in Delaware, Long Island, and southern Maine. New populations with significant levels of imidacloprid resistance were detected for the first time in 2005, including populations from several farms in central Maine and one from the Eastern Shore of Maryland. The most susceptible populations were collected from Canada and from the north central and northwest states, where population densities of Colorado potato beetles are generally lower and thus fewer applications of neonicotinoid insecticides have been used.

Fig. 3 depicts the overall concentration-mortality responses of Colorado potato beetle populations pooled from farms reporting good, fair and poor field performance of imidacloprid. These pooled responses are compared to the baseline response of susceptible populations established before imidacloprid was introduced. On farms where growers reported good control, the overall LC₅₀ of populations are about double that of the baseline response and the slope is shallower, indicating that susceptibility to imidacloprid has shifted, even on farms where soil treatments are still effective enough to achieve good control. For farms reporting fair and poor control, 50% lethal levels are between 3 to 4 ppm and 5 to 6 ppm, respectively, showing significant departure from baseline susceptibility.

Trends in imidacloprid susceptibility over time

Fig. 4 summarizes the changes in Colorado potato beetle susceptibility to imidacloprid that have taken place in different production areas of the U.S. and Canada since 1995. The average LC₅₀'s during 1995 to 1998 are based on a representative pool of Colorado potato beetle populations collected from each production area, whereas many populations assayed in 2001 through 2005 are biased towards populations from farms reporting reduced field efficacy of imidacloprid. The bars marked with an asterisk in Fig. 4 indicate that the mean probably overestimates the level of susceptibility for that production area. Concentration-mortality responses of the NJ reference population varied slightly but were not significantly different over the years of testing, indicating that the bioassay readings were consistent.

Growers in Delaware have extensively used soil and foliar treatments of imidacloprid since 1995 and have not been able to rotate fields far enough away to reduce population pressure. As a result, CPB populations have become increasingly more resistant to imidacloprid. Many growers have switched to thiamethoxam, which probably has exacerbated the problem due to cross-resistance (see below), but are now rotating modes of action with spinosad (Spintor) and novafluron (Rimon). Resistance levels have leveled off somewhat, although the population on the Cartanza farm at Little Creek, DE is one of the most resistant populations assayed thus far. This grower also has reported poor control with thiamethoxam.

For the first nine years of imidacloprid use, susceptibility did not change significantly on the Eastern Shore of Maryland due to strict crop rotation and the use of perimeter applications that created untreated refuges for susceptible beetles. The latter practice was discontinued in 2003 when growers switched to whole-field soil applications of thiamethoxam, after which there has been a definite shift upward in average LC₅₀'s values. Populations on Virginia's Eastern Shore are still susceptible to imidacloprid and this is partly attributed to crop rotation, use of insecticides with different modes of action, and temporal refuges afforded by the tomato acreage. In this area, most tomato transplants are treated with reduced rates of imidacloprid to control overwintered adults, and thus summer adults are generally exposed to non-selective titers of the systemic insecticide in the more mature plants. Since the potato crop is harvested by early July, the tomato crop serves as a refuge for summer adults and a place for gene mixing to occur.

On Long Island, Colorado potato beetles showed insensitivity to imidacloprid from the onset due to a general predisposition to resist toxic actions of all insecticides. After three years of soil applications, early season control could still be achieved with imidacloprid but its residual activity has been significantly reduced. Realizing the urgent need for resistance management, Long Island growers started to shift away from soil treatments in 1998 and began using foliar treatments of abamectin, cryocide, and spinosad to alternate modes of actions. Moreover, thiamethoxam was prohibited on Long Island, so growers could not switch to this insecticide as

was the case in other production areas. Currently, neonicotinoid resistance on Long Island appears to have slowed.

In New England, repeated use of imidacloprid at planting has been a common practice in many potato production areas, particularly the Connecticut River Valley in Massachusetts, central Maine, and the Fryeburg area in southern Maine. Populations collected from the Green Thumb and Hill farms in 2005 from the Fryeburg area continue to exhibit high levels of resistance to imidacloprid, despite the fact that no neonicotinoids has been used since 2003 (see more information below). Although most populations in central Maine are still susceptible, bioassay results indicated significant levels of resistance in three populations on farms where growers reported poor performance of imidacloprid. For example, the concentration-mortality response of one of these populations collected near Newport, ME showed a LC_{50} of 12.9 ppm. Considering the potato acreage in Maine, this area needs more extensive monitoring to detect early shifts in susceptibility.

Imidacloprid is still effective against Colorado potato beetles in the North Central states and Canadian provinces based on the limited sample of populations assayed. However, a few farms in Michigan and Minnesota have reported reduced control, and populations collected from these farms exhibited LC_{50} 's that range outside the baseline range. The Midwest is another potato production area that needs more extensive monitoring to detect resistance development.

In the Northwest, where the Colorado potato beetle is not a major pest of potato, populations remain very susceptible to imidacloprid.

Susceptibility to thiamethoxam

Of all the populations assayed for imidacloprid, 26 and 25 were also tested for thiamethoxam during 2004 and 2005, respectively. LC_{50} levels ranged from 0.06 to 0.84 ppm in 2004 (Fig. 5) and from 0.13 to 1.76 ppm in 2005 (Fig. 6). On a per active ingredient basis, thiamethoxam is clearly a more toxic insecticide to Colorado potato beetles. The average LC_{50} is about five times lower than that for imidacloprid, based on populations with no prior exposure to thiamethoxam and from farms reporting good control from imidacloprid. Assuming bioassay responses reflect the toxicity of field applications, soil applications of thiamethoxam may exert even greater selective pressure for neonicotinoid resistance when growers switch to this product because labeled rates are approximately 60% lower than the field rates of imidacloprid.

All populations from farms that reported fair or poor thiamethoxam control fell in the upper range of LC_{50} responses. Concentration-mortality responses in 2005 reflected a general decrease in susceptibility, with several populations with higher LC_{50} 's collected from farms with no history of thiamethoxam use. Moreover, the Little Creek (Cartanza) population in Delaware reported poor field control and had the highest LC_{50} for thiamethoxam. Establishment of a baseline response to thiamethoxam was confounded by the previous selection for resistance to imidacloprid, since both insecticides have the same mode of action. However, out of 59 populations assayed for thiamethoxam, 15 were very susceptible to imidacloprid and collected from farms reporting no previous use of thiamethoxam. The average LC_{50} level for these populations was 0.22 ppm.

Cross-resistance relationships

Fig. 7 is a XY plot of the LC_{50} 's for thiamethoxam in relation to the LC_{50} 's for imidacloprid for the same populations. Of populations from farms where thiamethoxam was not

used (solid squares), responses to thiamethoxam show a strong positive association with the 50% lethal concentrations of imidacloprid ($r = 0.78$, $p < 0.001$). This relationship is clearly the result of cross-resistance to imidacloprid because these populations were never exposed to field applications of thiamethoxam.

In 2005, four Colorado potato beetle populations representing opposite ends of the susceptibility response to imidacloprid were chosen to test for susceptibility to acetamiprid. Included were two susceptible populations from the Northwest, which were 14 times more susceptible to imidacloprid than the pair of resistant ones from Delaware and Maine. Relative responses to acetamiprid between these paired populations showed a 6.5-fold difference and matched closely with the lethal levels for imidacloprid (Fig. 8). Since none of these populations were previously exposed to acetamiprid, this suggests cross-resistance between the two neonicotinoids.

Spinosad is used as an alternative mode of action to control Colorado potato beetles, particularly on farms where populations have developed resistance to imidacloprid. To track susceptibility to this insecticide and examine possible cross-resistance with neonicotinoids, 49 populations were assayed for both spinosad and imidacloprid during 2004-05. Of the 21 populations that had no prior exposure to spinosad (Fig. 9, solid squares), there is a weak but significant positive association between spinosad and imidacloprid ($r = 0.43$, $p = 0.049$), suggesting possible cross-resistance. Of the populations that have been exposed to spinosad, a positive relationship could be the result of selection pressures exerted by both insecticides. Several populations exhibited LC_{50} 's above 5 ppm (about 2.5 times the baseline level of spinosad) and were collected from farms reporting fair control with spinosad. Thus, this may be evidence of early stages of resistance development to spinosad.

Monitoring changes in imidacloprid susceptibility in the Fryeburg, ME

In 2003, a Colorado potato beetle population from the Fryeburg area of Maine was assayed for imidacloprid susceptibility. The 50% lethal concentration was 12.2 ppm, indicating a significant 30-fold difference from the baseline susceptibility response (Fig. 10). Moreover, the two growers in the area reported a general lack of economic control with high rates of soil and foliar applications of imidacloprid. To prevent further resistance development, a neonicotinoid-free management plan was implemented in 2004 as a cooperative effort between the growers, Dr. Andrei Alyokhin at the University of Maine, and Bayer CropScience. Growers agreed to discontinue the use of neonicotinoid insecticides and switch to alternative modes of action to reduce selection pressure. During the 2004 growing season, and to a lesser extent also in 2005, early season beetle infestations were suppressed by soil treatments of disulfoton (Di-Syston) and then later populations were scouted and treated if necessary with foliar applications of oxamyl (Vydate) and spinosad (Spintor). On the assumption that fitness costs may be associated with resistance to imidacloprid, populations from several field sites in the Fryeburg area during each growing season were assayed to determine if resistance reverts and populations become more susceptible over time.

Fig. 10 summarizes the LC_{50} responses of overwintered and summer potato beetles from the Eastman and Anne fields during the 2004 and 2005 seasons. Overwintered beetles collected from the Eastman field in 2004 showed a LC_{50} response of 8.17 ppm, a one-third drop in resistance from the previous year. The summer population also showed a slightly lower LC_{50} of 5.76 ppm. Overwintered and summer populations from the Anne field showed similar levels of resistance in 2004. Thus, although 95% confidence limits of the LC_{50} estimates overlapped, the trend suggests some resistance reversion after the first neonicotinoid-free season.

In 2005, LC₅₀ responses of the overwintered populations from the Eastman and Anne fields showed no change in the level of resistance from the previous year. However, summer beetles from both field sites exhibited significantly higher levels of resistance, which were comparable to and even higher than the LC₅₀ responses recorded in 2003. It is unclear why levels of imidacloprid resistance have not changed after two neonicotinoid-free seasons. Laboratory studies (see below) and evidence from laboratory rearing indicate that imidacloprid resistant beetles are less fit than susceptible ones. Resistant populations tend to become more sensitive to imidacloprid after being reared for one generation or held in diapause at lower temperatures for an extended period.

One possible reason for the increase in LC₅₀ responses in 2005 could be due to cross-resistance with spinosad, which was used extensively as the recommended alternative insecticide in the Fryeburg area. For instance, the Anne field population was exposed to six applications of spinosad in 2005. Several lines of evidence support this hypothesis. First, the positive relationship depicted in Fig. 9 supports a pattern of cross-resistance between the neonicotinoids and spinosad. Secondly, both neonicotinoids and spinosyn insecticides interact with nicotinic acetylcholine receptors, but some binding assays indicate that they interact at different locations. Salgado and Saar (2004) have recently shown that imidacloprid interacts with two types of nicotinic acetylcholine receptors, one with high affinity and one with low affinity, and that spinosad binds with the low affinity receptor. The binding assays that indicated no interaction of imidacloprid with spinosad may have measured imidacloprid binding with the high affinity receptor and spinosyn binding with the low affinity receptor, thus missing the interactions between imidacloprid and spinosad at the low affinity receptor. Given this situation, it is possible, through unproven, that a single mutation of the low affinity receptor could cause resistance to both classes of compounds causing cross-resistance.

Proposed studies to examine potential cross-resistance between neonicotinoids and spinosad

Several studies are proposed to dissect the genetic correlations causing this disturbing pattern of positive cross-resistance and to determine if these observations indeed threaten the utility of spinosad as a replacement mode of action for the neonicotinoids. A laboratory selection will be conducted to induce resistance in the Fryeburg population to spinosad in order to determine if imidacloprid resistance simultaneously increases as a result.

Secondly, a bi-directional selection experiment has been initiated to measure the magnitude of the genetic correlation of resistance to imidacloprid and spinosad. This approach will document and measure, in a controlled setting, the genetic correlations that may be causing cross-resistance. The study will map the genetic factors causing individual tolerance to imidacloprid or spinosad to identify the chromosomal regions contributing to resistance to each compound. Resistance that maps to shared chromosomal regions would strongly suggest that there is a common genetic basis for the observed resistance and that there is also a common mechanism. This would implicate pleiotropy as the genetic cause of the observed correlations and such correlations are quite durable. Cross-resistance due to pleiotropy significantly limits the use of compounds with positively correlated resistance in a resistance management plan. Non-overlapping of the genetic location of genes contributing to resistance would suggest an independent genetic basis for resistance caused by the pattern of insecticide use and possible migration of Colorado potato beetles among farms. This result would indicate that spinosad would remain a viable alternative insecticide in a resistance management plan.

Fitness costs associated with imidacloprid resistance

Several laboratory experiments were conducted to compare the fitness components of the imidacloprid-resistant population from Fryeburg with a susceptible population. In one study, 20 replicate cohorts of ten neonate larvae of each population were reared on excised potato leaves in small arenas placed in environmental chambers. Mortality and changes in instars were recorded daily until pupation. Survival and development times to pupation and eclosion were determined. Of the newly-emerged adults, 20 pairs of males and females were randomly selected per population and reared for three weeks to measure adult survival and fecundity. In another study, the development time and hatchability of egg masses laid by resistant and susceptible beetles were recorded. Three egg masses from each of 10 females per population were reared under two different temperatures, 25° and 30° C.

Results showed no statistical significant differences in survival or time to pupation and eclosion between resistant and susceptible populations; however, development times were always slightly longer for resistant larvae. Due to high variance in the data, the only significant differences recorded were for second instars which took longer to develop and survived better than susceptible larvae. Adult survival of resistant beetles was also numerically higher but not statistically different from the susceptible beetles. Time to egg hatch and percentage hatchability was statistically the same for both populations; however, total fecundity per female was significantly different ($F = 8.37$, $df = 1,9$, $p = 0.018$), representing a major fitness cost associated with imidacloprid resistance. Resistant females produced 26.4% fewer eggs ($887 \pm SE 75$) than susceptible females ($1204 \pm SE 74$). When adjusted for differential adult survival, the net reproductive rate per resistant female (mean = 552) was an estimated 30.5% less than the rate per susceptible female (mean = 794). Together, when adjusted for generation time, the intrinsic rate of increase was estimated at 0.157 for the resistant population compared to 0.176 for the susceptible population. Reproductive differences between resistant and susceptible beetles may eventually remove resistant individuals in the absence of selection pressure; however, the question remains as to how long it will take for reversion to a susceptible population. An additional study will be conducted to examine the survival and change in susceptibility of resistant and susceptible beetles held at low temperatures to simulate for an extended period of diapause. Based on laboratory observations, overwintering survival may be a major fitness disadvantage for resistant Colorado potato beetles. If true, this would explain why bioassay responses of larvae from overwintered adults always show less resistance than the larvae of summer adults from the previous year.

References cited

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Table 1. Concentration-mortality responses of Colorado potato beetle populations to imidacloprid, thiamethoxam, and spinosad. 2004. Numbers following repeated farm population indicate the generation of larvae tested.

Farm	Location	Chemical	Reported field efficacy	Larvae tested	Slope +/- SEM	LC50 (ppm)	95% CL (ppm)	Goodness of fit	P value
Baldwin	Bridgeville, DE	imidacloprid	Not used	687	1.32 +/-0.36	1.56	0.38-3.76	23.19	-0.039
		thiamethoxam	Not used	480	1.56 +/-0.52	0.33	0.10-0.91	19.34	-0.013
		spinosad	Good	715	2.04 +/-0.66	2.71	0.76-6.43	58.62	<.001
Cartanza 1	Little Creek, DE	imidacloprid	Poor	962	1.27 +/-0.44	11.71	4.15-36.72	67.68	<.001
		thiamethoxam	Poor	720	1.83 +/-0.41	0.51	0.11-1.23	20.52	-0.083
		spinosad	Good	1034	1.33 +/-0.33	6.41	1.77-16.92	32.24	-0.021
Cartanza 2	Little Creek, DE	imidacloprid	Poor	657	1.77 +/-0.29	4.8	0.67-10.41	17.45	-0.180
Jackewicz	Magnola, DE	imidacloprid	Good	478	1.10 +/-0.34	4.34	1.40-12.60	7.32	-0.503
		thiamethoxam	Not used	607	1.91 +/-0.59	0.52	0.14-1.38	32.03	-0.002
		spinosad	Good	717	2.22 +/-0.69	4.43	1.04-10.55	53.3	<.001
Pries	Felton, DE	imidacloprid	Poor	843	1.00 +/-0.23	2.9	0.66-6.52	15.15	-0.651
		spinosad	Good	576	1.58 +/-0.57	4.35	1.17-11.04	34.39	-0.001
Wilson	Little Creek, DE	imidacloprid	Good	717	1.28 +/-0.23	1.37	0.25-3.03	12.58	-0.481
		thiamethoxam	Not used	754	1.84 +/-0.28	0.24	0.03-0.53	18.34	-0.145
		spinosad	Fair	590	2.16 +/-0.60	5.15	1.15-12.34	30.57	-0.002
Zimmerman	Little Creek, DE	imidacloprid	Not used	731	1.18 +/-0.36	2.55	0.74-6.88	23.55	-0.036
		thiamethoxam	Not used	711	1.38 +/-0.36	0.35	0.09-0.88	23.23	-0.039
		spinosad	Good	657	1.86 +/-0.72	3.93	1.20-9.86	56.5	<.001
Miller	Rupert, ID	imidacloprid	Good	700	1.20 +/-0.47	0.31	0.18-0.79	34.93	-0.001
Searle	Idaho Falls, ID	imidacloprid	Good	722	1.47 +/-0.53	0.29	0.16-0.73	39.2	0.000
		thiamethoxam	Good	697	1.45 +/-0.49	0.15	0.08-0.37	30.17	-0.005
		spinosad	Good	695	2.07 +/-0.33	1.73	0.25-3.71	18.4	-0.143
Vargas	Jerome, ID	imidacloprid	Good	720	1.43 +/-0.44	0.32	0.14-0.79	28.24	-0.008
		thiamethoxam	Good	733	1.84 +/-0.36	0.16	0.03-0.36	22.52	-0.048
		spinosad	Not used	815	2.12 +/-0.59	1.96	0.51-4.48	50.1	<.001

Chmiel	White Heath, IL	imidacloprid	Good	964	0.83 +/-0.36	0.12	0.12-0.49	26.35	-0.068
		thiamethoxam	Good	810	1.79 +/-0.31	0.23	0.04-0.50	13.59	-0.404
		spinosad	Good	767	1.87 +/-0.70	2.47	0.81-5.95	69.42	<.001
Kelly	Hadley, MA	imidacloprid	Poor	658	1.48 +/-0.79	3.96	2.00-11.58	105.71	<.001
		thiamethoxam	Fair	727	1.74 +/-0.56	0.72	0.22-2.10	33.2	-0.002
		spinosad	Good	719	2.20 +/-0.83	4.72	1.31-12.03	76.45	<.001
MacWilliams	Shiloh, MD	imidacloprid	Good	691	1.73 +/-0.29	1.45	0.20-3.17	18.87	-0.127
		spinosad	Not used	240	2.12 +/-1.12	3.98	2.47-28.28	11.39	-0.010
Ramirez	Herlock, MD	imidacloprid	Not used	1495	1.39 +/-0.21	2.25	0.35-4.92	44.91	-0.023
		thiamethoxam	Not used	761	1.90 +/-0.31	0.38	0.05-0.84	18.09	-0.154
		spinosad	Not used	992	0.93 +/-0.43	2.56	0.99-8.27	77.69	<.001
Reid	Beulah, MD	imidacloprid	Good	689	1.08 +/-0.36	1.14	0.35-2.81	25.41	-0.020
		spinosad	Not used	246	1.83 +/-1.00	3.12	2.45-19.48	11.23	-0.011
Rutoske	Suddersville, MD	imidacloprid	Good	1125	1.65 +/-0.59	4.53	1.50-14.08	106.38	<.001
		thiamethoxam	Not used	494	1.45 +/-0.79	0.67	0.32-6.31	38.48	<.001
		spinosad	Not used	710	2.16 +/-0.62	4.69	1.04-11.08	44.72	<.001
Schillinger	Glen Burnie, MD	imidacloprid	Not used	737	1.35 +/-0.35	3.01	0.70-7.15	22.78	-0.044
		thiamethoxam	Not used	537	1.54 +/-0.52	0.45	0.15-1.32	19.04	-0.015
		spinosad	Fair	776	1.57 +/-0.40	5.99	1.57-15.56	21.44	-0.065
Stevens	Pine Top, MD	imidacloprid	Good	716	1.49 +/-0.63	0.95	0.39-2.48	73.84	<.001
		thiamethoxam	Good	485	2.24 +/-0.48	0.22	0.05-0.48	5.36	-0.719
UM Farm	Beltsville, MD	imidacloprid	Not used	725	1.37 +/-0.33	0.18	0.07-0.43	4.56	-0.984
		thiamethoxam	Not used	742	1.49 +/-0.61	0.06	0.03-0.15	61.47	<.001
		spinosad	Not used	831	2.04 +/-0.36	2.07	0.32-4.55	21.42	-0.065
Admire	Fryeburg, ME	imidacloprid	-	470	1.33 +/-0.34	9.2	2.09-22.27	12.25	-0.507
Anne Field 1	Fryeburg, ME	imidacloprid	Poor	740	1.78 +/-0.32	6.71	1.13-15.18	14.1	-0.367
		thiamethoxam	Poor	750	1.47 +/-0.59	0.48	0.20-1.22	64.8	<.001
Anne Field 2	Fryeburg, ME	imidacloprid	Poor	635	1.52 +/-0.46	5.51	1.46-13.34	32.73	-0.002
		thiamethoxam	Poor	675	1.76 +/-0.44	0.73	0.17-1.68	30.97	-0.003
Eastman 1	Fryeburg, ME	imidacloprid	Poor	734	1.76 +/-0.35	8.17	1.55-18.99	7.45	-0.878

		thiamethoxam	Poor	254	1.46 +/-0.45	0.58	0.16-1.35	4.41	-0.221
		spinosad	Good	977	1.70 +/-0.44	3.88	0.87-9.19	57.5	<.001
Eastman 2	Fryeburg, ME	imidacloprid	Poor	676	1.35 +/-0.27	5.76	0.99-12.86	18.56	-0.138
		spinosad	Good	643	1.45 +/-0.73	2.98	1.28-8.10	65.94	<.001
Platinum	Fryeburg, ME	imidacloprid	-	576	1.34 +/-0.40	33.88	11.97-105.61	10.05	-0.690
		thiamethoxam	-	192	2.69 +/-1.21	0.84	0.47-2.89	9.26	-0.026
Tarr	Bridgton, ME	imidacloprid	Not used	727	1.25 +/-0.25	4.29	0.78-9.70	18.48	-0.140
		thiamethoxam	Not used	719	1.49 +/-0.35	0.36	0.10-0.82	20.98	-0.073
		spinosad	Not used	730	2.11 +/-0.52	2.73	0.58-6.17	35.81	-0.001
Tripp Field	Fryeburg, ME	imidacloprid	Poor	699	1.60 +/-0.45	4.39	1.14-10.39	35.5	-0.001
Sackett	Mecosta, MI	imidacloprid	Fair	576	1.76 +/-0.52	3.47	0.99-8.17	36.04	-0.001
		spinosad	Not used	580	1.74 +/-0.85	2.19	1.02-5.63	80.06	<.001
Walther	Decatur, MI	imidacloprid	Good	669	1.04 +/-0.37	0.27	0.16-0.70	23.41	-0.037
		spinosad	Not used	560	2.07 +/-1.18	1.03	0.75-2.71	103.25	<.001
Ferebee	Camden, NC	imidacloprid	Good	648	2.11 +/-0.63	0.58	0.17-1.35	45.42	<.001
Wood	Camden, NC	imidacloprid	Good	630	1.64 +/-0.48	0.59	0.19-1.38	32.52	-0.002
NJ Farm	Bridgton, NJ	imidacloprid	Not used	532	1.64 +/-0.52	5.33	1.71-16.09	20.2	-0.090
Reference (1)	NJDA, NJ	imidacloprid	Not used	763	1.74 +/-0.68	0.6	0.32-1.49	55.05	<.001
		thiamethoxam	Not used	489	1.71 +/-0.32	0.37	0.06-0.82	1.89	-0.984
		spinosad	Not used	721	1.99 +/-0.48	1.23	0.31-2.79	29.32	-0.006
Reference (2)	NJDA, NJ	thiamethoxam	Not used	451	1.71 +/-0.78	0.2	0.14-0.53	27.77	-0.001
		spinosad	Not used	481	2.27 +/-1.02	0.86	0.54-2.19	33.69	<.001
Van Meter	Bridgton, NJ	imidacloprid	Good	739	1.15 +/-0.26	2.8	0.62-6.73	19.06	-0.121
		spinosad	Good	587	1.93 +/-0.88	5.68	1.77-16.80	66.08	<.001
Kujawski	Riverhead, NY	imidacloprid	Fair	682	1.46 +/-0.37	4.62	1.06-10.84	23.67	-0.034
		thiamethoxam	Not used	722	1.72 +/-0.40	0.64	0.14-1.45	27.08	-0.012
		spinosad	Good	729	2.16 +/-0.72	4.5	1.14-10.95	62.25	<.001
Bailey	Everett, ON	imidacloprid	Good	691	1.20 +/-0.39	0.38	0.23-1.01	26.9	-0.013
		thiamethoxam	Not used	735	1.35 +/-0.42	0.14	0.06-0.34	37.75	<.001
		spinosad	Not used	722	2.03 +/-0.46	2.29	0.48-5.11	28.94	-0.007

Gerald	Cochranville, PA	imidacloprid	Fair	1009	1.43 +/-0.35	5.15	1.17-12.34	42.51	-0.001
		thiamethoxam	Good	538	1.65 +/-0.31	0.5	0.09-1.10	10.97	-0.204
		spinosad	Good	720	1.69 +/-0.30	5.36	0.89-12.08	16.61	-0.218
Huntsinger	Heggins, PA	imidacloprid	Good	1198	1.39 +/-0.28	5.05	0.94-11.45	43.74	-0.006
		thiamethoxam	Fair	960	2.00 +/-0.33	0.6	0.09-1.33	23.4	-0.176
		spinosad	Not used	720	1.55 +/-0.29	3.94	0.68-8.95	11.38	-0.579
Robinson	Albany, PEI	imidacloprid	Good	580	1.18 +/-0.65	0.26	0.25-0.92	28.23	-0.008
		spinosad	Good	581	2.12 +/-0.85	3.52	1.05-8.75	66.24	<.001
Holland	New Church, VA	imidacloprid	Blank	611	1.61 +/-0.59	0.72	0.27-1.76	50.96	<.001
Qualls	Ephrata, WA	imidacloprid	Good	592	1.35 +/-0.28	0.95	0.18-2.12	17.27	-0.187
		spinosad	Good	576	1.81 +/-0.51	2.01	0.53-4.58	27.51	-0.011
Schreiber	Eltopia, WA	imidacloprid	Good	199	1.26 +/-0.68	0.39	0.36-1.27	5.18	-0.159
		thiamethoxam	Good	200	1.76 +/-0.68	0.14	0.07-0.34	1.77	-0.621
		spinosad	Fair	205	1.88 +/-0.92	1.19	1.11-3.42	6.46	-0.091
Pine Bluff	Grand Marsh, WI	imidacloprid	Good	583	1.10 +/-0.32	0.78	0.38-1.94	12.85	-0.460
		spinosad	Not used	575	1.79 +/-0.69	2.17	0.77-5.24	51.04	<.001
West	Rice Lake, WI	imidacloprid	Fair	575	1.29 +/-0.31	1.59	0.46-3.64	12	-0.528
		spinosad	Not used	566	1.60 +/-1.04	9.32	4.16-129.11	85.41	<.001

Table 2. Concentration-mortality responses of Colorado potato beetle populations to imidacloprid, thiamethoxam, acetamiprid, and spinosad. 2005. Numbers following repeated farm population indicate the generation of larvae tested. Fryeburg populations collected from insecticide plots were tested as larvae from overwintered adults (OW), overwintered beetles after 2 weeks of feeding (OW2), and summer beetles (F1).

Farm	City	Chemical	Reported field efficacy	Larvae tested	Slope +/- SEM	LC50 (ppm)	95% CL (ppm)	Goodness of fit	P value
Cartanza	Little Creek, DE	acetamiprid	Not used	538	1.69 +/-0.33	2.32	0.45 - 5.10	1.92	-0.588
		imidacloprid	Poor	581	1.41 +/-0.31	10.27	2.09 - 24.11	4.66	-0.199
		thiamethoxam	Poor	374	2.21 +/-0.52	1.76	0.32 - 4.04	3.35	-0.341
		spinosad	Good	570	2.51 +/-0.38	2.09	0.25 - 4.46	4.73	-0.193
Zimmerman	Little Creek, DE	imidacloprid	Not used	767	1.50 +/-0.27	8.02	1.56 - 18.57	6.04	-0.302
		thiamethoxam	Not used	946	1.77 +/-0.83	0.89	0.40 - 4.24	53.52	<.001
		spinosad	Good	759	2.44 +/-0.32	1.55	0.16 - 3.28	2.75	-0.432
Jackewicz	Magnolia, DE	imidacloprid	Not used	563	1.13 +/-0.25	3.39	0.74 - 7.81	2.38	-0.794
		thiamethoxam	Not used	1003	1.71 +/-0.72	1.35	0.57 - 6.22	36.00	<.001
		spinosad	Good	572	2.54 +/-0.38	1.77	0.21 - 3.77	2.32	-0.509
Shire	Newark, DE	imidacloprid	Fair	529	1.47 +/-0.32	2.45	0.53 - 5.46	3.31	-0.346
Searle	Idaho Falls, ID	acetamiprid	Not used	977	2.31 +/-0.38	0.45	0.08 - 0.98	7.41	-0.192
		imidacloprid	Not used	578	1.23 +/-0.30	0.39	0.12 - 0.90	1.49	-0.684
		thiamethoxam	Not used	1156	2.27 +/-0.25	0.30	0.03 - 0.63	8.98	-0.110
		spinosad	Not used	964	2.35 +/-0.29	0.83	0.08 - 1.75	7.18	-0.207
Vargas	Jerome, ID	imidacloprid	Good	574	1.47 +/-0.30	0.91	0.16 - 2.00	2.03	-0.567
		thiamethoxam	Good	578	2.61 +/-0.39	0.13	0.02 - 0.29	1.06	-0.787
		spinosad	Not used	579	2.45 +/-0.44	0.64	0.10 - 1.37	2.91	-0.406
Kuhl	Winkler, MB	imidacloprid	Good	573	1.68 +/-0.51	1.71	0.62 - 4.98	7.53	-0.057
		thiamethoxam	Not used	778	2.00 +/-0.67	0.52	0.19 - 1.34	29.56	<.001
		spinosad	Not used	579	2.62 +/-0.39	2.05	0.23 - 4.38	4.94	-0.176
Stevens	Hurlock, MD	imidacloprid	Good	573	1.48 +/-0.32	2.72	0.52 - 5.99	4.23	-0.238
		thiamethoxam	Good	580	2.59 +/-0.39	0.48	0.06 - 1.03	3.95	-0.267
Brinsfield	Vienna, MD	imidacloprid	Good	591	1.38 +/-0.29	2.55	0.49 - 5.65	2.94	-0.567
		thiamethoxam	Good	574	2.47 +/-0.57	0.41	0.12 - 0.96	6.42	-0.093

Stevens	Waddell, MD	imidacloprid	Good	627	1.19 +/-0.43	5.87	2.59 - 19.81	7.25	-0.064
		thiamethoxam	Good	591	2.85 +/-0.67	0.61	0.15 - 1.43	10.55	-0.032
McCrum		imidacloprid	Fair	394	2.41 +/-0.45	5.50	0.79 - 11.97	0.99	-0.803
Finnemore	Caswell, ME	imidacloprid	Good	579	1.03 +/-0.28	5.43	1.24 - 12.72	0.41	-0.937
Crane	Exeter, ME	imidacloprid	Good	954	1.26 +/-0.23	13.67	2.44 - 31.11	4.13	-0.388
		AEF (OW)	Frysburg, ME	imidacloprid	Poor	571	1.84 +/-0.33	7.04	1.05 - 15.49
AEF (OW2)	Frysburg, ME	thiamethoxam	Not used	578	1.99 +/-0.65	0.93	0.37 - 2.68	11.52	-0.009
		imidacloprid	Poor	576	1.80 +/-0.32	6.05	0.90 - 13.26	5.54	-0.136
AEF (F1)	Frysburg, ME	thiamethoxam	Not used	584	2.38 +/-0.71	1.04	0.36 - 2.79	10.99	-0.012
		imidacloprid	Poor	1151	1.65 +/-0.24	17.23	2.59 - 38.25	2.26	-0.688
Admire (OW)	Frysburg, ME	imidacloprid	-	561	2.04 +/-0.62	5.85	2.14 - 16.22	9.97	-0.019
		thiamethoxam	-	565	1.97 +/-0.33	0.80	0.11 - 1.74	2.63	-0.453
Admire (OW2)	Frysburg, ME	imidacloprid	-	566	1.51 +/-0.32	9.61	1.82 - 22.14	3.09	-0.378
		thiamethoxam	-	575	1.94 +/-0.35	1.37	0.21 - 3.03	4.32	-0.229
Admire (F1)	Frysburg, ME	imidacloprid	-	584	1.65 +/-0.34	11.81	2.22 - 27.31	2.63	-0.452
Anne Field 1	Frysburg, ME	imidacloprid	-	566	1.86 +/-0.32	5.35	0.77 - 11.65	2.61	-0.455
		thiamethoxam	-	526	2.12 +/-0.73	1.28	0.49 - 4.02	10.76	-0.013
Anne Field 2	Frysburg, ME	imidacloprid	-	375	1.40 +/-0.43	17.60	5.38 - 49.87	0.43	-0.935
	Frysburg, ME	imidacloprid	-	192	2.31 +/-0.73	10.70	2.42 - 25.92	4.25	-0.236
	Frysburg, ME	spinosad	-	335	1.37 +/-0.40	3.53	0.88 - 8.83	4.2	-0.241
Anne Field 3	Frysburg, ME	imidacloprid	-	384	1.53 +/-0.37	6.28	1.27 - 14.45	0.1	-0.991
Control (OW)	Frysburg, ME	imidacloprid	-	577	1.93 +/-0.33	5.24	0.73 - 11.38	5.28	-0.152
		thiamethoxam	-	384	2.05 +/-0.41	0.91	0.15 - 2.00	1.82	-0.611
Control (OW2)	Frysburg, ME	imidacloprid	-	770	1.35 +/-0.26	7.06	1.18 - 15.78	3.63	-0.304
		thiamethoxam	-	579	2.23 +/-0.37	1.20	0.16 - 2.59	3.82	-0.282
Control (F1)	Frysburg, ME	imidacloprid	-	565	1.51 +/-0.54	9.58	3.92 - 38.44	8.36	-0.039
Eastman 1	Frysburg, ME	acetamiprid	Not used	574	1.50 +/-0.46	2.13	1.15 - 5.47	6.43	-0.092
		imidacloprid	Poor	590	1.99 +/-0.33	4.87	0.66 - 10.53	2.64	-0.451
		thiamethoxam	Not used	588	2.12 +/-0.55	0.64	0.22 - 1.59	8.1	-0.044
		spinosad	Good	572	1.90 +/-0.75	1.88	1.18 - 5.18	14.24	-0.003

Eastman 2	Frysburg, ME	imidacloprid	Poor	582	1.83 +/-0.36	10.37	1.72 - 23.36	0.93	-0.819
Platinum (OW)	Frysburg, ME	imidacloprid	-	575	1.56 +/-0.33	10.38	1.96 - 23.94	0.46	-0.927
		thiamethoxam	-	587	2.33 +/-0.78	1.25	0.45 - 3.66	12.07	-0.007
Platinum(OW2)	Frysburg, ME	imidacloprid	-	581	1.93 +/-0.38	12.37	2.10 - 28.07	5.24	-0.155
Poncho (OW2)	Frysburg, ME	imidacloprid	-	580	1.73 +/-0.33	7.83	1.25 - 17.44	1.68	-0.641
		thiamethoxam	-	581	2.68 +/-0.39	0.87	0.10 - 1.84	4.12	-0.249
Poncho (F1)	Frysburg, ME	imidacloprid	-	945	1.81 +/-0.27	11.73	1.51 - 25.47	6.8	-0.147
Greg Schools	Littleton, ME	imidacloprid	Fair	581	1.10 +/-0.29	8.05	1.83 - 19.34	0.96	-0.811
Aroostock	Monticello, ME	imidacloprid	Good	573	1.39 +/-0.30	4.07	0.75 - 9.05	0.89	-0.829
Smith	Newport, ME	imidacloprid	Fair-Poor	771	1.80 +/-0.32	12.89	2.09 - 28.99	1.91	-0.591
Walther	Decatur, MI	imidacloprid	Good	964	1.73 +/-0.55	0.80	0.40 - 2.01	23.07	0.000
		spinosad	Not used	348	2.35 +/-0.46	0.59	0.10 - 1.28	2.79	-0.424
Sackett	Mecosta, MI	imidacloprid	Good	575	1.75 +/-0.51	2.03	1.02 - 5.12	7.12	-0.068
		thiamethoxam	Not used	768	2.05 +/-0.75	0.67	0.32 - 1.93	20.21	0.000
		spinosad	Good	601	2.55 +/-0.39	1.71	0.22 - 3.64	2.43	-0.489
Hayse	Becker, MN	imidacloprid	Fair	573	1.37 +/-0.30	2.65	0.58 - 5.91	4.77	-0.189
		thiamethoxam	Not used	763	1.91 +/-0.51	0.94	0.31 - 2.47	9.6	-0.022
		spinosad	Fair	574	3.53 +/-0.51	1.44	0.14 - 3.04	1.79	-0.617
Dechene	Big Lake, MN	imidacloprid	Good	578	1.70 +/-0.51	1.43	0.53 - 4.05	8.06	-0.045
		thiamethoxam	Not used	1170	2.18 +/-0.55	0.58	0.15 - 1.42	22.41	0.000
		spinosad	Good	569	2.60 +/-0.39	1.71	0.20 - 3.66	2.01	-0.570
Young	Bedell, NB	imidacloprid	Good	579	1.05 +/-0.29	2.26	0.55 - 5.59	0.16	-0.984
		thiamethoxam	Not used	577	2.62 +/-0.47	0.40	0.05 - 0.87	4.72	-0.194
		spinosad	Not used	544	1.76 +/-0.41	0.88	0.27 - 2.01	2.34	-0.504
Hallmark	Milnor, ND	imidacloprid	Not used	572	1.00 +/-0.29	1.52	0.59 - 3.63	4.88	-0.181
		thiamethoxam	Good	588	2.10 +/-0.67	0.36	0.18 - 0.91	10.88	-0.012
		spinosad	Not used	570	2.24 +/-0.36	2.14	0.27 - 4.61	5.99	-0.112
Reference (1)	NJDA LAb, NJ	imidacloprid	Not used	554	1.00 +/-0.38	0.28	0.22 - 0.85	0.98	-0.807
		thiamethoxam	Not used	581	1.65 +/-0.30	0.17	0.03 - 0.36	2.74	-0.602
		spinosad	Not used	382	2.18 +/-0.87	0.41	0.23 - 1.02	0.75	-0.861
Reference (2)	NJDA LAb, NJ	imidacloprid	Not used	584	1.66 +/-0.37	1.09	0.31 - 2.48	1.58	-0.664

Reference (3)	NJDA LAb, NJ	imidacloprid	Not used	585	1.67 +/-0.36	1.29	0.33 - 2.90	0.11	-0.991
Kujawski	Riverhead, NY	imidacloprid	Fair	552	1.25 +/-0.31	6.61	1.37 - 15.5	1.25	-0.740
		thiamethoxam	Not used	1529	1.84 +/-0.52	1.24	0.39 - 3.53	27.8	<.001
		spinosad	Good	583	2.47 +/-0.38	2.30	0.28 - 4.90	1.16	-0.763
Bailey	Everett, ON	imidacloprid	Good	575	1.87 +/-0.32	1.03	0.15 - 2.24	0.33	-0.955
		thiamethoxam	Not used	575	2.57 +/-0.72	0.29	0.09 - 0.77	9.42	-0.024
		spinosad	Not used	576	2.32 +/-0.70	1.92	0.84 - 4.80	10.64	-0.014
Robinson	Albany, PEI	imidacloprid	Good	574	1.70 +/-0.31	0.95	0.15 - 2.08	0.77	-0.858
		thiamethoxam	Not used	573	2.60 +/-0.79	0.22	0.08 - 0.60	12.62	-0.006
		spinosad	Not used	580	2.37 +/-0.36	1.63	0.20 - 3.49	2.55	-0.467
Dore	Pont-Rouge, QU	imidacloprid	Fair	571	1.70 +/-0.31	3.21	0.53 - 7.01	0.09	-0.993
		thiamethoxam	Not used	1155	2.39 +/-0.51	0.66	0.14 - 1.53	17.47	-0.004
		spinosad	Not used	589	1.74 +/-0.43	0.57	0.20 - 1.34	1.58	-0.664
Long	CapeCharles, VA	imidacloprid	Good	620	1.21 +/-0.45	1.38	0.89 - 3.86	7.87	-0.049
		thiamethoxam	Not used	575	2.04 +/-0.55	0.23	0.08 - 0.56	10.06	-0.039
Hickman	Horntown, VA	imidacloprid	Good	582	1.16 +/-0.30	1.39	0.48 - 3.26	0.33	-0.955
		thiamethoxam	Good	588	1.53 +/-0.51	0.44	0.19 - 1.33	9.14	-0.027
		spinosad	Good	607	2.13 +/-0.56	1.21	0.55 - 2.94	6.57	-0.087
Brinn	Parksley, VA	imidacloprid	Not used	572	1.36 +/-0.28	3.77	0.70 - 8.44	5.48	-0.241
		thiamethoxam	Not used	386	2.51 +/-0.46	0.56	0.08 - 1.20	2.03	-0.566
Black	Seaview, VA	imidacloprid	Good	610	1.43 +/-0.28	1.97	0.36 - 4.36	4.88	-0.300
		thiamethoxam	Good	613	2.13 +/-0.55	0.29	0.09 - 0.69	11.22	-0.024
Qualls	Ephrata, WA	acetamiprid	Not used	587	1.55 +/-0.37	0.24	0.08 - 0.55	0.86	-0.834
		imidacloprid	Good	584	0.94 +/-0.29	1.24	0.55 - 3.01	3.07	-0.382
Anthony	WI	imidacloprid	Good	558	1.51 +/-0.33	1.73	0.44 - 3.89	0.29	-0.962
Barron	Rice Lake, WI	spinosad	Not used	586	2.60 +/-0.63	3.87	1.03 - 9.30	7.26	-0.064

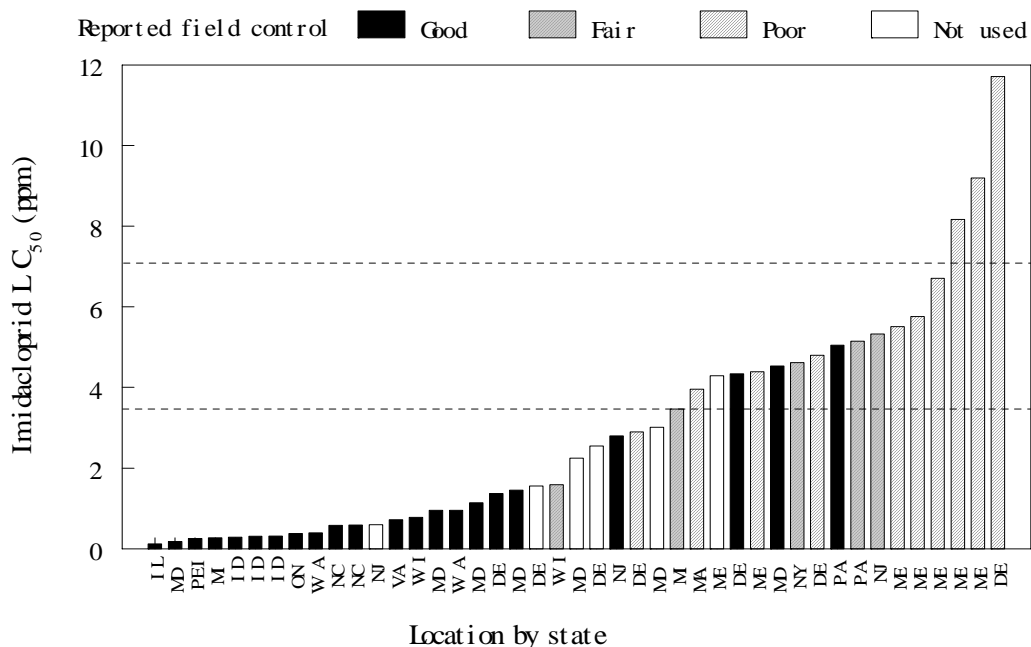


Figure 1. Range in LC₅₀ responses of Colorado potato beetle populations to imidacloprid with respect to the field efficacy reported by the grower. 2004.

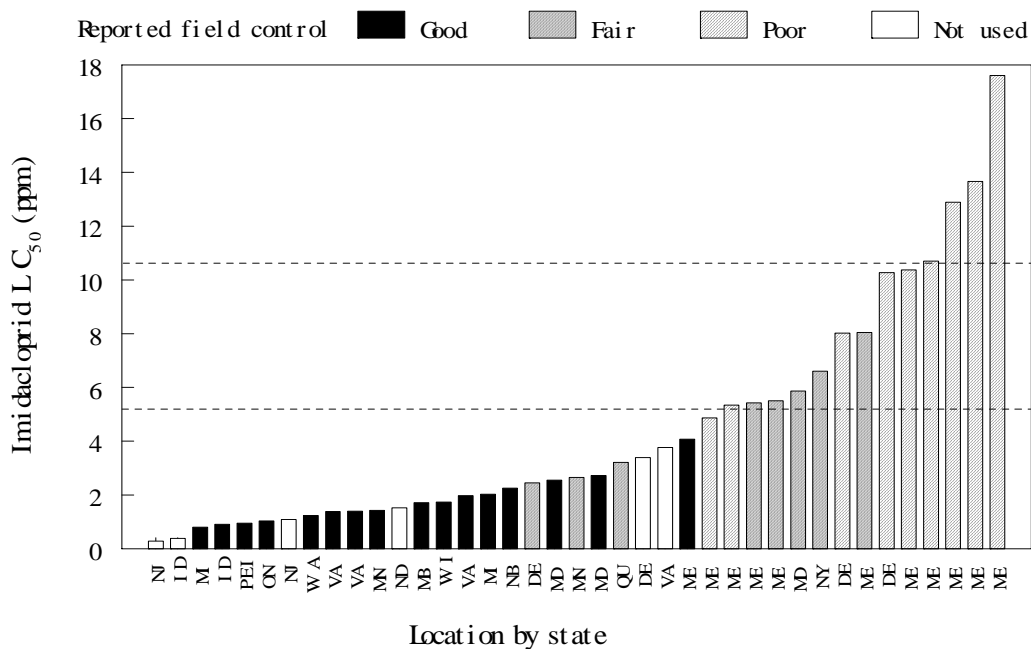


Figure 2. Range in LC₅₀ responses of Colorado potato beetle populations to imidacloprid with respect to the field efficacy reported by the grower. 2005.

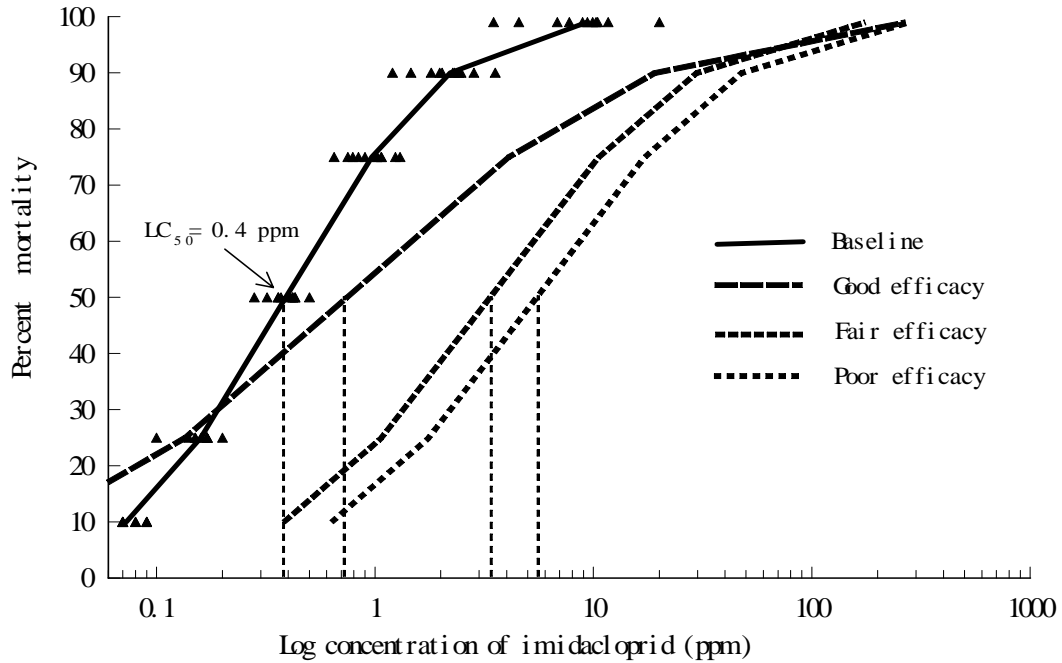


Figure 3. Overall concentration-mortality responses of Colorado potato beetle populations pooled from farms reporting good, fair and poor field performance of imidacloprid. Responses are depicted in comparison with the baseline response of susceptible populations.

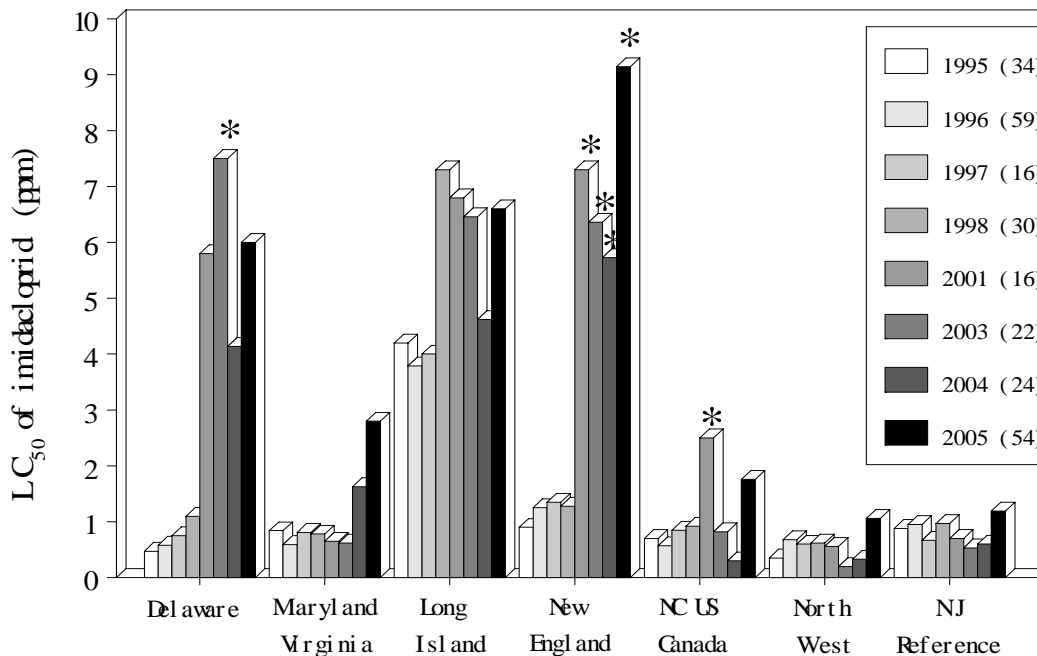


Figure 4. Trends in Colorado potato beetle susceptibility to imidacloprid in different production areas of the U.S. and Canada since 1995. Bars marked with an asterisk indicate that the mean overestimates the level of susceptibility because populations assayed for that production area were biased towards farms reporting reduced field efficacy of imidacloprid. Concentration-mortality responses of the NJ reference population are depicted to indicate the repeatability of the bioassay readings over time.

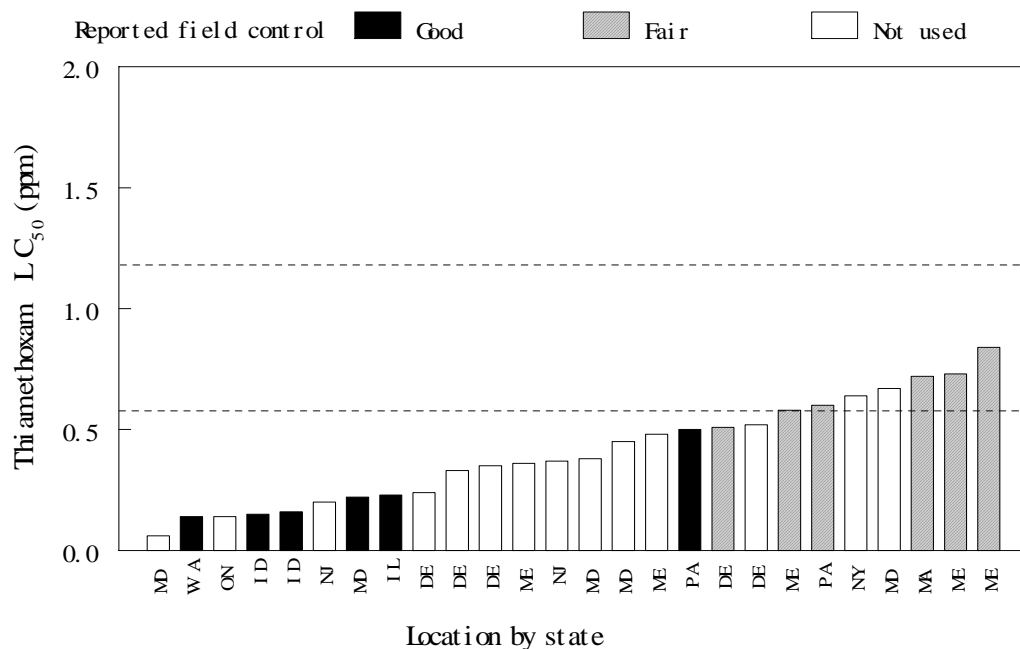


Figure 5. Range in LC₅₀ responses of Colorado potato beetle populations to thiamethoxam with respect to the field efficacy reported by the grower. 2004.

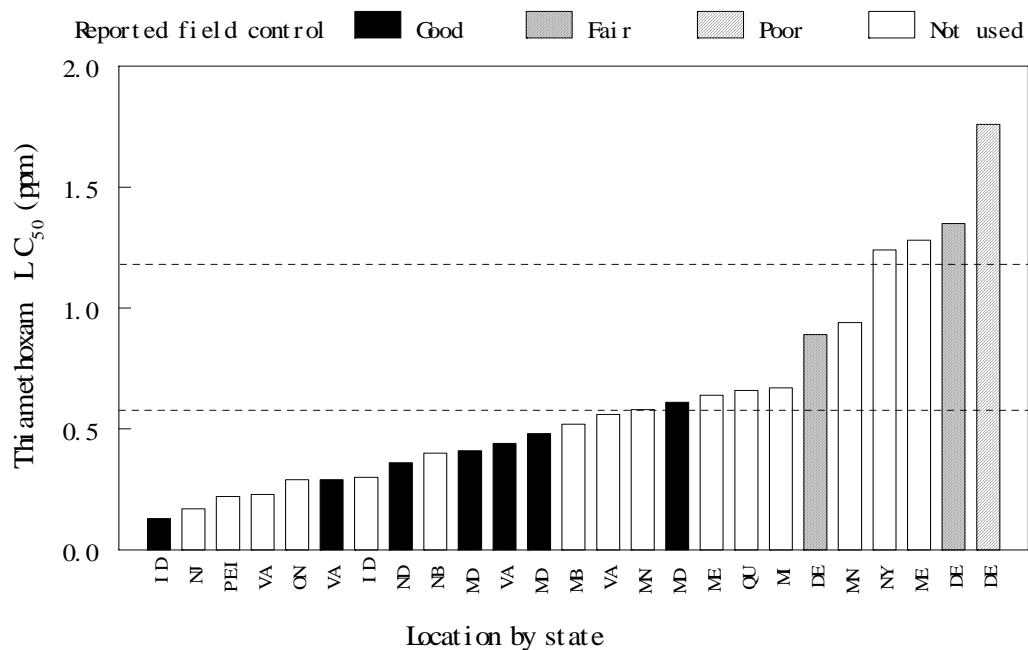


Figure 6. Range in LC₅₀ responses of Colorado potato beetle populations to thiamethoxam with respect to the field efficacy reported by the grower. 2005.

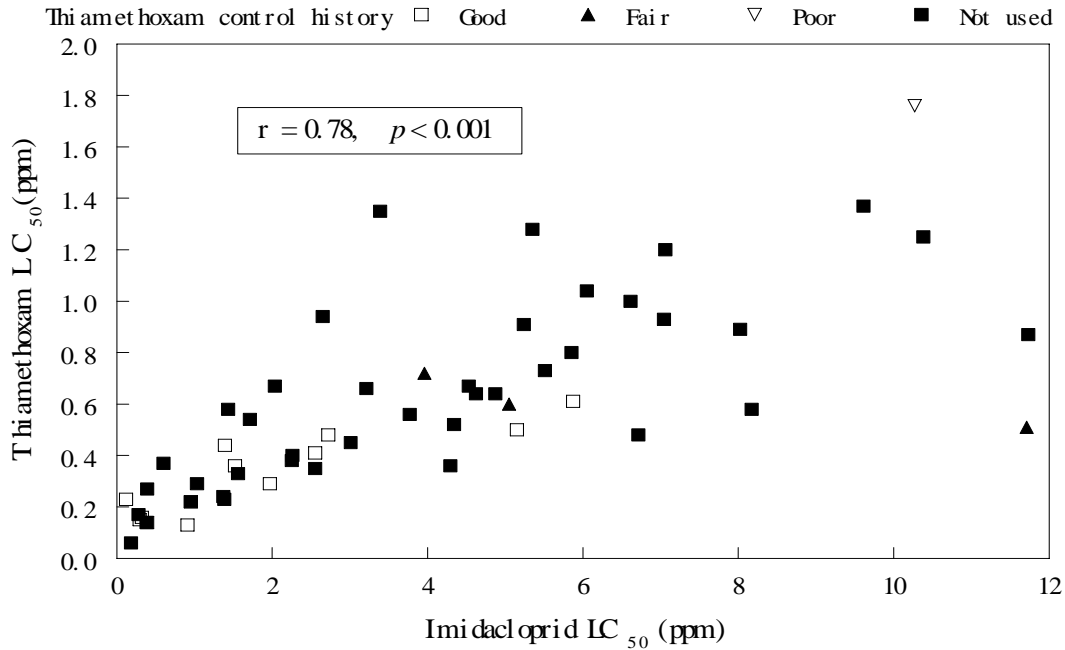


Figure 7. Plot of the LC₅₀ levels of thiamethoxam in relation to the LC₅₀ levels of imidacloprid for the same populations. Of populations from farms where thiamethoxam was not used (solid squares), responses to thiamethoxam show a strong positive association with the 50% lethal concentrations of imidacloprid.

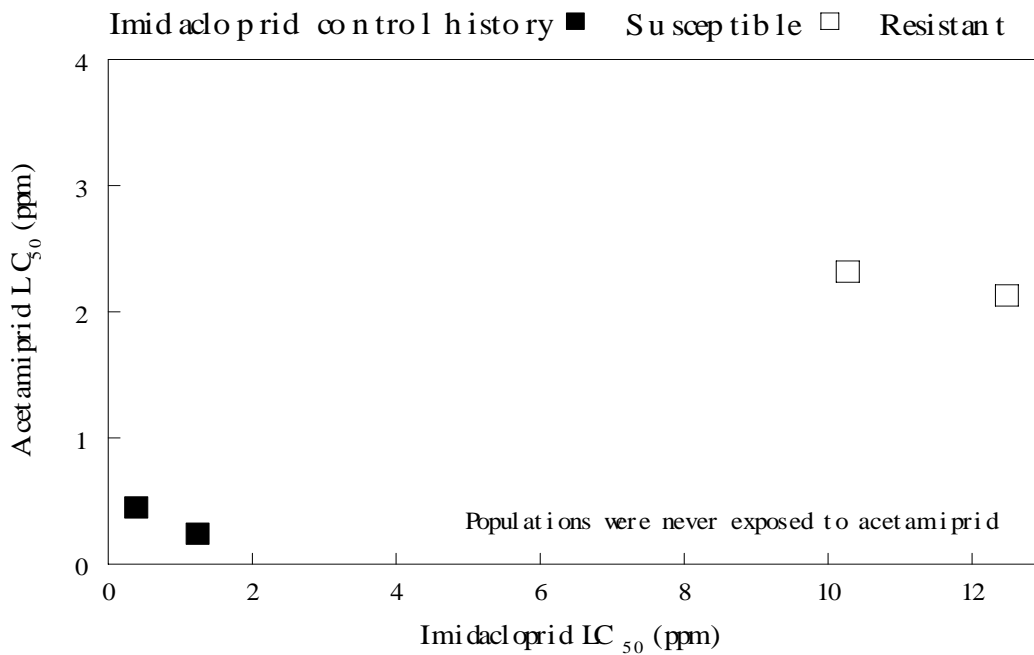


Figure 8. LC₅₀ responses to acetamiprid between pairs of populations representing opposite ends of the susceptibility response to imidacloprid.

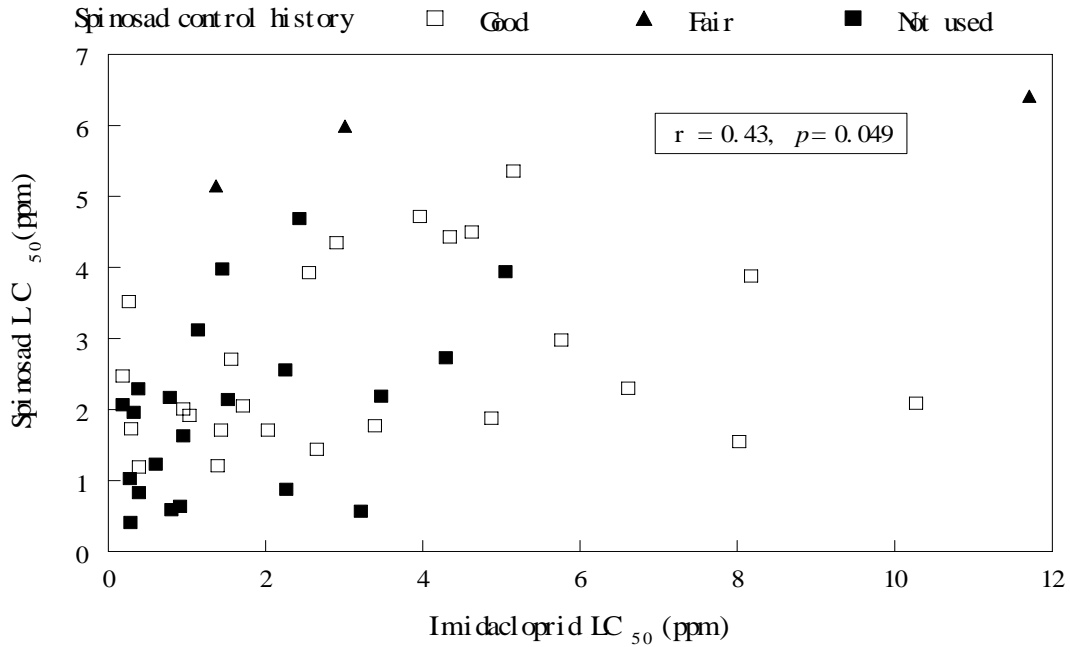


Figure 9. Plot of the LC₅₀ levels of spinosad in relation to the LC₅₀ levels of imidacloprid for the same populations. Of populations from farms where spinosad was not used (solid squares), responses to spinosad show a significant positive association with the 50% lethal concentrations of imidacloprid.

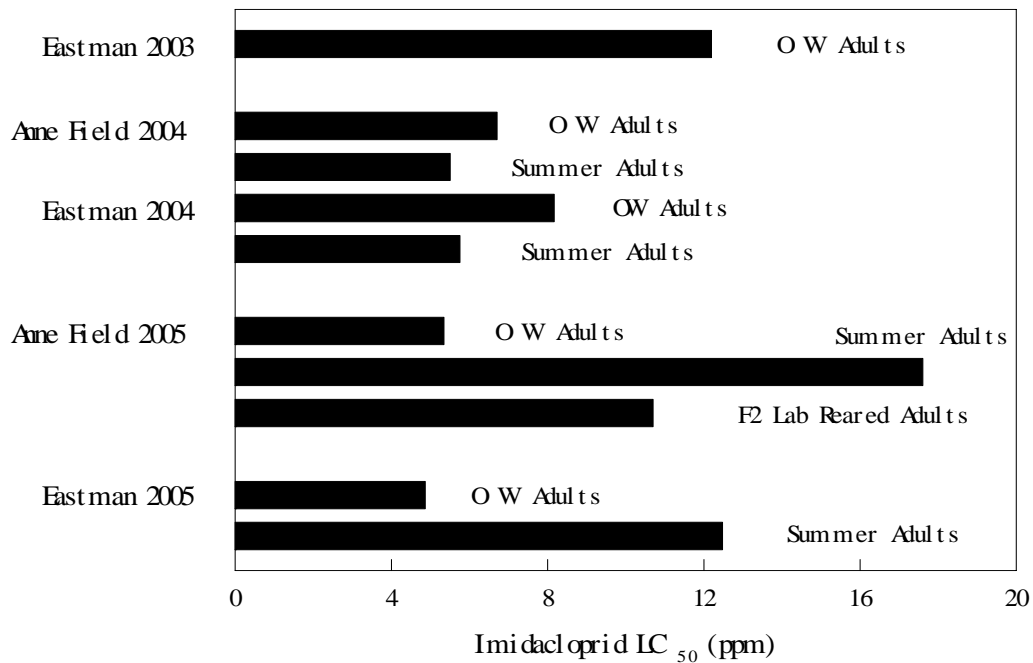


Figure 10. LC₅₀ responses of overwintered and summer potato beetles from the Eastman and Anne fields during the 2004 and 2005 seasons. Fryeburg, ME.