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Invasions on large and small scales: management of a well-established crop pest, the Colorado potato beetle

Mitchell B. Baker[1,2,*], David N. Ferro[1] & Adam H. Porter[1]
[1]*Department of Entomology, University of Massachusetts, Amherst, MA 01003, USA;* [2]*Department of Biology, P.O. Box 3003, Franklin and Marshall College, Lancaster, PA 17604, USA; *Author for correspondence (e-mail: m_baker@fandm.edu; fax: +1-717-358-4548)*

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Abstract

Understanding the movement of invading organisms is critical to predicting invasion dynamics. The Colorado potato beetle, *Leptinotarsa decemlineata* (Say), is an invasive species on multiple spatial and temporal scales, and can serve as a model for studies of invasion dynamics. It is the major insect defoliator of potato in North America, and successful management requires an understanding of CPB invasions of individual fields. Its origin, spread, and biology, especially the cycle of annual invasions of agricultural potato fields, are described. Approaches to reducing the size of colonizing populations include rotation, delay of planting, and treatments of field margins. Rotation and sub-lethal insecticide treatments can slow the establishment of invasions within fields. These approaches interact with a late season diapause switch away from reproduction to reduce the impact of CPB. The refuge approach to delaying the fixation of resistance alleles is designed to encourage alleles for susceptibility to invade treated areas. We present data from an experimental refuge crop planted adjacent to a field treated with imidacloprid, an insecticide for which there is high variation in resistance. The treated field was four times as resistant as the untreated side, and a cline in resistance was formed from the untreated to the treated portion of the field. The cline width of about 100 m provides an empirical basis for designing refuges to enhance the spread of alleles for susceptibility into treated areas and prevent fixation of resistance in the summer generation.

Introduction

The dynamics of invasions are not amenable to manipulative experimentation. The risk of escape of introduced exotics prohibit some experiments, and the spatial and temporal scales of many invasions prevent them from being replicated in the field for study. The Colorado potato beetle, *Leptinotarsa decemlineata* (Say), can serve as a model species for the study of invasions both for its history as an invader and because each year, cultivated potato fields are invaded from without by populations of beetles. Manipulations in planting and treatment can be used to test models of rates of spread and factors contributing to invasion dynamics.

Colorado potato beetle (CPB) is the major insect pest of potato in North America (Ferro and Boiteau 1993), and the greatest insect defoliator of potatoes worldwide. It has not only spread from its original range in Mexico to Europe and Asia through China (Zhang 1996) but each year it invades potato fields from overwintering sites along field margins (Weber and Ferro 1993) or more distant locations. Successful management of CPB requires treatment of the local invasion of each field every year. This is due not only to the periodic nature of agricultural crops in general, but is also due to the specific biology of CPB. The origin and spread of CPB from its original hosts and range is reviewed. Two types of smaller scale invasions are also discussed; the invasion of potato fields by CPB, and the invasion of CPB populations resistant to insecticides by susceptible CPB populations.

Concepts developed to describe the invasions of introduced species into novel areas are useful in understanding CPB dynamics in individual fields. Vermeij (1996) defines an invasion as 'the geographical expansion of a species into an area not previously

occupied by that species'. Though yearly CPB movements are of a shorter temporal and smaller spatial scale than typically studied invasions, understanding and manipulation of the colonization and invasion process links management to invasion biology. Vermeij (1996) divides the act of invasion into three stages; arrival, establishment, and integration. Successful management depends as much on altering the timing of these stages as on preventing any of them. Arrival depends on the dispersal capabilities of the invader, and the distribution of donor and recipient areas. Arrival can be seen as inevitable for any field, though rotation and planting schedules can influence the timing of invasions by CPB adults. The timing and numbers of arriving CPB strongly affects their pest status (Voss and Ferro 1992). Establishment, the persistence and reproduction of a local population, is slowed but not prevented in successful management of CPB. Factors influencing the rate of establishment of CPB in agricultural fields are described, especially those variables associated with the timing and initial size of the colonizing population.

The last stage for invasive species is integration, 'a process in which the species in the recipient biota and the invader respond to each other ecologically and evolutionarily' (Vermeij 1996). Managing the evolution of resistance to insecticides is a problem in influencing and predicting the integration of invasions, more specifically the invasion of resistance or susceptibility alleles among areas with different selective pressures. Current proposals and requirements for planting of refuge crops when using new transgenic cultivars are intended to manage the evolutionary response of insects to insecticides and prevent the fixation of resistance (Gould 1998). The refuge crop design for managing resistance seeks to maximize the invasion of resistant populations by susceptible beetles raised on untreated plants. The initial results of a new research technique to study the spread and evolution of resistance are presented, with the goal of designing optimal refuge strategies through the study of experimental clines in resistance in the field.

Origin and spread of CPB

The origin, spread, and host shifts of CPB are discussed by Hsiao (1981, 1985) and Casagrande (1985, 1987) and references listed therein. CPB most likely originated in Mexico on at least three natural hosts, *Solanum angustifolium*, *S. elaeagnifolium*, and *S. rostratum* (buffalo bur). Though initially thought to have spread eastward after adapting to cultivated potato (*S. tuberosum*), it is most likely that it initially spread with the cattle-facilitated spread of buffalo bur, and only shifted to potato after at least 40 years of range overlap (Casagrande 1987). CPB was first described by Nuttall in Iowa in 1811 but was first reported as a pest on cultivated potato in 1859 in Eastern Nebraska (Casagrande 1985). The inferred length of time between exposure to potato and adaptation to or exploitation of the new host is consistent with the current situation in Mexico, where potato has been cultivated for over 40 years (Casagrande 1985) and CPB has yet to act as a serious pest there. Beetles collected in Mexico on natural hosts performed poorly on cultivated potato (Hsaio 1981; Lu and Logan 1994). The first introductions in Europe were recorded in the 1870s (ref. in Hsiao 1985) and CPB was not a serious pest until the 1910s, but this delay must be attributed to other factors because the CPB strains were introduced through cultivation of *S. tuberosum*.

Several factors have facilitated spread and impact of CPB. Among members of the genus *Leptinotarsa* it is a relative generalist (Hsaio 1985). It certainly benefited from the spread of a native host, *S. rostratum*, which increased its range and the range of overlap with cultivated potato. It has also spread to local solanaceous weeds such as horsenettle, *S. carolinense* L, outside the range of its original hosts, which aid in colonization of newly planted and rotated fields (Hare 1983; Weber and Ferro 1996; Nault et al. 1997). Other contributing factors include its diverse migratory and mating patterns (Weber and Ferro 1994). CPB mate with several partners both before and after winter diapause, and have a mixed movement strategy where some offspring are sedentary and mate near where they hatched. Multiple mating combined with migration allows rare genes for resistance to spread rapidly, while having a portion of philopatric young that can inbreed means that a female heterozygous for resistance might have some of her offspring mate with each other, producing some homozygous resistant offspring.

Annual invasion of individual fields

CPB emergence, colonization, and establishment patterns

The phenology of CPB is illustrated in Figure 1. CPB overwinter as adults and emerge in late spring to colonize potato fields. The refractory phase of the diapause, during which the beetles do not react to the change in environmental conditions, lasts for approximately

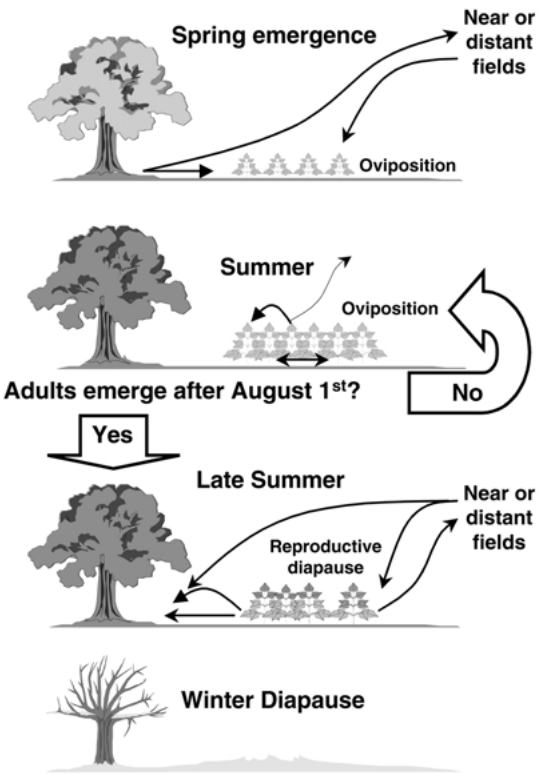

Figure 1. Phenology of CPB. Spring emergers take long or short distance flights or walk from overwintering locations to mate, feed, and lay eggs. Summer emergers feed for 5–7 days before developing wing muscles and ovaries. They primarily walk and take short flights but also can fly long distances, even if food is unavailable. If adults emerge after the diapause switch, they do not lay eggs, feeding and walking or flying to find feeding and overwintering sites. Diapause occurs in the adult stage, and can last one or more years.

3 months. After that, the beetles respond primarily to elevation of temperatures above +10 °C by emerging from the soil (de Kort 1990). The effects of temperature on emergence are modulated by soil moisture (Tauber et al. 1994), with low soil moisture preventing emergence. The beetles usually accumulate 50–250 degree days (DD) before they appear on the soil surface (Yang 1994; Ferro et al. 1999). Males and females terminate their diapause simultaneously (Yang 1994; Ferro et al. 1999), and require only 51–80 DD after emergence before they are able to oviposit (Ferro et al. 1999).

After emergence from the soil, overwintered CPBs colonize potato fields both by flight and by walking (Voss and Ferro 1990a). The beetles do not start flying until they accumulate 150–200 DD (amount required for flight muscle regeneration, Yang 1994), and beetle flight is strongly encouraged by the absence of food (Caprio and Grafius 1990; Ferro et al. 1991; Weber and Ferro 1996). If the fields are rotated, the beetles are able to fly up to several kilometers to find a new host habitat (Ferro et al. 1991; Weber and Ferro 1996). Mating starts before beetles leave for the host habitat, with at least half of the population mating within the overwintering sites (Ferro et al. 1999). Post-diapause females can also lay eggs utilizing sperm from the pre-diapause mating the previous fall. Post-diapause females not allowed to mate after emergence have fecundity 80% that of spring-mated females (Ferro et al. 1991), so even a few females could singly colonize a new field in the spring.

Even in fields planted with potato the previous year, most adult beetles invade in the spring from outside the planted fields. Colorado potato beetle overwinter in the soil as adults, with the majority of them aggregating in woody areas adjacent to fields where they have spent the previous summer (Weber and Ferro 1993), or along irrigation ditches (French et al. 1993). In Massachusetts 56–85% of beetles colonizing non-rotated fields were estimated to have emerged from woody borders surrounding fields (Weber et al. 1994).

Management of annual invasions

CPB invasions must be managed at both the arrival and establishment phases. Both the size of the arriving population and the timing of colonization have large effects on crop-loss, and approaches to manage both components of arrival are presented. Slowing the rate of population growth also reduces CPB impact, and two methods to minimize the impact of CPB during the establishment phase are discussed. These approaches are not exclusive, and successful management will address both the timing and the starting size of annual invasions, as well as the rate of population growth.

Arrival: minimizing the initial invasion

Crop rotation is an effective means of reducing the number of invading CPB (Casagrande 1987; Weisz et al. 1994, 1996). Rotated fields also require fewer insecticide applications, which delays the evolution of resistance (Roush et al. 1990). Rotation is more successful the greater the distance from the previously planted field, ideally 500 m or greater (Follett et al. 1996). An illustration of the potential of rotation is the distribution of CPB in a 0.7 ha field planted in South Deerfield, Massachusetts, along the Connecticut River (Figure 2). The field was 2.6 km from the

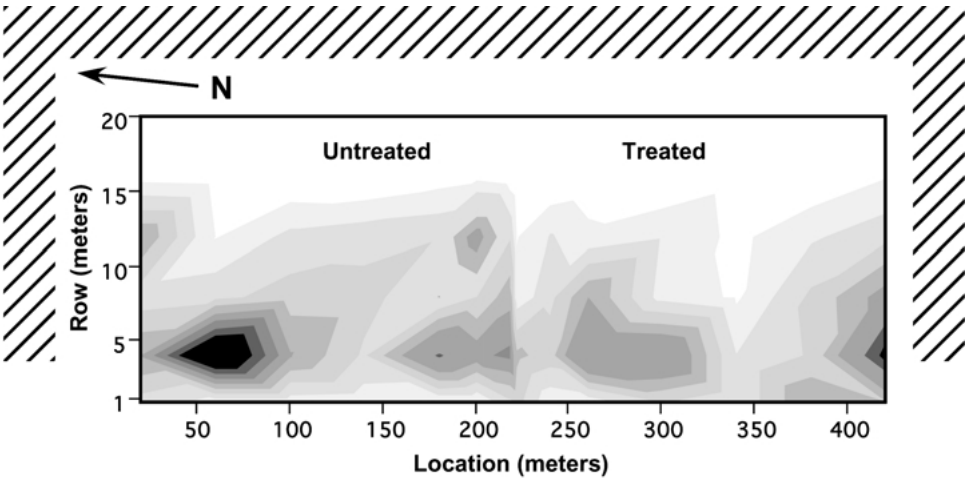

Figure 2. Filled contour plot (JMP®) of the cumulative densities over an entire season of adult CPB from 10 surveys of a 0.7 ha rotated and isolated field in South Deerfield, Massachusetts. Densities range from 0–3.75 adults/m (white) to 30–33.75 adults/m (solid black). The field was bordered on the north, east, and south sides by woodlot (hatched area), on the east side by the Connecticut river, and on the west side by a corn field. Colonization appeared exclusively to originate from the south–southwest, the direction to the nearest potato field (2.6 km away), onto the west and south edges of the field, though movement and reproduction extended the population eastward within the field.

nearest commercial potato field, and the river and surrounding woody margin isolated it on three sides. The adult population of immigrants and their descendents never reached past the first 15 rows, and significant infestation was limited to the first 4 rows.

Many agricultural crops are invaded yearly from without. This is due not only to the ephemeral nature of agricultural landscapes composed of annual or rotated crops, but can also result from changing needs or preferences of insects for mating or overwintering sites when not feeding on the crop hosts (e.g. Reynolds and Prokopy 1997). The preference of CPB to overwinter in woody margins surrounding fields has led to the development of effective physical approaches to minimize the size of invading populations in the spring (Boiteau et al. 1994; Ferro 1996). One of the most promising physical controls is the construction of plastic-lined trenches at the edges of fields surrounded by woody margins. Plastic-lined trenches placed between overwintering sites and experimental plots within potato fields reduced recruitment of adults by 85%. A larger scale experiment in a commercial field showed the same reduction in beetles colonizing the field (Ferro 1996). Insecticides can also serve as effective edge treatments to act as a barrier to colonization. In the same study, Ferro (1996) found that three outer rows treated with fipronil reduced initial colonization by 67%. Use of insecticides as a border treatment alone can reduce their potential environmental impact relative to comprehensive applications, and also allow untreated portions of fields to serve as refuges for natural enemies or susceptible beetles (below).

Establishment: slowing population growth

Population dynamics after colonization depend on the timing of colonization as much as on initial population size. This is primarily because of a late season diapause switch away from reproduction (Voss et al. 1988). Though they do mate (Voss and Ferro 1990b), most adults emerging after 1 August never attempt to lay egg masses (Figure 1). The time of colonization can determine what proportion of later generations lay eggs. This is important because successive generations within a season have the potential to be much larger than earlier ones. Simulations have shown (Voss et al. 1988) that a seven-day delay in the colonization of a field in Western Massachusetts leads to dramatically fewer larvae feeding late in the summer.

Delaying the time of invasion

There are two cultural ways the timing of annual invasions can be managed: through rotation and timing of planting. Rotation delays the colonization of fields by spring emerging CPB from one to three weeks (Lashomb and Ng 1984; Wright 1984). This is due

to the time needed for significant numbers of beetles to locate fields after emerging and leaving remote overwintering sites.

Late planting is designed to encourage early emerging beetles to migrate from non-rotated fields because the plants have not yet emerged, and also to delay colonization of rotated or non-rotated fields. The later plants emerge, the later the first oviposition is possible, and the greater the proportion of adults emerging after the diapause switch. Early planting and harvest might also reduce the impact of the second generation in northern climate fields (Casagrande 1987) but runs the risk of crop-loss because wet or cold conditions in early spring can encourage plant disease. Market incentives currently favor early planting and harvest (Weber and Ferro 1994). In addition, late-planted fields may act as sinks for beetles emigrating from earlier harvested fields looking for feeding and overwintering sites (Boiteau 1986). This could increase the regional CPB population by providing havens for beetles that might not otherwise find sufficient food to survive winter, and might also increase the emerging population at the late-planted field the following spring.

Lengthening development to reduce population growth rate

In addition to the direct effect of increased development time on population growth, any process that shifts portions of adult emergence after the diapause switch can greatly reduce pest status. Insecticides, even ones for which resistance has evolved, can delay development and reproduction sufficiently to dramatically reduce population growth rates. Liu et al. (1999) showed that a laboratory selected strain of corn bollworm resistant to Bt expressed in GM corn developed more slowly when raised on GM corn, though development times were similar on untreated plants. Earlier Alyokhin and Ferro (1999c) showed that CPB resistant to *Bacillus thuringiensis* subsp. *tenebrionis* Cry3A δ-endotoxin have slower development than susceptible beetles (Alyokhin and Ferro 1999a). The time spent in individual larval stages has also been shown to increase (Trisyono and Whalen 1997). Foliar applications of Bt Cry3A toxin, which are rarely strong enough to kill even susceptible adult beetles, can still slow invasions of fields by slowing development and postponing the bulk of summer emergence until after the diapause switch (Ferro et al. 1997).

The retardant effect shown by Bt endotoxin on development has been seen in several species and with insecticides with very different modes of action. For example, slowed development in strains resistant to synthetic insecticides was found in house flies and mosquitos (Ferrari and Georghiou 1981; Roush and Plapp 1982). CPB resistant to azinphosmethyl and azinphosmethyl/permethrin also showed slower development (Argentine et al. 1989). Unlike the family of Bt endotoxins, which act primarily by binding to sites in the gut lining, preventing absorption and lysing the lining (Gill et al. 1992), imidacloprid belongs to a new class of neurotoxins that bind to nicotinic acetylcholine receptors (Nishimura 1994). In the course of a study (described below) of movement between treated and untreated areas in fields, major effects were observed of imidacloprid on development time in the field.

A long, narrow (450 × 20 m) field was planted along the Connecticut river in South Deerfield, MA on 2 May 2000, and the southern side of the field was treated with the label recommended rate (32 ml/305 row meters) of imidacloprid (Admire® 2F [flowable], Bayer, Kansas City, Missouri). The other side was not treated for CPB, but did receive two sprays of Orthene® to control leafhoppers and aphids. The field was also treated alternately with Acrobat® or Penncozeb® for control of fungi and relatives. The field was censused 10 times during the summer from 6 June until 15 August for adults, egg masses, small (1st or 2nd instar) and large (3rd or 4th instar) larvae. The densities of each stage are illustrated in the contour plots in Figure 3(a–d). Oviposition by the spring emerging adults was later on the treated side, and development of the summer generation was slower as well. The peaks in density of egg masses were about 20 days apart on the treated and untreated ends, and the peak in density of large larvae on the untreated side of the field preceded the peak on the treated side by about 30 days (Figure 4). Lower concentrations of imidacloprid than are necessary to kill adult beetles can be used to slow the invasion of fields and prevent severe damage.

In addition to managing the arrival and establishment phases of CPB invasion, the integration of CPB must be managed for long-term reduction of CPB impact. Evolution of CPB to changing pesticide environments is inevitable, yet different management approaches can have very different effects on the rate of evolution of resistance and the fixation or resistance genes within populations. One approach to prevent or postpone

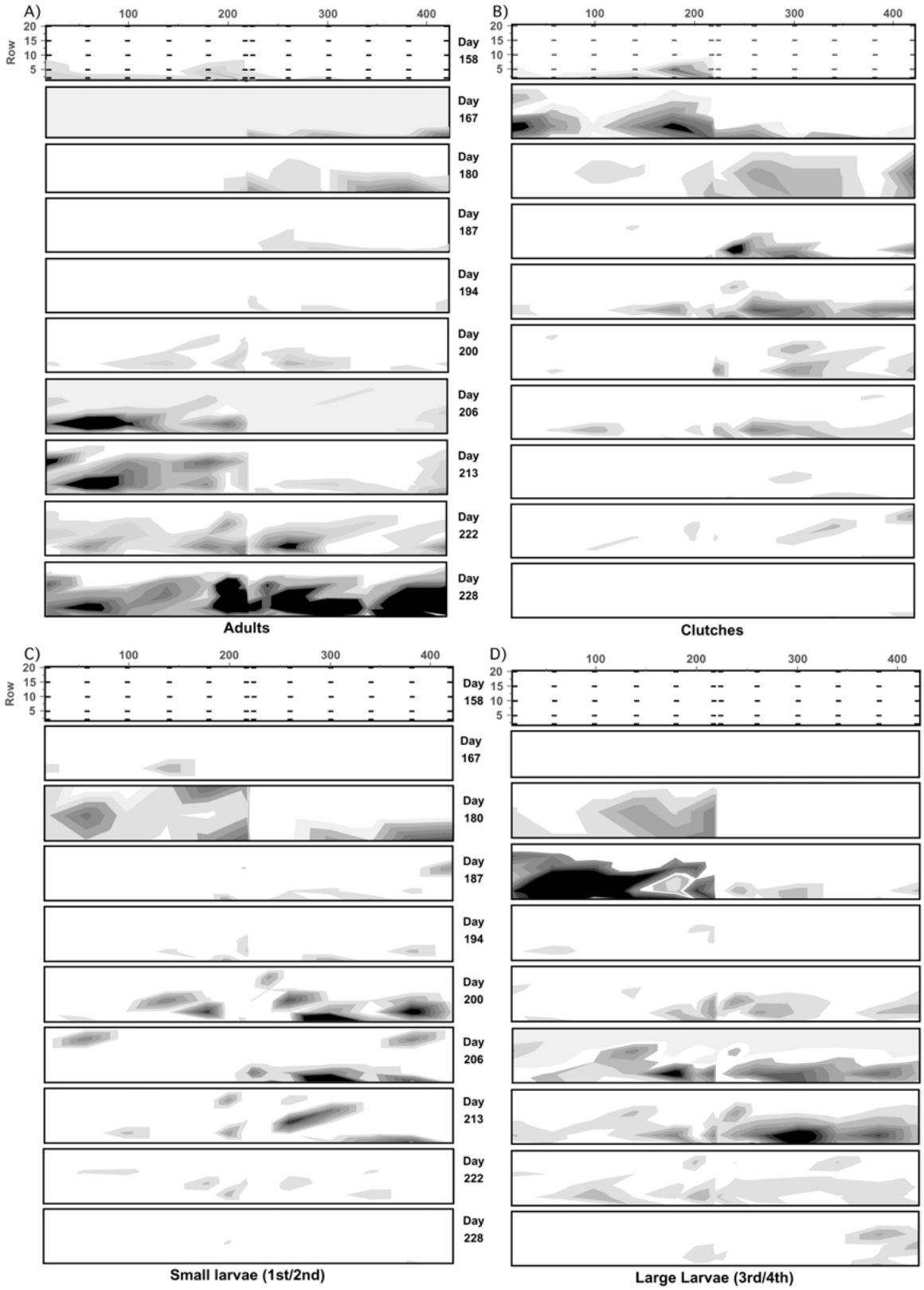

Figure 3. Filled contour plots (JMP®, SAS Institute) of the densities (numbers/row-meter) of (a) adults (0–>10/m), (b) egg masses (0–>5/m), (c) small (0–>20/m), and (d) large larvae (0–>10/m). Days are since 1 January.

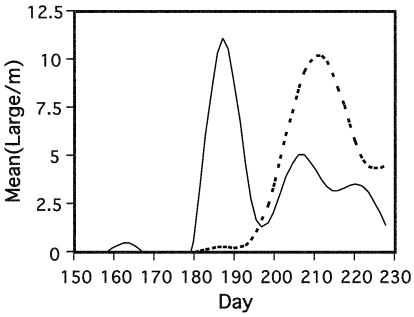

Figure 4. Mean density of large larvae/m on opposite ends (0–120 m from each end) of the South Deerfield field. Curves fit using smoothing spline function. Solid line is from the untreated side of the field, dashed line is from the side treated with imidacloprid at planting.

the fixation of resistance is the use of refuges for susceptibility alleles.

The refuge approach to managing the integration of CPB resistance

A combination of treated and untreated areas is hoped to delay the fixation of resistance in two ways: it is supposed to (1) reduce the strength of selection for resistance and (2) reduce the likelihood that resistant individuals will have resistant offspring (Gould 1998). Because the untreated refuge sustains a much higher population density than the treated area, only a small area is needed to raise a large population of susceptible individuals compared to the population on the treated area. In addition, the relative fitness of susceptible individuals may be higher in untreated areas, so the proportion of susceptible individuals should increase within the refuges. Fitness costs may be expected on theoretical grounds (Coustau et al. 2000; MacNair 1991), and costs to resistance have been found in CPB for Bt endotoxin (Alyokhin and Ferro 1999b,c), azinphosmethyl/permethrin (Argentine et al. 1989), and imidacloprid (Baker, Ferro and Porter, unpublished), but resistance does not always carry measurable costs (Tang et al. 1997). The second goal assumes that resistance is at least partly recessive, so that the offspring of a resistant and susceptible individual will still be susceptible. The dominance of a trait is affected by the environment within which it is expressed, and so greater genetic or physiological dominance will require treatments of higher toxicity in order to maintain the susceptibility of hybrids or heterozygotes. Resistance is expected to be recessive if the trait carries costs, because recessive inheritance is required to slow its loss in the absence of insecticides. However, novel resistance mutations, or non-costly ones should not be expected to be recessive, and some resistance traits show high levels of dominance. For example, Rahardja and Whalon (1995) found a dominance of 0.77 for resistance to Bt endotoxin in CPB, where -1 would indicate completely recessive expression, 0 complete additivity, and 1 complete dominance. A high degree of physiological dominance will not prevent refuges from slowing the evolution of resistance if high doses of insecticide can still kill hybrids of resistant and susceptible individuals (Gould 1998). We have found resistance to imidacloprid in CPB to be 0.31, close to an additive effect (unpublished data). The second goal also requires a high probability that resistant individuals in treated fields mate with susceptible individuals from the refuges, though too much mating can also speed the evolution of resistance (Caprio 2001; Mallet and Porter 1992).

Design of refuges to maximize the chance of hybrid matings requires greater knowledge of their movement than is currently available. Mark-recapture studies can suffer from biases due to recapture effort (Baker et al. 1995), density effects if large number of individuals are released, population effects if large numbers of individuals are removed to detect a marker or differences in detectability of different classes of individuals such as those that vary in resistance. In addition, gene flow and dispersal are not the same (Endler 1979), and dispersal data alone may not reflect the mating and fecundity that are needed to measure the exchange of genes between refuges and treated areas. The wide variation in resistance to imidacloprid currently found within and among fields (Olson et al. 2000, present study) is used as a natural marker to measure movement and spatial effects on gene flow from refuges in CPBs.

New approaches to studying the movement of CPB

Insecticides apply intense selective pressure on insect populations. Strong selection on a trait can differentiate even continuous populations. A cline is a geographic transition region where a trait (here, resistance to imidacloprid) goes from low to high frequency or vice versa (Huxley 1938; Endler 1977). A narrow cline is typically the result of the opposing forces of selection, which removes the genes brought by immigrants across the cline, and movement, which homogenizes differences and flattens the slope between the two sides (Slatkin 1973; Barton and Hewitt 1985).

Clines in traits due to anthropogenic changes in habitat have long been known (MacNair 1981). However, the use of clines to measure the movement of individuals or the levels of gene flow among populations has thus far been limited to naturally occurring transitions or accidental anthropogenic shifts in traits, such as heavy metal tolerance in plants and insecticide resistance in mosquitos (Lenormand et al. 1999). By creating a cline in resistance to imidacloprid in a 0.7 ha field in South Deerfield, Massachusetts, the distance from an untreated area that resistance is reduced due to gene flow from the refuge was measured.

The cline was created in the field that was described above in reference to the retardant effects of imidacloprid on development. Two surveys of resistance were conducted. Thirty egg masses were collected at 17 locations from the untreated end to the treated end that had been laid by the spring emergers. Second instar larvae weighing between 6.5 and 7.5 mg were assayed using direct applications of technical Admire® (Bayer) dissolved in acetone. One microliter at a range of seven concentrations from 1.3×10^{-6} through 1×10^{-4} g/l plus an acetone control were applied to the larvae, which were then placed on leaf cuttings, and mortality was observed after 24 h, defined as not moving legs for 5 s after being disturbed with brush hairs. We calculated the LD_{50} at each location using POLO for logit analysis (Le Ora Software 1987). Because of a cool, wet summer, and the late colonization of the rotated, isolated field, the summer adults emerged too late to lay egg masses. For this reason we conducted the second survey on adults instead of larvae. We collected 40 adults at each of 24 distances on 12 August. We fed the adults untreated foliage for 3 days in greenhouse cages. We applied 2 μl of a single concentration of imidacloprid at 1×10^{-2} g/l. We gave the adults access to water using moistened dental wick, and assayed mortality after 7 days, defined as not moving legs soon after being disturbed with a needle. We calculated resistance as the fraction surviving for each location. The shape of each cline was fit to an equation modified from the standard description of clines due to a balance of selection and dispersal (Slatkin 1973; Nagylaki 1975; Barton and Hewitt 1985). The modified equation is

$$r(x) = q_L + \frac{q_R - q_L}{2}\left[1 + \tanh\left(\frac{2(x-c)}{w}\right)\right],$$

where $r(x)$ is the resistance at location x, c is the location of the center of the cline, w is the cline width, q_L is the resistance at location 0, and q_R is the resistance at the treated end, as determined by a smoothing spline fit (JMP®, $\lambda = 5 \times 10^5$). The equation was fit by maximum likelihood (nonlinear fit, JMP®).

The clines in resistance caused by the presence or absence of imidacloprid on opposite sides are presented in Figures 5 and 6. In the 2nd instar larvae of spring emergers the LD_{50} at the untreated end of the field was 1.6×10^{-6} and the LD_{50} at the treated end was 6.9×10^{-6} (spline fit, $\lambda = 5 \times 10^5$, JMP®). In the summer adults the cline appears to be less steep, although a different measure of resistance was used. The widths of the two clines are similar, 105 m with a $CI_{0.95}$ from 35.3 to 222.4 in the 2nd instars, and 98 m with the lower $CI_{0.95}$ undefined and the upper $CI_{0.95}$ 412.3 in the summer adults. There are two non-exclusive likely sources for the less defined cline in the summer adults. The field had started to receive immigrants from at least one nearby (2.6 km distant) commercial field that had already been harvested. Also, imidacloprid concentrations may have been lower late in the season due to plant growth, heavy rains and diffusion through the soil.

The width of the observed cline suggests a scale for the size and placement of refuges. One concern with refuge design is that the strength of selection may be so strong that none of the susceptible individuals survive to mate with resistant individuals. By creating and measuring the clines created between treated and untreated areas we have an empirical measure of the distance over which movement can overwhelm strong selection. A conservative recommendation from the first year's results would be that no point on a treated

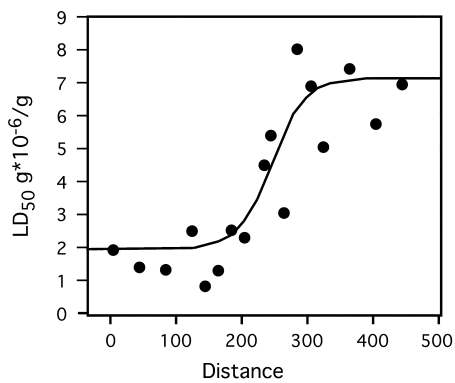

Figure 5. Resistance (LD_{50}) of first generation 2nd instar larvae as a function of position in the split field (0–220 m untreated, 220–440 m treated with imidacloprid).

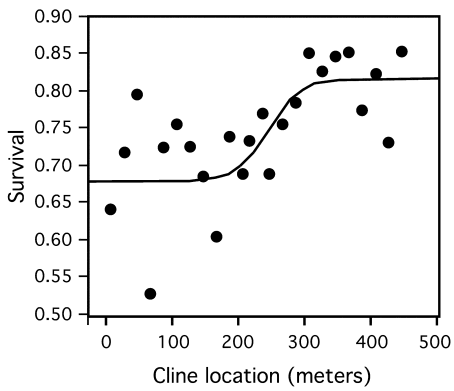

Figure 6. Resistance (survival at discriminating concentration of 2×10^{-2} g/ml) of second generation adults as a function of position in the split field (0–220 m untreated, 220–440 m treated with imidacloprid).

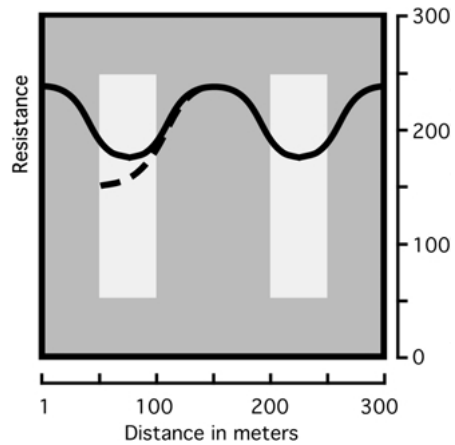

Figure 7. A suggested refuge crop design for preventing the fixation of resistance to imidacloprid in summer generation CPB. The shaded areas are treated, the unshaded areas are untreated. The solid curve describes the expected resistance inside and outside the refuge, with the dotted line showing the expected resistance in the untreated area had it shared only one border with a treated area.

field should be more than half a cline width from a refuge. This could be achieved by alternating 50 m strips of refuge with 100 m strips of treated field, with all four edges of the refuge surrounded by treated field (Figure 7). The resistance within the refuges will not be as low as on an untreated end of a field, because of the surrounding treated areas. The resistance at all points in the treated areas will be reduced however, due to gene flow from the refuges. In the experimental field one edge (the eastern side) was exposed to invasion from the outside. In a field where all the edges are treated, the densities within refuges will be much lower at the start of the season (Ferro 1996) and a narrower cline is expected to result. Lower initial density within in the center of a field will increase the area a refuge must be to reduce resistance in surrounding treated areas, but will also make an increase in refuge size less costly economically. Further modeling and empirical work is needed to show how refuge size should be adjusted in central versus adjacent refuges.

The requirements for management of resistance using refuges may not be that strict, because movement and selection should vary during life stages other than the ones tested thus far. There is no selection for resistance at overwintering sites, and there is a highly migratory phase prior to overwintering. Both of these factors will lead to wider clines at those stages. The next step is to measure how clines vary through the year, and changes similar to those observed by Lenormand et al. (1999) in *Culex pipiens* are expected, with wider clines associated with life phases showing greater migration or weaker selection. The requirements for refuges for treatments with higher levels of selection for resistance might be greater than those needed for imidacloprid. If selection is measured on both sides of the cline, the shape of the cline can then be used to estimate the movement rate of individuals (Barton and Hewitt 1985). The strength of selection measured with different treatments, such as GM potato, can then be used to adjust the refuge design to insure high rates of matings with susceptible individuals.

Conclusions

An understanding of movement is needed to manage colonization, establishment, and integration of CPB within single fields, and on a regional scale as well. Basic research on the life history and migratory strategies of CPB have shown how annual invasions progress. Managing both the size and timing of the arriving population, as well as slowing the rate of establishment leads to reduction of CPB impact. CPB and other insects that repeatedly invade agricultural systems, can be used as model systems for studying factors influencing invasions and spread of invasive species, such as the influence of the size of the initial colonizing population, synchrony with native predators or parasites, landscape connectivity and structure, and other factors. The ability to manipulate and replicate invasion events will allow for experiments that are not possible with novel exotic species. In this case, manipulation of selection for resistance created spatial

variation in resistance that allowed the measurement of the efficiency of invasion of alleles for susceptibility into a more resistant population.

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