Evaluation of Serbian commercial corn hybrid tolerance to feeding by larval western corn rootworm (Diabrotica virgifera virgifera LeConte) using the novel difference approach

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Evaluation of Serbian commercial corn hybrid tolerance to feeding by larval western corn rootworm (*Diabrotica virgifera virgifera* LeConte) using the novel difference approach

by

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ABSTRACT

Since the discovery of the pest in 1992, western corn rootworm (*Diabrotica virgifera virgifera* Le Conte (WCR)) populations in Serbia have successfully been kept low with crop rotation. This has reduced the efficiency of screening for WCR resistance. A cooperative project between Iowa State University and the Maize Research Institute, Zemun Polje evaluated 13 Serbian commercial corn varieties in Ames, Iowa over a two-year period. Corn hybrids were planted on trap crops where high WCR populations were assumed. Hybrids were evaluated for WCR resistance using a randomized complete block design with four replications. Treatments were paired rows arranged in split plots with one row in each pair treated with insecticide and the other row left untreated. WCR injury was evaluated using a rating of root size and root regrowth (1-6 scale), root injury (0-3 Node Injury Scale), root mass, lodging, and yield. The results indicated significant differences among the Serbian hybrids in the presence of moderate-to-high levels of western corn rootworms. The relative benefit of insecticide treatments for maize lines was a useful tool in evaluating resistant germplasm. However, conducting analyses on relative differences between insecticide treated and untreated plots was not as effective at detecting differences as comparing the plots independently.
CHAPTER 1. LITERATURE REVIEW

Corn

History of Corn in the United States. The word “maize” is derived from the Mayan word for “grain of life,” signifying its importance to the subsistence of life in ancient civilizations (Videnović and Drinić 2002). Corn, Zea mays Linnaeus, is a native of today’s Mexico (Chiang 1973) and is now one of the most important cultivated crops in the world. The domestication of corn is thought to have begun as long as 10,000 years ago, spreading relatively slowly due to the equally slow migration of people across continents. Introduction of corn into the present day United States (U.S.) is estimated to have occurred circa 2,500 years ago, only reaching Europe in the 16th century after Columbus’s discovery of America (Troyer 1999).

In the past, row spacing in the U.S. was 112 cm, a width that accommodated horse-drawn equipment (Olson and Sander 1988). With the mechanization of equipment, row-spacing began to decrease, resulting in increase in plant populations. Row-spacing in the U.S. Corn Belt is now typically 76 cm (Cardwell 1982, Porter et al. 1997). In the future, it is possible that equidistant spatial arrangements will be employed as they offer reduced competition for sunlight between individual plants (Bullock 1988, Nielsen 1988, Olson and Sander 1988, Porter et al. 1997). In equidistant spatial arrangement studies, plants have shown up to an 8% increase in grain yield (Nielsen 1988, Polito and Voss 1991, Porter et al. 1997), likely as a result of higher energy absorption. The variation associated with the increases in yield may be a result of hybrid adaptability or environmental conditions (Fulton 1970, Olson and Sander 1988, Nielsen 1988, Porter et al. 1997).
However, equidistant spacing results in greater transpiration which may be problematic in dry conditions, resulting in reduced grain yields (Fulton 1970, Porter et al. 1997).

**History of Corn in Serbia.** Corn has been planted on approximately 1.5 million hectares in Serbia (Sivčev and Tomašev 2002) since the Second World War: 99.6% in Serbia, and 0.4% (8,000 hectares) in Montenegro (Ćamprag et al. 1995, Videnović and Drinić 2002). As of 2002, most of this land was dedicated to continuous corn (Videnović and Drinić 2002). In 1999, the Food and Agriculture Organization (FAO) ranked Serbia as 18th in land area devoted to corn and 14th in grain production (Videnović and Drinić 2002). The total grain yield in 1947 was 2,153 million tons per year, which saw a consistent increase to 8,413 million tons by 1986 (Videnović and Drinić 2002). By the millennium, grain yield had tripled since WWII. In 1998, 11% of the corn land belonged to state farms while the rest was privately owned (Republički Zavod za Statistiku 1998).

By the 1930s, the USA had begun production of commercial hybrid corn, heavily influencing Serbia to follow their lead (Videnović and Drinić 2002). In 1953, Serbia sent a group of corn scientists from the former Yugoslav Republic to visit the USA to investigate advances in corn breeding (Videnović and Drinić 2002). The scientists were introduced to inbreeding and crossing, and were able to observe successful American hybrids (Videnović and Drinić 2002). The Serbians brought back to Yugoslavia both their training and the American dent lines (Schnell 1992). U.S. dent lines crossed with European flint lines created successful hybrids that were adapted to Europe and produced high yields (Videnović and Drinić 2002, Schnell 1992). Between 1964 and 1999, the Federal Commission for Variety Recognition recognized and approved 442 hybrids for
commercialization from national agricultural institutes. A main contributor was the Corn Research Institute, Zemun Polje (Videnović and Drinić 2002).

The current importance of corn is based on its role as livestock feed (80% of total corn usage) and human food and industrial uses (20%) (Videnović and Drinić 2002). Serbia has exploited the chemical and biological composition of the corn plant and its grain in fresh, stored, and dried forms. The country produces over 1,300 corn-derived products (Berkić 1997). Much like in the U.S., the value of corn use in the production of fuel continues to gain interest in Serbia (Videnović and Drinić 2002).

There is an increased demand for corn in the European and global markets, which inevitably boosts prices (F. Baća, personal communication). In order for corn production to meet high demands, larger areas of land need to be dedicated to corn grain production. Unavoidably, demands have arisen in Serbia to grow monocultured corn in continuous cropping (F. Baća, personal communication). This in turn brings with it the necessity of finding host plant resistance traits, that is, tolerance to Western Corn Rootworm (WCR) larval injury in corn (F. Baća, personal communication).

Additionally, the Serbian corn industry is faced with both political and economic issues. The country was once governed by socialistic rule where growers had restrictions limiting private land to 10 hectares (F. Baća, personal communication). Wages within the country did not increase as they did in Western Europe and the economy of former Yugoslavia, which is now Serbia, did not flourish (F. Baća, personal communication). Instead, low incomes and war tore the country’s already struggling economy down completely. When the land restrictions were lifted, growers who had adapted to the 10-hectare limit showed no interest in expanding their crop land for several reasons: age,
equipment, and economic factors. Many farmers were close to or of retirement age, had little to no mechanical equipment, or had very limited funds to spend on new seed, fertilizers, or pesticides (F. Bača, personal communication). In combination, all these factors have limited growers’ capabilities.

The younger generation of Serbian agriculturalists comprises the commercial growers. The commercial growers have more crop land and are continually expanding their land; their limitations are finances and available land for sale (F. Bača, personal communication).

Corporations with established capital are privatizing former agricultural producers by purchasing independently-owned land. The privatized individual farms, like commercial farms, embark on intensive production with high use of pesticide, fertilizer and mechanization at a robust, Western level. Such privatized farms collectively cover areas of 30 to 100 hectares at any given location (F. Bača, personal communication).

Western Corn Rootworm

Origin of the WCR. LeConte first documented the presence of galerucine chrysomelids (Branson and Krysan 1981), Diabrotica virgifera virgifera, the western corn rootworm (WCR), in 1868 in Kansas (Chiang 1973). The general consensus is that WCR does not predate corn in the U.S. (Krysan and Smith 1987) and that the insect was likely introduced into the U.S. along with corn from Central or South America (Branson and Krysan 1981, Tollefson 2007). However, corn was not grown in Kansas at the time (Goodman 1987, Weatherwax 1954). Moeser and Hibbard (2005) suggest that WCR may have in fact jumped hosts from some unknown ancestral host to corn in the Kansas area
much later and spread as a result of corn production in that region. Corn has proven to be the most suitable host for WCR, but scientists agree that it may not have been the original host (Clark and Hibbard 2004, Oyediran et al. 2004, Branson and Ortman 1967).

WCR has not been documented to naturally complete its development on any host other than corn, but a subspecies of *Diabrotica virgifera*, *Diabrotica virgifera zeae* Krysan and Smith (the Mexican corn rootworm) has (Branson et al. 1982a). In 1986 Witkowski reported damaging levels of MCR in areas over 250 km away from corn populations. This was later confirmed by Krysan and Smith (1987).

**Introduction and Spread of the WCR in the United States.** The first report of WCR as a pest was in 1909 (Gillette 1912), and the larval form of the beetle soon became one of the most economically damaging insect pests in agriculture (Branson and Krysan 1981). The western corn rootworm spread across the North American corn growing areas at the rate of 80 km/year, a rate that has been repeated in Central Europe (Tollefson 2007).

**Introduction and Spread of the WCR in Serbia.** WCR was first recorded in Europe in July of 1992 (Bača 1994) when damaged corn was found, suggesting that introduction must have occurred prior to the 1991 growing season (Čamprag et al. 1995). The pest quickly spread across Serbia, into Hungary and Croatia, and then the rest of Europe, making eradication unlikely and unfeasible (Kiss et al. 2005). The insect arrived in Veneto, Italy in 1998, spreading to Pordenone in 2002 and Udine in 2003, Switzerland in 2000, and near Paris, France in 2002. In 2003 the insect arrived in Eastern France, Belgium, UK, and the Netherlands (Kiss et al. 2005).

Miller et al. (2005) analyzed the genetic variation of adult WCR at several locations in Europe, determining with high certainty that, while a few introductions were linked,
several were independent of each other. The population in Northeastern Italy originated in Central Europe. The Eastern France population came from the Paris population, while the Northwestern Italy and Paris populations came directly from North America. The group also concluded that a minimum of three populations were introduced directly from North America (Miller et al. 2005). The group attributed the sudden rise of introductions to: adaptation of the insect, changes in control measures, and changes in transportation procedures (Miller et al. 2005).

Serbian surveying for the insect pest began in 1993 with visual scouting in corn fields. In 1996, use of sticky traps began; pheromone traps followed in 1997. In order to quantify the influence of degree days (daily temperature high added to the daily temperature low, divided by 2, subtracting the temperature threshold for WCR, 10, from the overall quotient) and the hydrothermal coefficient (HTC) (total precipitation divided by degree days) on WCR arrival, presence, and the total number of persistent adults, Baća et al. (2006) trapped the insects from 1996 through 2005 using sticky traps (Multigard, Csalomon, and Pherocon AM). They examined the influence of the degree days and the HTC sums on the dynamics of the arrival, duration of injury, and the total number of caught adults (Baća et al. 2006). During the eight-year study traps were put on corn plants in continuous corn during the last 10 days of the month of June. Traps were replaced every 4 weeks and discontinued 2-3 weeks after the last adult was captured. The HTC was calculated by adding the cumulative precipitation with the sum of the degree days (Baća et al. 2006).

The arrival of the adults can be expected when the sum degree days between April and June reaches 600°C. The first 10.4% of the population was registered when the sum
degree days reached 704°C. The dynamics of the arrival and the duration of the adults depend on the magnitude of both the degree days and the HTC. Increases in numbers were observed when the sum degree days was larger, and the duration of adult presence was longer when the HTC was higher. These results were most easily observed in 2000, when 21.4% of the total population of adults was present as early as June, and in 1999, with a recorded HTC of 0.31, adults were collected as late as October (Bača et al. 2006).

Uniform and dense populations of rootworms are necessary for proper evaluations of germplasm. In Serbia and the rest of Europe, this may be hard to achieve due to two primary factors: the clumped distribution of eggs by female adult WCR, which is also observed in the U.S., and low pest densities in the region. While artificial infestation is a solution to the non-uniform egg distribution, it requires special equipment and thousands of eggs, which are expensive to purchase if they are not able to be produced. Thus, varietal evaluations of corn may be performed on U.S. soil using European corn lines (Tollefson 2007).

**Life-History of the WCR.** WCR complete one generation per year. In the fall, eggs are deposited in the top 20 cm of soil (Hein and Tollefson 1985), but may be found as deep as 35 cm (Weiss 1983, Hein and Tollefson 1985). Eggs remain viable even through very cold winters. While documentation has shown that egg populations have been destroyed in laboratory freeze tests (Gustin 1981, 1986), similar situations in nature have not significantly reduced WCR populations. Eggs begin to hatch after 204 centigrade degree days have accumulated (Chiang 1973). Carbon dioxide levels and gradients in soil help first instars locate roots (Strand and Bergman 1987, Gustin and Schumacher 1989, Macdonald
and Ellis 1990, Bernlau and Bjostad 1998). However, extremely wet or dry soil may restrict movement by the larvae and thus increase mortality (MacDonald and Ellis 1990).

Western Corn Rootworm adults are sexually dimorphic, with differences in elytra coloration, antennal length, and size of the terminal abdominal segment (Sekulić and Kereši 1995). Both sexes can range from a yellow to greenish color with black stripes towards the edges of each of the elytra; in females, the black stripes of the elytra are usually straight and defined, while in the male the undefined stripes may bleed into one another (Sekulić and Kereši 1995). The antennae of the male are longer than the length of the body, while in the female the antennae are only about three-fourths as large (Sekulić and Kereši 1995). In males, the second and the third segment of the antennae are of the same length, while in females the third segment is noticeably longer than the second (Krysan and Smith 1987). The length of the body can span from 4.2-6.8 mm long depending on the gender, with the females the larger of the two sexes (Krysan and Smith 1987).

Immediately after larval emergence from the chorion they begin their host search. They may travel as far as 40 cm horizontally in search of food (MacDonald and Ellis 1990), but if host plants are not found within 12 – 24 hours, survival rates are dramatically reduced (Branson 1989, Strand and Bergman 1987). Specific studies have shown that development could be completed on several other plants including barley *Hordeum vulgare* Linnaeus and wheat *Triticum aestivum* Linnaeus, among others (Branson and Ortman 1967; Clark and Hibbard 2004). However, corn is the predominant dietary source.

First instar WCRs begin feeding on fine root hairs (Chiang 1973) and, in their second and third instars, progress to the youngest roots where they begin to tunnel into the cortex (Reidell and Kim 1990). Larval development almost always coincides with the
development of adventitious root tissue leading to detrimental effects if the larvae are in abundance (Reidell and Evanson 1993). Through larval feeding, secondary infections can be introduced via roots and lead to sickness or plant death (Levine and Oloumi-Sadeghi 1991). At ideal temperatures, larvae complete development in three weeks and progress to the pupal stage in which they remain for approximately 10-12 days (Jackson and Elliot 1988).

Field collected adults are able to be maintained in the laboratory and usually survive for 49-102 days (Ball 1957, Branson and Johnson 1973, Hill 1975, Boetel and Fuller 1997). Field-caught beetles that emerged earliest had the longest laboratory longevity (Boetel and Fuller 1997), but food quality also played an important role. Beetles fed on diets of young plants lived the longest (Elliott et al. 1990).

Both male and female adults feed on pollen, silk, and foliage (Branson and Krysan 1981), usually not causing significant damage or economic loss. Through their feeding, however, they may be vectors of corn stalk rot fungi (Levine and Oloumi-Sadeghi 1991).

Adults are active from July until the first frost (Levine and Oloumi-Sadeghi 1991, Branson and Krysan 1981, Tollefson 2007). Males emerge first (Ruppel et al. 1978, Branson 1987), beginning as early as late June. Emergence of both sexes continues for up to 70 days (Ruppel et al. 1978). Soon after emergence, mating begins (Ball 1957). Males mate an average of 8.2 times, while females mate only once (Branson et al. 1977) — but retain the ability to lay multiple clutches of eggs if food is available. The female pre-ovipositional period lasts 12-14 days (Branson and Johnson 1973, Hill 1975) and egg production is induced by the digestion of protein in corn silks and pollen (Elliott et al. 1990). In laboratory environments the average number of egg clutches laid is 13, consisting
of approximately 1,000 eggs total, and are deposited over a period of 60-70 days (Branson and Johnson 1973, Hill 1975). Oviposition occurs in the soil where the eggs remain until the following spring (Levine and Oloumi-Sadeghi 1991), when they hatch (Branson and Krysan 1981).

**WCR Interactions with Corn.** Plant damage caused by larval western corn rootworm feeding is manifested in several ways. The affected root systems have limited nutrient and water uptake, leaving plants without sufficient resources to complete proper development (Levine and Oloumi-Sadeghi 1991). Root pruning by larvae also provides a direct route for secondary infections and increases the likelihood of lodging.

An increase in a plant's tendency to lodge, as a result of root pruning, may reduce grain yield (Levine and Oloumi-Sadeghi 1991) up to 50% (Spike and Tollefson 1989). Unfortunately, it remains difficult to predict what kind of yield loss will occur, as environmental factors (moisture, soil type, nutrient availability, and weather), host plant characteristics (root-system size, compensatory growth capabilities, and plant population), and finally insect density all play a part in the ultimate damage to the plant (Levine and Oloumi-Sadeghi 1991).

**Host-Plant Resistance**

**Definition of Host-Plant Resistance.** Many researchers in Europe are now interested in the identification or development of corn lines that are resistant to WCR larval feeding. Resistance is a heritable trait (Bigger 1941, Painter 1951) and leads to a reduction in damage by the insect (Rausher 1992, Stowe et al. 2000). Painter (1951) described resistance as the heritable qualities in a plant that limit the amount of injury or damage
caused on that plant by insect attack. The term can be further broken down into its mechanisms: antixenosis; a plant’s ability to deter or decrease oviposition and colonization, antibiosis; the ability to reduce or affect metabolic, developmental, or reproductive pathways, and finally tolerance; the ability of a host plant to sustain injury, great enough to damage a susceptible variety, without a reduction in yield (Painter 1951, Kogan and Ortman 1978, Crawly 1983, Rausher 1992, Panda and Khush 1995, Stowe et al. 2000).

**Resistance to WCR in Corn.** As early as the 1920s, corn genotypes were shown to react differently to rootworm feeding (Bigger et al. 1938). By the 1940s, host-plant resistance research specific to WCR began in the Corn Belt states of the U.S. (Bigger et al. 1941).

Tolerance is not shown to be wide-spread, but it has been observed in some cultivars (Chiang and French 1980). No hybrid has been documented to possess complete tolerance (Rogers et al. 1975, Riedell and Evenson 1993, Gray and Steffey 1998). However, since the 1960s, there have been constant improvements in cultivars (Riedell and Evenson 1993, Gray and Steffey 1998). When present, the resistance mechanism manifests itself in large root systems, which provide abundant compensatory growth after injury (Chiang 1973, Branson et al. 1982a). Larger root-size and compensatory growth are often correlated (Owens et al. 1974).

Compensatory growth is an important factor in sustaining elevated yields (Spike and Tollefson 1988, Spike and Tollefson 1989) as it reduces the tendency to lodge (Levine and Oloumi-Sadeghi 1991) and the uptake of nutrients and water. On the contrary, abundant root tissue has been documented to negatively affect the yield by using any available
moisture for secondary root development in times of extreme drought (Gray and Steffey 1998).

**Breeding for Resistance.** Tremendous amounts of monetary resources are required to conduct a corn breeding program. The labor is often a limiting factor (digging, handling, and rating of roots) and results in small scale efforts by researchers. Advances in the discovery or breeding of resistance lines have thus occurred in small steps over time. Evaluations of resistance mechanisms have been conducted (Branson et al. 1982b, Kahler et al. 1985a) and have lead to the release of resistant lines (Kahler et al 1985b, Russell et al. 1971).

In 1997 and 1998 Hibbard et al. (1999) documented crosses sustaining less rootworm damage, but like other breeding programs they had limited success in developing resistant germplasm (Branson and Rueda 1983; Branson et al. 1969; Branson 1971; Branson et al. 1986; Riedell and Evan 1993; Owens et al. 1974; Wilson and Peters 1973; Wilson et al. 1995; Rogers et al. 1975; Rogers et al. 1976a, 1976b; Rogers et al. 1977). Tolerance has been documented as the typical resistance mechanism found in germplasm, but other mechanisms (antibiosis and antixenosis) of resistance have been suggested (Branson et al 1983, Kahler et al. 1985b, Assabgui et al. 1995).

There was limited success in developing or incorporating this germplasm into commercial lines. This may be due to findings that showed a tendency to lose any tolerance or resistance genes present in the germplasm when yield was targeted in selection trials (Welter and Steggall 1993, Rosenthal and Dirzo 1997).
Western Corn Rootworm Control

Biological Control. Biological control experiments using ground beetles (Coleoptera: Carabidae) suggested that the beetles were predators of WCR larvae (Tyler and Ellis 1979). This research was challenged, however, by other scientists who suggested that the ground beetles were mislabeled as predators and are not useful biological control agents (Best and Beegle 1977, Kirk 1982). Stinner and House (1990) suggested that certain non-insect arthropods such as mites in families Laelaptidae, Rhodacaridae, and Amerosiidae could serve as control agents. These arthropods were found to be significant predators of WCR larvae and eggs (Chiang 1970).

Susceptibility to a certain soil-dwelling nematode were found (Jackson and Brooks 1989, Steffey et al. 1987). However, inconsistencies were found in the results and were suggested to be due to mechanical and edaphic factors (Munson and Helms 1970, Poinar et al. 1983). No biological control methods have been shown to be sufficiently effective to be adopted by corn producers as part of their integrated pest management tactics.

Rotation and the WCR Variant. Historically, WCR injury was avoided through crop rotation with alfalfa, hay, clover, and small grains. In the 1940s, the utilization of corn for a growing number of purposes caused a farming shift. Corn producers began growing corn in fields where corn had been planted the previous season (continuous corn). This production increase expanded the range of the WCR (Krysan and Branson 1983, Nowatski 2001), elevating the WCR to pest status. Since the recognition of WCR damage, many growers have shifted back to crop rotation in combination with other control tactics in an attempt to control the insect.
Regular, annual rotation of corn with another crop, primarily soybeans, resulted in selection pressure against rotation and it become less effective. Finally, in the mid-1990s, a behavioral change occurred and WCR females began ovipositing in soybean fields that had been rotated annually with corn (Levine and Oloumi-Sadeghi 1996). In 1995 the first larval WCR occurrences in corn after soybeans were reported along with adult collections in both corn and soybean fields (Cook et al. 2005). This was just a few years after Levine and Oloumi-Sadeghi (1991) reported that WCR did not show the extended diapause adaptation to rotation that its close relative, the northern corn rootworm, (*Diabrotica longicornis* Say) did (Krysan et al. 1986).

The adult females of the variant WCR strain lay eggs in soybeans, and in lesser numbers in alfalfa, oat, and wheat, as well as corn (Cook et al. 2005). Eggs hatch the following season when corn is planted again (Levine and Oloumi-Sadeghi 1988). Since the mid-1990s, a dramatic increase in damage was observed in East-Central Illinois and Northwest Indiana (Levine and Oloumi-Sadeghi 1996). In Iowa, WCR surviving on corn planted after soybeans was recorded for the first time in 1999 (Rice and Tollefson 1999). Since then, WCR has been reported numerous times in the major corn growing states in cornfields planted after other crops (Levine et al. 2002). The presence of a WCR variant presents major problems as larval control through crop rotation is overcome and thus calls for stronger, alternative control measures.

**Insecticide Use.** The use of tolerant cultivars is not recommended as the sole management tactic. Even the most tolerant root systems may be overwhelmed when planted in areas with large populations of WCR (Levine and Oloumi-Sadeghi 1991). In lower insect populations, Chiang and French (1980) found that larval survival was higher on tolerant
varieties. Experts and growers have integrated many management tactics into the control of WCR including crop rotation, transgenic corn, and insecticide use. By 1986, insecticides and crop losses from WCR were costing corn producers an estimated $1 billion (Metcalf forward in Krysan et al. p260). Today, it is undoubtedly the most economically important corn pest insect (Tollefson 2007).

Once applied, the presently used soil insecticides remain active for 6-10 weeks, which coincides with primary larval feeding (Levine and Oloumi-Sadeghi 1991). The materials are formulated on absorptive material, like clay or sand, and then incorporated into the top layers of soil (Levine and Oloumi-Sadeghi 1991).

Cyclodiene were used in the late 1940s and were effective in their action against larval WCR, but in just over 10 years, resistance was so prevalent that the insecticides were abandoned (Meinke et al. 1998). By 1959, chlorinated hydrocarbon insecticides had been widely accepted by corn growers for a decade, even though the WCR’s resistance to them was documented (Ball and Weekman 1962) and was spreading 122 km per year (Metcalf 1983). As other classes of insecticides were developed, chemical control advanced beyond chlorinated hydrocarbons. However, in 1998, WCR beetles in areas where adults were controlled using the newest insecticides had already developed resistance (Meinke et al. 1998). Larval WCR have also shown elevated resistance levels to certain granular insecticides (Wright et al. 2000) in those same areas.

Pesticide use persists in the US (Tollefson 1990, Gray et al. 1993). Unfortunately, in some years, high numbers of emerging adults from treated areas have been reported (Gray et al. 1992). Larvae may be developing in areas of insecticide application because they may mature outside the treatment zone of insecticides, which are only applied in-furrow or in a
15 cm band over the row (Sutter et al. 1991, Gray et al. 1992). Due to the mechanical and chemical nature of soil insecticides they are only active in a certain zone around the roots (Felost and Steffey 1986). As a result, roots that grow outside this zone can be eaten by WCR and insect development can be completed (Felost and Steffey 1986).

Over-reliance on soil insecticides is not wise. Soil insecticide efficacy is influenced by numerous factors:

- Environmental conditions like moisture, humidity, and wind
- Chemical properties like soil composition and insecticidal stability (Felost and Lew 1989, Harris 1972)
- Cultural techniques and equipment (Levine et al. 2002)
- Biological factors like microbial insecticide degradation, extended diapause, and insect resistance (Meinke et al. 1998)
- Pesticide laws and regulations

All these issues make WCR management with soil insecticides challenging.

**Host-Plant Resistance.** Strauss and Agrawal (1999) further divided tolerance into the resultant mechanisms that occur in response to herbivore plant feeding: an increase in the photosynthetic rate after damage has occurred, elevated growth rates, increased branching of roots and/or shoots, and the ability to redirect stored carbon from undamaged to damaged plant parts. The mechanism(s) employed by a plant is dependent on the type and location of the feeding damage and the species and variety of the plant (Strauss and Agrawal 1999).

Researchers have found a negative association between nutrient availability and tolerance, mainly when environmental nutrient levels were highest (Gertz and Bach 1995,
Irwin and Aarassen 1996). Olff et al. (1990) attribute this association to the reduction in the root:shoot ratio by high nutrients as a reduction in the ratio reduces tolerance (Strauss and Agrawal 1999). Endophytic fungal infections were also negatively associated with tolerance (Strauss and Agrawal 1999), as infections were found to limit compensatory growth in all grasses (Belesky and Fedders 1996). Conversely, a positive association was found between water and light with tolerance (Maschinski and Whitham 1989).

**Transgenic Corn.** Transgenic corn is an alternative now offered to growers to alleviate the concern regarding insecticide resistance. However, laws, regulations, and technology fees accompany the use of transgenic seed. Additionally, WCR has already shown resistance potential. A greenhouse WCR colony confirmed that resistance to Bt corn can develop quickly in forced inbreeding that resulted in an 11.7 fold increase in tolerance (B. E. Hibbard, personal communication).

The U.S. MON 863 corn, developed by Monsanto in the 90s offered the first protection against WCR through expression of an insecticidal protein called Cry3Bb1 (Vaughn et al. 2005). Since the development of MON 863, Pioneer Hi-Bred International, Inc. (Johnston, IA), Syngenta (Basel and Stein, Switzerland), and Dow AgroSciences (Indianapolis, IN) have developed transgenic corn varieties that express insecticidal proteins that are modified or different from Cry3Bb1 (Moellenbeck et al. 2001). There has been varying control conferred by the different transgenic hybrids (Gray et al. 2007).

The European Union (EU) has not approved macro-scale use and production of genetically modified organisms (GMO), including corn. EU countries have begun experimentation on European soil, however, and are continuing the search for alternatives
to GMOs as no varieties are on the market or are approved for direct consumption (GMO Compass).

**Western Corn Rootworm Management**

**Sampling.** While in some regions WCR continues to show resistance to chemical control, insecticides are still in use. Turpin (1977) reported that 93% of soil-insecticide applications in the state of Indiana were applied without sampling the WCR population. Furthermore, although there were many areas of continuous corn production, approximately 50% of them did not have economically significant populations of WCR (Turpin et al. 1972, Gray et al. 1993). In 1986, Metcalf reported that 50-60% of land in the U.S. used for corn production was treated with soil insecticides to control WCR. Today, this percentage is presumably higher as states like Iowa, with its heavy corn production, concluded that soil insecticides should be used at all times until more accurate sampling techniques that predict damage for the following year are developed (Nowatski 2001).

Unnecessary applications of soil insecticides are avoided by sampling adult WCR beetles at the end of a season to predict larval damage the following year (Steffey et al. 1982, Tollefson 1990). Initially, one adult beetle per plant in a field of continuous corn was set as the standard to expect larval damage the following season (Pruess et al. 1974), warranting insecticidal treatment. Godfrey and Turpin (1977) suggested that the economic threshold would be attained when numbers reached 0.71 beetles per plant in first-year corn fields. Researchers later re-confirmed that a one-beetle-per-plant assessment was 83% accurate and that the associated economic damage was effectively estimated through the
use of adult sampling (Foster et al. 1986). Field sampling can be problematic because its precision level is not consistently as high as desired as it is a very rough estimate.

**Germplasm Management Programs.** Root-injury ratings have been the preferred method for evaluating corn tolerance (Tollefson 2007). Three root-injury scales have been developed and commonly used. The 1-6 scale was introduced in 1971 by Hills and Peters. The least amount of injury is represented by a rating of 1 and the greatest by 6; all other ratings fall in between (Hills and Peters 1971). A second scale, developed about the same time, also had categorical ranging from 1-9 (Musick and Suttle 1972) where the least amount of injury was also represented by a rating of 1, but with this scale, the greatest injury by 9; all other ratings again falling in between. The final and most intuitive scale is the Node-Injury scale developed by Oleson et al. (2005), which provides a linear representation of injury where 0 represents no injury and 1 represents a full node of roots destroyed. Three is the highest injury rating representing three or more full nodes of injury. Injury between full nodes is rated as a proportion of a node; for example, 1.25 represents one and a quarter nodes destroyed.

In the evaluation of corn varieties, root sizes are also recorded. These ratings are highly subjective due to variability in annual environments (Tollefson 2007). The Eiben root-size scale classifies the sizes of roots on a scale of 1 (smallest) to 6 (largest) (Rogers et al. 1975). A representative of the smallest and largest roots are chosen and assigned the numbers 6 and 1, respectively; roots that best represent the sizes between the 1 and 6 are then chosen and assigned ratings of 2-5. All other roots of that field are then rated against the representative samples.
The root-compensatory-growth scale uses a similar rating as the Eiben root-size scale, but assesses compensatory root growth (Rogers et al. 1975). A rating of 1 represents the least amount of secondary growth and a rating of 6 the most. Hills and Peters (1971) combined their own secondary root development rating (0-4 with 0 as least re-growth and 4 as most) with an injury rating (between 1 and 6) to calculate an index that predicts yield. The re-growth rating was subtracted from the injury rating and the value was a categorical index prediction of the yield.

Lodging, corn leaning or laying 30 or more degrees off the vertical, can be a clear indicator of WCR presence (Tollefson 2007). Pruned roots are the primary cause of lodging, leaving plants unsupported and leading to physiological and mechanical yield loss. Plants that are significantly lodged experience decreased photosynthetic efficiency due to the plant’s inability to receive adequate amounts of sunlight (Tollefson 2007). Mechanical yield loss results when harvesting equipment is unable to pick up lodged corn (Tollefson 2007). Lodging is not directly useful in selecting resistant germplasm as it does not allow for resistance mechanisms to be distinguished and, most importantly, it is also very dependent on environmental conditions (Tollefson 2007).

American lines with documented resistance appear to derive their resistance from large root systems and greater compensatory growth (Owens et al. 1974, Rogers et al. 1975). This finding indicates that the mechanism of resistance is most likely tolerance. Both root size and compensatory growth are affected by environmental conditions including, but not limited to, moisture and nutrients (Owens et al. 1974). Elevated levels of either antibiosis or antixenosis to WCR have not been found in corn (Tollefson 2007). Branson and Krysan (1981) suggested benefits in finding inbred and ancestral lines of corn that
exhibit antibiosis or antixenosis. However, the researchers predicted this as a difficult task as they are evolutionarily unfavorable and require more energy from the plant (Branson 1971). If they exist, the traits would likely be found in relatives of corn that over long periods of time were able to evolve.

Ultimately, growers are interested in the final grain yield (Tollefson 2007). Yield tolerance measures the yield outcome between a single variety planted with paired rows where one row is infested by an insect and the other is not. The difference between the two grain yields is termed the yield tolerance, with the smallest difference representing the greatest tolerance (Tollefson 2007). Yield tolerance might be used as an indicator of varietal vigor and performance under rootworm pressure.

Rogers et al. (1975) were the first to conduct field evaluations for tolerance to yield reduction as a result of WCR larval feeding. Their plots were planted on naturally infested soil, resulting in significant differences between treated and untreated plots (Rogers et al. 1975), which provided a solid foundation on which to repeat such evaluations on more naturally infested plots. The group found that feeding damage did not consistently result in lodging, and that yield loss alone could serve as indicators of feeding (Rogers et al. 1975). Corn on corn is still used today in both the USA (Branson and Krysan 1981, Krysan 1983) and Serbia (Videnović and Drinić 2002).

If stresses are minimal growers may find that damage may not lead to injury and thus see little or no yield loss (Levine and Oloumi-Sadeghi 1991). Control of adult population is recommended the previous summer; the task involves direct action, but can help the following season (Levine and Oloumi-Sadeghi 1991). Crop rotation and the elimination of volunteer corn (Steffy and Gray 1989, Levine and Oloumi-Sadeghi 1991) in
combination with other management tactics provide a robust integrated pest management strategy that confers results.
CHAPTER 2. EVALUATION OF SERBIAN COMMERCIAL CORN HYBRID
TOLERANCE TO FEEDING BY LARVAL WESTERN CORN ROOTWORM
(DIABROTICA VIRGIFERA VIRGIFERA LECONTE) USING THE NOVEL
DIFFERENCE APPROACH

Stephanie R. Kadličko

Introduction

The western corn rootworm (WCR), *Diabrotica virgifera virgifera* LeConte, was first described in the United States in the late 19th century. As its population grew over time, the insect became one of the most economically damaging agricultural insects in the country and persists today. Nearly 200 years later, in 1992, the first European incidence of corn damage by WCRs was reported in central Serbia close to the Belgrade airport in Surčin (Bača et al. 1995). Within just three years the insect spread to neighboring Hungary and Croatia; as a result of the rapid spread, eradication became unlikely and the insect is now present across Europe (Kiss et al. 2005).

Until very recently US corn growers have used two primary tactics in WCR control: crop rotation and insecticide application. While offering growers considerable control, the tactics have applied selective pressures on the insect resulting in the evolution of resistance. Genetically modified Bt-corn was later introduced to the North American market. It became immediately popular among growers, offering an effective and time-efficient method of protecting maize from WCR larvae. Europe, to date, has not approved of genetically modified organisms (GMOs) and thus does not grow transgenic corn.
In Serbia crop rotation continues to be used as the primary WCR control strategy, being found to be the most efficient and effective tactic (Bača et al. 1995). When rotation in Serbia is employed, corn is preceded by crops including wheat, sugar beet, and field pea. Unfortunately for subsistence growers, rotation is not always an option. Small land owners who grow corn to feed livestock, primarily cattle and hogs, do not have the option of rotating corn with other crops as the survival of their livestock and their farm depends on corn. Likewise, the option of insecticide is not always available due to their high costs.

In Serbia, resistance breeding remains a viable option because WCR populations are maintained at low to moderate levels with national, multi-crop rotation. Ideally a hybrid utilizing a mechanism of resistance that withstands a moderate level of insect feeding would provide the growers in the region with an option of producing corn in back-to-back seasons.

The Maize Research Institute, Zemun Polje, Serbia has several vigorous hybrids of various maturities that have been accepted and sold commercially for several years. Testing the performance of these hybrids under rootworm infestations would allow growers in Europe to make educated choices on seed that will offer protection from the devastating pest. Due to Serbia’s low rootworm levels, evaluation on domestic soil was impractical without employment of artificial infestation methods. To help determine whether resistance to WCR is present in any of the hybrids grown in Serbia, Iowa State University in Ames, IA and the United States Department of Agriculture - Agriculture Research Service in Columbia, Missouri entered into a collaborative project with the Maize Research Institute to evaluate Serbian germplasm. Specific objectives were to determine (1) how maize performance varied among commercial Serbian hybrids in the presence of moderate-to-high
levels of western corn rootworms, and (2) whether analysis on the relative benefit of insecticide treatments (performance differences between treated and untreated plots) for maize hybrids would be a useful tool to guide selection for resistance.

Materials and Methods

Thirteen commercial hybrids were evaluated in Ames, IA over a two year period. To ensure moderate-to-high levels of WCR, maize was planted onto land where corn had been planted late the previous year to attract gravid females (trap crops). For each hybrid, data on performance were collected in insecticide-treated and untreated plots. The difference between treated and untreated plots for each hybrid essentially measures the effect of insecticide use; smaller differences suggesting plant resistance or tolerance. If successful, the ‘difference approach’ would permit assessment of WCR resistance among the tested hybrids independent of differences not directly related to host-plant resistance.

Germplasm. Serbian commercial hybrids evaluated included ZP 341, ZP 360, ZP 434, ZP 42a, ZP 578, ZP 580 (2006 on), ZP 599, ZP 677, ZP 680, ZP 684, ZP 704, ZP 735 (2007 only), and ZP 737 (2006 only). Hybrid maturities ranged from 110 days in FAO 300s, to 130 days in FAO 700s. Hybrids were previously bred for various qualities (drought tolerance, yield, etc.) and were chosen for WCR evaluation based on performance in Serbian and European markets. Two public lines were used as control: CRW3 x LH51 as a naturally resistant control and B37 x H84 as a susceptible control. No controls were used in 2006. In 2007, ZP 580 and ZP 737 were unavailable; ZP 737 was replaced with ZP 735 due to genetic similarity, but the two hybrids were analyzed separately. ZP 580 was not replaced in 2007.
**Experimental Design and Field Use.** The experiment was arranged in a randomized complete block design with split plots and paired rows. One row in each pair received a single, in-furrow application of a granular pyrethroid insecticide (0.1465 kg (A.I.)/ha of Force® 3G, Syngenta Crop Protection Inc., Greensboro, NC, USA) at the time of planting. Four replications were planted with row spacing of 76.2 cm. In both seasons hybrids were planted at a single location in Ames, Iowa: in 2006 at Johnson Farm, Iowa State University and in 2007 at Bruner Farm, Iowa State University. Both locations had trap crops grown on them the previous season and were chosen because high natural WCR populations were assumed. Planting was conducted mechanically with a 4-row John Deere Max-Emerge™ 7100 integral planter. Seed was deposited with 12.7 cm seed spacing. Plots were subsequently thinned to 17.78 cm between plants when plants were approximately 30 cm tall. Each replication was divided into two sections that were separated by a narrow alley. One section, 7.01 m in length, was designated for individual plant evaluations from where representative plants were dug. The other section was left for yield measurements and was 7.62 m in length.

**Hybrid Evaluation.** In 2006 the first evaluation was conducted on 13 July and in 2007 on 11 July. All ratings were completed within 2 days of the initial root digging, except for root mass. Root mass was taken after roots had been cut from the plant, bagged, and dried for 4 days at 100 degrees Celsius. At each evaluation 5 representative plants from each row were chosen at random, dug, labeled, and cleaned with pressurized water. Plants were rated for root size using the Eiben Root-Size Scale (Rogers et al. 1975) and root injury using the Node-Injury Scale (Oleson et al. 2005). A root size rating of 1 indicated the smallest root system, and a rating of 6 indicated the largest root mass. The Node-Injury
Scale is a linear scale from 0-3 where 0 indicates no injury, 1 indicates one full node of pruning, and a partially pruned node is indicated as a percentage, i.e. a quarter node of pruning is given a score of 0.25. The second evaluation was conducted on 2 August in 2006 and on 15 August in 2007; all of the same evaluations were conducted and completed within 2 days of the root digging. Prior to harvest stand counts and lodging counts were taken. Corn was considered lodged if it leaned more than 30 degrees off the vertical. Corn was harvested on 30 October in 2006 and on 25 October in 2007.

**Statistical Analysis.** Preliminary tests were conducted using general linear models (PROC GLM, SAS Institute Inc. 2004) to evaluate whether maturity class predicted performance of varieties, and if study year interacted with other factors. To determine if the effect of insecticide application (difference between insecticide-treated and untreated plots) varied among commercial hybrids, models (PROC GLM) tested whether differences in measures of maize performance were influenced by variety, year and replicate (within year), with year and replicate treated as random effects. Separate analyses were conducted for root injury (second evaluation), root mass (second evaluation), size, lodging and yield; data from the first evaluations were excluded because it appeared that first evaluations of root injury and mass took place before all WCR injury occurred in both seasons. When an overall $F$-test indicated an effect of variety, $t$-tests were used to make pair-wise comparisons among means.

Because several apparent differences among lines were shown not to be statistically significant, two additional analyses were conducted. First, to determine whether the number of replicates used was sufficient to detect variability among the hybrids using the ‘difference approach,’ a power analyses was conducted for each of the five tested measures
of maize performance using a routine designed for analysis of variance with a block effect (NCSS 2002). Power \((1 – \beta)\) is the probability that a false null hypothesis will be rejected, thus avoiding a Type II error \((\beta)\) (the chances of a \(\beta\) decreases with the increase of power).

Required inputs for the power analysis included differences between insecticide-treated and untreated plots for each hybrid and an estimate of random error (square root of mean squared error). Data from 2007 were used in the power analyses because the ‘difference approach’ logically seems more likely to detect resistance when pest pressure is highest. Second, a more conventional analysis of hybrid performance was conducted. Separate models (PROC GLM) compare hybrids with or without insecticides. Each model tested whether performance (e.g., injury, lodging, yield) was influenced by variety, year and replicate (within year), with year and replicate treated as random effects. Dependant variables expressed as percentages were modified using the arcsine-square root transformation in order to make the data more normally distributed. Pair-wise comparisons were made among maize varieties as indicated for the ‘difference approach,’ which is equivalent to using Fisher’s Protected LSD.

**Results**

**Maturity and Year Interactions.** Variation among varieties in each maturity class appeared greater than differences among maturity classes, and a significant year × insecticide interaction suggested the impact of *Diabrotica* on maize varieties increased from 2006 to 2007. Difference analysis tested in models appeared to yield differences among lines. However, most were not statistically significant.
**Power Analysis.** The power analyses showed that, for three of five parameters, power could be increased significantly with the addition of replications. Root injury could apparently have been elevated from a power of almost 0.7 with the addition of 4 replications (Fig. 1). Size and lodging were shown to require approximately double the replication increase of injury, needing approximately 10 replications to achieve an acceptable power. An increase in the number of replications for mass and yield parameter measurements would apparently not gain any advantage, as an increase of 8 replications would still result in a power of approximately only 0.7 and 0.5, respectively.

**Lodging.** Lodging results for insecticide untreated plots are presented in Table 1 and treated plots in Table 2. Averages for untreated plots ranged from a transformed log of 0.5 for ZP 599 to a transformed log of 0.09 for ZP 735 (Table 1). For treated plots, averages ranged from a transformed log of 0.06 for ZP 580 to a transformed log of 0 for most of the other hybrids (Table 2). All hybrids resisted lodging significantly more than the negative control. Hybrids ZP 341, ZP 360, ZP 684, and ZP 735 resisted lodging significantly more than ZP 42A, ZP 599, and ZP 704.

**Yield.** Average yields for untreated plots ranged from 1.48kg/ha for ZP 42A to 2.22kg/ha for ZP 684 (Table 1). For treated plots, averages ranged from 1.82kg/ha for ZP 580 to 2.63kg/ha for ZP 680 (Table 2). All hybrids yielded significantly higher than the negative control. Hybrids ZP 578 and ZP 684 yielded significantly higher than 46% of the hybrids: ZP 341, ZP 360, ZP 42A, ZP 434, ZP 599, and ZP 737. ZP 42A yielded significantly less than 62% of the hybrids: ZP 341, ZP 434, ZP 578, ZP 580, ZP 677, ZP 680, ZP 684, and ZP 704.
**Mass.** Averages for untreated plots ranged from 9.48 g for ZP 599 to 16.05 g for ZP 578 (Table 1). For treated plots, averages ranged from 8.37 g for ZP 580 to 17.70 for ZP 578 (Table 2). All hybrids but ZP 599, had a higher mass (dry weight) than the negative control. ZP 578 had a higher mass than 46% of the hybrids, and ZP 677 had a higher mass than 31% of the hybrids. ZP 599 did not have significantly more root mass than the negative control or 46% of the hybrids.

**Size.** Averages for untreated plots ranged from 2.80 for ZP 360 to 3.64 for ZP 735 (Table 1). For treated plots, averages ranged from 2.90 for ZP 341 and 360 to 3.53 for ZP 580 (Table 2). All hybrids but ZP 42A were significantly larger than the negative control, but were not significantly different among each other.

**Injury.** Averages for plots with no insecticide ranged from 1.75 for ZP 704 to 0.67 for ZP 735 (Table 1). For treated plots averages ranged from 0.35 for ZP 434 to 0.06 to 0.10 for ZP 737 (Table 2). ZP 735 suffered significantly less injury than the negative control as well as ZP 434, ZP 578, ZP 580, ZP 677, ZP 684, and ZP 704. ZP 341 received significantly less injury than the control and ZP 704.

**Discussion**

**Insecticide Treatments as a Resistant Germplasm Evaluation Tool.** Although a preliminary analysis did not show significant differences among maturities, day length and maturity at evaluation locations should always be considered. The lines that performed best in this evaluation were of maturities that suited the region where they were tested.

The ‘difference approach’ is intuitively advantageous and theoretically would have been ideal for line evaluation. However, its field performance indicated otherwise. When
margins of error were considered, large differences between treated and untreated evaluation criteria for hybrids (the basis of the ‘difference approach’) were not statistically significant. The power analysis indicated that an investment of twice as many resources would be necessary to achieve high power and obtain significant results. Environmental differences in soil composition and quality, nutrition and moisture, among other factors, may account for the variability in yield and mass. Thus, the ‘difference approach’ was rendered impractical. However, with relatively little resources the ‘comparison approach’ was shown to be an effective tool. Comparing separate models for insecticide treated and untreated plots may be slightly more cumbersome, but shows distinct differences between hybrids.

By analyzing hybrids according to each variable in independent models (insecticide treated or untreated) significant differences become evident. Comparing hybrids that appeared to show resistance in untreated plots to insecticide treated plots clearly indicated the superior performance of certain hybrids. This method also allowed us to decipher whether apparent strengths in hybrid resistance are truly manifestations of host-plant resistance or just overall hybrid vigor, something that would not have been distinguishable through use of the ‘difference approach.’ In these experiments, the ‘comparison approach’ was able to detect differences with eight replications. However, as with any other data, statistical significance rises with an increase of replications over a wide range of environments.

**Hybrid Evaluation.** Individual traits are relatively meaningless when not combined with other traits for a specific hybrid. By considering multiple traits in the overall
evaluation certain lines were clearly exhibiting resistance, others demonstrated their breeding potential, while some hybrids were weak overall.

ZP 42A had an extremely low yield even with a significantly large root system. The yield was considerably larger when protected with insecticide, increasing 0.64 kg/ha, indicating severely reduced vigor in the presence of WCR larvae. ZP 42A also had an extremely low yield with a large root system, suggesting excess plant resources were diverted from ear development to compensatory root development. ZP 434 performed similarly to ZP 42A in terms of both root size as well as yield, gaining 0.64 kg/ha from insecticide protection.

ZP 578 and ZP 684 performed very well among the other hybrids. ZP 578 had the second largest yield of all the unprotected hybrids. Its yield did not increase by more than 0.16 kg/ha with insecticide protection, indicating tolerance to feeding by rootworms. Even with high injury ZP 578 resisted lodging likely due to its large root system. The large mass of ZP 578 in both the treated and untreated plots indicated that its large mass was not a result of post-injury compensation. ZP 684, which was competitive with ZP 578, resisted lodging and had the highest unprotected yield that did not increase with the application of insecticide. It did not benefit from insecticide application, suggesting both plant vigor and resistance to WCR damage. Its tolerance was likely conferred by its abundant root-mass.

ZP 580 did not have one of the highest yields, but did not receive additional protection from insecticide application. The mass increased significantly between the protected and unprotected plots, almost doubling. Resources devoted to growth apparently did not take away from ear development as may have been occurring in other hybrids; ZP 580 yielded higher without insecticide protection than with insecticides applied.
ZP 735 did not have competitive yields with many of the other hybrids but showed breeding potential. Its root size was the largest and among the heaviest. It also received the least amount of injury in the untreated plots suggesting that ZP 735 may be exhibiting an alternate resistance mechanism (antixenosis) than the other hybrids (tolerance). Even with lower yields, the breeding potential of ZP 735 should be considered.

The negative control was a very weak hybrid. Its yield, even when protected with insecticide, was 30 bushels lower than any other non-protected hybrid. The positive control proved to have low vigor in comparison to some of the other hybrids. However, when comparing insecticide treated and untreated plots it was evidently quite tolerant of significant feeding injury. Size did not increase with feeding, nor did mass (in insecticide untreated plots), and yield increased by only 0.08 kg/ha. This control demonstrated perfectly that relative assumptions across lines can be misleading in predicting the true tolerance of a given line.

One downfall to using this method of hybrid evaluation analysis is that it is less direct and may be time-consuming if many lines are involved in an evaluation. In the case of this study, the ‘comparison approach’ worked very well. The top hybrids (ZP 578, ZP 580, ZP 684, and ZP 735) were ranked high based on their ability to deliver unprotected yields that were as high or higher than protected yields (ZP 578, ZP 580, and ZP 684), or for their breeding potential (ZP 735). These hybrids appear to have natural host-plant resistance and are able to tolerate WCR feeding. Because the primary resistance mechanism observed was tolerance based, selection pressure by insects will likely be minimal and, as a result, the resistance is more likely to be preserved over time.
Thus, the conclusion can be made that the Maize Research Institute, Zemun Polje has several promising WCR resistant hybrids. These hybrids, in combination with crop rotation, will perform well for Serbian farmers and corn breeders without the use of insecticides for rootworm protection, suiting the current ecological WCR situation in the region.
References Cited


Table 1. Adjusted means of evaluated traits combined over two years for insecticide untreated plots.

<table>
<thead>
<tr>
<th>Hybrid</th>
<th>Root Size</th>
<th>SEM(^a)</th>
<th>Injury</th>
<th>SEM</th>
<th>Mass (g)</th>
<th>SEM</th>
<th>Lodging(^b)</th>
<th>SEM</th>
<th>Yield (kg/ha)</th>
<th>SEM</th>
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<td>670.99</td>
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</tbody>
</table>

\(^a\)SEM = standard error of the mean.

\(^b\)Log transformation of percentage of plants lodged.

Means in each column followed by the same letter are not significantly different (Least Significant Difference test; \(P \leq 0.05\)).
Table 2. Adjusted means of evaluated traits combined over two years for insecticide treated plots.

<table>
<thead>
<tr>
<th>Hybrid</th>
<th>Root Size</th>
<th>SEM&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Injury</th>
<th>SEM</th>
<th>Mass (g)</th>
<th>SEM</th>
<th>Lodging&lt;sup&gt;b&lt;/sup&gt;</th>
<th>SEM</th>
<th>Yield (kg/ha)</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>341</td>
<td>2.90 ab</td>
<td>0.23</td>
<td>0.18 abc</td>
<td>0.09</td>
<td>10.78 efgh</td>
<td>0.94</td>
<td>0.00 b</td>
<td>0.03</td>
<td>7918.67 def</td>
<td>423.29</td>
</tr>
<tr>
<td>360</td>
<td>2.90 ab</td>
<td>0.23</td>
<td>0.13 bc</td>
<td>0.09</td>
<td>12.76 cdef</td>
<td>0.94</td>
<td>0.00 b</td>
<td>0.03</td>
<td>8474.79 cd</td>
<td>454.64</td>
</tr>
<tr>
<td>42A</td>
<td>3.15 ab</td>
<td>0.23</td>
<td>0.11 bc</td>
<td>0.09</td>
<td>10.50 fgh</td>
<td>0.94</td>
<td>0.00 b</td>
<td>0.03</td>
<td>8344.86 d</td>
<td>423.29</td>
</tr>
<tr>
<td>434</td>
<td>2.93 ab</td>
<td>0.23</td>
<td>0.34 ab</td>
<td>0.09</td>
<td>15.44 ab</td>
<td>0.94</td>
<td>0.00 b</td>
<td>0.03</td>
<td>9824.28 ab</td>
<td>423.29</td>
</tr>
<tr>
<td>578</td>
<td>2.92 ab</td>
<td>0.33</td>
<td>0.26 abc</td>
<td>0.12</td>
<td>17.70 a</td>
<td>1.36</td>
<td>0.00 b</td>
<td>0.04</td>
<td>9279.46 abcd</td>
<td>613.30</td>
</tr>
<tr>
<td>580</td>
<td>3.53 a</td>
<td>0.23</td>
<td>0.14 bc</td>
<td>0.09</td>
<td>8.37 h</td>
<td>0.94</td>
<td>0.06 b</td>
<td>0.03</td>
<td>7151.66 ef</td>
<td>423.29</td>
</tr>
<tr>
<td>599</td>
<td>3.25 ab</td>
<td>0.23</td>
<td>0.21 abc</td>
<td>0.09</td>
<td>14.35 bcd</td>
<td>0.94</td>
<td>0.00 b</td>
<td>0.03</td>
<td>8589.65 cd</td>
<td>423.29</td>
</tr>
<tr>
<td>677</td>
<td>3.18 ab</td>
<td>0.23</td>
<td>0.42 a</td>
<td>0.09</td>
<td>12.69 cdef</td>
<td>0.94</td>
<td>0.03 b</td>
<td>0.03</td>
<td>9642.25 abc</td>
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<tr>
<td>680</td>
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<td>0.26 abc</td>
<td>0.09</td>
<td>12.81</td>
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<td>0.00 b</td>
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<td>10345.24 a</td>
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</tr>
<tr>
<td>684</td>
<td>3.20 ab</td>
<td>0.23</td>
<td>0.14 bc</td>
<td>0.09</td>
<td>13.16 bcde</td>
<td>0.94</td>
<td>0.00 b</td>
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<td>8688.82 bcd</td>
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<tr>
<td>704</td>
<td>3.48 a</td>
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<td>0.25 abc</td>
<td>0.12</td>
<td>14.68 abcd</td>
<td>1.36</td>
<td>0.00 b</td>
<td>0.04</td>
<td>7511.94 def</td>
<td>613.30</td>
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<tr>
<td>735</td>
<td>2.92 ab</td>
<td>0.33</td>
<td>0.18 abc</td>
<td>0.12</td>
<td>15.78 abc</td>
<td>1.36</td>
<td>0.00 b</td>
<td>0.04</td>
<td>8531.28 bcde</td>
<td>613.30</td>
</tr>
<tr>
<td>737</td>
<td>3.03 ab</td>
<td>0.23</td>
<td>0.10 c</td>
<td>0.09</td>
<td>12.18 defg</td>
<td>0.94</td>
<td>0.02 b</td>
<td>0.03</td>
<td>7859.67 def</td>
<td>423.29</td>
</tr>
<tr>
<td>neg</td>
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<td>0.33</td>
<td>0.35 abc</td>
<td>0.12</td>
<td>8.94 gh</td>
<td>1.36</td>
<td>0.69 a</td>
<td>0.04</td>
<td>3990.09 g</td>
<td>613.30</td>
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<tr>
<td>pos</td>
<td>3.28 ab</td>
<td>0.33</td>
<td>0.06 bc</td>
<td>0.12</td>
<td>11.94</td>
<td>1.36</td>
<td>0.00 b</td>
<td>0.04</td>
<td>6701.62 f</td>
<td>613.30</td>
</tr>
</tbody>
</table>

<sup>a</sup>SEM = standard error of the mean.

<sup>b</sup>Log transformation of percentage of plants lodged.

Means in each column followed by the same letter are not significantly different (Least Significant Difference test; \( P \leq 0.05 \)).
Fig. 1. Results of five post-hoc power analyses, conducted for each of the tested measures of maize performance, designed to determine whether the number of replications used was sufficient to detect variability among the hybrids using the ‘difference approach’ with a probability of 1-β (where β is the Type II error rate for testing a null hypothesis).
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