Effect of Insecticide Management History on Emergence Phenology and Neonicotinoid Resistance in *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae)

A. S. HUSETH AND R. L. GROVES[1]

Department of Entomology, University of Wisconsin-Madison, 1630 Linden Dr., Madison, WI 53706

ABSTRACT Emergence phenology and fitness attributes of several Colorado potato beetle, *Leptinotarsa decemlineata* (Say), populations were measured under field and greenhouse conditions. Anecdotal observations by producers and pest managers in many locations of the upper Midwest increasingly suggested that select populations of Colorado potato beetle were emerging over a longer period in the spring and were less sensitive to systemic neonicotinoids in cultivated potato. These changes in emergence phenology may be related to changes in systemic insecticide concentration over time. Specifically, a prolonged period of adult emergence in the spring increases the potential of low-dose chronic exposure to systemic neonicotinoid insecticides in potato. In 2010 and 2011, our objectives were twofold: 1) establish a common garden experiment to compare the emergence phenology of Colorado potato beetle populations uniquely managed with variable insecticide inputs, and 2) measure postdormancy fitness of emerged adult beetles from among these selected populations. Cumulative adult emergence was modeled with logistic regression. Results from this study found no clear evidence for direct relationships between phenology and management history or resistance. Differences in reproductive capacity, sex ratio, and body size were apparent in some instances. However, these results did not uniformly correspond to one specific form of potato pest management tested here. In this study, long-term reliance on systemic insecticides for Colorado potato beetle control did not serve as a strong predictor for variable life history for selected populations in Wisconsin.

KEY WORDS Colorado potato beetle, integrated pest management, diapause, phenology, insecticide resistance

Insect dormancy, more specifically diapause, is a physiologically driven interruption of development evolved to overcome long-term extreme conditions, often induced by environmental cues such as photoperiod, host-plant quality, and temperature (Lees 1955, Danks 1987, Koštál 2006, Yocum et al. 2011). In temperate potato production regions of the world, winter dormancy is a critical life-history strategy of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say), to avoid unfavorable winter conditions (Ushatinskaya 1978, Tauber et al. 1986, Tauber and Tauber 1993, Yocum et al. 2011). Colorado potato beetle remains dormant for ~3 mo, although several studies have documented long-term dormancy patterns, with some individual insects remaining in an arrested state for one or more years (Isley 1935, Tauber et al. 1986, Senanayake et al. 2000, Tauber and Tauber 2002). Often, variability in diapause duration can be closely associated with the presence of suitable host plants in the agroecosystem, especially for specialist herbivores like Colorado potato beetle (Xu and Long 1997, Skinner et al. 2004). Insects inhabiting more dynamic or unstable environments with unpredictable resources may extend diapause for longer periods to better synchronize with hosts, resulting in a delayed dormancy or protracted emergence phenology (Corley et al. 2004). Colonization of potato by Colorado potato beetle often occurs in a short window of time after crop emergence (Gibson et al. 1925), and this period corresponds to the postdormancy period of the pest (Boiteau 2001). For potato growers, synchronous Colorado potato beetle colonization is more advantageous, as it often results in a larger proportion of susceptible life stages present within fields at the same time. In contrast, asynchronous emergence and subsequent colonization result in extended egg deposition over a longer interval, leading to the presence of multiple larval stadia present simultaneously in the crop. Several of the new reduced-risk insecticides do not possess the same broad spectrum of activity against all Colorado potato beetle stadia. As a result, growers have continued to use foliar applications of the neonicotinoids, organophosphates, and carbamates for control of these asynchronous populations.

Understanding the environmental factors that influence insect dormancy and emergence phenology

has long been a goal for prediction of annual pest colonization of agricultural crops, and a critical component of effective integrated pest management programs (Tauber and Tauber 1993, Wissinger 1995, Noronha et al. 2002, Williams and Ferguson 2010). Descriptions of emergence phenology among agricultural pests have typically related accumulating temperature to insect abundance (Andrewartha 1952, Koštál 2006, Emerson et al. 2009). More recent research provides evidence to suggest other factors, such as insecticide resistance, may affect insect emergence phenology and crop colonization patterns (Carrière et al. 1995; Boivin et al. 2003, 2004).

Over the past two decades, the use of the systemic neonicotinoid insecticides has increased considerably in major and minor agricultural crops (Jeschke and Nauen 2008, Jeschke et al. 2011). Widespread adoption of these insecticides in commercial agriculture has resulted in reports of neonicotinoid-resistant insect populations in numerous taxa (Nauen and Denholm 2005; Whalon et al. 2008, 2012). Although resistance has been documented, little is known about how this widespread adoption may have had unanticipated effects on the biology and ecology of pests over time. To date, numerous studies have previously reported declining neonicotinoid concentrations over time in both annual and perennial cropping systems, often focusing on season-long population dynamics of pest species with high reproductive capacity and multiple generations per growing season (Byrne et al. 2005a,b, 2007, 2010; Castle et al. 2005). In cultivated potato, variable concentrations of systemic neonicotinoids in plant tissue after at-plant applications may be one additional factor contributing to the development of Colorado potato beetle resistance (Olson et al. 2004). To our knowledge, few studies have related emergence phenology of a specialist herbivore, such as Colorado potato beetle, to the method in which conventional insecticides are delivered. Moreover, limited information exists describing how insecticide delivery may affect insect life history in an insecticide-dependent agroecosystem.

Insecticides have been, and remain, a cornerstone for management of four key insect pests of cultivated potato in Wisconsin (WI): Colorado potato beetle; Potato leafhopper, *Empoasca fabae* (Harris); Green peach aphid, *Myzus persicae* (Sulzer); and Potato aphid, *Macrosiphium euphorbiae* (Thomas). For the past 15 yr, the majority of all commercial potato acres in WI used an at-plant systemic neonicotinoid application to manage early season Colorado potato beetle. Although measured resistance to the neonicotinoids has been well documented (Szendrei et al. 2012), WI growers continue to rely on in-furrow systemic neonicotinoids to manage potato insect pests. Advantages of this use-pattern include maximized plant coverage, longer residual within the potato foliage, and limited nontarget impacts to beneficial arthropods (Baker et al. 2007, Elbert et al. 2008). However, this uniformity and duration of insecticide use has strongly increased selection pressure over the 18 yr since the registration

of the neonicotinoid class (Olson et al. 2000, Zhao et al. 2000).

As a result of the long-term reliance on several insecticide classes, many Colorado potato beetle populations are now resistant to nearly all insecticide classes (Radcliffe et al. 1991, Alyokhin et al. 2008, Whalon et al. 2012). Furthermore, resistance development in Colorado potato beetle may be accelerated by multiple physiological mechanisms, interpopulation gene flow, and localized dispersal (Grafius 1995). Historically, management of resistant populations has resulted in more frequent applications and tank mixing of different insecticides, with considerable economic impact to growers (Grafius 1997). Effects of insecticide resistance are commonly expressed as costs of resistance within populations of insects. Baker et al. (2008) found fecundity to be one-third less in neonicotinoid (imidacloprid)-resistant Colorado potato beetle populations from Long Island, NY. In a review of *Bacillus thuringiensis*-resistant insects, Tabashnik (1994) concluded that insecticide tolerance in Colorado potato beetle may often result in reduced fecundity, increased overwintering mortality, and prolonged development times; resistance costs that when combined may have a considerable negative effect on population growth potential. In recent years, neonicotinoid resistance of Colorado potato beetle in WI has become apparent in two ways: first, resistance bioassay data reflect increasing 50% lethal dose ratio (LD_{50}) estimates over time, and second, the duration of field-level control has decreased significantly in recent years (Huseth and Groves 2011, Szendrei et al. 2012).

Furthermore, anecdotal observations by producers and pest managers further suggest a relationship between protracted emergence and resistant insects. Resistant populations of Colorado potato beetle populations may be better able to withstand the highest titers of in-plant insecticide by emerging early in the year where more susceptible populations may be emerging later to temporally avoid these high concentrations. Populations that seem to demonstrate variable emergence occur in highly intensified portions of the WI potato production agroecosystem, where systemic neonicotinoid adoption has been widespread since the registration of imidacloprid in 1995. Acceleration of resistance will occur as late-emerging portions of populations are exposed to sublethal systemic insecticide doses (Olson et al. 2004). Over time, the continual exposure of late-emerging insects to sublethal doses will generate greater resistance levels and compromise the efficacy of current and future systemic registrations. An improved understanding of how resistance may influence pest ecology, specifically emergence phenology, will provide new information to supplement our current understanding of resistance management strategies for systemic use-patterns. In this study, we examined the effects of long-term reliance on three common insecticide delivery methods (e.g., systemic, foliar, or mixed foliar–systemic application) on adult Colorado potato beetle emergence phenology, fitness, and insecticide tolerance. We hypothesized that 1)

Colorado potato beetle populations exposed to systemic neonicotinoids over several years would emerge from dormancy later and over a longer period; 2) overwintering mortality would be greater in populations experiencing systemic insecticide applications as a cost of neonicotinoid tolerance; 3) later-emerging insects would be, on average, smaller and weigh less, and as a direct result of fitness costs related to smaller body size; and 4) insects emerging later would be less fecund.

Materials and Methods

Neonicotinoid Bioassay. From 2007 to 2011, baseline imidacloprid susceptibility was measured at 41 locations throughout the seed and commercial potato production regions, near Antigo and Hancock, WI, respectively. Consecutive samples from individual fields were often difficult to obtain because of multiple-year crop rotation sequences (e.g., 2-, 3-, or 4-yr potato rotation intervals). As a result, potato fields <0.8 km in distance from each other were considered representative of a local Colorado potato beetle population for annual bioassays. Adult beetles were collected into plastic cups (0.94 liters), placed into coolers containing frozen ice packs, and transported to the University of Wisconsin (UW)–Madison campus, Madison, WI. On arrival, insects were fed greenhouse-grown potato foliage in screen cages maintained in an environmental chamber at 24°C and a photoperiod of 16:8 (L:D) h for 3 d.

Insensitivity to neonicotinoids was assessed by using topical imidacloprid bioassays. Technical-grade imidacloprid (97.5%, Bayer Corporation, Kansas City, MO) was dissolved into pesticide-grade acetone (Fisher Chemicals, Fair Lawn, NJ), and then serially diluted to a range of doses between 0.001 and 10 ppm. Between five and nine doses were chosen based on preliminary bioassays resulting in 0–100% mortality (Zhao et al. 2000, Mota–Sanchez et al. 2006). Adult beetles were divided into equal numbers per dose, each containing no fewer than 15 insects per replicate. Collected individuals were randomly selected and treated with 1 μl of insecticide solution applied to the first abdominal sternite with a 50-μl syringe equipped with a Hamilton PB-600 repeating dispenser (Hamilton Company, Reno, NV). Control insects received a 1-μl dose of pesticide-grade acetone alone. Treated insects were placed into 100 by 15-mm polystyrene petri dishes with filter paper (Fisher, Pittsburgh, PA). Insects were maintained on fresh greenhouse-grown potato foliage in an environmental chamber at 24°C and a photoperiod of 16:8 (L:D) h. Bioassay response was measured at day 3, 5, and 7 posttreatment. Insects were classified as alive, intoxicated, or dead. Intoxicated beetles were unable to grasp the tip of a pencil with all six legs and walk one body length up the pencil (Zhao et al. 2000). Insects that were incapable of coordinated movement (e.g., intoxicated) after day 7 were considered incapable of recovery (Zhao et al. 2000). Intoxicated and dead insects were pooled for subsequent statistical analyses.

Experimental Site. The site used for overwintering studies was situated in an unmanaged grassy area bordering a mixed deciduous field boundary with an easterly aspect at the UWs Hancock Agricultural Experiment Station, Hancock, WI. In 2010, the UW–Soil Testing laboratory analyzed 12 aggregate soil core samples (15 30-cm soil cores homogenized per sample) taken in an unaligned grid pattern from the study site. Soil texture was loamy sand that was, on average, composed of 83.3 ± 1.5% sand, 10.7 ± 2.4% silt, and 6 ± 1.1% clay. In 2011, the study site was shifted 50 m northward along the same field boundary to ensure no overlap of study areas between years.

Cage Study Populations. Sequential bioassay data from 2007 to 2010 was used to choose candidate field locations (and associated Colorado potato beetle populations) with known levels of neonicotinoid sensitivity. From this data set, sample locations were selected within 10 km of the Hancock Agricultural Research Station, Hancock, WI. Growers managing chosen field locations were asked to qualify their Colorado potato beetle management among three categories since first neonicotinoid registration in potato (1995–2010): 1) years of neonicotinoid use for beetle control, 2) annual method of pesticide application (e.g., systemic or foliar), and 3) calendar date of planting and first date of foliar application for Colorado potato beetle. Complete management records were available for some but not all locations for all 15 yr. Grower reports were compiled to classify three categories of early-season potato beetle management: systemic, foliar, or mixed application (i.e., systemic and foliar) strategies. Sample locations were chosen to include two systemic, one mixed, and one foliar population for each season.

In the autumn of 2009 and 2010, insects were collected from four potato fields separated by 4–10 km in the WI Central Sands potato production region. Three of four populations were represented in both study years. Adult beetles representing the second or summer generation from each sample location were collected at random in late August (2009: $n \approx 1,946$ individuals per population; 2010: $n \approx 2,416$ individuals per population). One population, Systemic-1, did not have potato within a 0.8-km radius of the field location in the fall of 2010. A fifth population, Systemic-2, was selected as a replacement for the 2010–2011 season with similar levels of measured neonicotinoid resistance. Insects were released into greenhouse screen cages and fed greenhouse-grown potato foliage (cultivar 'Russet Burbank') for 1 full week before release at the common garden location. In addition, a random sample ($n = 30$ insects) from each population was collected annually for autumn sex ratio and body size measurements.

Outdoor screen cages with open bottoms (1.8 by 1.8 by 1.8 m) were fixed in a randomized complete block design consisting of four populations in three replicated blocks (model #1406A, BioQuip Products, Rancho Dominguez, CA). Cage edges were buried 15 cm deep to limit insect entry or exit. Populations were evenly divided into groups of 750–900 beetles per

cage. Beetles were provided greenhouse-grown potato foliage (cultivar Russet Burbank) and were allowed to diapause naturally in undisturbed soil. To allow natural snow accumulation, field cages were removed after 1 full week of temperatures below 0°C.

Postdormancy Emergence. After snowmelt, cages were reinstalled and checked weekly for emerging adults. After the first recorded adult, insects were collected directly into plastic cups (0.94 liters) every other day from 1 May to 22 June 2010 (52 d) and from 17 May to 30 June 2011 (44 d). Sample cups were placed in coolers with freezer packs and driven to the UW–Madison campus. Individual insects were each weighed, sexed, and measured (total length and width). Daily collections were placed into an environmental chamber maintained at 10°C and photoperiod of 0:24 (L:D) h for 14 or fewer days after collection. At the end of the 14-d cohort sampling interval, pooled insects were moved to a greenhouse maintained at 21.3 ± 1.1°C (minimum 18.3, maximum 26.1) under natural light. Insects were placed into collapsible screen cages and blocked in an identical manner as the field study. Cages each received six potted potato plants, which were replaced as needed. Each 2-wk cohort of adults was assortively mated for a 14-d period. Although this period represents only a portion of reproductive capacity for postdormancy Colorado potato beetle, it did allow for a uniform oviposition period comparison among cohort groups and populations. Egg masses were removed from potato foliage daily. Total number of egg masses and eggs per mass were recorded for each cage.

After five consecutive observations with zero beetles collected in cages, the sampling frequency was changed to once every 7 days. Sampling concluded after four consecutive weeks of zero beetles collected. Each cage footprint then had a random 0.5 by 0.5 by 0.3 m (length by width by depth) soil sample extracted and sifted through 5.6-mm sieve-size market-grade screen (four meshes per linear inch), and all intact insects and insect parts were collected and counted.

Environmental Data. We used an accumulation of soil degree-days (SDDs) to account for between-year seasonal variation in sampling dates. Several studies have previously described overwintering phenology aligned over multiple years of postdiapause Colorado potato beetle emergence data by using accumulated simple SDDs (Lashomb et al. 1984, Tauber et al. 1994).

Simple SDDs were calculated as:

$$\text{soil degree-days} = \left[\frac{(T_{max} + T_{min})}{2} \right] - T_{base}$$

where T_{max} is the daily maximum soil temperature, T_{min} is the daily minimum soil temperature, and T_{base} is the soil temperature threshold (10°C) for Colorado potato beetles (Mailloux et al. 1988, de Kort 1990, McMaster and Wilhelm 1997, Boiteau et al. 2008). In the spring of each sample year, SDDs were calculated from soil temperatures logged at a depth of 10 cm by the UW-Extension's Automated Weather Observation Network station at the Hancock Agricultural Research

Station (UW-EX 2011). For each study year, downloaded data were used to calculate daily SDDs, beginning 1 January. In addition, by using the same basic formula, simple growing degree-days (GDDs) were calculated from 1 January for 1995–2010 by using downloaded minimum and maximum air temperatures. Cumulative SDDs and cumulative GDDs were calculated by using the *cumsum* function in R version 2.14.1 (R Development Core Team 2012). The duration of Colorado potato beetle control (e.g., first foliar application GDD minus planting GDD), at each grower location from which Colorado potato populations were collected, was estimated and used as a measure of the time until an economic threshold was reached over 15 yr at each field location. These estimates documented the erosion of neonicotinoid control producers have experienced over the interval of time since registration (e.g., 1995).

Statistical Analysis. Bioassay dose–response results were analyzed against imidacloprid concentration with a Log_{10} probit regression analysis (Finney 1971, Robertson et al. 2007; PROC PROBIT, SAS Institute). LD_{50} estimates and 95% CIs were calculated to determine relative neonicotinoid susceptibility of field-collected populations when compared with a neonicotinoid-susceptible field population collected at the UW Arlington Agricultural Experiment Station, Arlington, WI (Robertson et al. 2007). Imidacloprid tolerance was considered significantly different if 95% CIs of lethal dose ratios did not overlap (Robertson et al. 2007).

Cumulative GDDs from planting until the first nonsystemic insecticide application across 15 yr were compared with analysis of variance (ANOVA). Application records by year were considered a discrete categorical scale response and analyzed as factor-level responses, unless otherwise noted. Saturated GDD models considered all possible population by year interactions; nonsignificant parameter terms were eliminated by using sequential *F*-tests with the *drop1* function in R. Accumulated GDDs at vine kill was used as an end point in analyses for fields that received no further insecticide applications for control of Colorado potato beetle. Duration of control estimates were regressed directly with year as a continuous variable by using ANOVA. The predictor year was scaled to the first year of use of a neonicotinoid within our populations (e.g., year 1995 equals zero) to improve interpretation of regression coefficients. Parameters were selected backward from a saturated model containing all possible population by year interactions with sequential *F*-tests by using the *drop1* function in R. An ordinary least squares linear regression approach was used to compare the timing of first emergence for each population, gender, and year (Murtaugh et al. 2012). Emergence differences between genders within years were compared by using Welch two-sample *t*-tests (Murtaugh et al. 2012). Equality of proportional survivorship among populations was compared with χ^2 tests by using *prop.test* (Mead et al. 2002, Dalgaard 2008). Survivorship of insects between years was an-

alyzed with Pearson's χ^2 tests and Yates' continuity correction by using *chisq.test* in R.

To compare emergence patterns for each population and gender within each year, a nonlinear regression analysis was used to fit three-parameter logistic regression growth curves to cumulative Colorado potato beetle counts over time. The cumulative sum of male and female Colorado potato beetles captured in each cage was calculated for each sample date for all cages by using the function *cumsum* in R. The nonlinear model for the estimated cumulative Colorado potato beetle emergence (Y_{ij}) on the ith observation at SDD_{ij} after 1 January is:

$$Y_{ij} = \frac{Asym_i}{1 + \exp((Xmid_i - SDD_{ij})/Scal_i)} + \varepsilon_{ij}$$

where *Asym* is the asymptotic height of the growth curve (e.g., total beetle abundance), *Xmid* represents the inflection point of the curve (SDDs when cumulative Colorado potato beetle captures $= Asym/2$), and *Scal* is the SDDs surrounding peak emergence of a population ($\approx$25–75%). Preliminary visual examination of the data indicated differences among populations, genders, and years. Model fitting exercises revealed an overall nonlinear mixed model that failed to converge, thus individual nonlinear models were fit to each level of population, gender, and year for phenology comparisons.

Initial model fitting assumed within-group errors (ε_{ij}) were independently distributed as $N(0, \sigma^2)$. Examination of residual plots for these models indicated a serial accumulation of errors associated with cumulative Colorado potato beetle captures as SDD increased.

Within-group errors were allowed to be heteroskedastic with the variance model:

$$Var(\varepsilon_{ij}) = \sigma^2 x_{ij}^{2\delta}$$

which corresponds to the variance function $g(v_{ij}, \delta) = |v_{ij}|^\delta$. Here, δ was stratified by population and year, resulting in the variance function of $g(v_{ij}, \delta) = |v_{ij}|^{\delta s^{ij}}$ with s^{ij} allowing for year and population variation. Nonlinear mixed effects models were fit in R by using the *gnls* function (Package nlme, Pinheiro and Bates 2000), and parameter estimates and confidence intervals were extracted by using *summary*, *conf.int*, and *anova* functions. Parameter estimates were considered significantly different if 95% CIs did not overlap.

Male and female Colorado potato beetles were summated across all sample dates for each year and population by using the *tapply* function. Total counts for each population by year tested an equal sex-ratio hypothesis (e.g., one male:one female) for autumn and spring Colorado potato beetle samples. Likelihood ratio G-tests for categorical data tested the departure from the 1:1 sex-ratio hypothesis approximated on a χ^2 distribution (Sokal and Rohlf 1995, Wilson and Hardy 2002). To better understand how variability in size and weight may have influenced emergence patterns of each population, a simple linear regression modeling approach was used to associate differences in gender,

size, and SDD. Before response parameter selection, Pearson's product-moment correlation was used to determine similarities among measured individual traits: gender, weight, length, and width. Paired measures were all highly correlated, thus we chose to regress only insect weight as a response to individual size data by SDD. Emergence weight differences among populations and gender by SDD were determined by analysis of covariance. Finally, total number of females captured, total egg masses deposited over the 14-d oviposition period, and average eggs laid per female from each cohort were assessed by using simple linear regression for each population and year. Differences among populations by cohort group were determined by ANOVA. When appropriate, response variables were transformed (Log_{x+1}) to satisfy assumptions of normality. Means were separated post hoc by using Tukey's honestly significant difference tests. All linear model estimates and test statistics were extracted by using the *lm*, *aov*, *cor.test* (method "pearson"), *anova*, *TukeyHSD*, and *summary* functions in R (Venables and Ripley 2002).

Results

Neonicotinoid Bioassays and Grower Records. In recent years, potato producers statewide have noted that the number of days over which the at-plant systemic insecticides provide adequate control has steadily declined. Sequential imidacloprid bioassays for the five populations selected in this study showed a range of insensitivity from 2007 to 2011 (Table 1). Corresponding field-level control data showed a decline in duration of neonicotinoid control for the past 15 yr since initial registrations in potato (Fig. 1). From these data we were able to quantify, in a relative manner, reductions in efficacy of neonicotinoids in WI potato fields over time. On average, GDDs at planting for all locations was 43.9 ± 62.1 GDD (minimum 0; maximum 301.85). When locations were pooled, planting dates over 15 yr did not occur on significantly different GDDs in the spring ($F = 0.55$; df $= 15,44$; $P = 0.8949$). Independently, selected field locations were planted at significantly different GDDs over 15 yr ($F = 78.29$; df $= 4,55$; $P < 0.0001$). More important for growers, timing of first nonsystemic Colorado potato beetle application averaged 543.3 ± 207.7 GDD (minimum 201; maximum 1202.4), and timing of the first postplanting applications, over 15 yr, was significantly different when all locations were pooled, indicating changes in Colorado potato beetle phenology may result in annually variable colonization events ($F = 4.028$; df $= 15,44$; $P = 0.000153$). Over the same time interval, the length of residual control of the Colorado potato beetle was highly variable, occurring, on average, at 499.4 ± 200.9 GDD (minimum 194.7; maximum 1181.5) and differing significantly among years ($F = 5.894$; df $= 15,44$; $P < 0.0001$). When considered a continuous variable scaled to years of control since neonicotinoid registration (1995–2010), a significant main effect of population and year existed ($F = 8.211$; df $= 5,54$; $P < 0.0001$; Fig. 1), but a year by population interaction was not significant ($F = 1.154$; df $= 9,50$;

Table 1. Topical bioassay estimates for selected Colorado potato beetle populations

Population	Year	n	Slope ± SEM	LD_{50}	95% CI	χ^2	df	R-ratio[a]	95% CI
Systemic-1	2007	74	2.57 (0.81)	0.068	(0.021–0.099)	15.97	3	1.8	(1.05–3.21)
	2008	74	1.43 (1.1)	0.083	—	10.99	3	2.2	(0.52–9.43)
	2009	48	1.27 (0.65)	0.094	(0–0.19)	6.24	3	2.5	(0.96–6.69)
	2010	135	0.77 (0.38)	0.15	(0.076–1.06)	3.04	3	3.9	(1.48–10.41)
	2011	425	1.63 (0.14)	0.72	(0.59–0.87)	5.63	4	26	(24.55–26.5)
Systemic-2	2007	75	1.64 (0.54)	0.21	(0.046–0.34)	10.7	3	5.7	(2.84–11.46)
	2008	75	2.46 (0.8)	0.24	(0.049–0.37)	4.076	3	6.4	(3.26–12.73)
	2009	75	4.21 (1.5)	0.8	—	9.68	3	21.6	(14.66–31.92)
	2010	150	0.58 (0.17)	2.94	(1.21–28.8)	3.22	8	79.2	(23.87–262.57)
	2011	524	1.9 (0.27)	0.62	(0.41–0.94)	17.47	5	22.2	(21.01–23.43)
Systemic-3	2007	75	1.69 (0.51)	0.12	(0.05–0.18)	14.09	3	3.2	(1.92–5.37)
	2008	76	4.35 (1.1)	0.33	(0.21–0.41)	5.09	3	8.8	(2.15–35.78)
	2009	75	1.01 (0.53)	0.15	—	2.37	3	3.9	(1.93–8.29)
	2010	135	0.51 (0.17)	0.24	(0.058–0.76)	6.8	7	6.5	(2.32–18.06)
	2011	500	2.03 (0.26)	0.73	(0.51–1.04)	14.15	4	25.5	(24.55–27.98)
Mixed	2007	75	1.43 (0.58)	0.013	(0.0000204–0.03)	8.8	3	0.3	(0.08–1.52)
	2008	75	1.25 (0.43)	0.26	(0.15–0.7)	5.19	3	7.1	(3.85–12.95)
	2009	75	0.72 (0.93)	0.024	—	8.8	3	0.7	(0.02–17.73)
	2010	145	3.85 (0.65)	0.43	(0.34–0.51)	2.12	3	11.6	(8.92–15.2)
	2011	525	1.47 (0.11)	0.48	(0.4–0.6)	6.2	5	17.2	(16.8–17.66)
Foliar	2007	74	1.38 (1.46)	0.024	—	9.86	5	0.7	(0–479)
	2008	—	—	—	—	—	—	—	—
	2009	75	1.83 (0.15)	0.15	(0.1–0.48)	0.85	3	3.9	(2.22–6.96)
	2010	135	1.25 (0.22)	0.081	(0.05–0.12)	11.77	7	2.2	(1.32–3.56)
	2011	524	1.81 (0.25)	0.39	(0.27–0.58)	15.78	5	14	(13.5–14.62)
WI-susceptible[b]	2007	75	6.93 (1.69)	0.03	(0.025–0.036)	8.26	3	—	—
	2008	75	1.43 (0.39)	0.05	(0.025–0.31)	3.85	5	—	—
	2009	75	3.29 (1.36)	0.014	(0.0006–0.021)	3.68	3	—	—
	2010	135	4.38 (0.75)	0.037	(0.03–0.045)	3.34	7	—	—
	2011	600	3.13 (0.33)	0.027	(0.028–0.034)	14.11	6	—	—

[a] R-ratio: resistance ratio comparing LD_{50} of populations with response of WI-susceptible pop annually.
[b] Reference pop collected at the University of Wisconsin–Arlington Agricultural Research Station, Arlington, WI.

$P = 0.342$). On average, populations lost 34.88 GDDs of neonicotinoid control per year since 1995. When Julian days (e.g., calendar days after 1 January) were regressed against duration of control, populations lost an average of 3.32 d per year since 1995 or ≈50 d of control since neonicotinoid registration ($F = 15.28$; df = 5,54; $P < 0.0001$).

Emergence and Survival. First detections of post-dormancy adult Colorado potato beetles emerging from within replicate cages differed significantly between years, occurring, on average, at 74.5 and 37.9 SDDs on 1 May 2010 and 17 May 2011, respectively ($t = 9.706$; df = 32.01; $P < 0.0001$). Timing of first emergence of individual populations did not differ significantly in 2010 ($F = 0.46$; df = 3,20; $P = 0.7114$), whereas significant differences among populations were observed in 2011 ($F = 5.441$; df = 3,20; $P = 0.006692$). When Julian date was used as a time scale, emergence patterns differed greatly. A 16-d difference was observed in the average first emergence date between years, with first emergence date in 2011 observed to be 37.63 SDDs less than 2010. Overall, differences existed among years at all major points in cumulative emergence: a 14-d difference (24.55 SDDs cooler) at 25% emergence, an 11-d difference (28.25 SDDs) at 50%, a 10-d difference (40.45 SDDs) at 75%, and a 10-d difference (73.3 SDDs) at 100% emergence. Although annual differences in emergence were apparent, little variation in rate of emergence was observed among populations within the same year (Fig. 2). Furthermore, no significant gender differences in timing of first emergence occurred in either 2010 or 2011.
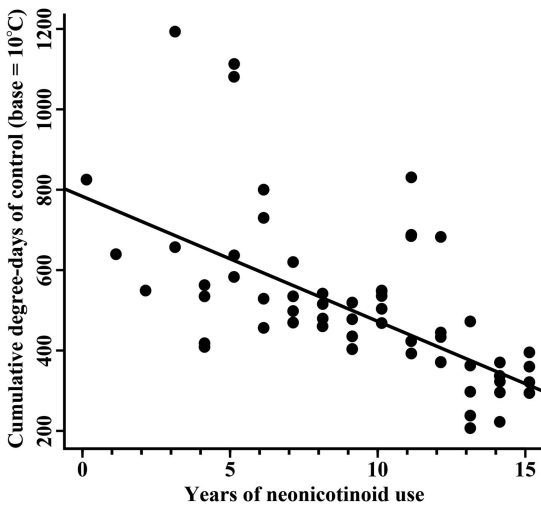

Fig. 1. Duration of Colorado potato beetle control with neonicotinoid insecticides over 15 yr of use (1995–2010) at five selected field locations in the Central Sands potato production region of Wisconsin. Duration of control is a relative measure calculated as: GDD of first Colorado potato beetle management minus GDD at planting.
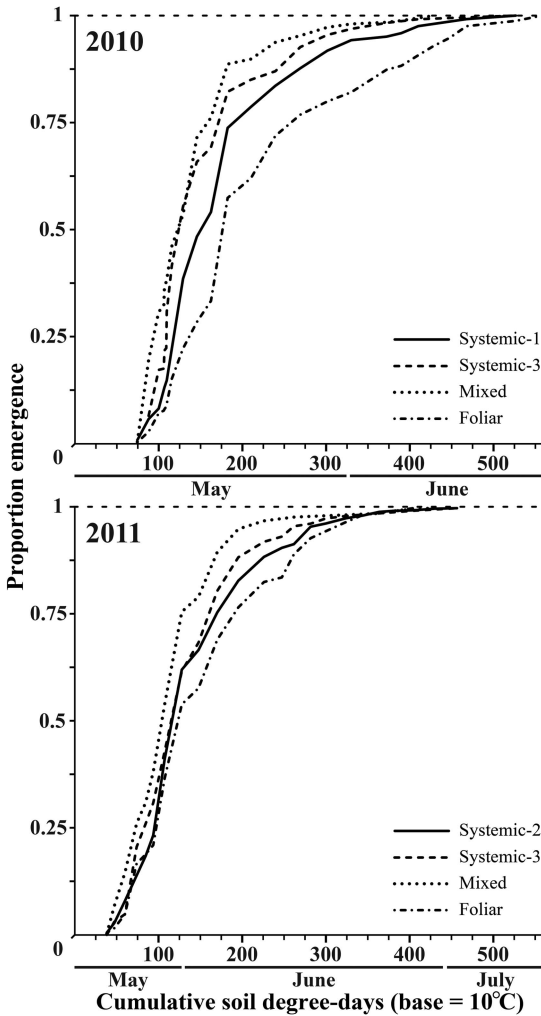
Fig. 2. Proportion emergence of adult Colorado potato beetle populations plotted against cumulative soil degree-days (base 10°C after 1 January).

Fig. 3. Percent survivorship for adult Colorado potato beetle populations released into the common garden experiment at the Hancock Agricultural Research Station, Hancock, WI, during 2010 and 2011.

Overwintering survivorship of adult Colorado potato beetle within the soil under cages was estimated to be $13.8 \pm 6.7\%$ (minimum 5.4%; maximum 23.5%) and $17.8 \pm 11.2\%$ (minimum 5.7%; maximum 34.7%) in 2010 and 2011, respectively, and these estimates differed significantly between years ($\chi^2 = 62.31$; df = 1; $P < 0.0001$). When compared only within sample year, survivorship was significantly different among populations in both 2010 ($\chi^2 = 173.69$; df = 3; $P < 0.0001$) and 2011 ($\chi^2 = 727.94$; df = 3; $P < 0.0001$) (Fig. 3). When comparing within population across the two years of the study, survivorship was significantly different in all populations: Systemic-3 ($\chi^2 = 98.62$; df = 1; $P < 0.0001$) and Mixed ($\chi^2 = 86.48$; df = 1; $P < 0.0001$) and Foliar ($\chi^2 = 107.78$; df = 1; $P < 0.0001$). Between-year differences were not calculated for Systemic-1 or Systemic-2 because of singular annual observations.
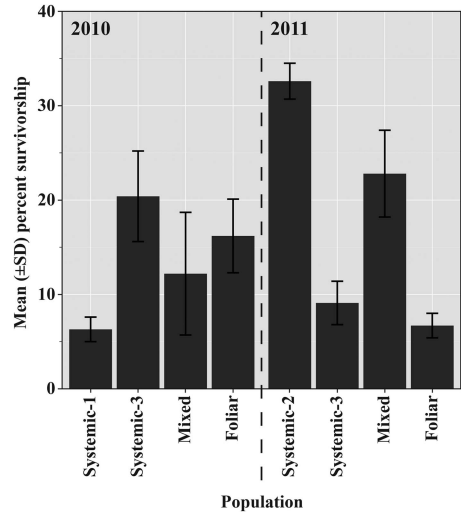
Logistic growth models were developed to describe emergence phenology of Colorado potato beetle populations with differing insecticide management histories in 2010 and 2011 (Fig. 4). Emergence of male and female insects was modeled individually for each population, and the average pseudo-R^2 value for all models was 0.76 (minimum 0.35; maximum 0.96). Model comparisons among populations and genders suggest peak emergence (*Xmid*) occurred at similar accumulated SDD in both years (Fig. 5B). In 2010, peak emergence for all populations occurred between 87.35–186.96 accumulated SDDs (6 May–23 May) and 85.16–139.11 SDDs (25 May–2 June) in 2011. Estimated SDDs for peak emergence were not different among subsets of male and female Colorado potato beetles in any population (Table 2; Fig. 4). Cumulative beetle abundance (*Asym*) estimates did, however, differ greatly among genders and populations (Table 2; Fig. 5A). Overall, survivorship was similar among populations and genders in 2010, whereas differences in survivorship were apparent in 2011 (Fig. 3). Sifted soil samples taken at the conclusion of each season from under cages produced no live or dead intact beetles.

Insect Size and Weight. The relationship among measured body size metrics demonstrated consistent patterns for each population and gender group. Correlation of width to length for all measured insects was highly significant ($r = 0.8523$; $t = 86.118$; df = 2793; $P < 0.0001$). Furthermore, insect weight was positively correlated to both length ($r = 0.8009$; $t = 70.698$; df = 2793; $P < 0.0001$) and width ($r = 0.8012$; $t = 70.78$; df = 2793; $P < 0.0001$). As a result of strong correlations among body characteristics, we chose to model only insect weight with predictors for gender, population, year, and SDD. The final analysis of covariance model examining these variables together described 33.13%
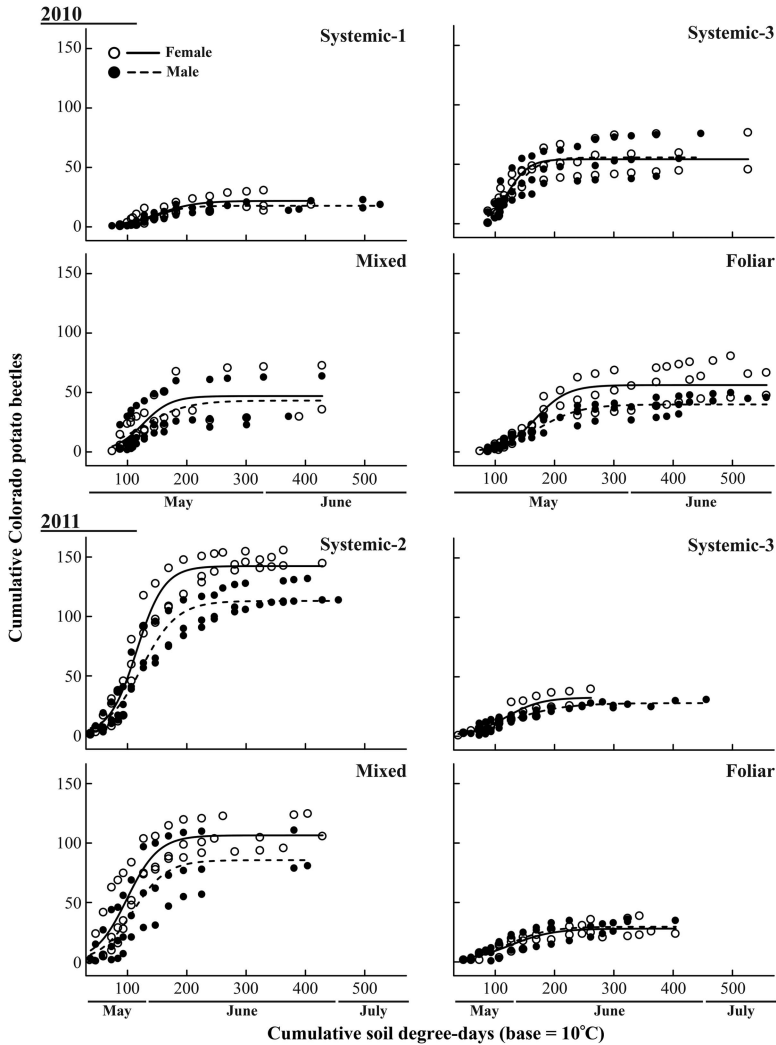
Fig. 4. Cumulative emergence of five Colorado potato beetle populations plotted against cumulative soil degree-days (base 10°C after 1 January). Results of simple logistic nonlinear least-squares regression analysis are shown as best-fit lines for each population and gender.

of the total variation in Colorado potato beetle weight (Table 3).

Gender Ratios and Fecundity. Colorado potato beetle gender ratios from autumn-collected populations did not differ significantly among populations (e.g., all one male:one female) in either 2009 ($\chi^2 = 2.5007$; df = 3; $P = 0.4752$) or 2010 ($\chi^2 = 0.6421$; df = 3; $P = 0.8867$) before release into cages. In the spring, populations again did not differ significantly in their observed gender ratios in either 2010 ($\chi^2 = 5.8801$; df = 3; $P = 0.1176$) or 2011 ($\chi^2 = 6.2654$; df = 3; $P = 0.09939$). When testing the distribution of genders within each population, only the population collected from the foliar-based insecticide program significantly departed from a 1:1 gender ratio in 2010 and was female-biased 1:1.57 (Table 4). In 2011, populations from the fields designated as Systemic-2 (1:1.25 male:female)

and Mixed (1:1.31 male:female) were again significantly female-biased (Table 4).

Total number of females, eggs, and egg masses all differed significantly among populations and populations within year (Table 5). In 2010, total eggs per female averaged 38.5 ± 17.3 (minimum 14.2; maximum 71.1) and 21.3 ± 26.2 (minimum 0.4; maximum 95.1). Eggs per female were not, however, significantly different between years ($t = 1.9024$; df = 19.1; $P = 0.07231$). Egg counts per female varied significantly as a result of a population by year interaction (Table 5). Average female fecundity differed significantly among sample cohort groups within populations for both 2010 and 2011 (Table 6). Only fecundity of the foliar population in 2011 was not significantly different among cohort groups (Table 6). Eggs per mass did not vary between years ($t = -0.3272$; df = 956.42; $P = 0.7436$),
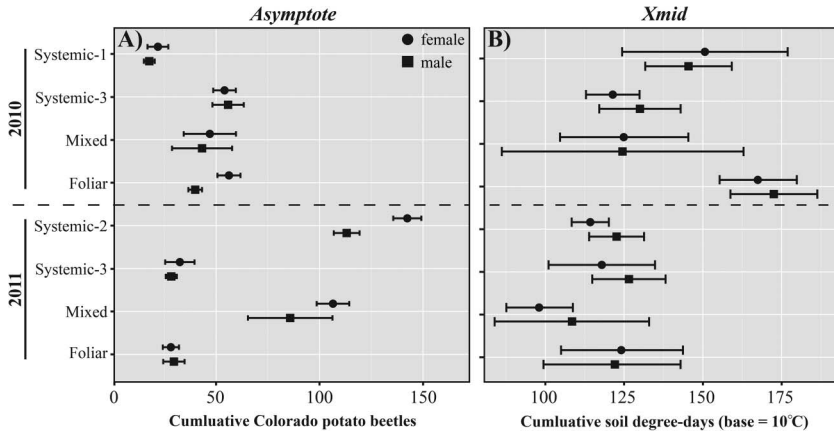
Fig. 5. Estimates (means ± 95% CI) for logistic curve models fitted to cumulative Colorado potato beetle counts for males (squares) and females (circles) for the spring of 2010 and 2011. *Asymptote* (A) represents an estimate of overall survivorship for each gender and populations. *Xmid* (B) represents estimated cumulative soil degree-days of peak emergence for each gender and population.

nor did they differ within population or among populations by year (Table 5). The average number of egg masses per female was not significantly different ($t = 1.9172$; 18.214; $P = 0.07103$) and averaged $1.9 ± 0.8$ (minimum 0.9; maximum 3.5) in 2010 and $1.1 ± 1.3$ (minimum 0.01; maximum 4.7) in 2011. However, egg masses per female were significantly different among populations and populations by year (Table 6).

Discussion

In this study, our common garden and greenhouse studies explored the ecology of Colorado beetle dormancy, documented this insect's seasonal phenology in WI, and related emergence patterns to long-term insecticide management history. Selected populations for our common garden study showed little relationship between management history and the potential for a multiple-year dormancy. Initially, low overall Colorado potato beetle survivorship observed among populations seemed to indicate the potential for multiple-season dormancy in WI. Soil extraction sampling, used to determine if any live insects attempted to remain in dormancy over multiple seasons, produced no live beetles, however. This finding departs from results obtained in the Northeastern and Western United States, where small proportions of

Table 2. Logistic regression parameter estimates (means ± 95% CI) fitted to cumulative postdormancy captures of Colorado potato beetles over the 2010 and 2011 growing seasons

Population	Gender[a]	Asymptote[b]		Xmid[c]		Scal[d]		AIC	R^{2e}
2010									
Systemic-1	Male	17.7a	(15.1–20.4)	146.3c	(132.6–159.9)	24.7ab	(17.0–32.4)	150.8	0.83
	Female	21.9ab	(16.8–26.9)	151.6bc	(125.5–177.8)	32.2ab	(15.1–49.3)	189.8	0.68
Systemic-3	Male	55.7e	(48.2–63.3)	131.0bc	(118.2–143.8)	21.2ab	(11.5–31.0)	331.1	0.72
	Female	54.3e	(48.8–59.7)	122.5b	(114.1–131.0)	17.7a	(10.7–24.8)	314.9	0.75
Mixed	Male	43.2bcde	(28.7–57.7)	125.5abc	(87.4–163.6)	36.8ab	(−.4–79.0)	297.9	0.35
	Female	47.0cde	(34.4–59.6)	126.1abc	(105.8–146.3)	23.6ab	(9.9–37.3)	275.9	0.57
Foliar	Male	39.2d	(36.6–43.3)	173.3c	(159.6–187.0)	35.7b	(27.5–43.9)	297.6	0.88
	Female	56.2e	(50.7–61.8)	168.3c	(156.1–180.5)	26.7ab	(21.2–32.3)	368.3	0.79
2011									
Systemic-2	Male	113.1g	(106.8–119.3)	123.6b	(115.0–132.3)	29.9ab	(23.6–36.2)	427.8	0.92
	Female	142.4h	(135.6–149.2)	115.5ab	(109.6–121.3)	23.3a	(19.4–27.1)	390.8	0.96
Systemic-3	Male	27.9b	(26.2–29.5)	127.5bc	(116.0–139.1)	45.0b	(32.6–57.3)	181.3	0.89
	Female	32.5bcd	(25.4–39.6)	119.1abc	(102.3–135.9)	29.4ab	(22.7–36.1)	155.9	0.82
Mixed	Male	85.6f	(65.2–106.0)	109.5abc	(85.2–133.9)	28.2ab	(11.8–44.7)	317.2	0.65
	Female	106.5fg	(98.6–114.3)	99.3a	(88.8–109.8)	26.6ab	(16.0–37.3)	359.6	0.83
Foliar	Male	29.6bc	(24.4–34.8)	122.2abc	(100.6–143.8)	33.9ab	(19.3–48.5)	220.3	0.73
	Female	28.0b	(24.1–31.9)	125.4abc	(106.1–144.6)	36.0ab	(21.9–50.1)	196.3	0.77

Parameter estimates followed by the same letter indicate overlapping 95% CIs.
[a] Simple logistic curve model: cumulative Colorado potato beetles = $Asym/(1 + \exp((Xmid - SDD)/Scal))$; where SDD is the cumulative soil degree-days (base 10°C) after 1 January when the cage was sampled.
[b] *Asymptote* of the curve (e.g. max Colorado potato beetles caught).
[c] Inflection point of the curve ($Asym/2$).
[d] Soil degree-days surrounding peak emergence of a pop (≈25–75%).
[e] Goodness of fit = model sums of squares/(model sums of squares + residual sums of squares), as a pseudo-R^2 in nonlinear modeling.

Table 3. Estimates for simple linear regression analysis evaluating the effect of cumulative soil degree-days on postdiapause Colorado potato beetle wt (g)

Parameter	Estimate[a]
(Intercept) Systemic-1	0.147‡ (0.00206)
Systemic-2	0.0000941 (0.00209)
Systemic-3	−0.00245 (0.00195)
Mixed	−0.000623 (0.00198)
Foliar	0.0091‡ (0.00197)
SDD	−0.0000976‡ (0.00000631)
Gender$_{male}$	−0.027‡ (0.00155)
Year$_{2011}$	−0.00382‡ (0.000965)
SDD by gender$_{male}$	0.0000283† (0.00000895)
ANCOVA evaluation	
R^2	0.3313
F-statistic	172.5
df	8, 2786
P value	<0.0001

Statistical analysis was performed following a $\mathrm{Log}_{(x+1)}$ transformation of the data.

Estimates are presented on the transformed scale.

[a] Parameter estimate differs significantly from zero (†$P < 0.01$; ‡$P < 0.001$).

insects (<7%) were found to diapause over multiple growing seasons (Biever 1990, Tauber and Tauber 2002). In those studies, screen cages were replaced annually to determine the proportion of individuals remaining in diapause for one or more years. Here, we sifted soil subsamples from beneath cages to determine presence or absence of Colorado potato beetles attempting to extend diapause. Although no multiple-year diapause patterns were observed for these Colorado potato beetle populations, we did observe variable emergence and survivorship patterns within sample years.

The spring of 2011 was, comparatively, a much cooler and wetter spring than 2010, resulting in a slower accumulation of SDDs. When sample years were standardized by Julian date, insects emerged later in 2011, yet fewer SDDs had accumulated than in

Table 4. G-tests for goodness of fit based on even proportions of male and female Colorado potato beetles (1:1) for spring beetle collections

Year	Population			
	Systemic-1	Systemic-3	Mixed	Foliar
2010				
Σ males	58	171	117	128
Σ females	64	183	139	196
Total	122	354	256	324
df	1	1	1	1
G	0.295	0.407	1.893	14.378
P value	0.5869	0.5236	0.1689	0.0001
2011				
Σ males	360	83	249	97
Σ females	451	83	327	89
Total	811	166	576	186
df	1	1	1	1
G	10.232	0	10.595	0.344
P value	0.00138	1	0.001134	0.55742

Goodness of fit G-tests follow a distribution of $G = 2 \ln L$ approximated by the χ^2 distribution. P values < 0.05 indicate significant departure from assumed 1 male:1 female sex ratio.

2010 (Fig. 2). Although calendar date of first emergence did not align precisely between sample seasons, cumulative SDD values did approximately align with other dormancy studies conducted in similar climatic regions (Lashomb et al. 1984, Mailloux et al. 1988, Tauber et al. 1994). When cumulative SDDs were aligned to account for seasonal variability, the rate of emergence from dormancy was comparable between 2010 and 2011 (Fig. 2). Selected populations in this study tended to achieve the first 50% of total emergence over a much shorter SDD interval than the remaining 50% of observations. Nonlinear modeling estimates showed that 2010 had greater variability in peak emergence with CIs ranging over 72.2 SDDs (19 d) when compared with an estimated 59.45 SDDs (8 d) in 2011. In this study, total Julian days over which peak emergence occurred varied considerably between years, but SDD estimates based on thermal accumulation generally aligned with the expected emergence phenology previously described in the Great Lakes region (Mailloux et al. 1988, Tauber et al. 1994). Furthermore, differences in peak emergence time between genders within population were not significantly different (Fig. 5B). Analyses of peak emergence suggest little evidence for within-season prolonged dormancy patterns.

Although there were no distinct relationships among the three management strategies and emergence phenology, Colorado potato beetle survivorship was different among populations, years, and genders. In 2010, survivorship was similar among populations, with the exception of the Systemic-1, where an overall survivorship of just 6.25% (Fig. 3) was observed. In contrast, survivorship in 2011 was more highly variable, demonstrating both population and gender-level differences (Figs. 4 and 5A). The two systemically managed populations in 2011 survived at differing rates, and the single systemic population, Systemic-2, emerged at a much faster rate and possessed the greatest overall survivorship among all populations tested. Furthermore, bioassays of this population revealed the highest estimated LD_{50} values of any population tested in our statewide survey (Table 1). Although fitness costs related to neonicotinoid tolerance may be one possible explanation for observed levels of mortality in this study, our highly resistant population demonstrated little indication of resistance-related mortality. Although management history did not uniformly explain survivorship differences in either year of the study, average survivorship was similar to a previous study of Colorado potato beetle dormancy conducted at the Hancock Agricultural Research Station, Hancock, WI (Milner et al. 1992). Survivorship estimates described by Milner et al. (1992) were considerably lower than those observed in other temperate potato-growing regions of the United States (Lashomb et al. 1984, Mailloux et al. 1988, Biever 1990, Tauber et al. 1994, Tauber and Tauber 2002). Colorado potato beetle survivorship in these studies exceeded 50% in many instances, far exceeding that of our WI common garden experiments. In some cases, high Colorado potato beetle mortality during the dormant pe-

Table 5.   Average fecundity (means ± SD) per Colorado potato beetle female

Population	Σ females	Σ eggs	Σ egg masses	Eggs per mass	Masses per female	Eggs per female
2010						
Systemic-1	64	2957	145	20.3 ± 0.2	2.3 ± 0.6	47.6 ± 12.5
Systemic-3	183	5594	266	21 ± 3.7	1.5 ± 0.2	30.9 ± 9.5
Mixed	139	6933	328	21.1 ± 0.8	2.6 ± 0.9	54.8 ± 17.3
Foliar	198	4302	216	19.2 ± 3.5	1.1 ± 0.1	20.9 ± 6
2011						
Systemic-2	451	718	33	23.2 ± 9.3	0.1 ± 0.1	1.6 ± 2.1
Systemic-3	83	3931	188	21.3 ± 1.2	2.6 ± 1.9	53.5 ± 36.8
Mixed	327	2871	136	21.3 ± 2.4	0.4 ± 0.2	8.5 ± 4.2
Foliar	89	1940	95	20.4 ± 1.5	1.1 ± 0.1	21.7 ± 2.9
ANOVA results						
Population[a]						
R^2	0.598	0.598	0.595	0.056	0.528	0.698
F-value	7.056	7.078	6.975	0.282	5.318	10.96
P value	0.00117	0.00115	0.00124	0.886	0.00479	0.0000886
Population by year[b]						
R^2	0.873	0.714	0.731	0.0745	0.83	0.884
F-value	15.75	5.702	6.212	0.184	11.16	17.36
P value	0.00000437	0.00192	0.00123	0.985	0.00000422	0.0000027

Summary results are reported as original means and SDs; statistical analysis was performed following $Log_{(x+1)}$ transformation of the data.
[a] *P* values calculated using df = 4, 19.
[b] *P* values calculated using df = 7, 16.

riod has been related to soil temperatures below 0°C for several consecutive days. Kung et al. (1992) documented that sustained temperatures below −6°C were required for significant winter-kill of Colorado potato beetles in dormancy and further noted the strong temperature buffering capacity of snow cover, which may considerably dampen extreme fluctuations in ambient air temperature. In this study, winter conditions at the common garden location did not approach the estimated killing threshold for Colorado potato beetle. At the common garden site in the winter of 2009–2010, there were 80 total days where the minimum soil temperature (5 cm) was below zero; however, none of these temperatures fell below the −6°C threshold (−0.86 ± 0.82; minimum −4.18; maximum −0.02) reported previously. By comparison, in the 2010–2011 winter period, there were 130 total days with minimum soil temperatures below zero, none of which fell below the −6°C threshold (−0.77 ± 0.68; minimum −3.42; maximum 0) (UW–EX 2011). Similarities between results of this and previous overwin-

tering studies in WI suggest diapause mortality may potentially be a more significant factor affecting populations than in other potato production regions.

Differences in size and weight of emerged Colorado potato beetles collected from cages in this overwintering study indicated some potential costs of resistance as a function of management history. Results suggest that the neonicotinoid-susceptible foliar-managed populations were, on average, heavier over time than populations exposed to longer-term systemic insecticide management programs (Table 3). Body size has been closely related to mating success and fecundity in several Chrysomelid species; such results further indicate that smaller insects of either gender tended to be less fit (Boetel and Fuller 1997; Baker et al. 2007; Alyokhin et al. 2008; French and Hammack 2010, 2011). Connecting declining insect body size and weight with fecundity was a critical component of this study, developing an emerging picture of risk periods for egg deposition within the growing season. Average fecundity of populations in our study was

Table 6.   Average (means ± SD) Colorado potato beetle fecundity per female by cohort group

Population	Cohort group					Evaluation		
	1	2	3	4	5	df	*F*	*P*
2010								
Systemic-1	310.3a ± 128.8 (6)[a]	39.1ab ± 26.8 (25)	16.5b ± 15.2 (32)	—	—	2,6	6.954	0.0274
Systemic-3	67.4a ± 33.9 (34)	27ab ± 12.6 (86)	8.4b ± 6.5 (57)	0b ± 0 (4)	198.7a ± 95 (3)	4,10	52.21	<0.0001
Mixed	122.5a ± 66.4 (39)	47ab ± 6.1 (56)	15.3b ± 24.6 (41)	0b ± 0 (3)	—	3,8	13.52	0.00169
Foliar	176.9a ± 118.3 (14)	52.8a ± 25.1 (39)	1.2b ± 1.3 (109)	7b ± 6.6 (26)	0b ± 0 (10)	4,10	19.18	0.00011
2011								
Systemic-2	0b ± 0 (187)	1b ± 1.3 (234)	13.9a ± 10.2 (30)	—	—	2,6	13.34	0.00619
Systemic-3	16.4b ± 7.6 (38)	117.2ab ± 161.1 (45)	137.5a ± 22.6 (7)	—	—	2,6	3.77	0.0869
Mixed	0.6b ± 0.7 (184)	15.3ab ± 9.2 (135)	101.8a ± 101.4 (8)	—	—	2,6	21.83	0.00176
Foliar	7.9 ± 6.9 (33)	41.9 ± 27.7 (47)	48.8 ± 72 (9)	—	—	2,6	1.57	0.284

Means within a row followed by the same letter are not significantly different (*P* < 0.05; Tukey's HSD). Data are reported as original means and SDs; statistical analysis was performed following $Log_{(x+1)}$ transformation of the data.
[a] Number of females collected per cohort group.

significantly greater in earlier cohort groups; this pattern corresponded with greater weight of insects in 2010; however in 2011, cohorts of two populations (Systemic-3 and Mixed) were more fecund as time progressed (Tables 3 and 6). When cohorts were pooled, eggs per female did not present a clear trend with respect to management or resistance estimates (Table 6). Interestingly, the population with the greatest survivorship, Systemic-2, produced the fewest eggs per female and also possessed the greatest estimates of neonicotinoid insensitivity of all populations (Tables 1 and 5). In Colorado potato beetle, connections between insecticide insensitivity and estimated fecundity have been well documented for several other insecticide modes of action classes (Argentine et al. 1989, Trisyono and Whalon 1997, Alyokhin and Ferro 1999). Moreover, Baker et al. (2007) found imidacloprid-resistant Colorado potato beetle populations had fewer fitness costs (fecundity, fertility, and larval development rate) when populations were resampled after 5 yr. Such reductions in fitness costs associated with resistant populations may partially explain our inability to infer any direct relationship between insecticide tolerance and long-term management strategies. This stabilization, or reduction in measurable fitness costs within individual populations over time, may be one possible explanation for observed fecundity differences among populations over time in this study.

Quantitative thermal accumulation models are useful tools to describe temperature-dependent arthropod life cycles in seasonally variable environments across different populations, locations, spatial scales, and times (Pruess 1983, Tauber et al. 1994, Murtaugh et al. 2012). Several studies of potato agroecosystems have previously reported on the spring emergence patterns of Colorado potato beetle in relation to accumulating spring temperatures as a potential method of predicting patterns of colonization (Lashomb et al. 1984; Tauber et al. 1986, 1994; Mailloux et al. 1988). In some field-collected Colorado potato beetle populations, adults have demonstrated considerable variation in the duration of dormancy, with some individual insects extending diapause over multiple years (Biever 1990, Tauber and Tauber 2002). Over 10 consecutive growing seasons, Tauber and Tauber (2002) characterized prolonged dormancy of adult Colorado potato beetles within several populations collected from cultivated potato in New York. The authors concluded that small portions of most beetle populations remain in dormancy for one or more years as an evolutionary bet-hedging response that improves persistence of beetles in environments with unpredictable host-plant resources. Flexible insect herbivore life histories containing traits such as prolonged dormancy are critical adaptions to variable host-plant resources or quality (e.g., plant defense compounds or agricultural toxins) in unstable annual agroecosystems (Hare 1983, Wissinger 1995, Xu and Long 1997, Hoy et al. 1998, Kennedy and Storer 2000). Here, we considered insecticide management to be a contributing factor to overall host-plant quality of cultivated potato that, in turn, may have had direct effects on Colorado potato beetle life history, specifically the duration of dormancy. To our knowledge, no previous study on this species has attempted to directly relate pest management history to selection for modified dormancy or emergence intervals, either within or between seasons. Although long-term systemic neonicotinoid exposure explained only a limited portion of variation in emergence phenology, we feel that this body of work demonstrating a limited effect of long-term management furthers our current understanding of implications for insecticide management decisions on Colorado potato beetle biology and ecology.

In other agricultural pest species, insecticide resistance traits have been closely related to variation in population-level life histories, specifically trade-offs between dormancy and fitness costs (e.g., diapause behavior, weight, development time) in obliquebanded leafroller, *Choristoneura rosaceana* (Harris), and codling moth, *Cydia pomonella* (L.) (Carrière and Roff 1995; Carrière et al. 1994, 1995; Boivin et al. 2003, 2004). In the Upper Midwest, substantial diapause plasticity of variant, western corn rootworm, *Diabroctica virgifera vigifera* (LeConte), has been widely documented and attributed to insect pest management strategies in simplified agricultural landscapes (Levine et al. 1992, 2002; Gray et al. 2009 and references therein). Variable population-scale life histories in western corn rootworm have been linked to both behavioral and possibly genetic resistance mechanisms to maize–soy crop rotation strategies, presenting considerable challenges to growers attempting to manage this significant economic pest of maize with cultural controls (Onstad et al. 2003, Knolhoff et al. 2006). Furthermore, many rotation-resistant rootworm populations are increasingly insensitive to transgenic insecticidal traits in maize, generating considerable concern as to the durability of this valuable technology (Onstad and Meinke 2010). A growing body of literature surrounding western corn rootworm, Colorado potato beetle, and several other significant arthropod pests emphasize the importance of placing insecticide resistance into an ecologically based, management framework supported by a union of information, not only limited to bioassay data but also contemporary findings about pest life cycles, agronomic practices, and structure of agroecosystems (Gould 1983, Onstad et al. 2003, Gray et al. 2009, Onstad and Meinke 2010, Carrière et al. 2012).

In conclusion, reduction of neonicotinoid control has been observed in several localized populations within the potato production agroecosystems for the past 5 yr in WI (Huseth and Groves 2011). Furthermore, widespread concern about reduced neonicotinoid efficacy within the grower community suggests that effective and economical management of Colorado potato beetle, as a result of resistance, may be more affected than simple bioassay estimates indicate. In comparison with other regions of the United States, current levels of neonicotinoid tolerance in WI are not exceptionally high (Szendrei et al. 2012), yet trending LD_{50} estimates coupled with grower observations of reduced field-level control indicate significant erosion

in efficacy of this widely used, chemical mode of action class. Several studies have documented the emergence phenology patterns and fitness costs associated with developing resistance in the Colorado potato beetle. To our knowledge, no studies have attempted to combine the effect of long-term insecticide management history with resistance. We hypothesized that survivorship and abundance of selected populations would be directly related to insecticide delivery decisions over the past 15 yr. We saw no clear evidence for these relationships between beetle phenology and insecticide delivery or neonicotinoid resistance. Differences in body size and fecundity were apparent, but did not uniformly correspond to one specific insect management method. From a pest management perspective, long-term reliance on systemic insecticide application methods does not serve as a strong predictor of variable phenology within insects colonizing cultivated potato in WI.

Acknowledgments

References Cited

Alyokhin, A. V., and D. N. Ferro. 1999. Relative fitness of Colorado potato beetle (Coleoptera: Chrysomelidae) resistant and susceptible to *Bacillus thuringiensis* Cry3A toxin. J. Econ. Entomol. 92: 510–515.

Alyokhin, A., M. Baker, D. Mota–Sanchez, G. Dively, and E. Grafius. 2008. Colorado potato beetle resistance to insecticides. Am. J. Pot. Res. 85: 395–413.

Andrewartha, H. G. 1952. Diapause in relation to the ecology of insects. Biol. Rev. 27: 50–107.

Argentine, J. A., J. M. Clark, and D. N. Ferro. 1989. Relative fitness of insecticide-resistant Colorado potato beetle strains (Coleoptera: Chrysomelidae). Environ. Entomol. 18: 705–710.

Baker, M. B., A. Alyokhin, A. H. Porter, D. N. Ferro, S. R. Dastur, and N. Galal. 2007. Persistence and inheritance of costs of resistance to imidacloprid in Colorado potato beetle. J. Econ. Entomol. 100: 1871–1879.

Baker, M. B., S. R. Dastur, B. D. Jaffe, and T. Wong. 2008. Mating competition in Colorado potato beetles (Coleoptera: Chrysomelidae) does not show a cost of insecticide resistance. Ann. Entomol. Soc. Am. 101: 371–377.

Biever, D. 1990. Prolonged dormancy in a Pacific Northwest population of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae). Can. Entomol. 122: 175–177.

Boiteau, G. 2001. Recruitment by flight and walking in a one-generation Colorado potato beetle (Coleoptera: Chrysomelidae) environment. Environ. Entomol. 30: 306–317.

Boiteau, G., J. D. Picka, and J. Watmough. 2008. Potato field colonization by low-density populations of Colorado potato beetle as a function of crop rotation distance. J. Econ. Entomol. 101: 1575–1583.

Boetel, M. A., and B. W. Fuller. 1997. Seasonal emergence-time effects on adult longevity, fecundity, and egg viability of northern and western corn rootworm (Coleoptera: Chrysomelidae). Environ. Entomol. 26: 1208–1212.

Boivin, T., J.-C. Bouvier, D. Beslay, and B. Sauphanor. 2003. Phenological segregation of insecticide-resistant genotypes in the codling moth *Cydia pomonella* (Lepidoptera: Tortricidae): a case study of ecological divergences associated with adaptive changes in populations. Genet Res. 81: 169–177.

Boivin, T., J. Chadœuf, J.-C. Bouvier, D. Beslay, and B. Sauphanor. 2004. Modeling the interactions between phenology and insecticide resistance genes in the codling moth *Cydia pomonella.* Pest Manage. Sci. 61: 53–67.

Byrne, F. J., S. J. Castle, J. L. Bi, and N. C. Toscano. 2005a. Application of competitive enzyme-linked immunosorbent assay for the quantification of imidacloprid titers in xylem fluid extracted from grapevines. J. Econ. Entomol. 98: 182–187.

Byrne, F. J., N. C. Toscano, A. A. Urena, and J. G. Morse. 2005b. Quantification of imidacloprid toxicity to avocado thrips, *Scirtothrips perseae* Nakahara (Thysanoptera: Thripidae), using a combined bioassay and ELISA approach. Pest Manage. Sci. 61: 754–758.

Byrne, F. J., N. C. Toscano, A. A. Urena, and J. G. Morse. 2007. Toxicity of systemic neonicotinoid insecticides to avocado thrips in nursery avocado trees. Pest Manage. Sci. 63: 860–866.

Byrne, F. J., R. D. Oetting, J. A. Bethke, C. Green, and J. Chamberlin. 2010. Understanding the dynamics of neonicotinoid activity in the management of *Bemisia tabaci* whiteflies on poinsettias. Crop Prot. 29: 260–266.

Carrière, Y., and D. A. Roff. 1995. Change in genetic architecture resulting from the evolution of insecticide resistance: a theoretical and empirical analysis. Heredity 75: 618–629.

Carrière, Y., J.-P. Deland, D. A. Roff, and C. Vincent. 1994. Life-history costs associated with the evolution of insecticide resistance. Proc. Biol. Sci. 1351: 35–40.

Carrière, Y., D. A. Roff, and J. P. Deland. 1995. The joint evolution of diapause and insecticide resistance: a test of an optimality model. Ecology 76: 1497–1505.

Carrière, Y., P. B. Goodell, C. Ellers–Kirk, G. Larocque, P. Dutilleul, S. E. Naranjo, and P. C. Ellsworth. 2012. Effects of local and landscape factors on population dynamics of a cotton pest. PLoS ONE 7: e39862. (doi: 10.1371/journal.pone.0039862).

Castle, S. J., F. J. Byrne, J. L. Bi, and N. C. Toscano. 2005. Spatial and temporal distribution of imidacloprid and thiamethoxam in citrus and impact on *Homalodisca coagulata* populations. Pest Manage. Sci. 61: 75–84.

Corley, J. C., A. F. Capurro, and C. Bernstein. 2004. Prolonged diapause and the stability of host-parasitoid interactions. Theor. Popul. Biol. 65: 193–203.

Dalgaard, P. 2008. Introductory statistics with R, 2nd ed. Springer, New York, NY.

Danks, H. V. 1987. Insect dormancy: an ecological perspective. Biological Survey of Canada No. 1. Ottawa, Canada.

de Kort, C.A.D. 1990. Thirty-five years of diapause research with the Colorado potato beetle. Entomol. Exp. Appl. 56: 1–13.

Elbert, A., M. Haas, B. Springer, W. Thielert, and R. Nauen. 2008. Applied aspects of neonicotinoid uses in crop protection. Pest Manage. Sci. 64: 1099–1105.

Emerson, K. J., W. E. Bradshaw, and C. M. Holzapfel. 2009. Complications of complexity: integrating environmental, genetic and hormonal control of insect diapause. Trends Genet. 25: 217–225.

Finney, D. J. 1971. Probit analysis, 3rd ed. Cambridge University Press, Cambridge, United Kingdom.

French, B. W., and L. Hammack. 2010. Reproductive traits of northern corn rootworm (Coleoptera: Chrysomelidae) in relation to female and male body size. Ann. Entomol. Soc. Am. 48: 688–694.

French, B. W., and L. Hammack. 2011. Multiple mating, fecundity, and longevity in female northern corn rootworm (Coleoptera: Chrysomelidae) in relation to body size. Ann. Entomol. Soc. Am. 104: 834–840.

Gibson, A., R. P. Gorham, H. F. Hudson, and J. A. Flock. 1925. The Colorado potato beetle, *Leptinotarsa decemlineata* (Say) in Canada. Can. Dep. Agric. Bull. 52.

Gould, F. 1983. Genetics of plant-herbivore systems: interactions between applied and basic study, pp. 603–614. *In* R. F. Denno and M. S. McClure (eds.), Variable Plants and Herbivores in Natural and Managed Systems. Academic, New York, NY.

Grafius, E. J. 1995. Is local selection followed by dispersal a mechanism for rapid development of multiple insecticide resistance in the Colorado potato beetle? Am. Entomol. 41: 104–109.

Grafius, E. J. 1997. Economic impact of insecticide resistance in the Colorado potato beetle (Coleoptera: Chrysomelidae) on the Michigan potato industry. J. Econ. Entomol. 90: 1144–1151.

Gray, M. E., T. W. Sappington, N. J. Miller, J. Moeser, and M. O. Hohn. 2009. Adaption and invasiveness of western corn rootworm: intensifying research on a worsening pest. Annu. Rev. Entomol. 54: 303–321.

Hare, J. D. 1983. Seasonal variation in plant-insect associations: utilization of *Solanum dulcamara* by *Leptinotarsa decemlineata*. Ecology 64: 345–361.

Hoy, C. W., G. P. Head, and F. R. Hall. 1998. Spatial heterogeneity and insect adaption to toxins. Annu. Rev. Entomol. 43: 571–594.

Huseth, A. S., and R. L. Groves. 2011. Colorado potato beetle insecticide resistance in Wisconsin: characterizing diapause physiology and behavior associated with neonicotinoid resistance, pp. 149–151. *In* Proceedings of Wisconsin's Annual Potato Meeting, UW- Madison College of Agriculture and Life Sciences, Research Division and UWEX, February 1–3, Steven's Point, WI.

Isley, D. 1935. Variations in the seasonal history of the Colorado potato beetle. J. Kans. Entomol. Soc. 8: 142–145.

Jeschke, P., and R. Nauen. 2008. Neonicotinoids–from zero to hero in insecticide chemistry. Pest Manage. Sci. 64: 1084–1098.

Jeschke, P., R. Nauen, M. Schindler, and A. Elbert. 2011. Overview of the status and global strategy for neonicotinoids. J. Agric. Food Chem. 59: 2897–2908.

Kennedy, G. G., and N. P. Storer. 2000. Life systems of polyphagous arthropod pests in temporally unstable cropping systems. Annu. Rev. Entomol. 45: 467–493.

Knolhoff, L. M., D. W. Onstad, J. L. Spencer, and E. Levine. 2006. Behavioral differences between rotation resistant and wild-type *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae). Environ. Entomol. 35: 1049–1057.

Koštál, V. 2006. Eco-physiological phases of insect diapause. J. Insect Physiol. 52: 113–127.

Kung, K.-J. S., M. Milner, J. A. Wyman, J. Feldman, and E. Nordheim. 1992. Survival of Colorado potato beetle (Coleoptera: Chrysomelidae) after exposure to subzero thermal shocks during diapause. J. Econ. Entomol. 85: 1695–1700.

Lashomb, J. H., Y.-S. Ng, G. Ghidiu, and E. Green. 1984. Description of spring emergence by the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae), in New Jersey. Environ. Entomol. 13: 907–910.

Lees, A. D. 1955. The physiology of diapause in arthropods. Cambridge University Press, Cambridge, United Kingdom.

Levine, E., H. Oloumi–Sadeghi, and J. R. Fisher. 1992. Discovery of multiyear diapause in Illinois and South Dakota by northern corn rootworm (Coleoptera: Chrysomelidae) eggs and incidence of the prolonged diapause trait in Illinois. J. Econ. Entomol. 85: 262–267.

Levine, E., J. L. Spencer, S. A. Isard, D. W. Onstad, and M. E. Gray. 2002. Adaption of the western corn rootworm to crop rotation: evolution of a new strain in response to its management practice. Am. Entomol. 48: 94–107.

Mailloux, G., M. A. Richard, and C. Chouinard. 1988. Spring, summer and autumn emergence of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae). Agric. Ecosyst. Environ. 21: 171–180.

McMaster, G. S., and W. W. Wilhelm. 1997. Growing degree-days: one equation, two interpretations. Agric. For. Meteorol. 87: 291–300.

Mead, R., R. N. Curnow, and A. M. Hasted. 2002. Statistical methods in agriculture and experimental biology, 3rd ed. Chapman & Hall/CRC, Boca Raton, FL.

Milner, M., K.-J. S. Kung, J. A. Wyman, J. Feldman, and E. Nordheim. 1992. Enhancing overwintering mortality of Colorado potato beetle (Coleoptera: Chrysomelidae) by manipulating the temperature of its diapause habitat. J. Econ. Entomol. 85: 1701–1708.

Mota–Sanchez, D., R. M. Hollingworth, E. J. Grafius, and D. D. Moyer. 2006. Resistance and cross-resistance to neonicotinoid insecticides and spinosad in the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera : Chrysomelidae). Pest Manage. Sci. 62: 30–37.

Murtaugh, P. A., S. C. Emerson, P. B. McEvoy, and K. M. Higgs. 2012. The statistical analysis of insect phenology. Environ. Entomol. 41: 355–361.

Nauen, R., and I. Denholm. 2005. Resistance of insect pests to neonicotinoid insecticides: current status and future prospects. Arch. Insect Biochem. 58: 200–215.

Noronha, C., G. M. Duke, and M. S. Goettel. 2002. Damage potential and phenology of the Colorado potato beetle (Coleoptera: Chrysomelidae) on potato in Southern Alberta. Phytoprotection 83: 89–98.

Olson, E. R., G. P. Dively, and J. O. Nelson. 2000. Baseline susceptibility to imidacloprid and cross resistance patterns in Colorado potato beetle (Coleoptera: Chrysomelidae) populations. J. Econ. Entomol. 93: 447–458.

Olson, E. R., G. P. Dively, and J. O. Nelson. 2004. Bioassay determination of the distribution of imidacloprid in potato plants: implications to resistance development. J. Econ. Entomol. 97: 614–620.

Onstad, D. W., and L. J. Meinke. 2010. Modeling evolution of *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae) to transgenic corn with two insecticidal traits. J. Econ. Entomol. 103: 849–860.

Onstad, D. W., D. W. Crowder, S. A. Isard, E. Levine, and J. L. Spencer. 2003. Does landscape diversity slow the spread of

rotation-resistant western corn rootworm (Coleoptera: Chrysomelidae)? Environ. Entomol. 32: 992–1001.

Pinheiro, J. C., and D. M. Bates. 2000. Mixed-effects models in S and S-Plus. Springer, New York. NY.

Pruess, K. P. 1983. Day-degree methods for pest management. Environ. Entomol. 12: 613–619.

R Development Core Team. 2012. R: a language and environment for statistical computing, (version 2.14.1). R Foundation for Statistical Computing, Vienna, Austria. (http://www.R-project.org/).

Radcliffe, E. B., K. L. Flanders, D. W. Ragsdale, and N. M. Noetzel. 1991. Pest management systems for potato insects, pp. 587–621. *In* D. Pimental (ed.), CRC Handbook of Pest Management, 2nd ed. vol. III. CRC, Boca Raton, FL.

Robertson, J. L., R. M. Russell, H. K. Preisler, and N. E. Savin. 2007. Bioassays with arthropods, 2nd ed. CRC, Boca Raton, FL.

SAS Institute. 2009. SAS 9.3.1 Help and documentation. SAS Institute, Cary, NC.

Senanayake, D. G., E. B. Radcliffe, and N. J. Holliday. 2000. Oviposition and diapause behavior in Colorado potato beetle (Coleoptera: Chrysomelidae) populations from East Central Minnesota and the valley of the Red River of the North. Environ. Entomol. 29: 1123–1132.

Skinner, L. C., D. W. Ragsdale, R. W. Hansen, M. A. Chandler, and R. D. Moon. 2004. Temperature-dependent development of overwintering *Aphthona lacertosa* and *A. nigriscutis* (Coleoptera: Chrysomelidae): two flea beetles introduced for the control of leafy spurge, *Euphorbia esula.* Environ. Entomol. 33: 147–154.

Sokal, R. R., and F. J. Rohlf. 1995. Biometry: the principles and practice of statistics in biological research, 3rd ed. W. H. Freeman and Company, New York, NY.

Szendrei, S., E. Grafius, A. Byrne, and A. Ziegler. 2012. Resistance to neonicotinoid populations of the Colorado potato beetle (Coleoptera: Chrysomelidae). Pest Manage. Sci. 68: 941–946.

Tabashnik, B. E. 1994. Evolution of resistance to *Bacillus thuringiensis.* Annu. Rev. Entomol. 39: 47–79.

Tauber, M. J., and C. A. Tauber. 1993. Adaptions to temporal variation in habitats: categorizing, predicting, and influencing their evolution in agroecosystems, pp. 103–127. *In* K. C. Kim and B. A. McPheron (eds.), Evolution of Insect Pests: Patterns of Variation. Wiley, New York, NY.

Tauber, M. J., and C. A. Tauber. 2002. Prolonged dormancy in *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae): a ten-year field study with implications for crop rotation. Environ. Entomol. 31: 499–504.

Tauber, M. J., C. A. Tauber, and I. Masaki. 1986. Seasonal adaptions of insects. Oxford University Press, Oxford, United Kingdom.

Tauber, M. J., C. A. Tauber, and J. P. Nyrop. 1994. Soil moisture and post dormancy emergence of Colorado potato beetles (Coleoptera: Chysomelidae): descriptive model and field emergence patterns. Environ. Entomol. 23: 1485–1496.

Trisyono, A., and M. E. Whalon. 1997. Fitness costs of resistance to *Bacillus thuringiensis* in Colorado potato beetle (Coleoptera: Chrysomelidae). J. Econ. Entomol. 90: 267–271.

Ushatinskaya, R. S. 1978. Seasonal migration of adult *Leptinotarsa decemlineata* (Insecta, Coleoptera) in different types of soil and physiological variations of individuals in hibernating populations. Pedobiologia 18: 120–126.

(UW–EX) University of Wisconsin–Extension. 2008. Automated Weather Observation Network (AWON). (http://www.soils.wisc.edu/uwex_agwx/awon).

Venables, W. N., and B. D. Ripley. 2002. Modern applied statistics with S, 4th ed. Springer New York, Inc., New York, NY.

Whalon M. E., D. Mota–Sanchez, and R. M. Hollingworth. 2008. Global pesticide resistance in arthropods. CAB International, Oxfordshire, United Kingdom.

Whalon M. E., D. Mota–Sanchez, and R. M. Hollingworth. 2012. The MSU arthropod pesticide resistance database. (http://www.pesticideresistance.org).

Williams, I. H., and A. W. Ferguson. 2010. Spatio-temporal distributions of pests and their parasitoids on the Oilseed Rape crop. *In* I. H. Williams (ed.), Biocontrol-Based Integrated Management of Oilseed Rape Pests. Springer, The Netherlands.

Wilson, K., and I.C.W. Hardy. 2002. Statistical analysis of sex ratios: an introduction, pp. 48–92. *In* I.C.W. Hardy (ed.), Sex Ratios: Concepts and Research Methods. Cambridge University Press, Cambridge, United Kingdom.

Wissinger, S. A. 1995. Cyclic colonization in predictably ephemeral habitats: a template for biological control in annual crop systems. Biol. Control 10: 4–15.

Xu, G., and G. E. Long. 1997. Host-plant phenology and Colorado potato beetle (Coleoptera: Chrysomelidae) population trends in Eastern Washington. Environ. Entomol. 26: 61–66.

Yocum, G. D., J. P. Rienhart, and M. L. Larson. 2011. Monitoring diapause development in the Colorado potato beetle, *Leptinotarsa decemlineata,* under field conditions using molecular biomarkers. J. Insect Physiol. 57: 645–652.

Zhao, J., B. A. Bishop, and E. J. Grafius. 2000. Inheritance and synergism of resistance to imidacloprid in the Colorado potato beetle (Coleoptera: Chrysomelidae). J. Econ. Entomol. 93: 1508–1514.