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FLIGHT INITIATION BEHAVIOR AND HOST PLANT ATTRACTION IN
THE COLORADO POTATO BEETLE, LEPTINOTARSA DECEMLINEATA (SAY)
(COLEOPTERA: CHRYSOMELIDAE)

By

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ABSTRACT

FLIGHT INITIATION BEHAVIOR AND HOST PLANT ATTRACTION IN THE
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Adult CPB were released in 25m diameter circular arenas with plots of potatoes at each cardinal direction. Movement was examined by trapping beetles on plants in the plots and an outer circle of plants. Post-diapause adults aggregated on individual plants while summer adults were distributed randomly. No correlation was found between the orientation of beetle movement and wind direction (anemotactic behavior). Flight initiation and behavior were studied in the field by releasing treatments of beetles and observing behavior for 20 min. Beetles were found to initiate flight at temperatures as low as 15° C, with maximal initiation at 20° C. Starved beetles flew more readily than recently fed beetles, and flight behavior differed. Starved beetles flew higher and further (migratory), while fed beetles flew lower and shorter distances. Experiments in a laboratory wind tunnel confirmed that flight initiation increases with increasing radiation.

To my parents, who prepared me for all this, and to
Lois, who helped me go through it all.

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INTRODUCTION

Leptinotarsa decemlineata (Say), the Colorado potato beetle (CPB), is the most important insect pest of potato in the eastern United States. Originally from areas near the Rocky Mountains and as far east as the Nebraska-Iowa border, CPB fed on the native plant Solanum rostratum. By 1859 the beetle had apparently shifted its behavior and began to feed on potato, Solanum tuberosum, and the beetle then began a rapid expansion eastward, advancing most rapidly when aided by prevailing winds (Fig. 1).

The potato beetle has a long association with pesticides. The first spraying equipment utilized in agriculture was used to spray paris green (an arsenite) against the CPB. Various forms of arsenates provided erratic control of the beetle until the early 1940's. In 1939, Swiss entomologists tested samples of DDT against both larvae and adults of the CPB, which was decimating the potato crop of Switzerland (Gauthier et al. 1981). DDT provided effective control of the beetle until 1952, when Quinton (1955) noted resistance in New York State. Since that time, numerous pesticides have been utilized against this pest, but the time span over which these insecticides have been effective has become progressively shorter

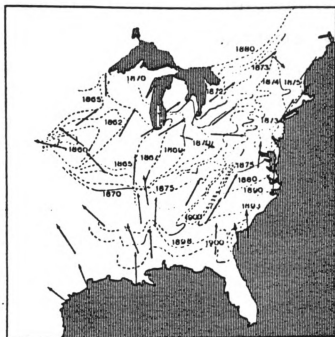


Figure 1. Spread of the Colorado potato beetle in North America. Dashed lines represent spread, arrows represent prevailing winds (Johnson 1967, after Tower 1906).

(Gauthier et al. (1981). In Long Island recently, there were no registered pesticides effective against the potato beetle (Gauthier et al. 1981).

The inadequacies of insecticide-dominated CPB control programs have become increasingly apparent, especially in the Northeast United States where insecticide resistance and population densities are more severe. Pesticide application have been increased, which, combined with porous soil types, has led to concerns about groundwater contamination. Biological studies of the CPB are required to enable the addition of effective bio-control and cultural practices to potato crop protection schemes.

With the increasing resistance of CPB to many insecticides, it is more important to conserve the efficacy of those compounds which remain effective. The development of resistance depends upon the relative frequencies of resistant and susceptible genes in a population. To properly understand how it develops, information is needed on the flow (dispersal) of individuals and genetic information between field populations. Immigration of susceptible pests from non-crop hosts will also affect gene frequencies.

Crop rotation can potentially reduce CPB populations and decrease the number of insecticide applications required for effective control. However, to predict how much impact rotation will have, more information is required to know the conditions under which potato beetles will disperse. Beetles can disperse by walking or flying,

and it is important to understand when each type of dispersal occurs and over what distances. The final step of dispersal involves host-plant location, or at least location of a habitat suitable for the host plant.

Dispersal of insects has usually been separated into two behaviorally different types of motion, migration and trivial or appetitive movement (Southwood 1962). Whalon and Croft (1985) define migration as flight with suppression of reactions to vegetative stimuli. This allows for long distance directed movement which is limited only by morphological restraints. Migration also involves an ecological component, as a migrating insect moves beyond its original breeding habitat. Trivial motion (appetitive, vegetative) is contained within the breeding habitat and is involved with the search for food, oviposition site or mate.

Hanski (1980) found that with coprophagous beetles, long distance migration varied with the population size of the beetles, indicating that at least in these species there is no important difference between long and short distance movements. It is, in any case, difficult to work with the migration/trivial motion dichotomy in the field. Delineation of habitat and internal motivation of an insect are, for example, extremely hard to quantify. A more useful and working definition of dispersal is any movement away from or to an aggregation.

The view that dispersal is merely the ridding of

surplus insects from a defined area has been replaced by an understanding that it plays an important role in the population dynamics and genetics of many species (Taylor and Taylor 1977, Dingle 1972). It is now apparent that, at least for many species, the dispersal characteristics must be included in any description of its population dynamics, along with the more traditionally recognized life table parameters. While there are many examples of dispersal resulting from habitat overcrowding, recent work has emphasized the colonizing potential of dispersing insects. In a population, the best genetic material (the most fit individuals) migrate out, while less fit individuals remain behind and oviposit in the original habitat (Finch 1980). This selection process would insure maximal use of all food resources in an area.

Most work with dispersal has been done with agricultural systems which tend to be ephemeral in nature. Because of this habitat characteristic, research has focused on insects which are r-strategists (Southwood 1962), and less emphasis has been placed on dispersal of k-strategists.

Most insects dispersing over longer distances must have the ability to enter a pristine habitat and build up a large population before that habitat begins to deteriorate to compensate for mortality in finding new patches (Hughes 1980). In its native territory, the CPB find patches of Solanum rostratum and reproduce before this habitat becomes unsuitable (a patch may last several

months). Alternatively, a species may develop search mechanisms which reduce mortality en route to the new habitat.

Stinner et al. (1984) point out that dispersal must be seen as one of several alternatives to an evolving insect. An insect in a habitat which is becoming unsuitable has the option of diapausing (or estivating) or escaping by dispersing. Similarly, instead of colonizing new areas, it may evolve to utilize its present habitat more efficiently or it may adopt a wider host range.

The hypothesis that dispersal is a random motion has been disputed in many papers. Dobyhansky and Wright (1943) found that a random walk model did not adequately describe the dispersal they observed in Drosophila pseudoobscura. Taylor and Taylor (1977) have suggested that within populations there are two antithetical sets of behavior. The first is repulsion, where individuals seek to separate and maximize their resources, and aggregatory behavior, where individuals congregate to maximize use of available resources. Taylor (1978) re-analysed data from many previous experiments in dispersal and found that the general equation:

$$N = e^{(a+bXc)}$$

N=number

X=distance

a, b and c are constants found when fitting the equation

gave good fits to observed distributions in most cases. The values of c computed ranged from -1 to 4, with the conclusion that random dispersal (where c=2) is just one of

a continuum of distributions and hence quite rare.

Baars (1979) found that marked carabid beetles showed periods of random motion with small distances covered per day interspersed with periods of directed movement with large distances covered. Similar patterns were observed in the click beetle Agriostes obscures (Brian 1947) and in blowflies (Macleod and Donnelly 1963). Grum (1965, 1971) found that those beetles which moved quickly were hungry while the slow-moving ones were satiated.

Hanski (1980), in a study of dung beetles, reported results similar to those of Taylor and Taylor, but suggested that increased spatial variance is not caused by increased density, but that both are caused by a third external factor. Conditions for reproductive success are not uniformly distributed in space, and those individuals in an area of high success will leave more offspring, and these offspring will be aggregated. Thus, Hanski argues that Taylor and Taylor's model of aggregation/repulsion is not an adequate description of the dynamics involved in dispersal.

Clark et al. (1978) noted that directional, aggregative flight is observed in many natural systems and proposed, somewhat tongue-in cheek, that the following rules are more realistic than random diffusion.

- 1) The proportion of animals leaving a habitat is a function of per capita habitat quality.

- 2) The exodus population as determined by (1)

moves as a patch, not as independent individuals.

3) The population of (2) will go off in a single direction and will settle together.

4) The migrating population will not settle within a sizeable refractory distance of their original location.

5) Settlement of different populations will be contagious in space, due to biological and physical aggregative factors.

The authors admit that this is an extreme position, but hope that it might illustrate the spectrum of migratory possibilities.

Whalon and Croft (1985) present an idealized dispersal study model, utilizing three different planes to monitor dispersal. The first plane lies within the original habitat and represents a measure of trivial dispersal. The second plane lies beyond this habitat and is intercepted only by dispersing individuals (though others may accidentally appear there). A third plane lies beyond the breeding habitat and measures the suitability of this habitat to dispersing insects.

An important consideration in any dispersal study is the partitioning of the pest between crop and noncrop host plant communities. Most research is based on single-crop single-pest interactions, and little attention has been paid to the impact of alternative crop and noncrop host mixes on movement (Barfield and Stimac 1979). The CPB is known to be as attracted to several wild Solanum species as

to potato (Hsiao and Fraenkel 1968, Hsiao 1974), but little is known about how these plants affect local population dynamics of the beetle. Lashomb (cited in May and Ahmad 1982) noted, however, that one wild species S. dulcamara is rarely heavily infested despite nearby aggregation of CPB. A field should receive pest inoculum proportional to the size of the inoculum at the crop level and to the number of fields competing for this regional pool (Barfield and Stimac 1979).

Dispersal behavior is triggered by factors operating within the original habitat. Little is known of the nature of these cues. Monocultures, however, have been shown to have communities of insects with higher rates of emigration than other habitats (Stinner et al. 1984). Many of these studies have been on oligophagous species. Southwood (1962) points out that most insect pests are r-strategists. If the maximum number of migrants are to be produced, then the population must build up rapidly to the carrying capacity of the habitat. Southwood (1977) notes the relationship between habitat suitability and generation time and necessity for escape.

Once en route, dispersing individuals may aggregate due to innate behavior or converging winds and physical barriers such as mountain ranges (Hughes 1979). Mortality en route may also be selective, and the average size or sex distribution may be different after dispersal. Little is known about the effects of dispersion on the phenomena

of pesticide resistance. Resistant insects have undergone a selection which presumably would make them less vigorous under normal conditions (as is evidenced by the decrease in frequency of resistant genes when selection pressure is decreased). This decreased vigor would make them less likely to survive the ordeal of dispersal. In any case, the genetic and phenological makeup of the population would be altered following dispersal.

The ultimate success of a dispersing individual is measured in the amount of offspring produced, which is determined to a large part by factors present in the new breeding habitat. Weather may play a large role here, but since insects disperse over longer distances on wind systems, the initial and final habitats are likely to be attached by the same wind system and hence likely to be experiencing similar weather (Hughes 1979). Natural enemies of the pest are also not likely to arrive at the new habitat with the first migrants, and these individuals will experience a temporary respite from predation and parasitism. The natural enemies, however, will likely have some efficient means of finding their hosts. Generalist predators may already be present in the new habitat, but these may or may not be efficient in reducing pest numbers. Ecological studies have shown that these types of predators may be most efficient at controlling low level pest populations, and while they may not be important in controlling pest outbreaks, they could eliminate numerous small populations (such as those resulting from dispersal)

before reaching pest status.

Host Plant Attraction

Attraction to host plants can be the result of either visual or chemical stimuli, working alone or together, and also interacting with other physiological and abiotic factors, such as starvation or temperature. The role of chemical attraction has been given the most attention in the literature, particularly within the Cruciferae, but visual cues must also be given serious consideration.

The attraction of insects to host plants is presumed to have evolved through long periods of coevolution. The plants utilize so-called secondary plant substances to form repellents, and specialist insects then break the repellent qualities of the plants and locate plants by focusing in on specific cues of the host plant. These cues may or may not be those compounds first involved in the repellency.

Characteristics of ecosystem texture affects the behavior of insects. Patch size, for example, has been shown to affect attraction. Flea beetles, Phyllotreta cruciferae(Goeze) achieved higher population densities in large plots, while Artogeia rapae (L.) showed the opposite trend. Plant apparency will also affect the ability of the insect to find its host. Vulnerability of the plant to attack depends on its size, growth form and permanence, in addition to the relative abundance of the plant in the community (Pimental 1961). Perennial plants can afford to produce relatively expensive products to protect

themselves, while ephemeral plants tend to rely on escape and metabolically "cheap" materials. Monocultural practices increase apparency.

Volatile chemicals released by plants can also lead to increased plant apparency. Most plant volatiles have chains of fewer than 20 carbons, usually with a molecular weight under 300 (Finch 1980). The lower the molecular weight, the more volatile the compound, while olfactory efficiency decreases. At the same time, due to differences in weight, different gases will diffuse at different rates, though similarity between weight of the various compounds released by a plant may diminish this effect. Individual plants will also vary in chemical composition due to age, environment and genetic makeup. Hence the insect must be responsive to a wide variety of chemicals and cannot be too precise in its plant recognition tactics.

Problems exist in determining the makeup of the volatiles emanating from a plant since the odors around a plant may not be the same as those extracted from within the plant. Air from the headspace of cotton, Gossypium hirsutum, contained only 6 of the 58 chemicals known to occur in cotton seed essential oil (Hedin et al. 1975).

It is unknown how many primary odors an insect must respond to effectively to locate a host plant. Wright (1964) suggested that sensitivity to 6 primary odors would give the insect the possibility of recognizing 2^6 or 64 combinations. Ma and Visser (1974) hypothesized for the CPB that there is no single chemical responsible for

attraction, but it is rather the summation and integration of neuronal impulses from at least five different olfactory receptors.

An insect may respond in different ways to a chemical stimulus. An alignment to a chemical gradient is chemotaxis, while movement along that gradient is kinesis. Changes in rate of movement are referred to as orthokineses, while a change in turning rate is klinokinesis. Finally, odors can act as releasers, and the insect will respond by moving upwind (anemotaxis) or at an angle to the wind (anemomenotaxis). The latter two responses require complicated orientation mechanisms while in flight.

Host plant chemicals may be classified as primers or releasers (Wilson and Bossert 1963). A primer chemical alerts the insect that a food source is located in the vicinity, but no response beyond a heightened sensitivity to releaser chemicals is produced. A releaser chemical releases the behavioral response, and causes the insect to begin to actively search for food.

Wilson (1963) defined active airspace as that volume of air downwind from an odor source where the concentration of volatile chemical stimulants is great enough to produce a behavioral response in the insect. As these airspaces are located within the boundary layer, any insect actively searching for plants by odor plumes must fly low over the vegetation. Plant apparency becomes diminished during

periods of strong winds due to rapid dilution of plant volatiles.

Tracking of the odor plume is accomplished either with an anemotactic response or with an anemomenotactic response. Linsenman (1972) found that dung and tenebrionid beetles tracked 30 off of an upwind course. Such a zigzagging course would help to keep the insect from flying too far out of the plume and to locate the plume if lost. Kennedy (1977) suggests that reversing anemomenotaxis might be the most important component in leading insects up odor plumes.

Wright (1958) proposed an alternative hypothesis of orientation based upon the filamentous nature of an odor cloud. An insect flying upwind would encounter pulses of stimulants at increasing frequencies and this leads the insect upwind.

The size of the active airspace of a plant is determined primarily by

1. Wind and terrain properties.
2. Diffusion properties of the chemicals involved.
3. Amounts of the volatile chemical released by the plant.
4. Efficiency of the insect olfactory apparatus.

The olfactory sensitivity varies, but in general the behavioral threshold is 1/10 that of the electrophysiological threshold. For Delia brassicae, the latter was found to be 10⁻¹¹ g/liter while the former

may occur at concentrations as low as 10^{-12} g/liter (Finch 1980). The sensitivity of the CPB olfactory apparatus may also lie within this region (Finch 1980).

Douwes (1968) suggested that most host plant finding is the result of starvation rather than the direct effect of any host plant volatile chemicals. Hence, the insect must be in a physiological state receptive to the volatiles before any response will occur. This receptive state is not a simple on/off switch, but is the result of integration of a number of internal and external inputs (Dethier 1982, Miller and Strickland 1984).

Colorado Potato Beetle - Biology

The Colorado potato beetle (CPB) was an obscure insect in the early part of the last century. It was found on natural weeds, mostly buffalo bur, Solanum rostratum, and was known to occur in Mexico and the eastern foothills of the Rocky Mountains. S. rostratum is found in open, semi-arid grassland (Whalon 1979), where it is adapted to the exploitation of shifting and patchy habitats.

The CPB emerges from diapause in the spring on the basis of temperature and humidity cues. Alfaro (1943) gave $10-11^{\circ}$ C as the average ambient temperature at the onset of emergence, with $14-15^{\circ}$ C as the optimum. Grison (1962) reported that 590-610 DD were accumulated when 75% of the beetles had emerged in France (no base temperature was provided). In New Jersey, Lashomb et al (1984) found

that 50% of the beetles had emerged after the accumulation of 70 DD and 75% after approximately 100 DD. A temperature base of 9.8°C was used in their study, starting on March 1. They derived the following equation to predict beetle emergence

$$Y = (100) \left[1 + e^{-0.7991 - 0.0234X - 10.62} \right]$$

y = percent beetles emerged

x = DD accumulation

If the beetles fail to find food sources after emergence, they must disperse by walking or flying to locate food resources. De Wilde and Hsiao (1981) reported that dispersal in the spring is accomplished mainly by walking, while in the summer, when temperatures are higher, most beetles disperse by flight. These results are from within-field dispersal observations of adult beetles. When dispersing by walking, the beetles are sensitive to both micro- and macro-terrain variations. The types of vegetation covering the soil are also important. Ng and Lashomb (1983) showed that the time it took for beetles to walk 3.04 m varied from 5 min to 2 h 30 min on bare soil, while in a wheat field the corresponding times were 82 min and 8 h.

Dispersal by flight can be either micromigration over short distances by flying into the prevailing wind, or macromigration, flying with the wind for long distances. Brcaak (1950, cited by Wegorek 1959) calls the long flights semipassive.

When the adults have located a suitable food source,

they begin to oviposit. Several studies have indicated that feeding is necessary for both copulation (Gibson 1925) and oviposition (Grison 1947). Grison (1950) has indicated that 22°C is the optimum temperature for maximum fecundity. The eggs develop in 5 to 21 days. The larvae pass through 4 instars in 21 to 43 days. The fourth instar is responsible for over 75% of the food consumed during larval development.

Pupae develop in 7 to 21 days and the adults which emerge earliest may lay additional eggs while those beetles which emerge later merely feed. Those females which do not oviposit are better prepared physiologically for diapause (Wegorek 1957).

The beetles enter diapause when the photoperiod drops below a critical length. This critical time depends on the climatic conditions in the region where the race was collected.

To effectively exploit its natural host plant, the CPB must be able to locate isolated stands of S.rostratum and optimal host plant finding should be important. While exact flight costs for the CPB are unknown, many beetles experience high energy use during flight due to high wing loading and wingbeat frequency (Johnson 1969).

McIndoo (1926) demonstrated attraction of CPB to potato plant odors in a Y-shaped olfactometer. Schanz (1953) found that this response was largely eradicated when the terminal four antennal segments were amputated.

Wind tunnel experiments (de Wilde 1969, Visser 1976,1978) have indicated an odor-conditioned anemotaxis orientation in the CPB. Visser et al. (1979) isolated several of the components of potato plant odor. These were collectively termed green leaf volatiles by Visser and Ave (1978).

Ma and Visser (1978) studied olfactory coding at the single unit level. Evidence was found of at least five different types of receptors. Green leaf volatile sensitivity was demonstrated in four of these while the fifth was primarily sensitive to methylsalicylate. While electro-antennagram responses of the beetle were not plant specific (Visser 1979), the responses and balance between the individual receptors showed potential for discriminating between species. One pair, for example, could potentially discriminate within the Solanaceae between the genus Solanum and crushed tomato leaves (genus Lycopersicum).

No single green leaf volatile proved to be attractive to Colorado potato beetle (Visser and Ave 1978), and the addition of any one of several of these substances into an airstream passed over potato plants reduced the response of the beetles. They concluded that the response of the beetle is due to the precise ratio of green leaf volatiles given off by S. tuberosum. Since green leaf volatiles are common in many families of plants, a highly diverse community, where many plants contribute to the total concentration of such volatiles in the airstream would

complicate host plant finding by the CPB. S. dulcamara has been shown to be as attractive as potato in the lab (Hsiao 1978), but in the field it is rarely heavily infested despite its close proximity to large populations of CPB. The position of S. dulcamara in diverse ecosystems may serve to mask its odors, while large stands of S. tuberosum are more easily found.

Very little work has been done on the effects of visual stimuli on CPB. De Wilde (1957) found that larvae prefer red to green. This preference may be dependent on the physiological state of the beetle. Gotz (1958) has shown that the larvae of Vanesse urtica L. are attracted to green in the feeding stage, but this preference changes to brown shortly before pupation. The same is possible for both larvae and adults of the CPB.

To date, little work has been done in the field on these phenomena. Hawkes (1979) has pointed out some of the pitfalls involved with the use of wind tunnels. In the case of the cabbage root fly, Delia brassicae (L.), tests done in small wind tunnels showed no odor-conditioned anemotaxis, while the opposite was true in larger wind tunnels. Field work is needed to address the applicability of wind tunnel experiments to the field.

Ng and Lashomb (1983) found that post-diapause beetles preferentially orient towards the northwest in the field in New Jersey. The hypothesis is suggested that in its native habitat, CPB will often emerge from diapause in

an area without food. The northerly orientation would bring beetles in mountainous areas to warmer microclimates on south facing slopes. These beetles would be warmer than those not moving onto slopes and would tend to fly earlier and find suitable hosts sooner.

While crop rotation is not an uncommon practice in potato production, there is little data on its affects on CPB. Wright (1984) and Lashomb and Ng (1984) both demonstrated that rotation will delay population buildup of CPB. Lashomb and Ng reported that in fields rotated out of potatoes and planted in wheat, beetles did not begin to walk until temperatures reached 15° C. Conditions for flight from the field, which were assumed to be exposure to 6 h. of intense insolation and temperatures above 25° C, would only be obtained for 2-3 h/day. The wheat acted as both a mechanical and environmental barrier to beetle movement.

To effectively predict pest outbreaks and population growth within fields, it is necessary to determine how immigration and emigration will affect the population dynamics of this pest. Any attempt to model the system will need information on the dispersal from nearby crop and noncrop host plant communities.

The objectives of this study were to:

- 1) determine movement patterns of CPB in response to small isolated potato plots. A long range goal was to determine mode and speed with which beetles migrate from one field to another, and how directed this movement is.

2) determine if CPB in Michigan migrate by flight in the spring and if so, what parameters affect flight initiation. This information was to be used as a first step in evaluating the effects crop rotation would have on CPB movement.

CHAPTER 1

The behavior of the Colorado potato beetle, Leptinotarsa decemlineata (Say), in response to isolated potato plots in circular arenas.

Complete reliance on insecticides for management of the Colorado potato beetle (Leptinotarsa decemlineata (Say)) has proven unsuccessful as the beetle has become increasingly resistant to pesticides, and interest has developed in alternative methods of control for this insect. Wright (1984) and Lashomb and Ng (1984) both reported that crop rotation was effective in retarding CPB population buildup. Since the potato plant is most sensitive to beetle defoliation during tuber initiation and bulking (Beresford 1967, Hare 1980), which occurs 30-45 days after planting, delays in beetle buildup can be important in reducing insecticide applications on rotated fields.

The effectiveness of crop rotation as a management tool for the control of CPB depends upon the ability of beetles to find new patches of host plants. The use by CPB of plant odors (green leaf volatiles) to locate plants was

reported by Visser (1976, 1978) in wind tunnel studies. The addition of additional amounts of any single of these green leaf volatiles to an airstream passed over potato plants reduced beetle response, apparently because a precise balance of volatiles is required for beetle response.

Schanz (1953) reported that in olfactometers the odor-conditioned anemotactic response ceased when the terminal 4 segments of the antennae were amputated. Wegorek (1959) and Caprio and Grafius (Chapter 3) were both unable to demonstrate host-plant attraction by the CPB in wind tunnel studies.

The role of visual cues in host plant attraction of the CPB has been little investigated. De Wilde (1957) examined larval behavior and found a preference for red over green. No investigations have been made on adult response to color.

De Wilde and Hsiao (1981) and Tower (1906) reported that CPB moves primarily by walking in the spring and flight occurs more frequently in the warmer summer months. In Europe, where CPB flight was of concern when the beetles were invading new territories, flight frequently occurs in the spring (Johnson 1969). Mass spring migrations across the English Channel were reported by Small (1948).

Ng and Lashomb (1984) reported that post-diapause beetles moved in a predominantly northwesterly direction after emergence. The authors hypothesized that this is an adaptation by beetles to move onto southerly mountain slopes where increased temperatures would allow for early

spring flight following emergence.

The objectives of this study were: 1) to examine CPB movement in circular arenas and to determine if beetles move to small, dispersed plots of plants by walking or by flight; 2) to study if beetles would move primarily to upwind plots as suggested by Visser's data, or if plots in all directions received equal numbers of beetles, suggesting the use of other (visual) cues or even random motion; and 3) to study the importance of visual cues examined.

Materials and Methods

1985. Each arena was 70 ft in diameter, with a plot of 16 potato plants (var. Atlantic) in each of the four ordinal directions. Between the plots, a circular pitfall trap was constructed using 4" drainage tile laid on its side with the upperside removed and dug into the soil (Figure 2). Oil was placed in the bottom of the tile to prevent beetles from escaping. A similar pitfall trap was placed on the inside edge of two randomly selected plots in each arena. Beetles were released in the center of each arena and the plots and tile were sampled daily. Sampled beetles were removed from arenas.

Weed control in the arenas was accomplished by tillage and applications of metribuzin (preplant) and spot treatment with glyphosate (postplant) at normal field rates.

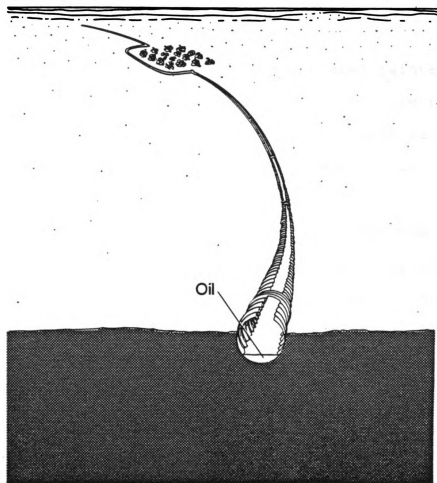


Figure 2. A sectional diagram of a 1985 beetle release arena.

Beetles used in this experiment were collected at the Michigan State University Montcalm Potato Research Farm (Entrican, MI). Tests were conducted with post-diapause and first generation (summer) adults. Releases were replicated over three arenas.

Beetles were marked with Testor's enamel paints. Two dots of each of two colors were applied to the elytra after they were washed with acetone. These marks were sufficient to identify beetles to the date and arena in which they were released.

1986. Observations in 1985 indicated that beetles may have been following the pitfall traps around the circumference until potato plots were located. Three new arenas were constructed, each with four plots of 16 plants arranged in a 70 ft diameter pattern. These plots were surrounded by a 100 ft diameter circle of potato plants, which functionally replaced the circular pitfall traps used in 1985.

Each plant in both the plots and the outer ring was sampled daily or twice daily, and the number and identity of the beetles were recorded.

Post-diapause beetles were marked as in 1985, while summer adults were marked using 1.5x2.5 mm paper labels which were glued to the left elytra (appendix A). These marks were unique to each individual, and beetles were allowed to remain in the field after sampling. Multiple recaptures were therefore made for most beetles.

Weather parameters in 1986 were recorded using a

Campbell Scientific Inc. CR21 Micrologger. Hourly measurements of temperature, windspeed, wind direction and a calculated wind vector were recorded.

In order to estimate the number of beetles in diapause below the potato plots, following plant senescence (in September and October), the soil beneath 8 plants in each plot was excavated to a depth of 12" and checked for the presence of marked potato beetles. Similar samples were dug randomly within the arenas.

One randomly selected plot in each arena was surrounded by a single layer of cheese cloth, 1 m high and raised 4-6 cm off the ground (Figure 3). This was designed to reduce visual cues potentially affecting beetle orientation to these plots. Five releases of summer adults were made in each arena. Results were analysed with an ANOVA test, treating each plot as a separate treatment and by the use of orthogonal contrasts to compare covered versus uncovered plots.

Statistical analysis: The percentage of beetles arriving daily to each plot (of total beetles arriving at all plots in that arena on that day) was correlated with an index of wind speed, direction and duration (Figure 4). To determine this index, only those wind vectors which lay within a 90° arc of the direction of the plot were considered (i.e., for the east plot, wind vectors from 0° through 180° were included). These vectors were then weighted by the cosine of deviation of that vector from a

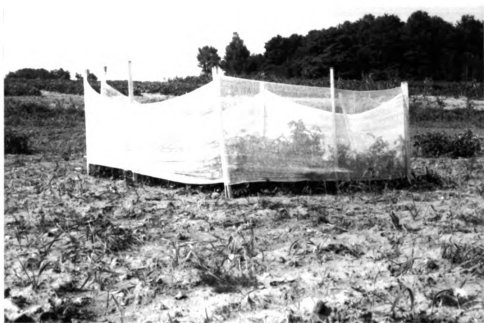


Figure 3. Potato plot covered by cheesecloth to reduce visual cues.

Wind Correlation Method

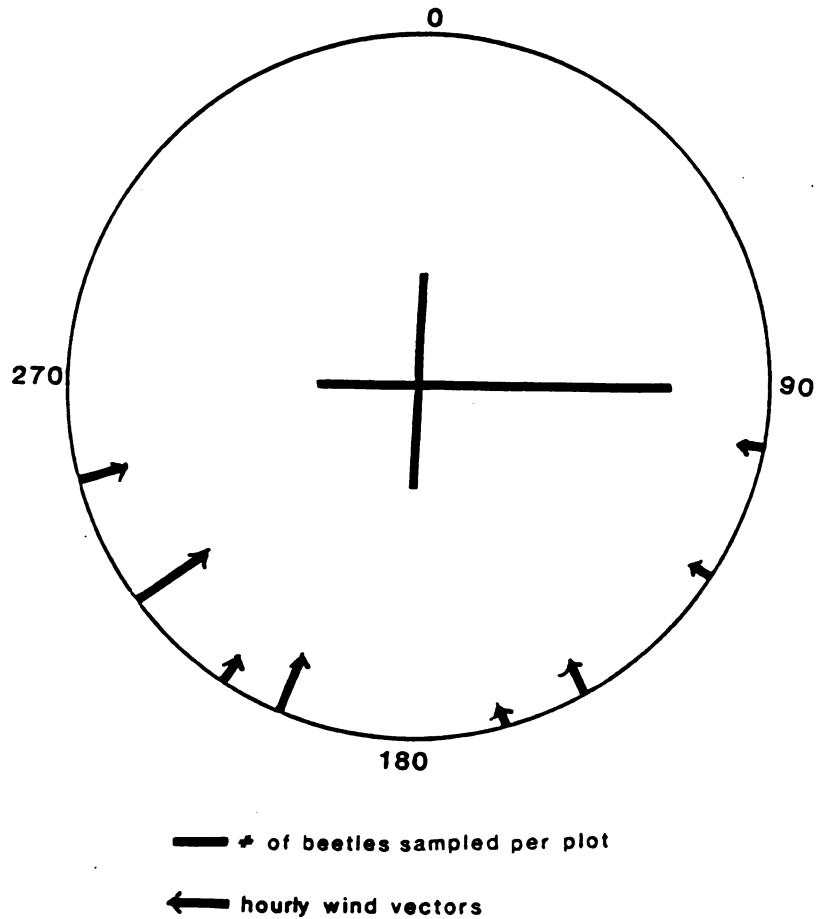


Figure 4. Wind correlation method. Correlation was made between percentage of total beetles sampled at a plot and wind index value. The value of the wind index was based on a weighted value of all wind vectors with an upwind component. Weights were assigned as the cosine of the wind vectors deviation from directly upwind.

vector oriented directly towards the plot, giving the time velocity component of the wind toward the plot. Winds blowing directly over the plot were therefor weighted more heavily than winds which blew at an angle over the plot. In the previous example, a vector from the north would be weighted with a zero, while a vector from the northeast would be weighted by the cosine of 45° ($= 0.707$). These indices would be higher on days with stronger winds, while the number of beetles arriving at a plot was a function of when and how many beetles had been released. Therefore, the daily wind indices were transformed into percentages based on the sum of all four directional indices for that date.

A percentage wind index for each 24 hr period in each plot indicated the relative wind speed and duration toward the plot. These values were correlated with the percentage of newly-recaptured beetles found in each plot.

Results

1985. The testor enamel marks used to mark beetles tended to flake off, but greenhouse tests (Figure 5) indicated that on the average only 0.5 marks per beetle were lost per week, and 90% of the beetles were still marked after 2 weeks. In the field, 66% of the beetles were recaptured within one week of release. One thousand two hundred eighty two overwintering beetles were released into the 3 arenas, and 830 (64.7%) were recaptured (Table 1). Of the recaptured beetles, 630 (75.9%) were recaptured at

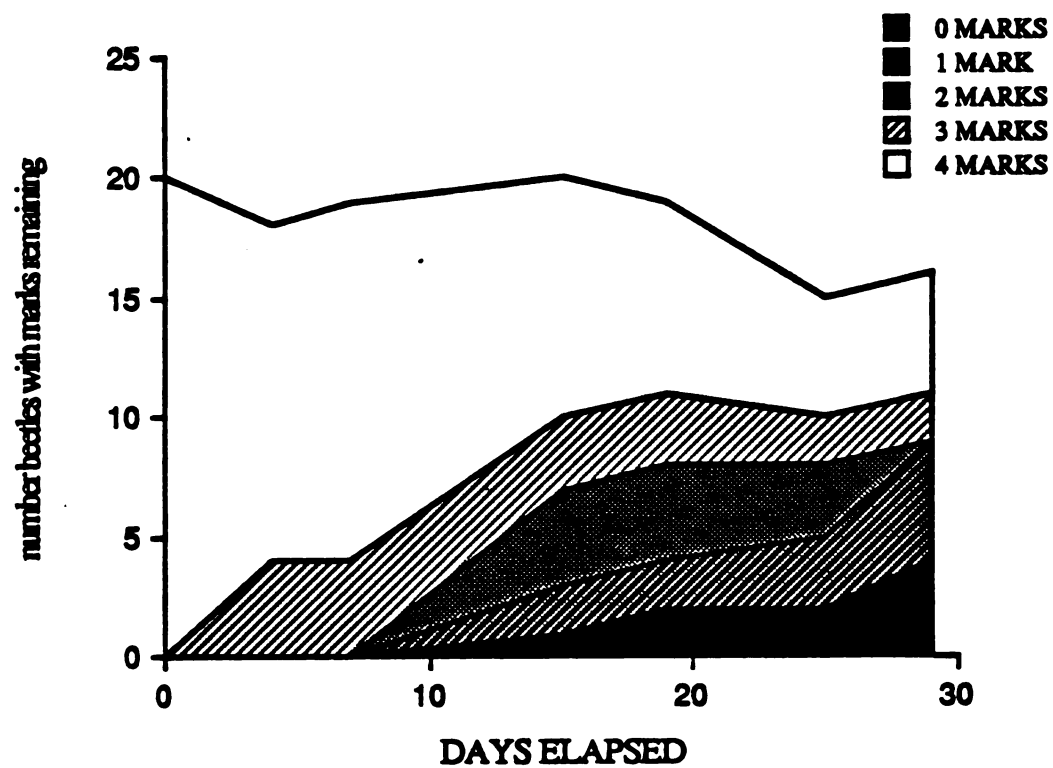


Figure 5. Marking success with enamel marks on CPB in greenhouse trials. All beetles initially had 4 marks.

the plots (either in the trap surrounding the plot or on the plants).

Recapture rates for summer adults were similar (Table 2). Releases BW, LR and BM were made in an arena where two plots were surrounded by linear pitfall traps made from gutters. At plant recaptures were low for these 3 releases, indicating that the beetles may have been avoiding these traps.

The recapture distribution of beetles found at plots is presented in Table 3. If an external factor, such as wind or geomagnetism affects the orientation of the beetles, releases made on the same day should have similar distributions. The distributions from simultaneous releases were analysed in a chi-square contingency table (Table 4) and the results indicate that differences between distributions were significant. There is, therefore, no indication of an external factor involved with beetle orientation to the plots.

The object of placing the tile around two of the plots in each arena was to determine the amount of flight activity over this barrier and to test the hypothesis that beetles predominantly disperse by walking. Field observations later indicated that this was not a suitable method for testing this hypothesis. Of approximately 100 post-diapause beetles observed flying by these arenas, only one was observed to land on a plant within the arena. All others flew outside the arena and were not recorded in the data. The estimate of flight activity from trap records

Table 1. Total post-diapause beetle recapture rates for 1985. Releases made on the same day were in different arenas.

Beetle code	Date of release	# released	% recapture	# at plants	% recaptured at plants
BG	6-14	104	84.6	58	65.9
RY	6-14	100	83.0	68	81.9
WR	6-14	101	73.3	66	89.2
LG	6-17	108	85.2	72	78.3
MO	6-17	163	81.0	102	77.3
GM	7-01	101	73.3	60	81.1
OW	7-01	98	35.7	26	74.3
YE	7-01	98	42.9	24	57.1
BO	7-01	25	16.0	4	100.0
GW	7-01	24	54.2	11	84.6
LY	7-01	25	40.0	9	90.0
EG	7-02	106	34.0	22	61.1
EL	7-02	108	80.6	76	87.4
MB	7-02	121	49.6	32	53.3
Totals		1282	64.7	630	75.9

Table 2. Total 1985 summer adult recapture rates for CPB releases made in circular arenas.

Beetle code	Date of release	# released	% recapture	# at plants	% of recaptured at plants
YL	8-16	55	89.1	27	55.1
BW	8-16	83	77.1	15	23.4
GB	8-19	84	44.0	14	37.8
LR	8-19	55	58.2	1	3.1
EO	8-20	47	42.6	9	45.0
BM	8-20	114	57.0	11	16.9
Totals		438	61.2	77	28.7

would consequently be low. The traps used were also not 100% effective, and it was noted that beetles on one side of the trap would spend considerable time probing the trap for a path across to the potato plants. Given these conditions, it is possible that a number of beetles either flew across the barrier after locating the plants or found spots in the traps where it may have been possible to walk onto the plants. In either case, the mode of dispersal was walking, yet it was interpreted in the results as flight. With these limitations it was felt that the only comparison which could be made was between short distance flight activity of post-diapause and summer adults in the single arena utilized for releases of both generations (Table 5). The differences in flight activity between post-diapause and summer adults were not significant (ANOVA, $F=1.3$, $df=1,16$).

1986. One of the three arenas was washed out in heavy rains, so experiments were only replicated in the remaining two arenas.

The recapture rates for 1986 post-diapause beetles (Table 6) show that fewer beetles were recaptured on the plants in 1986 than in the previous year. This was due to the aggregation of beetles on plants in the outer ring which was stronger than any beetle orientation to the plots. Recapture rates for summer adults are similar between years (Tables 2 & 7). In comparing between generations, more of the summer adults were recaptured on the plots. Again this was due to the aggregation of post-

Table 3. Distribution of 1985 beetle recaptures from potato plots in a circular arena.

Beetle code	Date released	<u>Number beetles recaptured in plot</u>			
		North	East	South	West
BG	6-14	12	19	7	17
RY	6-14	33	19	12	3
WR	6-14	24	6	4	24
LG	6-17	30	25	8	9
MO	6-17	13	23	15	48
GM	7-01	0	2	46	8
BO	7-01	0	0	3	0
GW	7-01	0	3	5	1
LY	7-01	0	0	9	0
OW	7-01	4	5	10	6
YE	7-01	3	7	3	9
EG	7-02	8	5	5	2
EL	7-02	33	12	9	11
MB	7-02	1	3	8	15

Table 4. Chi-square analysis of beetle recapture distributions on a per release basis. Significant chi-square values indicate that replicates released on the same day do not have similar recapture distributions.

Date of releases	# of releases	# beetles released	# beetles recaptured	X	df	p
6-14	3	305	233	35.19	6	<.01
6-17	2	271	221	32.16	3	<.01
7-01	6	371	166	51.84	15	<.01
7-02	3	335	170	28.94	6	<.01

Table 5. Comparison of flight activity between post-diapause and summer adults in one arena.

Generation	# at plant	# on plant	Mean proportion on plants ($\pm$ SE)
post-diapause	195	45	0.202 $\pm$ 0.068
summer	23	3	0.154 $\pm$ 0.081

a - Mean calculated from individual plot observations using a standard arcsin transformation.

Table 6. Total post diapause adult recapture rates, 1986

Beetle code	Date of release	# released	% recapture	# at plants	% of recaptured at plants
MG	7-21	32	40.6	4	30.8
YL	7-21	24	45.8	1	9.1
SJ	7-21	36	44.4	1	6.3
AS	7-23	48	33.3	11	68.8
LI	7-23	50	62.0	9	29.0
BO	8-01	43	58.1	22	88.0
GR	8-01	68	29.4	15	75.0
Totals		301	43.9	63	47.7

Table 7. Total summer adult recapture rates, 1986

Beetle code	Date of release	# released	% recapture	# at plants	% of recaptured at plants
W1-100	8-13	47	59.6	19	67.9
G1-100	8-14	98	65.3	47	73.4
P1-100	8-15	93	65.6	36	59.0
WAA-DE	8-18	97	74.2	41	56.9
WRA-SZ	8-21	47	44.7	17	89.5
WEO-FY	8-21	41	48.8	16	84.2
WII-MM	8-24	43	14.0	5	83.3
WF7-II	8-24	45	40.0	11	64.7
WFG-KB	8-29	46	26.1	6	60.0
GAA-BZ	8-29	48	52.1	18	90.0
Totals		605	54.0	216	68.4

Table 8. Recovery of marked summer adults from diapause in soil under potato plots.

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Beetle code	Release date	Estimated % recovery

W1-100	8-13	60.0
G1-100	8-14	22.2
P1-100	8-15	34.1
WAA-DE	8-18	28.6
WRA-SZ	8-21	94.7
WEO-FY	8-21	22.2
WII-MM	8-24	100.0
WF7-II	8-24	80.0
WFG-KB	8-29	100.0
GAA-BZ	8-29	21.0

Average		43.8

diapause adults on plants in the outer ring.

In 1985, post-diapause adults showed little tendency for aggregation in contrast to similar adults in 1986. These discrepancies may be explained by different sampling techniques (data from 1985 is on a per plot basis) and by different physical constraints imposed by arena architecture.

Three typical distributions of initial recaptures are shown in Figure 6. Maximum recapture rate occurred 1.5-2 days after release. Direct observation showed that actively-walking beetles required only ca. 1 hour to reach the plants, so this delay time must be related to the time it takes for beetles to begin to move out of the unfavorable center of the arena and the frequency and length of walking bouts.

Distribution of all (both initial and repeat) recaptures following release was at a maximum at 5.5 days following release (Fig. 7). Neither dispersal out of the arenas nor mortality within was evaluated in this study, but surveys of soil under the plots showed that an estimated 43.8% of the beetles recaptured on the plots went into diapause directly below the plots (Table 8). The number of individual beetles sampled in each plot was totaled and divided in half (only half of each plot was sampled for diapausing beetles) and compared to the number of beetles dug up from the soil below the plot. This suggests that a large portion of summer adult beetles diapause in potato fields. This is consistent with the

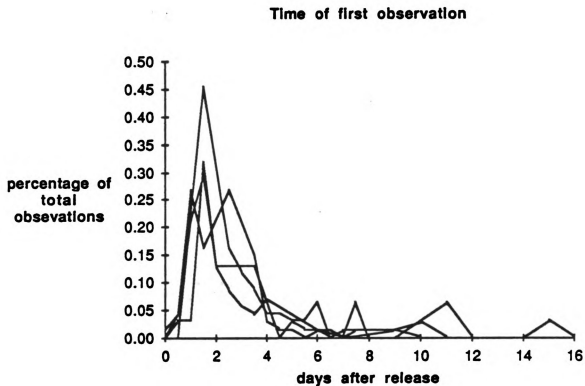


Figure 6. Three typical initial recapture distributions for summer adults in circular arenas

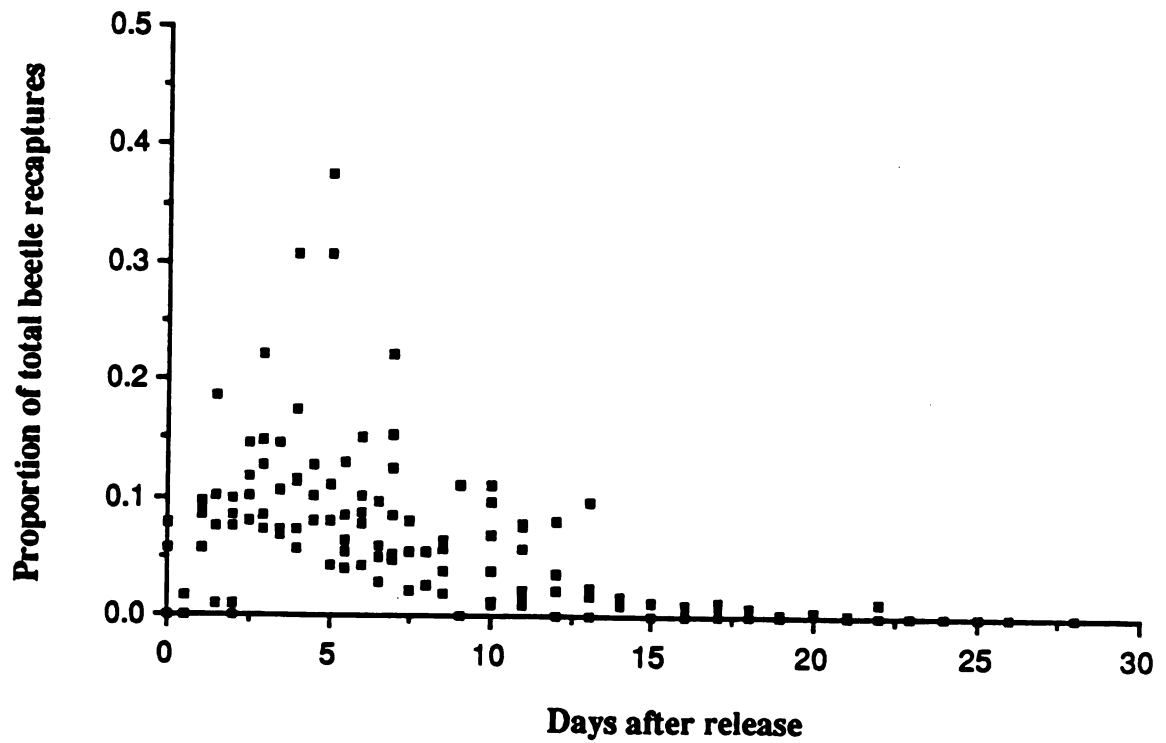


Figure 7. Distribution of daily beetle recaptures as a proportion of total beetle recapture for each release. Summer adults, 1986

observation that CPB disperses mainly in the spring in Michigan.

The residency time of individual beetles averaged $\bar{x} \pm SE$ days in the arena (Figures 8,9). Residency time decreased with later releases as beetles went more rapidly into diapause.

Differences in aggregation were observed between post-diapause and summer adults. The variance to mean ratios based on single plant samples and analysed using Taylor's power law showed significant differences (Figure 10), with the post-diapause beetles tending to be more aggregated. No mechanism which would lead to such aggregation is suggested by this research. The hypothesis that it is a result of beetles orientating in a geomagnetic direction is not, however, supported. Aggregations occurred to the east, north and west, not in one compass direction.

Adult beetle initial recapture distributions were generally not highly correlated with wind direction indices (Figure 11). In general, summer adult correlations (those after julian date 225) were higher, at least until JD 237, after which diapause effects may have become important. The low correlation for post-diapause beetles may be explained by the high aggregation of this generation, which tended to override wind effects.

Percent of total daily recapture was calculated for each plot and summed over arenas to determine if beetles had a preferred orientation (Table 9). An analysis of variance showed no significant differences, indicating that

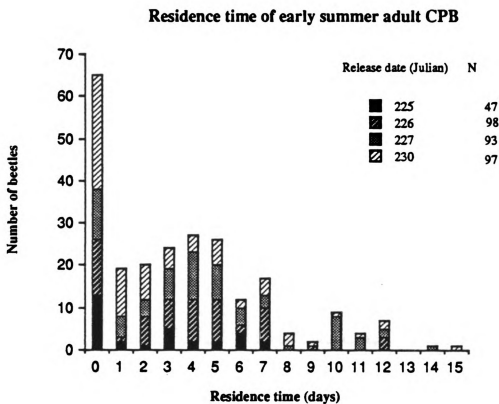


Figure 8. Residence time of early summer adult CPB.

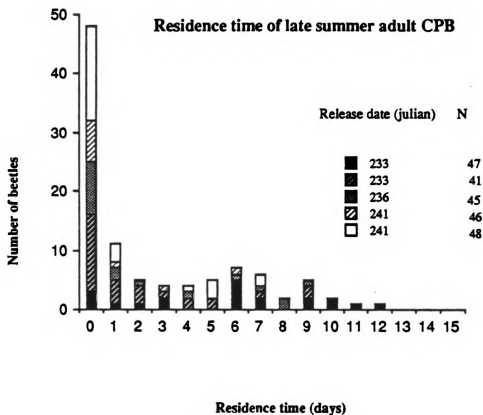


Figure 9. Residence time of individual late summer adult beetles (1986).

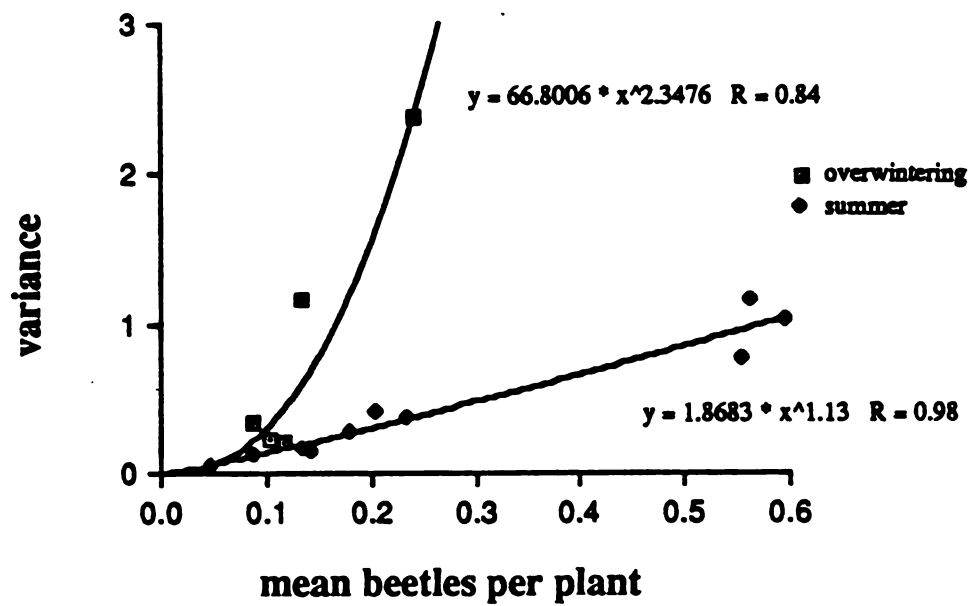


Figure 10. Aggregation of post-diapause adult beetles in circular arenas, 1986.

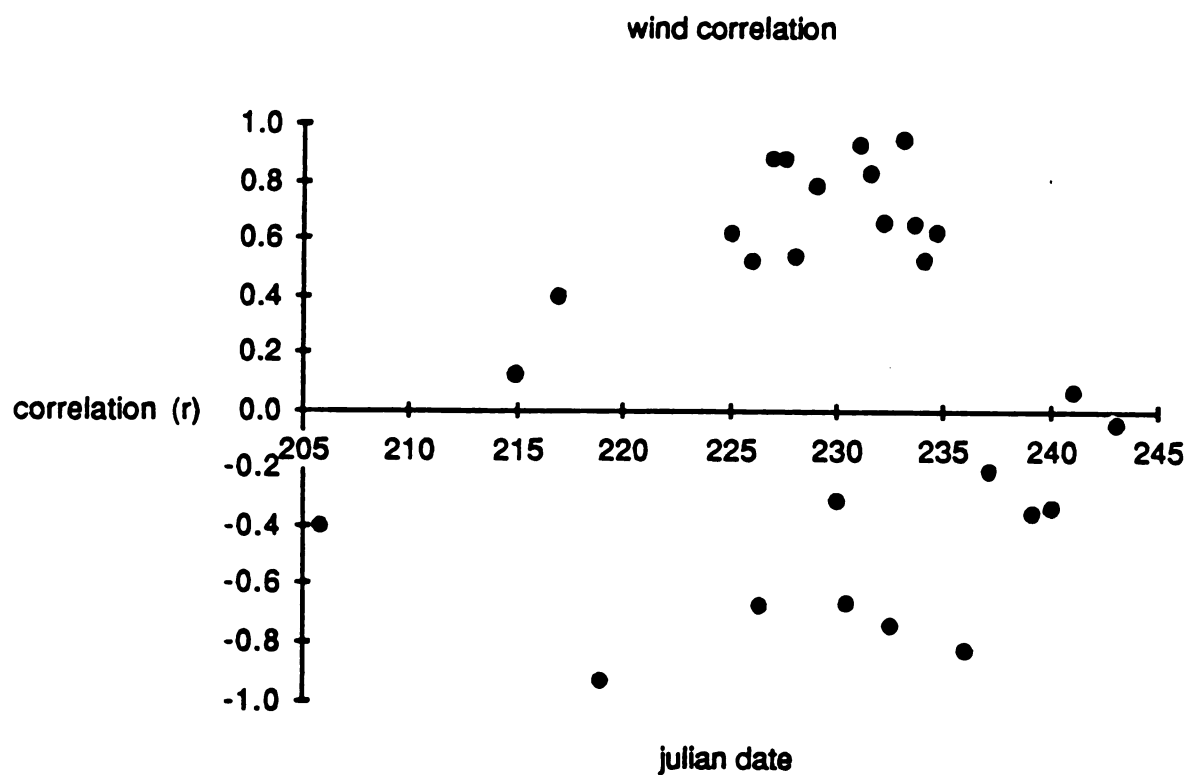


Figure 11. Correlation of hourly wind vectors to beetle orientation. Each point represents the correlation of 4 plots to 4 corresponding wind indices.

over the course of the summer beetles oriented to all the plots approximately equally.

Beetles did show reduced orientation to plots that were concealed with cheese cloth (Table 10), but the differences were not significant. This treatment did not remove all visual cues (plant color and shape were still somewhat visible - Figure 4), but relative to other plots they were certainly reduced, and it was expected that beetle numbers in covered plots would be lower if visual cues were important.

Discussion

These results indicate that the anemotactic response of CPB noted in wind tunnel studies is not sufficient to explain a large part of the variation found in field orientation. As each plot had the same percent of daily recapture rate, a significant amount of CPB orientation behavior may be random, at least at distances 15 m from host plants.

Directed orientation of the beetles when they are closer to the plants is not ruled out by these results. Indeed, positive host plant attraction is indicated by the recapture of 66% of recaptured beetles (in 1986) in plots. These plots only accounted for 11.6% of the circumference of the arena. Assuming that the beetles cross out of the arena only once, then 12% should have been found on the plots. If the attraction cues are not active from 50 ft, then beetles may be committed to a particular direction on a random basis and only after covering some distance become

Table 9. Average percentage of daily summer adult beetle recapture by plot (1986).

=====	
Plot	Recapture (%)

North	24
East	27
South	23
West	26

Pooled SE	02

Table 10. Average plot recapture over 5 summer adult releases. Starred plots were covered by cheesecloth (had reduced visual cues).

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Average plot recapture (over 5 releases)		
Plot	Arena 1	Arena 2

North	5.2	5.0*
East	5.8	7.0
South	4.6	6.0
West	3.6*	6.8

Pooled SD	6.4	4.1

attracted to nearby plants.

The post-diapause adults released in 1986 showed strong tendencies to aggregate when measured on a single-plant sample basis. As CPB females are probably unmated before diapause, it is reasonable that dispersing adults would have a mechanism to aggregate on plants in the spring. It is, however, unusual for an insect to disperse, perhaps over long distances, without having mated.

The results of this research indicate that olfactory and visual cues are probably not active over distance of 15 m, at least for plot sizes of 16 plants. In field situations, directed movement between fields has been noted (Gibson 1925), but it is not certain what the causative factors were in these cases. There is also evidence that CPB does overwinter in large numbers in potato fields and that rotation of potato fields can be important in reducing CPB infestations. The use of trap cropping, in conjunction with the aggregatory behavior of post-diapause beetles, is suggested as a possible means of further delaying beetle dispersal. To maximize gains from crop rotation, rotated fields should be more than 400m apart, as data reported by Skuhravy (1968) indicates that this may be a critical distance beyond which beetles will disperse by flight (Figure 12).

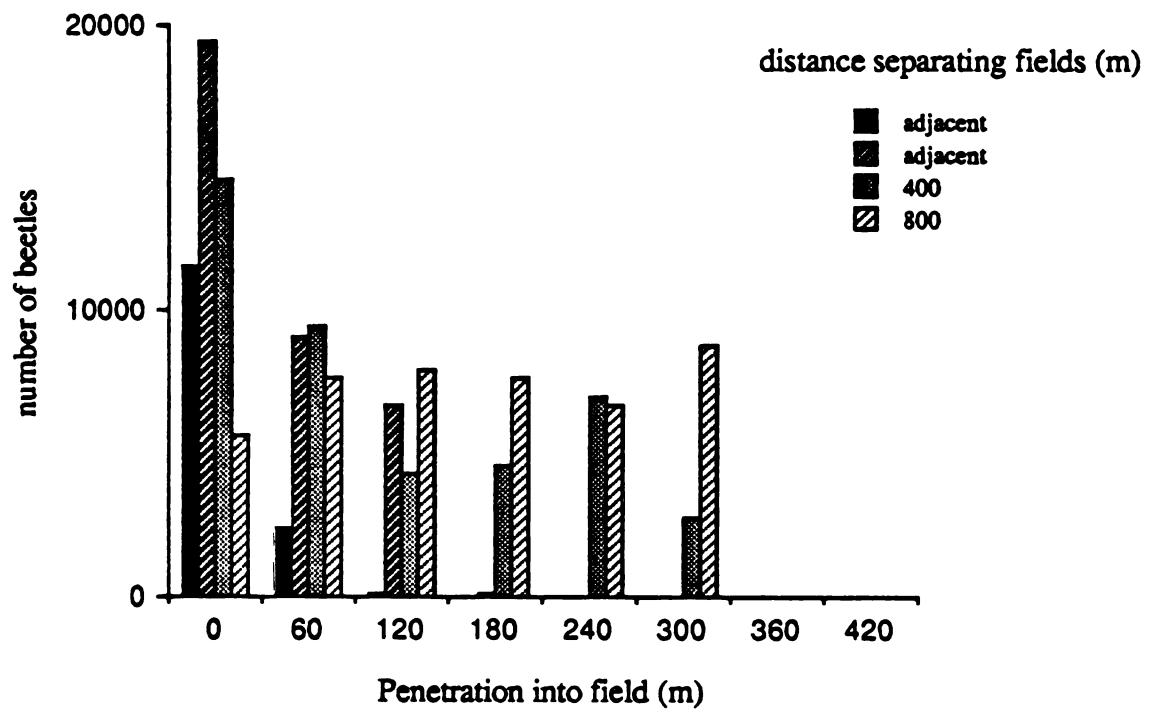


Figure 12. Spread of CPB into fields at varying distances from initial release point (Skruhavy et al. 1968).

CHAPTER 2

The effects of light, temperature and feeding status on flight initiation in post-diapause Colorado potato beetle, Leptinotarsa decemlineata (Say).

The Colorado potato beetle is known to disperse both by walking and by flight. De Wilde and Hsiao (1981) reported that spring dispersal occurs mostly by walking, while flight becomes predominant during summer. Recent results in Massachusetts (Voss 1986) support this view. Tower (1906) suggested that most migration in North American beetles occurs in the fall prior to diapause, while de Wilde (1962) found similar results in southern Europe. In Canada, Gibson et al. (1925) reported that flight "in search of food plants is one of the first acts of the beetles when they emerge naturally".

In Europe, the need to predict CPB migrations into uninfested areas prompted research into beetle flight. Wegorek (1959) and Johnson (1969) both reported mass spring migrations in northern Europe. The invasion of the island of Jersey by spring migrants was detailed by Small (1948). In late May, large numbers of post-diapause potato beetles flew over the Channel and were washed up on the shore.

Johnson (1967) has proposed that the physiological status of the beetles prior to diapause affects the time of dispersal (Figure 13). If the beetles emerge from pupation in early fall, dispersal and feeding will occur in the fall and spring dispersal will be reduced because of early sexual maturity. Conversely, if the beetles emerge in the late fall, they go into diapause while still sexually immature and most dispersal will occur in the spring. This assumes that the oogenesis flight syndrome (Johnson 1969), where sexually immature females tend to disperse, adequately describes the condition of the CPB.

Grison and Le Berre (1953) reported that beetles use up glycogen energy reserves after emergence and lose the capacity to fly after a few days. Both temperature and solar insolation affect flight initiation in the CPB. Maximum flight occurs in the 20-25°C range (Johnson 1969, Wegorek 1959), with a minimum temperature of 17°C before flights were initiated. The percentage of beetles flying increases with increasing daily solar insolation (Le Berre 1962).

Little research has been done on the duration of CPB flight, but beetles have been captured 12 miles out to sea (EPPO 1951, 1957). Mayne (1931) reported beetles traveling at least 150 kilometers, though several flights may have been used to cover this distance.

The objectives of this study were to determine how temperature affects flight initiation in Michigan beetles and to assess the effect of starvation/feeding on flight

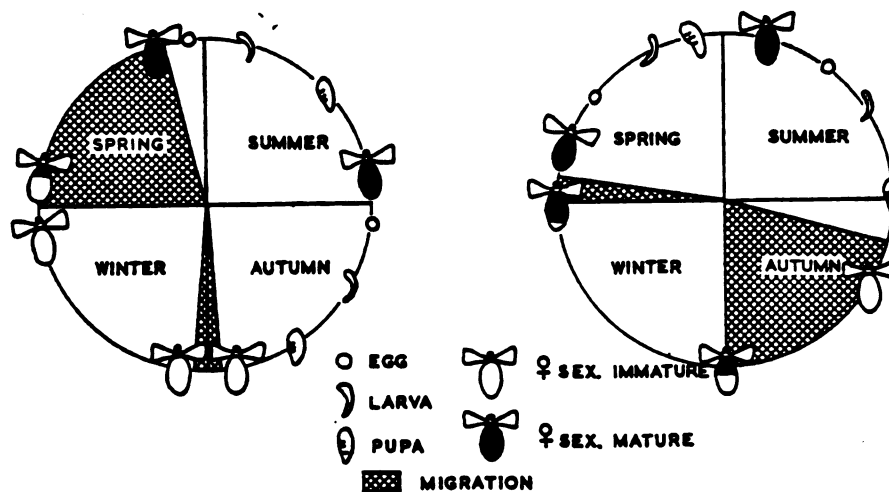


Figure 13. Hypothetical relation between developement of ovaries and seasonal migration. In the left diagram, adults which emerge in the fall are overtaken by winter before dispersing, and most dispersal occurs in the spring. In the right diagram, beetles disperse before being overtaken by winter (from Johnson 1967).

behavior (flight initiation and distance of flight).

MATERIALS AND METHODS

CPB were dug from the soil at the Michigan State University Montcalm Potato Research Farm (Entrican, MI) while still in diapause in April of 1985 and 1986. The beetles were stored in soil in 0.5 liter containers at 5°C. Ten days before use the beetles were transferred to a growth chamber at 27°C and were allowed to emerge. The containers were checked daily and emerged adults were placed in a 20°C growth chamber (16:8 hr photoperiod). The adults were not fed except where stipulated as a treatment. Studies were conducted in June 1985 and June/July of 1986.

The effects of temperature and feeding status on flight initiation were studied in the field. CPB were released 5 at a time on barren soil and their behavior noted for 20 minutes. Beetles remaining after 20 min. were removed and not used again. Flight characteristics such as height, distance, and orientation relative to wind direction were recorded.

A randomized complete block design was used with each treatment replicated once per block and blocks repeated over time to reduce variability associated with time and temperature differences.

Temperature effects: Field experiments on temperature effects were conducted by starting beetle releases at sunrise when temperatures were low. Continued releases were made every half hour until late afternoon.

Feeding effects: To examine the effects of feeding on flight behavior, an initial experiment was run comparing the behavior of beetles starved 2 wks following emergence to beetles which had been fed 1 wk. prior to release.

A second experiment in which the behavior of unfed beetles (10 days after emergence) was compared to the behavior of beetles provided with food (potato leaves) for 7, 3 and one day prior to release. Each experimental unit consisted of five beetles fed the same length of time and released as explained above. Results were analysed with regression analysis.

To determine if starvation following feeding affected beetle flight, beetles emerging from diapause were fed 7-10 days and then starved 7, 3 or 1 days. Releases were made in the field using methods noted above. The behaviors of these beetles were compared with control (fed) beetles.

To study the effects of light intensity, laboratory experiments were conducted using a wind tunnel. The tunnel, 5.5' x 3.5' and 3.5' high was constructed using 3M stretch plastic (patio door insulating kit) for the walls stretched over an angle iron frame (Figure 14). Two layers of baffling, each consisting of 4 sheets of cheese cloth and separated by 4", were used between a 21" floor fan and the wind tunnel to create laminar air flow. Windspeed, measured using an AEI type 3002 velometer, averaged 9 cm/sec. The base of the tunnel was made of plywood. 7 wooden blocks (5x5x10cm) were added to provide terrain for the beetles. Light was provided by four 3200K tungsten



Figure 14. Rotatable wind tunnel.

lamps, placed over the corners of the tunnel. Light intensity was 0.45 lux (measured using a Belfort Instrument Company helical pyronometer model 5-3850. placed in the center of the wind tunnel).

An experimental unit consisted of 5 beetles released in the center of the wind tunnel and observed for 15 minutes. Records for each beetle were kept on flights (tarsi left the base of the tunnel) and attempts to fly (wing spreading but tarsi remain on base).

Light effects: To determine if the amount of light affected flight initiation, groups of beetles were released in the wind tunnel in a completely randomized design. Treatments involved varying light intensity by turning on two, three or four of the floodlamps. Beetle response was measured as above.

RESULTS

Temperature effects: Beetle flight initiation was strongly correlated with ambient air temperature (Figure 15). No beetle flight was observed when the temperature was below 15°C, while all of the observed beetles flew when temperatures rose above 20°C. Between these two temperatures a linear relationship was observed. Optimal temperature for flight in this study was 5°C lower than that reported by both Wegorek (1959) and Johnson (1969).

Lower temperatures in our study generally occurred early in the day when solar insolation was less.

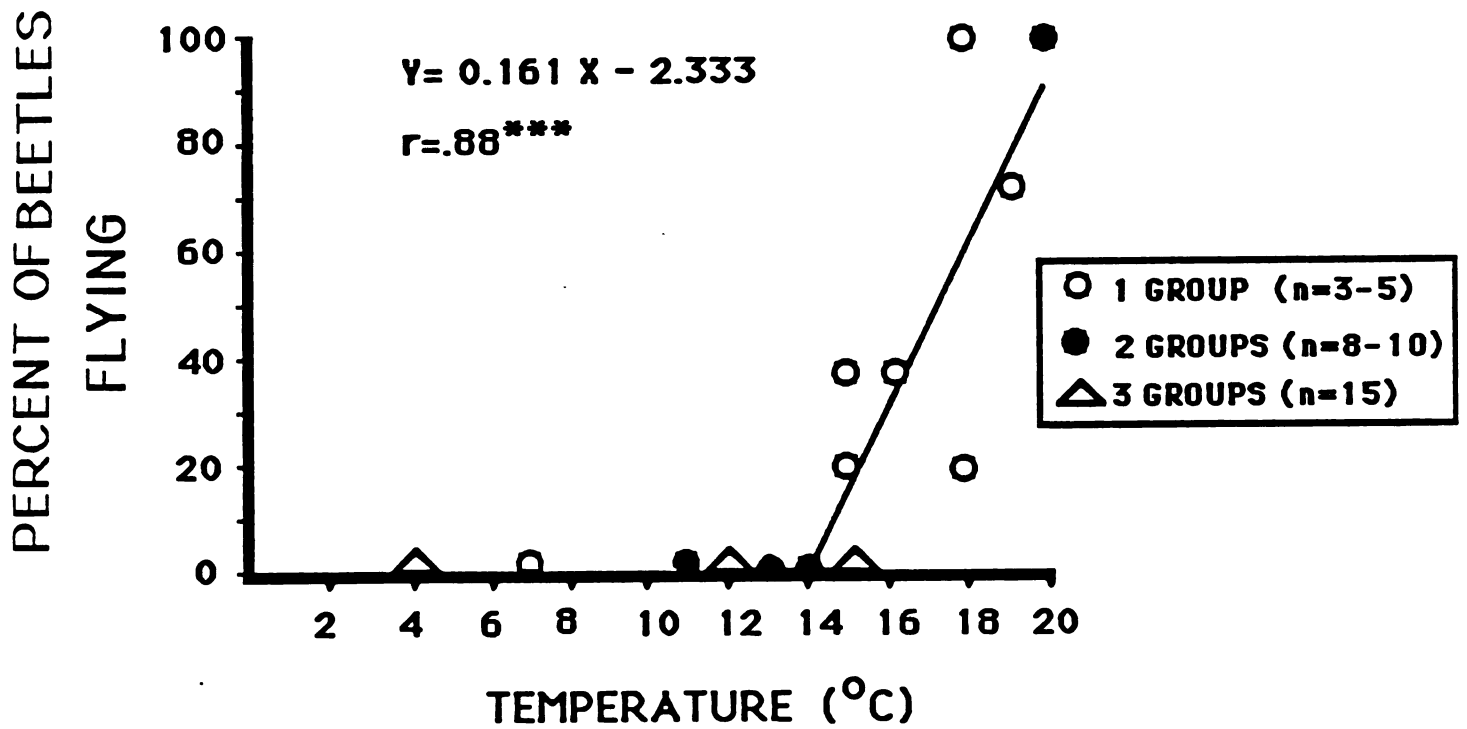


Figure 15. Effect of ambient air temperature on beetle flight initiation in the field.

Observations indicate, however, that the beetles flew when minimal temperatures were reached, not at any particular hour at a given insolation level. Temperatures above 15°C were generally not reached on cloudy days, so it was not possible to determine if both insolation and high temperatures were required for flight.

When air temperature rose to 25°C, soil surface temperatures, especially sandy soils, rose to over 35°C and beetle mortality rose markedly. Grafius (1986) reported a lethal temperature for CPB of 35-40°C in the lab.

Feeding effects: In the initial starvation experiment in the field, 70% of beetles which had not been fed after emergence from diapause (starved 10 days) flew, while only 30% of the fed beetles flew. In the second feeding experiment, starved beetles again flew most frequently, but no significant differences in flight among beetles fed different lengths of time was noted (Figure 16). There was, however, a significant difference in flight behavior between groups of beetles which had not been fed and those which had when analysed as orthogonal contrasts ($F=3.34$, $p = 0.041$).

Starvation effects: In the starvation experiments there was a tendency for the starved beetles to fly more frequently, but no significant differences were noted (Figure 17). Frequent changes in ambient air temperature, soil surface temperature and cloud cover apparently caused large variations in the results.

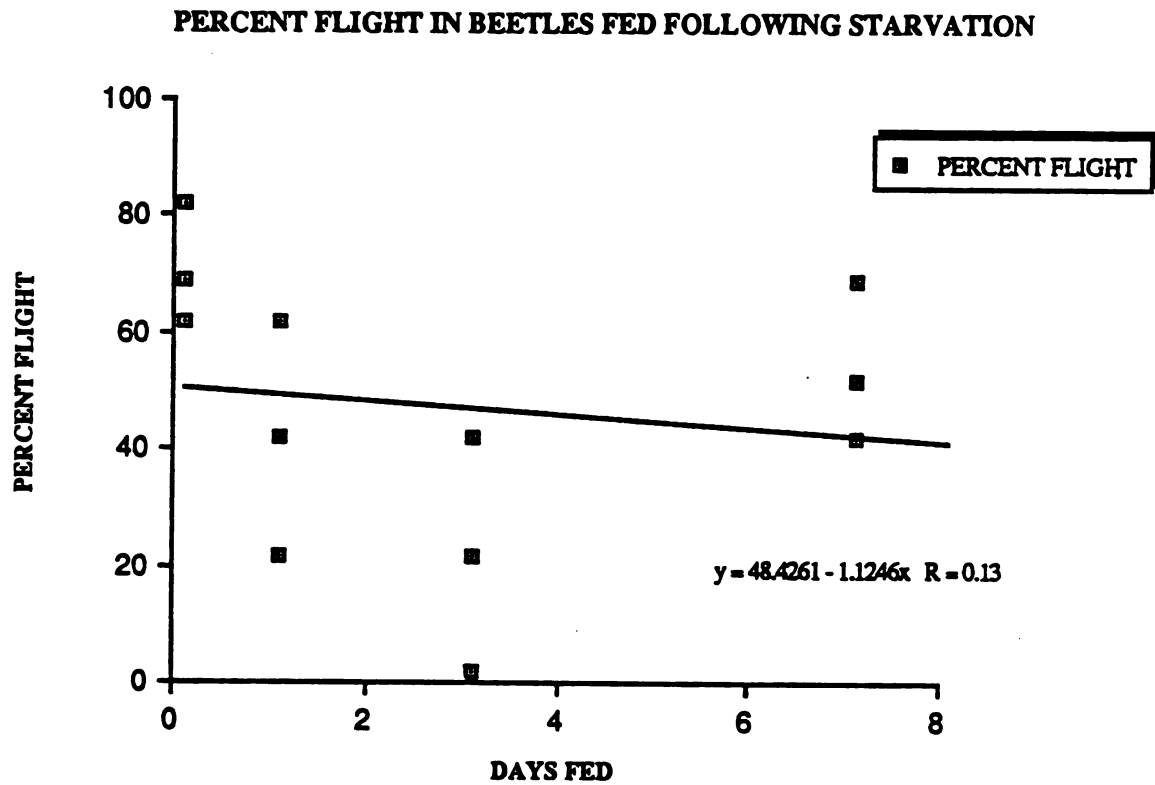


Figure 16. The effect of feeding on beetle flight initiation in the field following 2 weeks of starvation.

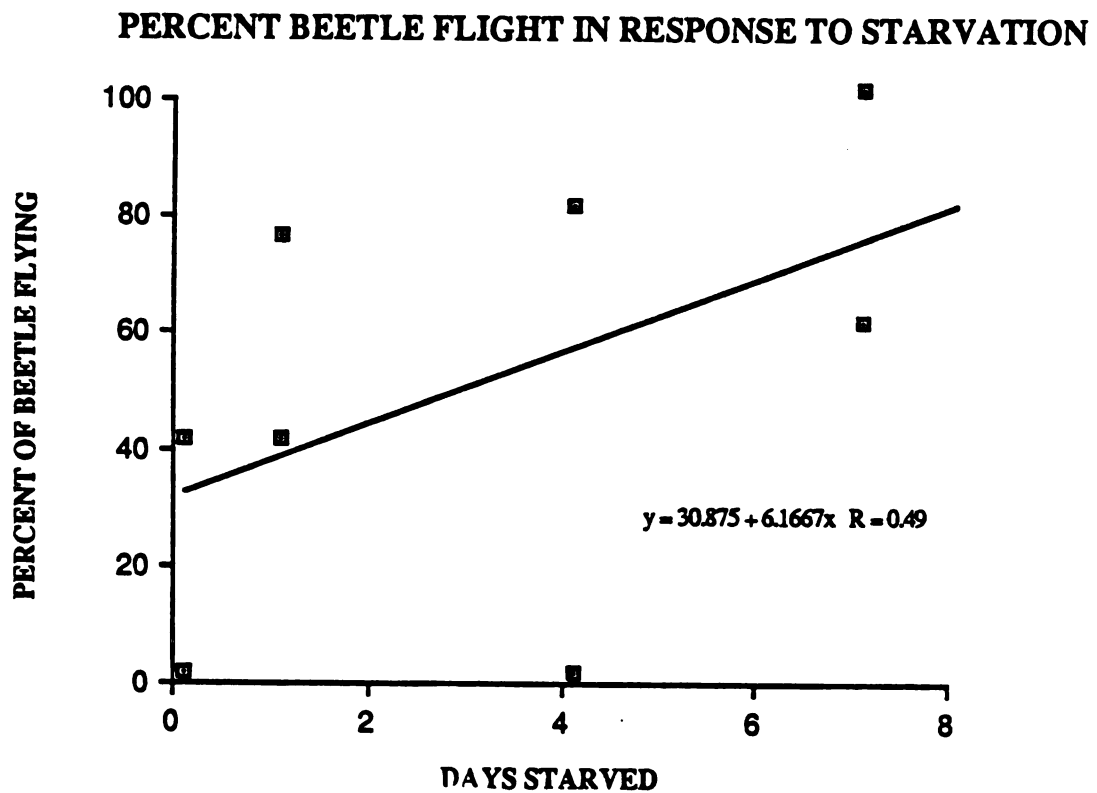


Figure 17. The effect of starvation on beetle flight initiation behavior in the field following 1 week of feeding.

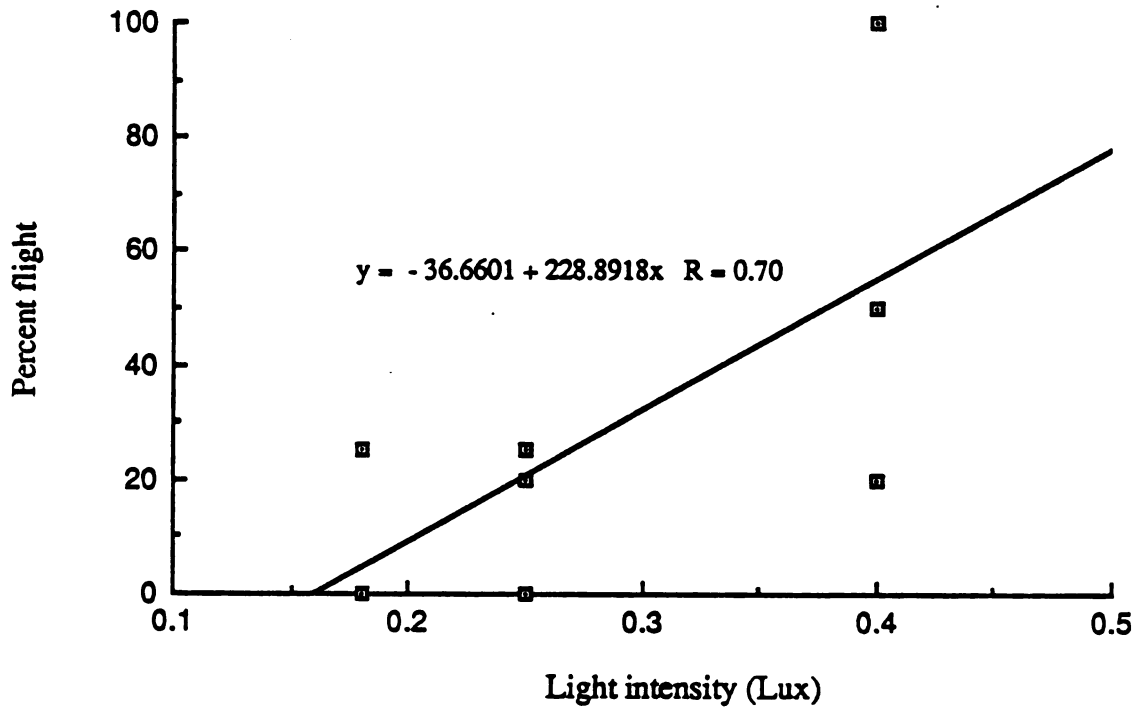


Figure 18. The effect of light intensity on beetle flight initiation in the laboratory.

The feeding status of the beetles had a definite effect on flight behavior. Starved beetles had an increased tendency to take migratory flights when compared to beetles that were recently fed (Table 11). Any flight over 15m in height was called a migratory flight, while others were indentified as short distance flights. This necessarily was an artificial division, but those beetles which reached 15m often flew much higher (>50m), flew downwind, and it was never possible to track the entire flight (flew >100m in distance)., i.e., were migratory. In contrast, it was usually possible to follow entire flights of beetles which did not gain this altitude (heights generally 1-4m) and these flights showed a greater tendency to be against or across the prevailing wind.

Light effects : The beetles flew more frequently when light intensity levels were high (regression analysis, $R^2 = 0.49$, $T = 2.59$, $p = 0.035$, Figure 18). The air temperature in the tunnel did not vary between treatments, but the increased light intensity may have elevated the beetles' body temperatures. These results support the results of Le Berre (1953) and Voss and Ferro (Univ. Mass., per. com.) where flight actvivty increased with increasing insolation.

Table 11. Type of beetle flight in response to feeding.

Treatment	N	flights >15 m high	flights <15 m high	migratory flights (%)
starved	33	19	14	57.6
fed	18	3	15	16.7

DISCUSSION

The Colorado potato beetle in Michigan is capable of migrating on warm days in the spring, possibly over long distances. This dispersal could occur at temperatures as low as 15°C, although maximum dispersal by flight would be expected at temperatures from 20° to 25° C. If Johnson's hypothesis on CPB seasonal migration (Figure 13) is correct, then Michigan beetles are overtaken by winter or short day photoperiod before migrating and most of the females in diapause should be unmated. Voss (PhD. thesis, in press) reported that in Massachusetts females stopped laying eggs in late July/early August due to photoperiodic effects. Similar daylength-controlled switches may be active in control of migratory behavior.

Beetles in Michigan were also able to fly after being starved at 20 C for as long as 3 wks following emergence (and possibly longer). This may indicate that beetles overwintering in Michigan have greater glycogen fuel reserves than the beetles investigated by Grison and Le Berre (1953).

Wegorek (1959) found that spring dispersal in rotated potato fields could be reduced through the use of trap crops. These results indicate that trap cropping might also be effective in Michigan. Beetle flight activity might not actually be reduced, but the frequency of dispersive flights, at least initially, would be significantly less. Manipulation of flight activity and

dispersal could also be used to increase or decrease gene flow between populations and help manage insecticide resistance. Susceptible populations should be encouraged to disperse while highly resistant populations should be retained locally and subjected to intensive non-insecticide management pressure.

CHAPTER 3

The behavioral response of the Colorado potato beetle, Leptinotarsa decemlineata (Say), to wind and potato plant odors in a wind tunnel.

INTRODUCTION

The Colorado potato beetle (CPB), Leptinotarsa decemlineata (Say), was in the early part of the last century an obscure insect found on natural weeds, mostly buffalo bur, Solanum rostratum. It was known to occur in Mexico and the eastern foothills of the Rocky Mountains. S. rostratum is found in open, semi-arid grassland (Whalon 1979), where it is adapted to the exploitation of shifting and patchy habitats.

To effectively exploit its original host plant, the Colorado potato beetle must be able to locate isolated stands of S. rostratum. While exact flight costs for the Colorado potato beetle are unknown, many beetles experience high energy use during flight due to high wing loading and

wingbeat frequency (Johnson 1969). Selection pressure on the Colorado potato beetle on its original host would be for optimization of host plant finding.

McIndoo (1926) demonstrated attraction of Colorado potato beetle to potato plant odors in a Y-shaped olfactometer. Schanz (1953) found that this response was largely eradicated when the terminal four antennal segments were amputated.

Wind tunnel experiments (De Wilde 1969, Visser 1976,1978) have indicated an odor conditioned anemotaxis orientation in the Colorado potato beetle. Visser et al. (1979) isolated several of the components of potato plant odor. These were collectively termed green leaf volatiles by Visser and Ave (1978). No single green leaf volatile proved to be attractive to Colorado potato beetle, and the addition of any single one of these substances into an airstream passed over potato plants reduced the response of the beetles. They concluded that the response of the beetle is due to the precise ratio of green leaf volatiles given off by potato plants.

The objectives of this study was to: 1) test the hypothesis that both chemical and visual stimulants play a part in the host plant attraction of the potato beetle, and 2) to test the hypothesis that increased periods of starvation would affect behavior leading to host plant finding.

METHODS AND MATERIALS

Insects and wind tunnel: The Colorado potato beetles used were from a lab culture collected in Antrim County, MI. The beetles had been raised in the lab for 8 months. Adult beetles were placed in cages after emergence and allowed to feed for 2 to 3 weeks. Beetles were then separated and placed into vials while starved. Filter paper in each vial was moistened every second day.

The wind tunnel used was constructed of plexiglass with baffles to provide laminar airflow. Illumination was provided by a halogen lamp placed over the center of the walking plate. A 1.2 x 0.9 m wooden walking plate with a 5 cm grid, covered by two glass plates, was centered in the tunnel. These plates were washed with a damp paper towel after each trial. The beetles were released in random directions at the center of the walking plate (Figure 19).

All experiments were conducted in a climate-controlled room with a temperature of 25°C and a RH of 40-50%.

Response to starvation for one day: This experiment was designed to test responses of the beetles starved for 1 day. Test beetles were subjected to : 1) no wind, no plants, 2) wind of 25 cm/sec and no plants, or 3) wind (25cm/sec) that had passed over potato plants. Treatments were ordered in a randomized complete block design, blocked over time. Traces were made of movement with 15 second intervals marked off. Each trial lasted 3 min after initiation of motion by the beetle.



Figure 19. Stationary wind tunnel with walking plate.

Results were analyzed using circular distributions. Angles were equated from the starting point to either the beetle's position at the end of the experiment if it had not reached an edge of the plate, or to the point where the beetle first reached an edge. Rayleigh's R was used to test for nonrandomness ($H:p=0$). Differences between two populations were tested using Watson and William's test (1956).

Response after starvation of 7 days: This experiment was identical to experiment 1 except that the beetles were starved 7 days.

Response to polarized light: This experiment was designed to test the effect of the rotation of the light source on the orientation of the beetles. It was thought that polarization of the light source might be contributing to the orientation of the beetles. Thirteen beetles were split into two groups. One group was run as above with no wind and no plants. The light was then rotated 90 degrees and the beetles released again. The same experiment was then completed on the second group of beetles. Analysis was as above.

Orientation: To determine if a preferred orientation by the beetles in the wind tunnel was a result of visual cues in the tunnel or actual orientation to geomagnetic cues, a second tunnel was constructed. This tunnel was 5.5'x3.5'x3.5' and was mounted on a rotatable base. The walls of the tunnel were 3M stretch plastic stretched over an angle iron frame. Light was provided by a single 400W

metal metal halide lamp (General Electric, # MV 400/BU/I), centered over the tunnel (0.25 lux). Wind speed was 9.0 cm/sec, and baffles between the 21" floor fan and the tunnel created near laminar airflow.

Beetles used in this experiment were collected in Antrim County, MI and had been in culture for 2.5 years. Prior to release, all had been starved 24-48 hrs. Initially, the beetles were released individually in a random order while the wind tunnel was held stationary. Responses were recorded as above, except that the beetles were tracked for 5 min. Following this, the experiment was repeated, but with the wind tunnel oriented to a new randomly selected direction after every third beetle.

The results were analysed as before, but with two sets of orientations generated from the randomly-oriented set of beetles, one with respect to the tunnel frame of reference, the other with respect to magnetic north.

Results

Response to starvation: No significant differences were found between the mean angles for beetles starved 1 or 7 days (Table 12) and these experiments were pooled for further analysis.

Beetles run under control conditions (no wind, no plants), showed a significant preferred orientation towards the northeast (Table 13, $\alpha=65$ degrees for combined experiments). The R values indicate that there was a definite non-random orientation.

Table 12. Colorado potato beetle responses to potato plant odors. Comparisons between like treatments (see Table 13 for treatments) and two different periods of starvation. (Experiment 1 starved 1 day, experiment 2 starved 7 days)

Treatments		N	Mean Angle	Angular deviation	Rayleigh's R	F value
Exp 1	Exp 2					
1	1	28	25	64	14.9**	.1222
2	2	29	334	80	10.9**	.1477
3	3	29	316	85	9.7**	.429

Table 13. Responses of Colorado Potato Beetle to potato plants in a wind tunnel. Experiment 1 used beetles starved 1 day, experiment 2 used beetles starved 7 days. Treatment 1: no wind, no plants. Treatment 2: wind (25 cm/min), no plants. Treatment 3: wind, 5 plants.

Exp	Treat-ment	N	Mean angle	Angular deviation	Rayleigh's R
1	1	15	30	71	6.9**
1	2	15	330	63	8.2**
1	3	15	309	69	7.3**
2	1	13	21	56	8.0**
2	2	14	345	103	2.8
2	3	14	335	105	2.6

The presence of wind shifted the median angle in that treatment to 116 degrees , but an increase in variability was also noted. In treatment 3 (wind and plant), the median angle was 134 degrees. The treatments summed over both experiments showed nonrandom distributions, but the individual treatments with beetles starved 7 days had nonsignificant R values, reflecting the increase in variability.

Treatments wind with no plants and wind with plants were not found to be significantly different ($F=.6069$; $df=1,56$).

Response to light: Comparison of results from the two treatments of the rotated light experiment show no significant differences (Table 14, $F= .0727$, $df=1,23$), so polarized light evidently did not play a role in the preferred orientation of the beetles.

The average angle of the pooled result of these two experiments was 65 , the same as the average angle for the no wind, no plant treatments in the starvation experiments. An F test comparing these treatments gave a value of .0008 ($df= 1,51$), indicating that the preferred orientation was not random.

A final test was done by examining all treatments in terms of either no wind or wind only. The second and third treatments from the starvation experiments were pooled and compared with the first treatment from the same experiments and the results from the polarized light experiment. The results show a significant positive

anemotactic behavior (Table 15).

Preferred orientation: The orientation of the beetles with respect to the tunnel frame of reference was not significantly different from the stationary tests and the R values for both were significant at the $p=0.1$ level, indicating nonrandom distributions (Table 16). The results with respect to magnetic north were significantly different from the stationary tests and the R value was not significant. The preferred orientation noted in the stationary wind tunnel was apparently a response to cues within the wind tunnel, not to geomagnetic cues.

Discussion

Adult Colorado potato beetles showed a definite anemotactic response to air flow, but there was no indication of an increased upwind response in the presence of potato plants. In the present experiment, whole plants in plain view of the beetles were used, and this indicates no response to either visual or chemical stimuli. Wegorek (1959) found a similar lack of response in potato beetles from Poland. Both de Wilde (1969) and Visser (1976,1978) found a similar upwind bias, but both also found that potato plant odors enhanced this response. Visser used only newly-emerged unfed beetles, and the physiological state of the beetles may affect the changes in response. Jermy (1958) found, using an olfactometer, that beetles only oriented to potato odors if they had

Table 14. Responses of Colorado potato beetles (starved 2 days) in a wind tunnel with no wind and no potato plants. Treatment difference involved the rotation of the light source by 90 degrees .

Treat- ment	N	Mean angle	Angular deviation	Rayleigh's R
1	13	29	76	5.4
2	12	20	65	6.3**

Table 15. Summary of all results with data pooled over all wind and no-wind treatments.

Treatment	N	Rayleigh's R	F value
wind vs. no wind	111	40.8***	15.04***

Table 16. Comparison of beetle orientation in a stationary wind tunnel vs. a rotated tunnel (results in rotated and geographic frame of reference).

Treatment	N	Mean angle	Rayleigh's R	F test (vs. stationary)
stationary	18	313.2	8.427**	
rotated	18	311.9	6.195*	.0018
geographic	18	209.2	2.785	7.456***

previously been allowed to feed on potatoes . De Wilde's experiments used beetles 2-3 weeks after emergence (as in the present experiment), but the beetles' positions were only recorded after 5 min., and during this time changes in the beetles orientation due to border effects (running into sides) may have affected the outcome.

The preferred orientation noted by Ng and Lashomb (1983) may also affect the outcome of wind tunnel experiments. They found beetles orienting to the northwest in the field, and neither wind nor sun were responsible for this effect. The beetles in the controls of the original experiment oriented to the northeast. However, the results from the preferred orientation study indicate that the beetles are not responding to geomagnetic cues under the conditions present in the wind tunnel study.

The results of this study indicate that CPB does have an anemotactic response to wind in wind tunnels, but that this response is not enhanced by the presence of odors from potato plants, either whole plants or damaged plants. The period of starvation did not affect the host plant finding of the laboratory-reared beetles. Finally, no preferred orientation was noted in walking tests with post-diapause beetles.

DISCUSSION

THE ROLE OF CPB MOVEMENT IN POTATO CROP PROTECTION STRATEGIES

The objectives of crop protection strategies for control of Colorado potato beetle (CPB) at the farm level are to reduce CPB numbers under an economic injury level while at the same time reducing short- and long-term costs associated with frequent insecticide use. On a larger scale, increasing concern is being given to the development of insecticide resistance in CPB and potential strategies should be evaluated in terms of their effect on resistance development.

Non-rotated crops

In fields where crop rotation has not been practiced, alternative strategies for CPB control are limited. Alternating pesticides may slow resistance development and maintain efficacy of chemicals, but only if the insecticides used have different modes of action. The selection pressure from each chemical should be neutral or negative with regard to the allele(s) affected by the second chemical. Hence the use of one chemical would relax or reverse selection pressure for development of resistance to the second chemical. Very little testing has been done

to determine how different pesticides interact in this manner.

Timing potato planting so that plant emergence does not coincide with CPB emergence from diapause is also an available strategy. Results reported in this thesis indicate that starved beetles will leave the field if temperatures are high enough. In the spring of 1986 at the Montcalm Potato Research Farm, potato planting was delayed due to heavy rains. Many beetles emerged before the plants and high flight activity was observed. However, since beetles emerge until mid-late June, this option is not exceptionally well suited to the varieties of potatoes now available to growers in Michigan. Further development of this strategy will depend in large part upon breeding programs to develop varieties which will mature rapidly and be more tolerant of photoperiod changes in late summer.

Rotated crops

Crop rotation in potatoes has been practiced primarily because of its importance in soil nutrition, reducing fungal diseases and nematode problems. The practice also impacts CPB populations by delaying buildup of damaging populations (Wright 1984) and by inducing higher mortality associated with locating host plants. Delaying host plant location reduces the number of beetles in the field when plants are smallest. Beresford (1967) has shown that potatoes are most sensitive to defoliation at tuber initiation (35-45 days after planting). The same number of beetles will defoliate smaller plants much faster than

larger plants, and EIL (economic injury levels) should be lower (on a per plant basis) for younger plants. Spraying in nonrotated fields at this time is usually ineffective as more beetles are continually emerging to replace those killed by pesticides. In rotated fields, the plants are larger and more tolerant of defoliation before beetle numbers increase. Ideally, spraying in rotated fields could be delayed until after emergence is complete, reducing the number of sprays required for effective control.

Rotation may also have important consequences for development of resistant genotypes. Each spray application will presumably increase the frequency of resistant genes in the field population. In non-rotated fields, these beetles will go into diapause and emerge in the spring as a cohesive group onto new potato plants. Any new genetic material arriving in the field in the form of dispersing beetles will be diluted by the dominant field genetic frequencies. Crop rotation would force beetles to move and decrease the cohesiveness of the field population, even when dispersing to nearby fields. This in turn would increase the relative abundance of beetles dispersing from other sources (with differing and possibly less resistant genotypes). An accurate prediction of the effects of rotation on resistance development would therefore depend upon reasonable estimates of beetle dispersal from nearby fields and from noncrop hosts and upon knowledge of the

genotypes of the various source populations.

CPB movement between fields is not well documented. Lashomb and Ng (1984) studied dispersal between adjacent (separated by a road), rotated and nonrotated fields. Oviposition in the rotated field began 54 degree-days (base 10° C) later than in the nonrotated field and peak eggmass density never exceeded 10% of the nonrotated field. The nonrotated field in this case received 3 sprays compared to 1 spray applied to the rotated field. In comparisons of rotated and nonrotated fields on Long Island, Wright (1984) found that CPB numbers were reduced substantially in rotated fields and that growers used on the average 1 additional spray on first generation beetles in the nonrotated fields. Effects of distance to new food sources on CPB dispersal are not well studied, in terms of numbers dispersing from one field to another, mortality during dispersal, or mode of dispersal (walking vs. flight). Movement between adjacent fields appears to be by walking, with some short distance flights. Long distance dispersal occurs by flight, primarily of starved beetles (Chapter 2). Once these beetles have begun to fly, they frequently fly over nearby food patches without response.

A critical factor which is still unknown is the distance beetles will walk to find new food sources and how far apart fields must be before individuals will switch over to flying. Beetles will frequently walk to nearby fields in large numbers (Lashomb and Ng 1984, Caprio, personal observation). Dispersal by walking decreases with

increasing distance (and is modified by intervening ground cover), while dispersal by flight would increase. Skuhravy et al. (1968) reported on beetle penetration into rotated potato fields (Figure 12). In fields adjacent to the previous year's fields, penetration was very small with the majority of the beetles found within 60 m of the border, consistent with the hypothesis that beetles walk between nearby fields. In rotated fields 800m apart, beetle penetration was uniform throughout the field, suggesting that beetle dispersal had occurred by flight.

Observations of flight behavior indicate that starved beetles ignore nearby sources of food and fly over 50m high passively with the wind. This would decrease the number of beetles immigrating into a field from nearby fields (though it may increase immigration from other sources) and would increase genetic mixing on a regional level.

Wind tunnel studies (de Wilde et al. 1968, Visser 1976, 1977) indicate that beetles will walk upwind, especially if potato plant odors are present in the air stream. Wilusz (in Wegorek 1959) reported that beetles in the field moved more readily to potato trap crops placed in the open than trap crops placed contiguous to a forest edge. Results obtained from studies reported in this thesis indicate that such upwind tendencies are not of primary importance under field conditions as beetles oriented to all of the plots with equal likelihood.

Some consideration should also be given to the crop

which replaces potatoes in the rotation scheme. Ng and Lashomb (1984) reported that beetles took longer to locate potato slices placed 10m away when the ground cover was wheat than on bare ground, with times for grass turf being intermediate. Lashomb and Ng (1984) reported that temperatures under a canopy of wheat are high enough to support flight initiation for 2-3 hrs/day. They used data from Johnson (1969) which indicated that beetles will only fly when temperatures exceed 25°C and after 6 hrs of solar insolation. These results indicate that beetles will initiate flight at significantly lower temperatures (15°C), and that long periods of solar insolation are not required. Never-the-less, decreased temperatures under the canopy will reduce beetle flight. Of greater importance is the mechanical barrier presented by the alternative crop. Observations were made of beetles attempting to initiate flight in a 33cm tall field of corn. While these beetles flew no less frequently than beetles released on bare ground, none of the flights resulted in dispersal because the beetles inevitably flew into corn plants and were knocked back down to the ground (they are clumsy fliers).

Trap crops may also be used to reduce beetle dispersal from a rotated field. Arena experiments reported here (Chapter 1) demonstrated that post-diapause beetles aggregate following emergence in the spring. This behavior may have evolved because the beetles originally fed on patchily distributed food plants and because females going into diapause are not mated. Following emergence, females

must be able to find new food sources and mate. The present paper does not attempt to suggest the mechanism causing these aggregations. There are several reports of sex pheromones in CPB (Tower 1906, Levinson et al. 1979), but these are all short distance sex recognition chemicals and are not thought to be effective over long distances.

The effective active space about potato plants (the area over which potato plants are attractive to CPB) must be determined to estimate the optimal spacing of the trap crop. In the arena experiments we conducted, 66% of the beetles were recaptured on potato plots, although these plots only accounted for 12% of the circumference of the arena. Plots were separated in a straight line by 16 m, and for two-thirds of the beetles to arrive at the plots, an idealized active space of 5 m must extend out from each plot. This is at best a vague estimate, and several assumptions are made in its estimation. The first is that beetles walk in straight lines and cross out of the arena once. Personal observation of beetle movement in the arenas suggests that this assumption is not violated dramatically. A second assumption is that there is no wind or that wind does not affect the active space (as would be the case if visual cues were being utilized). Light to moderate wind would increase the size of the active space, resulting in an increase in beetles locating the plants and hence an overestimate of the actual active space. However, since no correlation was found between wind direction index

and beetle orientation, there is little evidence to support the view that wind affects beetle orientation. A final assumption is that once beetles enter the active space they locate the plot. If this assumption were not true, the result would be that our estimate of the active space was too small, i.e., we have chosen a conservative estimate.

These results indicate that spacing rows of potato plants 8-10 m apart as a trap crop would be effective as a means of delaying post-diapause beetle dispersal from a rotated field.

Taylor and Georgiou (1979) suggest that dispersal and genetic dominance are the two most important factors affecting development of resistance. Effective dominance of susceptible genes can be obtained by adjusting spray dosages so that heterozygotes are killed before arrival of susceptible immigrants. The decay rate of the pesticide is important, as susceptible immigrants will not survive if pesticide levels are too high. An additional problem with the potato beetle is the promiscuity of the females. A homozygous female may mate with resistant males before spray application and primarily with susceptible males following the spray. If, however, the initial matings are most important in terms of fertilization, then this female may continue to lay homozygous resistant eggs and reduce the effectiveness of immigration. We do not as yet, however, understand how repeated matings in this insect affect fertilization.

Data presented here and in the literature on mode and factors affecting CPB dispersal indicate that there are opportunities for manipulating dispersal and for management of population densities. Techniques such as crop rotation and trap cropping would also affect gene flow and possibly development of insecticide resistance. More research is needed to determine the genetics of resistance in CPB and to determine rates of inter-field and wild-host immigration before adequate models of resistance development in this insect can be developed.

APPENDIX

Appendix A

A new method for marking the elytra of Colorado potato beetle which allows for identification of individual beetles for use in behavioral and mark-recapture studies.

The study of dispersal and movement in the field is essential for understanding of pest population dynamics and understanding how to manipulate these populations with such cultural techniques as crop rotation and trap crops.

The Colorado potato beetle (Leptinotarsa decemlineata (Say)) has always presented a problem to mark for field studies and mark recapture type analyses. The waxy elytra contribute to the flaking off of enamel paints, while the lack of dense hairy regions precludes the use of fluorescent dusts. Hare (1983) marked the hind wings with felt tip pens, but this technique requires extensive handling. Attempts were made to mark the beetles with rubidium fluoride but the marker was excreted within 5 days (Voss and Ferro, 1985).

The objectives of this study were to develop and test a system for marking CPB that would be relatively stable, quick to use, and allow beetles to be identified individually. The system tested has wide applicability beyond the CPB, and has potential use for most adult

Coleoptera, Orthoptera and other insect orders where there is sufficient hardened cuticle to attach the marks.

MATERIALS AND METHODS

First generation adult beetles (summer adults) were collected from potato fields at the Michigan State Montcalm Potato Research Farm in late August and stored with potato foliage in a cooler (5°C) for six weeks. A second set of 1-2 week old adults was obtained from a lab culture. This culture had been in the lab for 2.5 years and was originally collected in Antrim County, MI. These beetles were considered to be healthier and less stressed than the stored field beetles.

Marks were produced on a dot matrix printer with the aid of a computer program which printed out all possible 2 character alpha-numeric combinations. These were edited to reduce confusion between similar marks, and photocopy reduced to produce labels 2.5mm x 1.5 mm.

Each treatment unit consisted of 10 beetles housed in a cylindrical wire mesh cages 1 ft in diameter and 2 ft tall (photophase 16h light:8h dark, 20°C). Beetles were in a semi-natural condition, free to move about on plant foliage and soil surface or to burrow into the soil. One potato plant was kept in each cage and changed weekly. Counts of mark retention and beetle mortality were made every second day over a four week period.

The experimental design for the experiment with field beetles was a randomized complete block with 5 blocks of 3

treatments each: (1) a control with no marking, (2) marks glued directly to the elytra with no pretreatment, or (3) marks glued to the elytra after a pretreatment. The pretreatment consisted of wiping the elytra with acetone and roughing up the left elytron with fine (180 grit) sandpaper. Krazy glue^r, a cyanoacrylate-based glue, was used to attach the marks.

For the lab-reared beetles there were four blocks of 2 treatments: (1) an unmarked control, or (2) marking with the acetone + sanding treatment described above.

RESULTS

The acetone+sanding treatment significantly increased the adherence of the marks. The proportion of beetles still marked after 4 weeks was 91.7% ($\pm$ 14%) with the acetone-sanding treatment vs 38.9% ($\pm$ 40%) without pretreatment (Figure 20, means significantly different on day 31, $t = 3.026$, $p < .05$).

However, the pretreated marked beetles from the stressed field population had higher mortality than controls during the first two weeks of the study (Figure 21), though the differences were never statistically different (ANOVA on day 14, $F=2.37$, $p=0.125$). Differences between treatments decreased after this point. The pretreatment apparently eliminated the weaker beetles more rapidly.

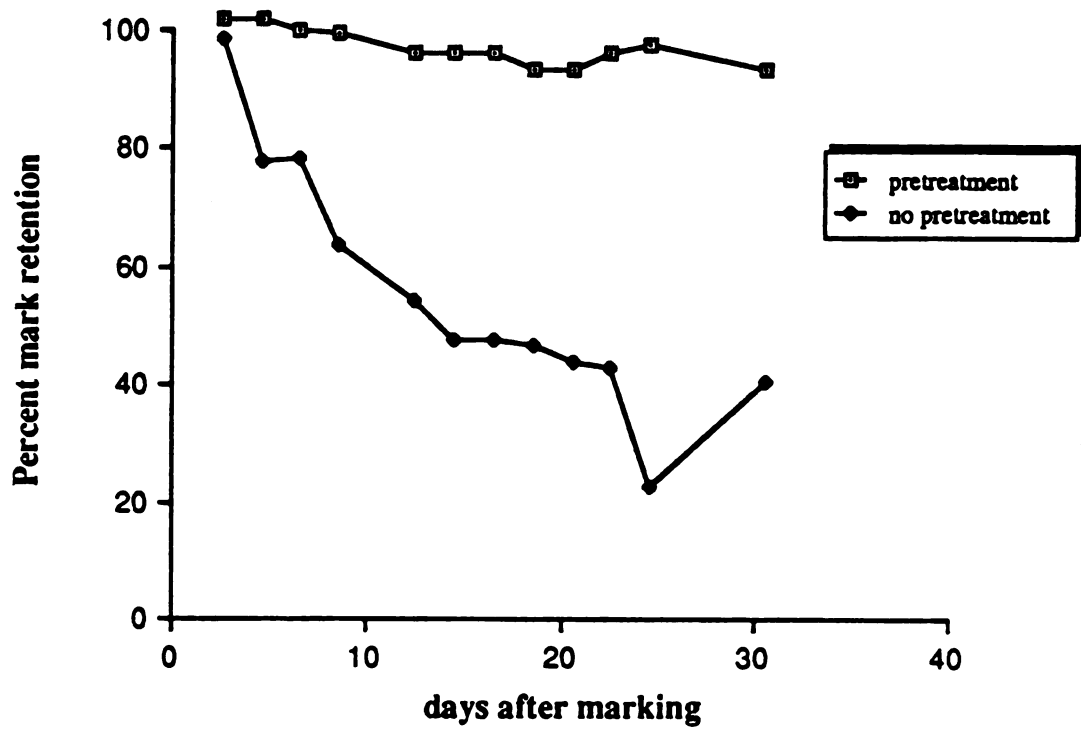


Figure 20. Mark retention of field-collected beetles marked after an acetone/sanding pretreatment vs. beetles marked without any pretreatment.

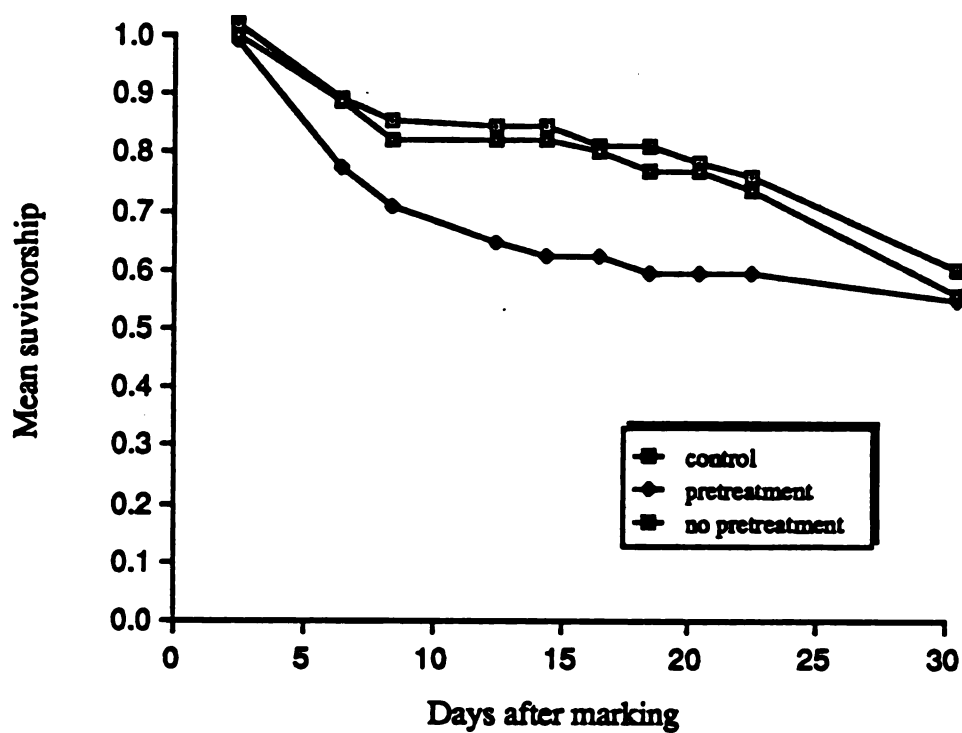


Figure 21. Mean survivorship of marked field-collected beetles (with and without pretreatment) vs. control (unmarked) beetles.

In the less stressed laboratory-reared beetles, little mortality (under 3%) was found in either the control or treated beetles over a 3 week period (Figure 22).

Discussion

This method of marking has great potential for CPB and possibly other beetles as well. It allows for large numbers of beetles to be marked individually and relatively efficiently (50-100/hr). The marks are small and light enough to be of minimal interference with flight and can be colored to blend or contrast with the insect's natural coloration. By using different colored marks and attaching marks to left or right elytron, it is possible to individually mark over 10,000 beetles. The marks were attached well enough that we were able to dig up diapausing beetles in the field and identify them (Chapter 1). Some caution must be exercised, however, when using this method in mortality studies, as beetles under stress may have increased mortality.

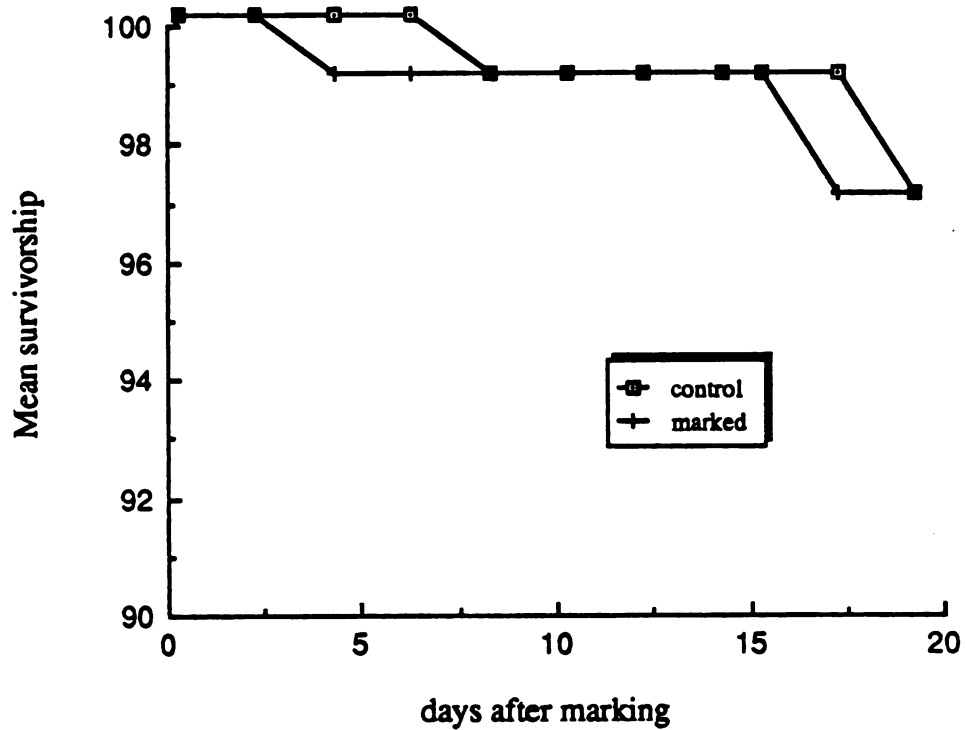


Figure 22. Mean survivorship of marked laboratory-reared beetles (with an acetone/sanding pretreatment) vs. control (unmarked) beetles. Note that the scale runs from 90% to 100%.

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