Characterization of selected life history traits of western corn rootworm, *Diabrotica virgifera virgifera*, LeConte populations resistant and susceptible to methyl-parathion

By

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CHARACTERIZATION OF SELECTED LIFE HISTORY TRAITS OF WESTERN CORN ROOTWORM (*DIABROTICA VIRGIFERA VIRGIFERA*, LECONTE) POPULATIONS RESISTANT AND SUSCEPTIBLE TO METHYL-PARATHION

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University of Nebraska-Lincoln, 2003

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Research was conducted to characterize the relative fitness of organophosphate resistant versus susceptible western corn rootworm populations. Tethered flight behavior was similar among resistant and susceptible beetles with the exception that susceptible beetles flew more frequently than resistant beetles. Average fecundity per female was significantly greater in susceptible than resistant populations, but female longevity and egg viability were similar. Net replacement rate was significantly greater in a susceptible population than a resistant population and fecundity appeared to be the key factor affecting net replacement rate. Results of resistant by susceptible reciprocal cross experiments indicate that fecundity and net replacement rate were negatively influenced in a sex-linked fashion by the resistant population. The mean developmental time from neonate larva to adult was similar across parental and reciprocal crosses.

After sub-lethal exposure to methyl parathion, resistant beetles exhibited both trivial and sustained flight behaviors, however, mean total flight time, mean

total trivial flight time, and mean number of flights per beetle significantly declined. Resistant females maintained fecundity at a similar level in insecticide and insecticide-free environments; however, age and reproductive state at the time of insecticidal exposure significantly affected reproductive potential.

Data collectively indicate that in the absence of insecticide, the relative fitness of susceptible beetles is greater than that of resistant beetles. In spite of this, fitness costs incurred when western corn rootworms are resistant to organophosphate insecticides do not appear to be severe enough to prevent movement, colonization, and reproduction to occur at levels that enable population densities to be maintained in either insecticide or insecticide-free environments. Data from this study complement what is known about the mechanisms and inheritance of resistance which increases our general understanding of the evolution and geographic spread of resistance in Nebraska.

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Introduction and Literature

Biology

The genus *Diabrotica* Chevrolat is strictly New World and largely neotropical (Krysan and Smith 1987). There are 354 described species in the genus (Krysan 1999), some are of economic importance. Pest species within the genus *Diabrotica* have been the subjects of many studies on life history (Ball 1957; Branson and Johnson 1973; Hill 1975; Branson 1976, 1976b; Branson et al. 1977; Krysan and Branson 1977; Metcalf 1979; Branson and Krysan, 1981; Krysan 1982, Coats et al. 1986; Naranjo 1990, 1994). *Diabrotica* are of greatest diversity in tropical areas, although areas north of Mexico have a higher proportion of pest species. *Diabrotica* is generally separated into three species groups: *signifera, fucata*, and *virgifera* (Krysan 1986). Identified pest species belong to the latter two groups. The range of *Diabrotica* in North America is limited by climatic conditions defined along group lines. No species in the *fucata* group successfully overwinter in the temperate north, although several *virgifera* group species do (Krysan 1986). The western corn rootworm (*Diabrotica virgifera virgifera* LeConte) and northern corn rootworm (*D. barberi* Smith and Lawrence) are known to be widely distributed pests of corn throughout the Midwestern United States (Chiang 1973, Levine and Oloumi-Sadeghi 1991).

The western corn rootworm was first discovered as a pest of corn in Colorado in 1909 (Gillette 1912). By the 1940's, the western corn rootworm was noted as a major corn pest in Nebraska and Kansas (Ball 1957). In the Corn Belt of the United States, adult rootworms may be present in corn from late June through frost, where they feed on corn pollen, silks, immature kernels, and

foliage (Krysan and Branson 1983, Branson and Krysan 1981). Oviposition occurs in soil, primarily in cornfields from July through September. Eggs overwinter in the soil until hatch (Branson and Krysan 1981). The egg hatch period may occur between late May to early July in the U. S. Corn Belt.

The western corn rootworm exhibits a period of obligatory diapause in the egg stage. The intensity of diapause differs by geographic regions. Branson (1976b) demonstrated that diapause intensity is under genetic control by selectively reducing diapause intensity to the point of developing a "non-diapausing" strain. Both parents contribute to the inheritance of diapause intensity, with a larger portion being inherited from the female (Krysan and Branson 1977).

Eggs of western corn rootworm from northern latitudes tend to hatch sooner under controlled temperatures, than eggs from more southern latitudes (Wilde et al. 1972). Western corn rootworms are able to terminate diapause regardless of environmental conditions (Krysan 1978, 1982). Branson (1976a) found that egg hatch lasted a period of 5 weeks, which is similar to the 4 week period reported by Levine et al. (1992) and Musick and Fairchild (1971).

Beetles prefer to lay eggs in corn (Painter 1951, Smith 1966). Western corn rootworms prefer an ovipositional site that is composed of moist dark soil with large aggregates and soil cracks (Kirk et al. 1968, Krysan and Miller 1986). Typically eggs are found heavily concentrated at the base of plants in non-irrigated fields (Patel and Apple 1967), and between rows in fields with irrigation (Lawson 1964, Pruess et al. 1968). Several studies have shown that the majority

of eggs are found in the top 10 cm of soil (Patel and Apple 1967, Ball 1957, Chiang 1965). However, Weiss et al. (1983) discovered that only ca. 80% of the eggs were recovered in the top 10 cm of soil in irrigated fields and that number decreased to 45 % in non-irrigated fields. Research later documented that 60% of western corn rootworm eggs can be found at depths of 20-30 cm in dryland fields and the suggestion was made that soil samples be taken to a depth of 30 cm when sampling for eggs (Gray et al. 1992).

The host range of western corn rootworm larvae is restricted to a few species of grasses. Branson and Ortman (1970) found that immature stages of western corn rootworm could complete development on grass hosts other than corn, but corn is the preferred host for larval development. Larvae pupate in the soil and may be found up to 63.5 cm away from the main corn root, (Chiang 1973). Emerging adults may be required to move some distance before reaching the soil surface.

Soon after emergence western corn rootworm beetles begin to mate (Branson et al. 1977), and initial oviposition occurs approximately 14 days later (Branson and Johnson 1973, Hill 1975). Female western corn rootworms live an average of 80 days and may lay as many as 1000 eggs under laboratory conditions (Branson and Johnson 1973, Hill 1975).

Injury and Management

The western corn rootworm is one of the most economically important pests of corn in the United States (Levine and Oloumi-Sadeghi 1991). Larvae, which feed extensively on corn roots, tunnel and prune roots (Branson 1986,

Riedell 1990) which disrupts water relations (Riedell 1990, Hou et al. 1997, Urías-López et al. 2000) and alters nutrient content of the grain (Kahler et al. 1985). Larval injury makes corn plants susceptible to lodging (Sutter et al. 1990, Spike and Tollefson 1991b) and may facilitate infection by root and stalk rots (Palmer and Kommedahl 1969). Larval feeding can also reduce plant vegetative biomass production (Chiang 1973, Apple et al. 1977, Spike and Tollefson 1991a, Godfrey et al. 1993, Dunn and Frommelt 1998, Gray and Steffey 1998, Urías-López and Meinke 2001) and grain yield (Turpin et al. 1972, Apple et al. 1977, Spike and Tollefson 1991a, Godfrey et al. 1993, Gray and Steffey 1998, Urías-López and Meinke 2001).

It has been difficult to establish a general western corn rootworm injury-yield relationship in corn, because the relationship is influenced by biological, environmental, and agronomic factors (Mayo 1986). Studies on the relationship of effects of corn hybrids and rootworm injury on yield (Allee and Davis 1996, Gray and Steffey 1998, Urías-López and Meinke 2001) indicate that corn hybrids may inherently differ in their ability to tolerate injury and partition biomass in response to injury and other stresses.

Current corn rootworm management options include crop rotation and insecticidal control of larvae or adults (Levine and Oloumi-Sadeghi 1991). Management practices for *Diabrotica* species in continuous corn have focused on the use of insecticides. Soil insecticides are typically applied at the time of planting or at first cultivation to reduce the number of larvae in the root zone (Mayo and Peters 1978). Seed treatments have recently been introduced as an

alternative to soil insecticides (Gustafson 2002). Foliar applications of insecticide may be used in late summer to suppress beetle populations, thereby reducing oviposition and larval damage the following year (Pruess et al. 1974).

Annual application of soil insecticides without an assessment of corn rootworm populations and their potential damage to corn is commonly practiced among growers. Growers do not always weigh the costs of materials and equipment against the possible benefits of control, but use prophylactic applications as "insurance" against rootworm damage (Turpin and Maxwell 1976).

When soil insecticides are used, selection pressure exerted by this control strategy is less likely to be as severe as that exerted by adult management programs. Soil applications of insecticides are targeted to protect only a portion of the root zone, which leaves untreated refugia and allows a significant number of larvae to develop to the adult stage (Sutter et al. 1991). In contrast, the goal of adult management is rootworm population suppression over the whole field.

Historically, crop rotation to a nonhost crop (i.e. soybean) has provided excellent larval control because corn rootworms oviposit almost exclusively in corn fields. Larvae must feed on corn roots (or related grasses) in order to complete their development cycle. The practice of growing continuous corn began in the 1940's and aided in the range expansion of western corn rootworm (Branson and Krysan 1981, Krysan and Branson 1983). Hill and Mayo (1980) present evidence that continuous corn increases populations of *D. virgifera*.

In recent years, crop rotation has failed as a management tactic for western corn rootworm in areas of the eastern Corn Belt (Onstad et al. 2001). Levine and Oloumi-Sadeghi (1996) initially suspected that the use of pyrethroid insecticides, commonly used in seed corn for control of corn earworm (*Helicoverpa zea* (Boddie)), repelled female western corn rootworm beetles from treated corn to untreated soybeans where they laid their eggs. Later studies ruled out prolonged egg diapause and the repellency of pyrethroid insecticides as explanations, leaving the possibility that the corn-soybean rotational practice had selected for a western corn rootworm behavioral variant that oviposits in soybean (Levine et al. 2002). In 1997 it was reported that a western corn rootworm variant was found in Indiana, with a greater host preference for soybeans (Sammons et al. 1997). Spencer et al. (1999) presented evidence suggesting that the mechanism may be a greater capacity to disperse and refutes any preference for a specific crop. The authors also suggest that the variant rootworm has less affinity for corn, exhibits considerable movement back and forth between crops, and oviposits many eggs outside of corn.

Cucurbitacins are strong feeding stimulants for adult Diabroticites (Metcalf et al. 1980). These bitter tetracyclic triterpene compounds, common to many *Cucurbita* species, are toxic or repellent to most nonadapted phytophagous arthropods (DaCosta and Jones 1971, Nielson et al. 1977, Gould 1978, Nielson 1978, Metcalf et al. 1980, Weissling and Meinke 1991). They are not volatile enough to act as attractants (Branson and Guss 1983). A large number of kairomones have been identified that function as *Diabrotica* attractants (Ladd et

al. 1983, Andersen and Metcalf 1986, Metcalf and Lampman 1989 a,b). The western corn rootworm sex pheromone (8-methyl-2-decyl propanoate) has been isolated from western corn rootworm females (Guss et al. 1984). Semiochemicals have been successfully used to alter the spatial distribution of adult corn rootworms (Lance 1988, Weissling and Meinke 1991).

In the last two decades, a behaviorally-based management concept using semiochemical-based baits has been developed in which adult control could be achieved while greatly reducing the amount of insecticide applied per hectare (Metcalf et al. 1987, Weissling and Meinke 1991, Sutter and Hesler 1993, Meinke 1994, Chandler and Sutter 1997). The concept originally involved the use of attractants to lure beetles to bait particles, and cucurbitacins to induce beetles to feed specifically on a small amount of insecticide. Later research indicated that attractants were not needed in baits to achieve adequate control. Currently, a cucurbitacin source is sold as an adjuvant that can be tank-mixed with insecticides (Invite®, Sidetrack®), or encapsulated with carbaryl as a stand-alone product (Slam®).

Tolerance to some larval feeding has been found in a few corn cultivars (Branson 1976, Chiang and French 1980). Riedell and Evenson (1993) defined rootworm tolerance as the ability of a corn plant to grow and produce higher grain yields than a susceptible plant when both are exposed to the same level of injury. Size of the root system is commonly associated with tolerance, the larger the root system the more tolerant a plant is to feeding by the western corn rootworm (Branson 1986). Ortman et al. (1968) indicated that the root volume ratios

between protected and unprotected corn plants were correlated with the level of western corn rootworm injury. Research by Godfrey et al. (1993) and Gray and Steffey (1998) suggests that some corn hybrids differ in their ability to tolerate injury and partition biomass in response to injury.

Current research is focusing on sources of antibiosis, either in native germplasm or for use in transgenic plants. Various companies are developing rootworm protected transgenic plants (e.g. Mollenbeck et al. 2001, Ostlie 2001) which may eventually be important tools in rootworm management programs.

Movement

Movement is an important life history trait where resources are discontinuous in time and space (Southwood 1977, Drake and Gatehouse 1995, Dingle 1996). This is especially true in the Corn Belt of the central United States, where the practice of annually rotating corn and soybean crops has been widely adopted over the past 30 years (Isard et al. 2000). Spatial and temporal uncertainty has recently increased the importance of movement in the life history of the western corn rootworm.

Western corn rootworm larvae are subterranean and oligophagous. It is critical to their survival that they locate host roots. Suttle et al. (1967) and Short and Luedtke (1970) reported that the combined net displacement of the three larval instars reached up to 50 cm. Western corn rootworm are attracted to and orient towards CO_2 sources (Strnad et al. 1986, Hibbard and Bjostad 1988, MacDonald and Ellis 1990, Strnad and Dunn 1990, Jewett and Bjostad 1996, Bernklau and Bjostad 1998). Western corn rootworm larvae can move at least 25

cm in soil toward a source of CO_2 (Strnad and Bergman 1987), but this movement can be inhibited to varying degrees by changes in soil bulk density and soil porosity (Ellsbury 1994, Guss et al. 1984). It has been suggested that some soil textures (i.e. high sand content) increase desiccation and reduce survival of western corn rootworm larvae (Turpin and Peters 1971).

Adult corn rootworms are very mobile and dispersal is an important part of their life history and ecology (Cinereski and Chiang 1968, Hill and Mayo 1980, Godfrey and Turpin 1983, Coats et al.1986, Grant and Severs 1989, Lance et al. 1989, Naranjo and Sawyer 1987, Naranjo 1990). The primary means of spreading infestations among western corn rootworm populations appears to involve beetle movement (Grant and Seevers 1989). Female western corn rootworms have been observed to emigrate away from continuous corn at specific times during the growing season (Godfrey 1980, Godfrey and Turpin 1983). Specifically, movement from field to field by mated females tends to increase during the preovipositional period (Hill and Mayo 1980, Godfrey and Turpin 1983). Female beetles appear to be attracted to cornfields for oviposition whether or not food is present (Cinereski and Chiang 1968). First year corn fields that lack populations of soil-dwelling lifestages of western corn rootworm may gain adult populations by the immigration of beetles. Movement of western corn rootworm beetles into first year corn may not be a reflection of random dispersal. Thus sex ratio, age, or density of beetles in continuous corn is often different than that of first year corn (i.e. first year corn often has a greater female/male ratio than continuous corn) (Godfrey and Turpin 1983).

Female western corn rootworm beetles more readily move from cornfields than male beetles (Hill and Mayo 1980). However, male beetles also have demonstrated the capability for sustained flight in laboratory studies (Naranjo 1990). Godfrey and Turpin (1983) found that males were abundant in first year corn throughout July and August. Isard et al. (2000) also suggest that both male and female beetles are able to frequently fly between corn and soybean in Illinois throughout an extended period from mid-July through early September.

Preferred food sources for western corn rootworm beetles have been identified as pollen, corn silks, and corn kernels (Forbes 1882, Ball 1957, Hintz and George 1966, Ludwig and Hill 1975). Beetles make directional movements toward pollinating corn and associated semiochemicals (Naranjo 1994, Prystupa et al. 1988, Darnell et al. 2000). Hill and Mayo (1974) found that the use of a late planted corn trap-crop reduced the injury potential the following season in corn surrounding the trap crop. Meinke (1995) describes late pollinating corn fields, in relation to surrounding fields, as "receiver fields" due to their attractiveness to beetles, causing beetles to move away from "donor fields" that have finished pollinating (relatively less attractive).

Corn phenology also appears to be an important factor that influences the spatial dynamics of beetles in field corn (Darnell et al. 1999). Whole plant count samples are more likely to be independent of each other when spaced at least 30 to 40 m apart during pollination (Darnell et al. 1999). Similarly, Midgarden et al.

(1993) found that unbaited sticky traps should be placed at least 30 m apart in order for samples to be independent of each other.

Western corn rootworm founder populations have been shown to move as far as 112.6 km (70 miles) (Balshaugh 1980). Grant and Seevers (1989) found a significant correlation between the number of beetles washed ashore on Lake Michigan with the passage of synoptic scale weather systems. In 1975, range expansion of western corn rootworm in Michigan was shown to progress in relation to the prevailing winds (Ruppel 1975).

Tethered western corn rootworm female beetles in the preovipositional life stage have been found to fly continuously for up to 4 h with the ability to travel up to 24 km in one flight (Coats et al. 1986). Coats et al. (1986) observed the occurrence of sustained flights during early morning or evening hours and field data from VanWoerkom et al. (1980, 1983) supports this pattern.

Western corn rootworm life history is synchronized to seasonal changes in environmental conditions and the temporal and spatial arrangement of host crops. Changes in environmental conditions favor a diel cycle of activity and quiescence and flight appears to be on a predictable schedule. Bimodal peaks observed in Iowa appear 2-3 hours after sunrise and before sunset (Witkowski et al. 1975). Laboratory studies have also revealed that sustained flight is confined to the early morning and evening hours, and trivial flights occur throughout the day (Coats et al. 1986, Naranjo 1990). Grant and Seevers (1990) found that a wide range of radiation loads, air temperatures, and wind speeds occur during peak periods of flight activity.

Western corn rootworm flight activity in corn fields peaks at air temperatures between 22 and 27 °C and beetles initiate flight at wind speeds up to 2.0 m s^{-1} (VanWoerkom et al. 1980). Grant and Seevers (1990) have shown that flight is most common when air temperatures within the canopy and wind speeds above the canopy ranged from 18 to 26 °C and 1.0-2.5 m s^{-1}, respectively. The flight of western corn rootworm on the edge of soybean fields appears to be correlated to a greater extent with atmospheric stability than air temperature or wind speed alone (Isard et al. 1999). The influence of air temperature and wind speed on movement is complex when data are averaged over multiple days conducive to flight (Isard et al. 2000).

Naranjo (1994) reported random orientation of beetles at locations within corn fields, but both northern and western corn rootworms show bias toward corn when released between corn at all developmental stages versus soybeans, wheat, sweet clover, or weeds. In choice experiments within corn, beetles oriented toward flowering over vegetative corn, suggesting that a combination of olfactory and visual cues aid in host selection (Naranjo 1994). Naranjo (1994) suggests that orientation changes at habitat interfaces and associated visual cues may be more important for movement between fields than within corn fields.

Insecticide Resistance Evolution

During the late 1940's, Nebraska growers began to apply soil insecticide at planting time for the control of corn rootworm larvae (Hill et al. 1948). By 1954, large-scale soil applications of organochlorine insecticides were used (Metcalf 1986b). Ball and Weekman (1963) noted ineffective western corn rootworm

control in 1959 in south-central Nebraska and high levels of resistance to aldrin were documented by 1963. Cyclodiene-resistant rootworms rapidly infested non-resistant areas from a focal point in southeastern Nebraska in 1961 to much of the corn growing areas of North Dakota, South Dakota, Nebraska, Iowa, Kansas, Missouri, Wyoming, and Colorado by 1964 (Metcalf 1983). By 1980 most of the United States Corn Belt had become infested with the resistant strain (Metcalf 1986b). Metcalf (1986a) has suggested that increased fitness found in the cyclodiene-resistant beetles, and behavioral changes associated with the resistance (i.e. increased mobility) made the cyclodiene-resistant beetles superior competitors (Metcalf 1986a). Western corn rootworm populations are still resistant to organochlorine insecticides (Parimi et al. unpub.) including populations from geographic areas where establishment of the western corn rootworm occurred after organochlorine use was suspended (Siegfried and Mullin 1989, Parimi et al. unpub.).

Organochlorine insecticides were eventually replaced with carbamate and organophosphate insecticides, which have been used extensively in the 1970's through the 1990's, providing control of both adults and larvae (Meinke et al. 1998). From 1986-1998 the product most commonly used to control adults in beetle spray programs was Penncap M® (encapsulated methyl-parathion). This was because of its low price and long residual efficacy in the field.

In areas of south-central Nebraska where beetle spray programs had been used annually for many years, reports of insecticide control failures began to occur in the early 1990's. Research, which began in 1994 at the University of

Nebraska, documented the presence of insecticide-resistant western corn rootworm populations in Nebraska (Meinke et al. 1998, Miota et al. 1998, Scharf et al. 1999).

Initial topical bioassays estimated that 10-17 fold more methyl-parathion was required to kill 50% of the beetle populations collected near York and Holdrege NE, than the most susceptible populations bioassayed. LD_{50} resistance ratios were 8-9 fold for carbaryl and 2.5-3.5 fold for bifenthrin (Meinke et al. 1998).

A diagnostic vial bioassay was developed to identify methyl-parathion resistant corn rootworm populations (Meinke et al. 1997) and has been used to monitor the spread of resistance and to map the distribution of resistant populations in south-central Nebraska. The intensity of resistance has increased in many populations, and the geographic distribution of resistance has expanded over time based on bioassay results and control failures in the field. Resistant populations are located primarily in or near the Platte River valley. However, many populations outside of the Platte River valley now include a low to moderate percentage of individuals that survive the diagnostic concentration and exhibit elevated resistance-associated esterase isoenzymes (Zhou et al. 2002). Counties in Nebraska that have been documented as containing one or more resistant populations include: Gosper, Dawson, Custer, Sherman, Buffalo, Phelps, Harlan, Kearney, Franklin, Adams, Clay, Hamilton, York, Fillmore, Thayer, Polk, Seward, and Butler (Zhou et al. 2002).