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Relationship between corn stalk strength and southwestern corn borer penetration

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RELATIONSHIP BETWEEN CORN STALK STRENGTH AND SOUTHWESTERN CORN BORER PENETRATION

By

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Studies were conducted to determine if corn stalk strength had an effect on southwestern corn borer (*Diatraea grandiosella* Dyar) survival during different growth stages. In 2006 southwestern corn borer larvae were placed on corn during the tassel stage near the ear and base of the plant. Survival was higher near the ear than near the base of the plant. In 2007, five varieties of corn were planted at three locations in Mississippi. Plants were infested with five 3rd instar larvae at the ear zone during tassel, dough and dent development stages. After five days stalk strength and borer survival were measured. Survival decreased as the corn progressed from tassel to dent stage. Survival varied among corn varieties. The relationship between stalk strength and borer survival was not consistent, indicating that there are likely factors more directly limiting borer survival than physical stalk strength.

Key words: *Diatraea grandiosella*, *Zea mays*, stalk strength, penetration resistance

DEDICATION

This thesis is dedicated to Jerry and Fredna Gibson in memory of their son, Bradley Kyle Gibson. Brad, the primary author of this thesis, died in an automobile accident on March 22, 2009, about two months before he was expecting to complete this thesis. The final preparation of this thesis was completed by Brad's primary adviser, Fred Musser.

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CHAPTER I

INTRODUCTION

Pest management is a very important aspect of modern agriculture, which constantly requires updating information and exploring new ideas and technology in order to keep up with changing pest problems. Since the 1960's the use of pesticides has declined because of increased awareness of social, environmental, and economical issues (Boethel and Eikenbary 1986). Boethel and Eikenbary (1986) stated that because of the increased concern in these areas, there has been a resurgence of interest in integrated pest management and host plant resistance.

Plants naturally develop resistance to some pests such as herbivores, nematodes and pathogens (Norris et al. 2003). If this natural resistance did not occur there wouldn't be many of the plants around today due to the plant's inability to move or escape certain pests in their area. Because plants do not move to evade pests much like many vertebrates or other organisms do when endangered, the pressures to evolve characteristics that tolerate pests are increased (Norris et al. 2003). These defenses, which result from this natural selection, may be physiological, chemical or behavioral (Pedigo and Rice 2009).

Host plant resistance is a well-founded science and can be defined as the ability of a plant to reduce the development and reproduction of a pest that is using the plant as a host, and to tolerate the damage of that pest (Norris et al. 2003). As time has passed, manipulations in the ability of plants to naturally defend themselves against pests have been a focus of many plant breeders. Advancements in breeding programs, which artificially manipulate resistance to insects and other arthropods, are some of the most important advancements in agriculture and are at the forefront of breeding in any organism (Pedigo and Rice 2009).

Host Plant Resistance

Host plant resistance basically falls under three categories: ecological, induced, and constitutive resistance (Kogan 1994). Ecological resistance is defined as the ability to escape or avoid herbivores in time or space. Since many plants are only suitable hosts for herbivores during small periods of development, this can be achieved by growing in a manner where plants are not easily infested by herbivores. An example would be growing crops at an earlier or later time than normal so the crop's development is not suitable to the requirements of the herbivore. This is not genetically controlled and can be considered a cultural control practice.

Both induced and constitutive resistance are genetically controlled and are the primary goals of breeders seeking host plant resistance. Induced resistance occurs when plants, which are stressed by some mechanical, chemical or environmental factor, change themselves so that they are less desirable or suitable for herbivore feeding (Kogan 1994). Constitutive resistance is not brought about by environmental factors and is always expressed in the plant. There are three subcategories of genetic resistance: antixenosis, antibiosis and tolerance. Antixenosis and antibiosis have negative effects on the insect while tolerance doesn't harm the insect but allows the plant to overcome herbivore damage. Antixenotics characteristically disrupt normal host selection behavior by directing insects away from the host, affecting the movement of herbivores, discouraging biting or piercing insects, or preventing feeding and the insect's ability to lay eggs on the host. Antibiotics harm the insect by disrupting the growth and development of insects. This is achieved by the use of toxins, chemicals which reduce the digestibility of plant tissues, or hormones, which disturb the insect's endocrine functions. Tolerance, as previously stated, does not harm the insect feeding on the plant. This type of resistance occurs when the plant has the ability to repair the damage which has been caused or grow at such a pace that the insect doesn't cause economic damage at densities that cause damage in less tolerant varieties (Kogan 1994).

One of the earliest accounts of host plant resistance was found in the late eighteenth century when the 'Underhill' variety of wheat was found to be resistant to the Hessian fly, *Mayetiola destructor* (Diptera: Cecidomyiidae) (Pedigo and Rice 2009). This is considered to be the first insect resistant plant variety. The first significant contribution of host plant resistance to crop protection came in 1890 when European grape vines were grafted onto American grape vine root-stocks which were resistant to root feeding insects recently introduced into Europe from America (Waiss et al. 1977). Since then, host plant resistance has been studied in many crops. Examples of host plant resistance being used for insect management in corn (*Zea mays* L.) include the use of DIMBOA and certain enzymes found in corn plants to control the European corn borer (Lepidoptera: Pyralidae) (Buendgen et al. 1990, Norris et al. 2003). Corn earworm (Lepidoptera: Noctuidae) feeding was discouraged by the presence of long tight cornhusks (Waiss et al. 1977). Ng (1988) found that leaf morphological characteristics deterred southwestern corn borer feeding. These characteristics included more vascular bundles per unit of corn leaf and a greater combined thickness of the cuticle and outer cell walls of the epidermis of both the upper and lower leaf surfaces.

Regardless of the plant property used to deter insect damage, host plant resistance has proven to be a valuable method of pest management. In this paper, strength of corn stalks as related to their ability to deter feeding by the southwestern corn borer will be evaluated. This potential source of host plant resistance has previously been evaluated for lodging resistance, (Flint-Garcia et al. 2003) but not directly for resistance to the southwestern corn borer.

Corn Development

Corn has several vegetative and reproductive stages of development from emergence of the plant to physiological maturity (Ritchie et al. 1997). The first stage is VE, which stands for emergence of the plant and is followed by a numerical set of stages beginning with V1, which is when the collar of the first leaf appears. The first leaf of the emerging corn plant is characteristically oval shaped.

Each stage of development following V1 is determined when the collars of additional leaves appear: V2 when the second leaf collar appears, and V3 when the third leaf collar appears (Ritchie et al. 1997). At the V3 stage, the plant's growing point is still under the soil line making it vulnerable to flooding and soil insects. When the plant reaches the V6 stage the growing point is most likely above the soil line and the stalk has a reached a point when it begins to elongate. Normally the uppermost one or two ear shoots begin to develop by the V9 stage. Ear shoots will eventually develop into a harvestable ear. The tassel begins to rapidly develop at V9 and the stalk continues to elongate. Time between stages at this point begins to shorten to about two to three days. The number and size of potential kernels begins to be determined at the V12 stage. The V15 stage marks the point when the plant is about 10 to 12 days from silking or the first reproductive stage of development. This stage is a very important point in the plant's development for determining yield. Nutrient uptake, temperature, insect damage, pollination and other factors of overall plant health all play crucial roles during this period of growth and development. This process of vegetative growth continues until the plant begins to tassel (VT). Tasseling has been reached when the last branch of the tassel is visible while the silks still are not visible. This period marks the end of vegetative development and the plant begins pollen shed, also known as pollen drop (Ritchie et al. 1997).

The first reproductive stage (R1) is referred to as silking. This stage begins when silks are visible outside the husks (Ritchie et al. 1997). As pollen drops from the tassel, it is intercepted by the silks. Generally it takes about 24 hours for the pollen grains to grow from the end of a silk to the ovule where fertilization will take place. The next stage in reproductive development is blistering (R2) and occurs approximately 10-14 days after silking. This stage is known as blistering because the kernels are small, white and resemble a blister in appearance. At this point, the cob has reached its full size, the silks have served their purpose and are now beginning to dry out and turn brown. R3 marks a stage called milk and occurs approximately 18-22 days after silking. The kernels, once white, take on the characteristic yellowish appearance. The inside of the kernels is now full of a white, milky, starch fluid. Milk stage is followed by the dough stage (R4), which typically occurs 24-28 days after silking. The inner portion of the kernel has now progressed from a fluid starch material to a thickened starchy dough. During the final periods of the dough stage the kernel begins to harden and dent on the top of the kernel. This leads into the dent stage (R5), which occurs 35-42 days after silking. During this stage the kernel has hardened and the starch material in the kernel is solidifying. A line between the liquid and solid starch moves during the stage toward the base of the kernel. R6 is the point in which the corn plant and kernels have reached physiological maturity, usually 55-65 days after silking. Kernels have now reached their maximum dry weight and the starch mixture has sunk down completely in the cob. Stalks have progressed from herbaceous to semi-woody and the concentration of lignins and other chemicals have changed to increase stalk strength. This is when corn may be harvested for grain (Ritchie et al. 1997).

Southwestern Corn Borer

H.G. Dyar first described the southwestern corn borer in 1911 from a specimen collected in Guadalajara, Mexico (Davis et al. 1933). The southwestern corn borer at that time was included in the literature with *Diatraea crambidoides* and *D. lineolata*, both species being pests of corn and found in the southeastern part of the country. Dyar and Heinrich (1927) first identified southwestern corn borers as a separate species, *D. grandiosella.* It is not known how long the southwestern corn borer has lived in the United States. The spread of the southwestern corn borer is believed to have occurred northward from Mexico, and it entered via Arizona, New Mexico and Texas around the same time. C.H. Gable and R.A. Epperson in unpublished records observed the southwestern corn borer moving eastward from Lakewood, New Mexico to Carlsbad, New Mexico in 1914 (Dyar and Heinrich 1927). Arizona county agents observed the southwestern corn borer in 1916 and the first observation of damage by the insect occurred in 1917 in the state (Dyar and Heinrich 1927). The southwestern corn borer probably occurred unnoticed in the southwest for many years prior to its discovery. As agriculture began to grow in these states and irrigation allowed for more land to be cultivated for agriculture cropping systems, the desert barriers that once excluded areas from each other gradually became joined together. This allowed the southwestern corn borer to move further east (Davis et al. 1933). Chippendale and Sorenson (1997) calculated that the southwestern corn borer migrated eastwardly at an estimated rate of 13 miles per year from 1913 to 1931, 20 miles per year from 1932 to 1953 and 35 miles per year from 1954 to 1964. As of 1997, the northern limits of distribution were south-central Kansas to western Georgia, and these limits appear to be determined by low temperatures and natural enemies (Chippendale and Sorenson 1997).

Since the southwestern corn borer was first introduced in the United States, it has been a serious and destructive pest of corn (Chippendale and Sorenson 1997). The southwestern corn borer is also considered a pest on several other hosts (Davis 1965), namely sorghum, sugarcane, broomcorn, Sudan grass and Johnsongrass (Metcalf and Metcalf 1993). It is also found in Texas in various types of sorghum: milo, feterita, hegari, orange-top cane, and kafir (Davis 1965). The southwestern corn borer causes serious crop losses in the southern corn producing states ranging in the millions of dollars annually (Chippendale and Sorenson 1997). Davis (1998) reported that southwestern corn borer densities were steadily increasing in Mississippi during the 1990's. An estimated 60,700 ha of Mississippi corn were affected by the southwestern corn borer with most acreage requiring two to three insecticide treatments per growing season. This infestation cost producers between $20-25/ha per application in insecticides. At the end of the 1998 growing season, a survey indicated that an average of 65% of stalks within the Mississippi delta were infested. Because of this infestation, a 6% yield loss was estimated, costing Mississippi corn producers over $7,000,000 in yield losses plus application costs (Davis 1998).

The land area planted in corn and the value of corn has dramatically increased in the southern corn producing states from 2005 to 2007 (USDA NASS), so the costs of southwestern corn borer damage have likely also increased. In 2005 the price of corn was about two dollars per bushel, but by 2007 it had doubled to four dollars per bushel. Nationally the value of production increased from 22.2 to 54.7 billion dollars during 2005 to 2007. The increase in corn production and price in Mississippi increased the value of corn production from 102 to 496 million dollars during 2005 to 2007 (USDA NASS). This increase in corn value makes pest management of yield-reducing pests such as the southwestern corn borer even more important.

The southwestern corn borer undergoes three generations per year and has four growth stages: egg, larva, pupa, and adult. The egg is flattened, elliptical, and ranges from 0.8-1.3 mm in length. The top of the egg is convex in shape and the bottom assumes the shape of whatever it is laid on (normally a leaf or stalk) (Davis et al. 1933). Eggs are laid in clusters on the leaf or stalk surface usually on the undersides of leaves (Metcalf and Metcalf 1993). When the eggs are first laid they are translucent and white in appearance but shortly after they have a greenish-white appearance because of the green background onto which the egg was laid. After a period of 24 hours it has a cream color and three orange-red lines dividing the egg into four parts giving it a characteristic candy-stripe appearance. Approximately 24 hours before the egg hatches the egg turns yellowish and the head of the larva inside becomes visible. Parasitized eggs take on a blackish color and remain black after the parasite exits the egg. The blackish color stands out more than a normal egg on the surface of the leaf. Parasitized eggs also differ in that the parasite exits through a circular hole in the top of the egg while southwestern corn borer larvae exit thru slits in the margin of the egg (Chippendale and Sorenson 1997).

Larvae of the southwestern corn borer have a summer form and an overwintering form. The summer form is white with brownish-black dots on the body and lives inside the host plant above the soil level. Full-size larvae are typically about 25 mm in length. The overwintering form is a light yellow color or slightly darker and the head is not quite as brown as the summer form of the larva. This form lasts until early spring when the insect changes inside the lower part of the stalk into a small pupa. The brownish-black dots also disappear from the body of the insect (Metcalf and Metcalf 1993).

Once the larva has completed its development the larva will change into a prepupal stage followed by a pupal/resting stage. The prepupal stage is the same color as the larval stage; however, the wing pads and appendages take on a darker yellow color. At this point the pupa is very delicate and velvety. After a period of 24 hours the pupa darkens until the entire pupa is brown in appearance. The pupa is usually between 13 and 25 mm in length. Male pupae are smaller than female pupae, and the genital openings of the female pupa are located on the anal end (Chippendale and Sorenson 1997).

The emerging adult escapes the lower part of the roots by squeezing through a previously made escape tunnel which is protected by a thin layer of corn tissue that was not eaten by the larva (Chippendale and Sorenson 1997). The adult stage of the insect is a cylindrical dull white moth (Davis et al. 1933). The moth has a wingspan of 32mm and typically lays from 300 to 400 eggs in a lifetime. Male moths are smaller than female moths and are slightly darker in appearance (Chippendale and Sorenson 1997). The fore wings are darker than the hind wings and their edges are parallel to one another. The labial palpi of the insect extend forward resembling a beak and its antennae are filiform proximad (Chippendale and Sorenson 1997). Typical development from egg to adult may take 36+ days depending on temperature and diet (Metcalf and Metcalf 1993).

The southwestern corn borer moth is only active at night (Davis et al. 1933). The moths can be found in the daytime hours on corn plants in the shade, most likely on the open leaves or in the whorl of the plant. The moths are not easily disturbed and the leaves on which they are resting may be moved without disturbing the moths. The moths become active late in the afternoon and when disturbed will fly in a zig-zag motion about 3-10 meters before resting on another plant. No feeding takes place in the adult stage of the insect. Adult moths are usually inactive the first night of emergence and begin mating the second day after. As the moths mate they form a straight line facing away from each other with the female's wings overlapping the males. Mating may last from one and a half to three and a half hours. Males tend to mate with two females. Oviposition of the eggs usually begins the night after mating. Females may not move around the field as readily just before first oviposition because of the weight of the eggs, but as the eggs are laid, their movement in the field increases. More eggs are laid in the first two days of oviposition than the rest. Oviposition lasts 3-4 days until the female moth becomes sluggish and eventually dies. Therefore adult females live approximately five days while the male lives only about three days, usually dying soon after mating (Davis et al. 1933).

Southwestern corn borer larvae damage corn in three major ways (Henderson and Davis 1969, Moulton et al. 1992). First generation larvae of the southwestern corn borer lay their eggs on the leaf tissue of young corn plants. First generation larvae feed on leaf material before moving down into the growing point of whorl stage corn plants. This type of feeding may occur around the V6 period of development when the growing point is above the leaf surface and there are adequate amounts of leaf material for the corn borer to feed on until reaching the growing point located inside the whorl of the corn plant. This feeding causes a condition known as “dead-heart” in which the growing point of the plant dies and can result in yield loss due to low plant population (Henderson and Davis 1969, Moulton et al. 1992).

The second type of damage is stalk feeding, which is caused by second generation larvae tunneling into and down the corn stalk (Moulton et al. 1992). This generation attacks corn during the V12 to VT period of development. As discussed earlier, this is a very important time of plant growth and development of the corn plant because the plant is requiring the highest amounts of nutrients and water uptake. This feeding causes a reduction in yield because it removes xylem and phloem tissue in the pith of the stalk and interrupts the translocation of plant nutrients and water to the ears.

Third generation larvae cause a type of damage known as lodging (Henderson and Davis 1969). Once the larva enters the corn stalk in the same ways as the prior generations, it tunnels to the base of the stalk where it girdles a groove inside the stalk, weakening the base of the plant which leads to the stalk falling under its own weight or from high winds. Once the stalks have fallen it becomes very difficult to mechanically harvest the ears. The ears also become increasingly susceptible to losses from bacterial and fungal diseases (Henderson and Davis 1969). Second and third generation southwestern corn borers can attack corn anytime from late whorl stage until harvest depending on when the corn was planted.

Management of the Southwestern Corn Borer

Control of the southwestern corn borer can be achieved by many different methods. Since the overwintering form of the insect occurs in the lower portion of the stalk, destruction of plant material by cultivation or burning after harvest is a very effective measure to reduce overwintering populations. Rotation with a non-host crop is also an effective method of control (Metcalf and Metcalf 1993).

Scouting for the southwestern corn borer during the growing season includes visually looking for eggs, larvae and feeding damage (MSU Extension 2007). Eggs are laid on the leaves around two to three leaves from the ear and can be difficult to see. Larvae are seen feeding on leaf tissue and typically cause windowpane damage to the leaves along with pinhole scars. The distribution of larvae is random or uniform which is particularly important when developing sampling plans and thresholds (Overholt et al. 1990). Overholt et al. (1990), found that in order to achieve a sampling accuracy of 0.20 for a threshold of 25% infestation of small larva or eggs, 19 plants should be sampled. The sample size for scouting southwestern corn borers was increased to 25 plants per field because the study observed plants under laboratory conditions while scouting in a field situation can have a greater sampling error. Any infestation below 25% is considered to be below economic importance and infestations above this level are considered to be yield-reducing.

In season control of the insect can be achieved by timely insecticide applications (MSU Extension 2007). The applications must be timely because the insect is a stalk borer and once the larva has reached the inner portion of the stalk it is nearly impossible to get an insecticide to reach the insect. The critical time in which an application must be made is the period from oviposition through second instar. During this 10-12 day period larvae feed on the leaf surface and insecticidal control can reach as high as 80 percent. However, eggs can be laid over several weeks due to varied adult emergence time, so scouting must be maintained over an extended period and multiple insecticide applications may be needed.

Current insecticides labeled for treatment of corn for southwestern corn borers include Warrior T (lambda-cyhalothrin), Asana XL (esfenvalerate), Ambush/Pounce (permethrin), Fury (zeta-cypermethrin) and Intrepid 2F (methoxyfenozide) (MSU Extension 2007). A method used to determine when to apply insecticides is the use of pheromone traps which attract male southwestern corn borers with a blend of (Z)9-hexadecenal, (Z)11-hexadecenal, and (Z)13-octadecenal, a chemical blend that mimics the female sex pheromone. Pheromone traps have proved to be an effective means of determining when moths are flying, but counts are not currently used as a threshold (Hedin et al. 1986). Daves (2006) states that another tool that can help optimize insecticide application timing is a temperature-dependent computer model which predicts the periods of southwestern corn borer emergence and oviposition.

In addition to insecticide applications, the use of genetically modified crops is an effective means of control. The major toxin group used for control in genetically modified plants is from *Bacillus thuringiensis* (Allen 2002). The bacterium, *Bacillus thuringiensis*, (Bt) contains toxins that either kill or greatly reduce the health of the European and southwestern corn borers and some other Lepidoptera. Thuricide was the first form of the bacterium used for testing in 1957 with the foliar insecticide 'Dipel' following it in 1970 as the first commercial Bt-based product. Overall, Bt foliar insecticides never found a substantial place in the market because their cost was comparable to chemical insecticides, but they degraded rapidly in the field and provided low or inconsistent control of target insects. Recent advances in genetic engineering have allowed DNA from Bt that codes for toxins to be inserted into crop DNA so the toxins are expressed in the crop tissue. These crops, known as transgenic crops, overcame the limitations of the Bt based foliar insecticides and have been widely adopted by growers. Southwestern corn borers have been found to cause limited damage to these transgenic corn varieties compared with their non-Bt counterparts (Allen 2002). In addition to low larval survival, larvae found to survive on Bt transgenic corn weighed significantly less than larvae on non-transgenic varieties (Williams et al. 1997).

Davis et al. (1933) state physical and climatic conditions that cause mortality include subjecting the overwintering larvae in the stalk to cold weather, moisture or the lack of moisture, rotting of the stubble, termites and disease. During the growing season, rain may wash away eggs, and water standing in the whorl of the plant can cause death of the early instar larvae located inside. Early instars of later generations are affected by the tough fibrous tissue of the corn leaves and subsequently die of starvation (Davis et al. 1933).

Natural enemies include parasitoids, predators, and diseases (Davis et al. 1933). Eggs of parasitoids of the southwestern corn borer include *Trichogramma minutum* (Hymenoptera: Trichogrammatidae) and *Apanteles diatraeae* (Hymenoptera: Braconidae) which oviposit in the eggs of the southwestern corn borer. *Solenopsis geminate* (Hymenoptera: Formicidae) and *Crematogaster spp.* (Hymenoptera: Formicidae) are species of ants that attack the larvae of the southwestern corn borer by tearing it apart and carrying it away piece by piece. General predators of southwestern corn borer include species of termites, mites and thrips.

Corn Stalk Strength

Several studies have measured corn stalk strength and looked at its relationship to plant lodging. Some methods of measuring stalk strength include bending, crushing, penetration, shearing, and measuring of rind thickness (Schertz et al. 1978). Berzonsky and Hawk (1986) concluded in their study that as stalk-crushing strength (pressure it took to crush a segment of a corn stalk) increased, lodging decreased. Another method of measuring stalk strength is the measuring of the stalk's thickness using a micrometer caliper and then using a hydraulic press to crush two-inch sections of stalk (Zuber and Grogan 1961).

Stalk strength has been indirectly measured by counting lodged stalks over several locations (Sibale et al. 1992). However, Sibale et al. (1992) point out that the variability of environmental conditions such as wind gusts, soil moisture, different plant genotypes, etc. makes this a poor method of measuring lodging resistance in corn. Sibale et al. (1992) compared the cost and variation in readings for crushing and penetration methods. It was found that even though the rind penetrometer penetrated into the plant, compromising the corn stalk tissue, it did not affect yield and cost the user around $800 for a penetrometer while the crushing method cost over $7,500 for the unit and destroyed the plant being sampled. Changes in the physiology of the plant such as the plant becoming harder or larger over time also favored the rind penetrometer readings over the stalk crushing method (Sibale et al. 1992).

Sibale et al. (1992) explored how to use the rind penetrometer best by comparing two different penetrometers. One penetrometer was a manual penetrometer which had a probe attached to a mechanical force gauge. The second penetrometer was a modified penetrometer that recorded force digitally and had a stop bar attached to its probe. The penetrometers were used on opposite sides of the same plant and the readings were compared. The modified electronic rind penetrometer was found to be the best method for measuring stalk strength because it was simpler and more rapid while maintaining consistency and accuracy.

While the rind penetrometer has proven to be useful in evaluating stalk strength, most studies using the rind penetrometer focused on breeding for overall lodging resistance and the impact of certain bacterial and fungal infections, and did not evaluate insect damage. The one exception is a study by Martin et al. (2004), which compared rind penetrometer resistance and the ability of European corn borer larvae to feed in corn. Using a penetrometer similar to the one used by Sibale et al. (1992), Martin et al. (2004)

evaluated different varieties of corn at different times in the year. Early in development, varieties were found to have no significant differences in penetrometer resistance or in first generation European corn borer damage or lodging. However, there were differences among varieties in second-generation damage and penetrometer resistance during reproductive stages. Feeding damage and stalk strength had a significant negative correlation. It may also be noted that penetrometer resistance was correlated to the amount of certain lignins and fibers. Therefore it was hypothesized that lignins and fibers may provide both physical and chemical resistance as the constituents increase stalk strength and reduce the digestibility of the stalk for the European corn borer. Significant correlations were made in this study that demonstrated rind penetrometer resistance, stalk composition and second-generation European corn borer resistance were related to each other.

This current study evaluates the relationship between the penetration resistance of plant tissue and insect feeding. More specifically the objective is to relate corn stalk strength to southwestern corn borer larvae's ability to penetrate the corn plant. Several practical applications from this research could prove beneficial to southwestern corn borer management. Breeders could use a penetrometer to identify or breed for a corn stalk strong enough to reduce southwestern corn borer feeding. This procedure could also be used to screen for new host plant resistance traits in varieties. Resistant varieties could be planted by producers to reduce the need for insecticides on non-transgenic crops and to reduce selection pressure in transgenic crops. Another application of this experiment could be to determine a stalk strength at which southwestern corn borers cannot penetrate. This would potentially allow producers to avoid insecticide applications when the corn is not vulnerable to southwestern corn borers.

CHAPTER II

MATERIALS AND METHODS

Penetrometer Measurements

From the numerous methods used to measure stalk strength (Schertz et al. 1978), the rind penetrometer method was selected for this study since it seems to better approximate stalk strength from the perspective of southwestern corn borer larvae than the other methods.

A digital force gauge, DFG51, manufactured by the Omega Technologies Company (Stamford, CT), was used to record the force necessary to penetrate a corn stalk. This model measures force up to 25 kilograms in 0.02 kg increments. The machine consisted of a hand held gauge attached to a steel probe affixed to a threaded bolt on top of the housing. Several attachments for the rod were included with the machine including pointed, rounded and flat attachments. It was found that the rounded and flat attachments gave erratic measurements. When using these attachments the stalk was bent or folded more than it was penetrated. In contrast, the pointed attachment slid easily into the stalk each time it was used.

Three pointed probing rods of varying diameter were screened for their ability to consistently measure corn stalk strength: 0.24, 0.48 and 0.95 cm in diameter. The 0.95 and 0.48 cm diameter rods were so large that they split the stalk fibers rather than cleanly piercing the rind. The 0.24 cm diameter rod pierced the rind cleanly, providing more consistent measurements than the 0.48 and 0.95 cm probes. A stop bar was added to the machine to allow only one cm of the probing rod to penetrate the corn stalk. Similar to the stop bar used by Sibale et al. (1992), a piece of acrylic glass was screwed onto the back of the force gauge using the predrilled holes in the gauge. The edge of the glass stopped the probing rod from going further into the stalk. The stop bar enabled the user to probe the stalk at a consistent depth and provided more consistent measurements.

To take rind strength measurements, the stalk was held firmly and the probing rod was quickly thrust perpendicular into the stalk until the stop bar touched the stalk. The highest force exerted during penetration was displayed on the meter and recorded. The meter was then reset to zero before sampling the next plant. Measurements are reported in kg rather than kg/cm^2. All measurements used the same probe which had a surface area of 0.045 cm^2. Because our experience showed there was not a clear relationship between probe diameter and penetration resistance, expressing the force required to penetrate the stalk in kg is as informative as expressing it per unit area and is less likely to be extrapolated inappropriately.

2006 Trial

During the 2006 growing season, one variety of corn was evaluated to determine the consistency of the force gauge and also the best method of infesting corn with southwestern corn borer larvae. A single variety was randomly chosen because it was not known how much variation would be between each stalk in the field. Corn was planted on 97-cm rows at the Brown Loam Experiment Station located in Raymond, MS on

March 23, 2006 and standard agronomic practices were maintained throughout the growing season.

Two hundred plants were evaluated while the corn was in the dough stage. The internodes immediately above the ear and between the lowest two nodes of the plant were probed. Southwestern corn borer eggs were obtained from the USDA rearing lab located in Starkville, MS. They were then taken to the Brown Loam facility where they were placed in a 86.7 L plastic container measuring 58.2 cm long, 43.2 cm deep, and 34.5 cm wide. The container was placed in a temperature controlled room and held at 27° C. The southwestern corn borers were allowed to grow until they reached 3rd instar. Third instars were chosen for infestations because approximately 5 – 31% of tunneling occurs between the third and fourth instars (Whitworth et al. 1984).

Two methods of infesting third instar southwestern corn borers were tested to determine the best infestation method for this experiment. The first method used 0.63 cm diameter PVC pipe cut into 2 cm long pieces with their edges rounded to fit onto the stalk. The pipe was placed against the stalk with a larva inside the pipe. The end of the pipe facing away from the stalk was closed off using a strip of breathable athletic tape. A second method of infestation involved placing 4-5 corn borers directly onto the leaf sheath just below the primary ear and below the second internode above the ground. Survival and stalk penetration were evaluated after three days. Penetration was determined by peeling back the leaf sheath on which the larvae were placed and examining the stalk for holes or signs of feeding. Force to penetrate the corn stalk was measured using the penetrometer with the methods described previously.

Individual plant data were ranked according to penetrometer resistance in the ear zone and at the base of the plant separately. Mean resistance and southwestern corn borer survival were calculated for each group of 20 plants at each location. There was no attempt to keep ear and base readings from the same plant together. Southwestern corn borer survival was analyzed as a function of penetrometer resistance using PROC GLM (SAS Institute 1999) for both stalk locations independently as well as overall.

2007 Trial

Corn was planted at the MSU research facilities located in Starkville, MS on April 9 (NF1) and April 30, 2007 (NF2) and in Raymond, MS (BL) on April 23, 2007 in a randomized complete block design with 4 replications at each planting. Each plot was 4 rows wide, 15.2 meters long with a 2-meter gap between replicates and a four-row planted border around the entire plot. Corn was planted on 97-cm rows and standard agronomic practices were maintained throughout the growing season.

Five varieties of corn were chosen for their range in stalk strength based on seed company reports of stalk strength or stalk standability. Ratings of strength or standability were either on a scale from 1-10 with 10 being the strongest or on a scale from poor-excellent. The five corn varieties tested (company and strength ratings in parentheses) were: 23R31 (Terral, 10), 25R31 (Terral, 9), P33-10 (Pioneer, average), 32R25 (Pioneer, poor), and 668 (Dekalb, excellent). All these varieties were approximately the same maturity (113-116 days relative maturity) so that they would reach each maturity stage at approximately the same time.

Stalk strength is generally associated with plant maturity (Ritchie et al. 1997). So, stalk strength was evaluated at VT-R1 (tassel), R4 (dough) and R5 (dent) growth stages. During each growth stage, 20 consecutive plants were sampled in each variety of corn. By sampling over a period of time from tassel to dent stages at different locations and over a range of varieties, the data were expected to test a wide range of stalk strengths.

Southwestern corn borer eggs from the USDA rearing lab located in Starkville, MS were received as needed on 3-4 wax paper sheets with eggs on both sides. The eggs were kept in a rearing room maintained at 16:8 light: dark, 27°C and 60% relative humidity until the eggs began hatching. Upon hatching, the egg sheets were divided equally and placed into 12.9 L rectangular plastic containers measuring 25.5 cm wide, 39 cm long and 13 cm deep. Approximately 240 ml of sterilized water agar was put in each container in order to keep a moist environment. A moist paper towel was placed on top of the agar followed by 4-5 fresh corn leaves, and a sheet of eggs. Additional layers of paper towel, leaves and egg sheets were added until they reached the top of the container. A hole was cut into the lid and covered with cheesecloth to allow air circulation. The upper leaves needed to be replaced every 2-3 days due to desiccation. The larvae were allowed to feed 5-6 days until they reached third instar. These larvae were taken to the field plots and placed on the leaf sheaths using a fine paint brush immediately above the ear leaf at a rate of five larvae per stalk on 20 consecutive plants per plot. After five days the stalks were examined to determine if the larvae were able to penetrate through the rind and enter the pith. At this time the force required by the force gauge to penetrate through the rind in the internode above the ear was also recorded for each infested plant.

Probing was done in a region that showed no damage from southwestern corn borer feeding.

The same experiment was conducted at three locations during 2007. Data were analyzed for each planting independently and together using location as a class variable. Experimental units were 20 consecutive corn stalks and the sampling unit was a single plant in each plot. Differences in mean force required to penetrate the stalk were compared for crop maturities, varieties and locations using PROC GLM. Southwestern corn borer tunneling success in the numerous varieties, locations and maturities were also evaluated using PROC GLM. Differences were considered significant for $\alpha = 0.05$.

CHAPTER III

RESULTS AND DISCUSSION

2006 Trial

There was no survival of southwestern corn borer larvae after three days using the pipe method. Placing the larvae directly onto the leaf sheath proved to be a better method of infesting southwestern corn borer larvae, so larvae were placed on the leaf sheath for all data reported.

Comparison of the mean (±SEM) upper and lower force measurements showed that the lower portion of the stalk required more force to penetrate than the upper portion of the stalk (Figure 1). Larval survival was 53% and 30% survival on the upper and lower portions of the stalk, respectively (Figure 1). These results were consistent with expectations that larval survival would be lower where stalk strength was greatest.

A closer examination of the data explored the relationship between penetration resistance and southwestern corn borer survival within each corn stalk location. Contrary to expectations based on larval survival at the base and ear zone, at the base of the stalk southwestern corn borer survival increased 18.6 ± 3.2% for every kg increase in penetration resistance ($F = 34.70$; df = 1, 8; $P < 0.001$) (Figure 2). There was no relationship between penetration resistance and southwestern corn borer survival in the ear zone ($F = 1.90$; df = 1, 8; $P = 0.205$), nor when both stalk locations were combined ($F = 0.52$; df = 1, 18; $P = 0.481$). Penetration resistance on the lower stalk ranged from 1.07

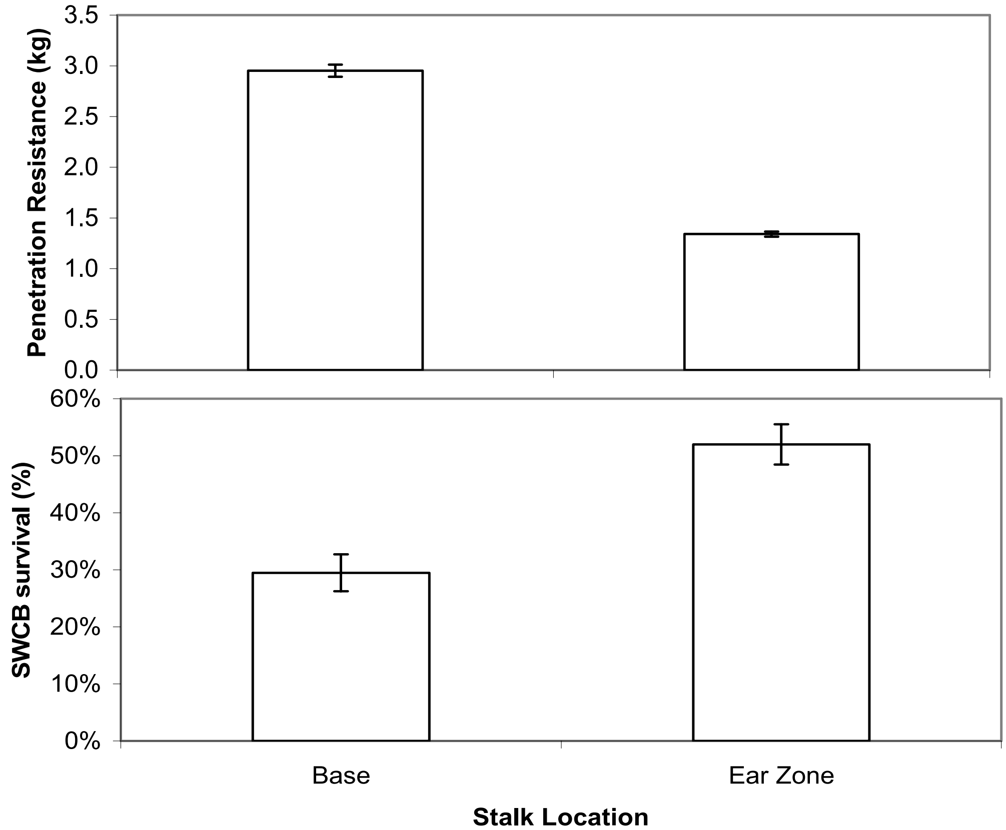

Figure 1 Mean stalk penetration resistance and southwestern corn borer (SWCB) survival (± SEM) on corn during dough stage. Raymond, MS during 2006.

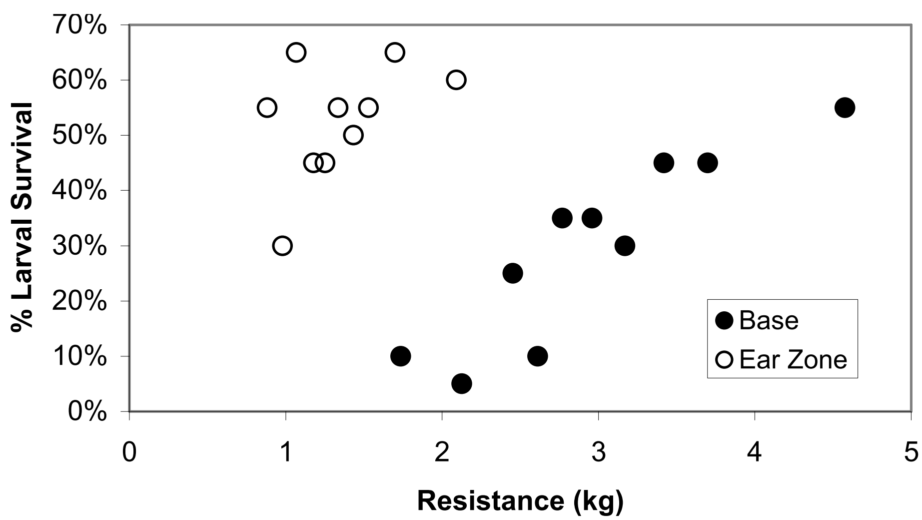

Figure 2 Southwestern corn borer larval survival after five days when placed in the ear zone or at the base of the plant. Each point is the mean of 20 plants after being ranked by stalk resistance. Raymond, MS during 2006.

to 6.68 kg, but resistance in the ear zone had a smaller range and lower values from 0.78 to 2.50 kg. Based on this analysis, stalk strength does not appear to be limiting factor of southwestern corn borer survival within the range tested. Rather, stronger stalks, at least at the plant base, contained some trait or traits that increased survival. This may have been higher moisture content or more nutritious tissue as the stronger stalks tended to be the larger plants that were not quite as mature (personal observation). The major conclusion drawn from these data is that stalk strength within one commercial variety is not a limiting factor for southwestern corn borer survival, although it may be associated with plant characteristics that could impact southwestern corn borer survival.

2007 Trial

Data from 2006 showed that the force required to penetrate the stalk was not a significant mortality factor in the ear portion of the corn stalk. However, all 2006 data were collected from a single corn variety at a single point in its development. Most southwestern corn borer tunneling begins near the ear zone, and stalk boring can occur during most stages of reproductive growth (Davis et al. 1933), so practical southwestern corn borer crop protection requires resistance in the ear zone over several weeks. To evaluate a broader range of stalk strengths in the ear zone over a longer period of time, five different varieties were compared in 2007 at three different locations during three growth stages.

Stalk penetration resistance was analyzed using the effects of location, maturity and variety and their interactions. Location and several interactions with location were significant factors when all locations were analyzed together, so to facilitate interpretation, the three plantings were analyzed independently. At Brown Loam, maturity, variety and the interaction between maturity and variety were all significant factors affecting penetration resistance (Table 1). Penetration resistance was greatest during tassel stage and least during the dough stage (Figure 3). The varieties at Brown Loam expressing the greatest resistance overall were 668 and 23R31. The interaction of maturity and variety was significant, largely due to 668 and P33-10. At tassel stage P33-10 had the most resistance and 668 had the least resistance, but by dough stage this had completely reversed. At the two North Farm locations (NF1 and NF2), maturity and variety were significant factors influencing penetration resistance as at Brown Loam. However, unlike the Brown Loam location, the interaction was not significant at either North Farm locations (Table 1). At NF1, average penetration resistance over all varieties was greatest during tassel and least during dent (Figure 3). However, at NF2, resistance was least during dough stage and similar during the other stages. At both locations, varieties 23R31 and 668 had the highest penetration resistance, and 32R25 and P33-10 had the lowest penetration resistance. Variety differences in stalk penetration resistance at all locations were consistent with the ratings provided by the companies, indicating that hybrid selection was effective in achieving a range of stalk strengths for testing our hypothesis that stalk strength can impact southwestern corn borer survival.

Table 1 Analysis of variance using type 3 sums of squares for penetration resistance at all locations in 2007.

Location	**Factor**	**df**	**F**	**Pr > F**
Brown Loam	Maturity	2, 42	63.30	<0.001
	Variety	4, 42	2.92	0.032
	Maturity * Variety	8, 42	4.36	0.001
North Farm 1	Maturity	2, 42	17.72	<0.001
	Variety	4, 42	6.41	<0.001
	Maturity * Variety	8, 42	0.45	0.862
North Farm 2	Maturity	2, 42	30.95	<0.001
	Variety	4, 42	3.54	0.014
	Maturity * Variety	8, 42	0.80	0.604

The factors of location, maturity and variety plus the factor of penetration resistance were evaluated for their impact on successful penetration and stalk boring by southwestern corn borer larvae. Location was a significant factor with survival much lower at Brown Loam than at the other locations (Table 2). Several factors could have attributed to the lower survival at Brown Loam, including the longer transportation time of the larvae to Brown Loam (2 hr) compared to the North Farm (10 min), different weather conditions, and more highly drought-stressed corn since the field was not irrigated at Brown Loam. Because southwestern corn borer survival was poor at Brown Loam, there were no significant differences in survival among varieties at this location ($F = 0.25$; df = 4, 47; $P = 0.906$). There were no significant factors involving

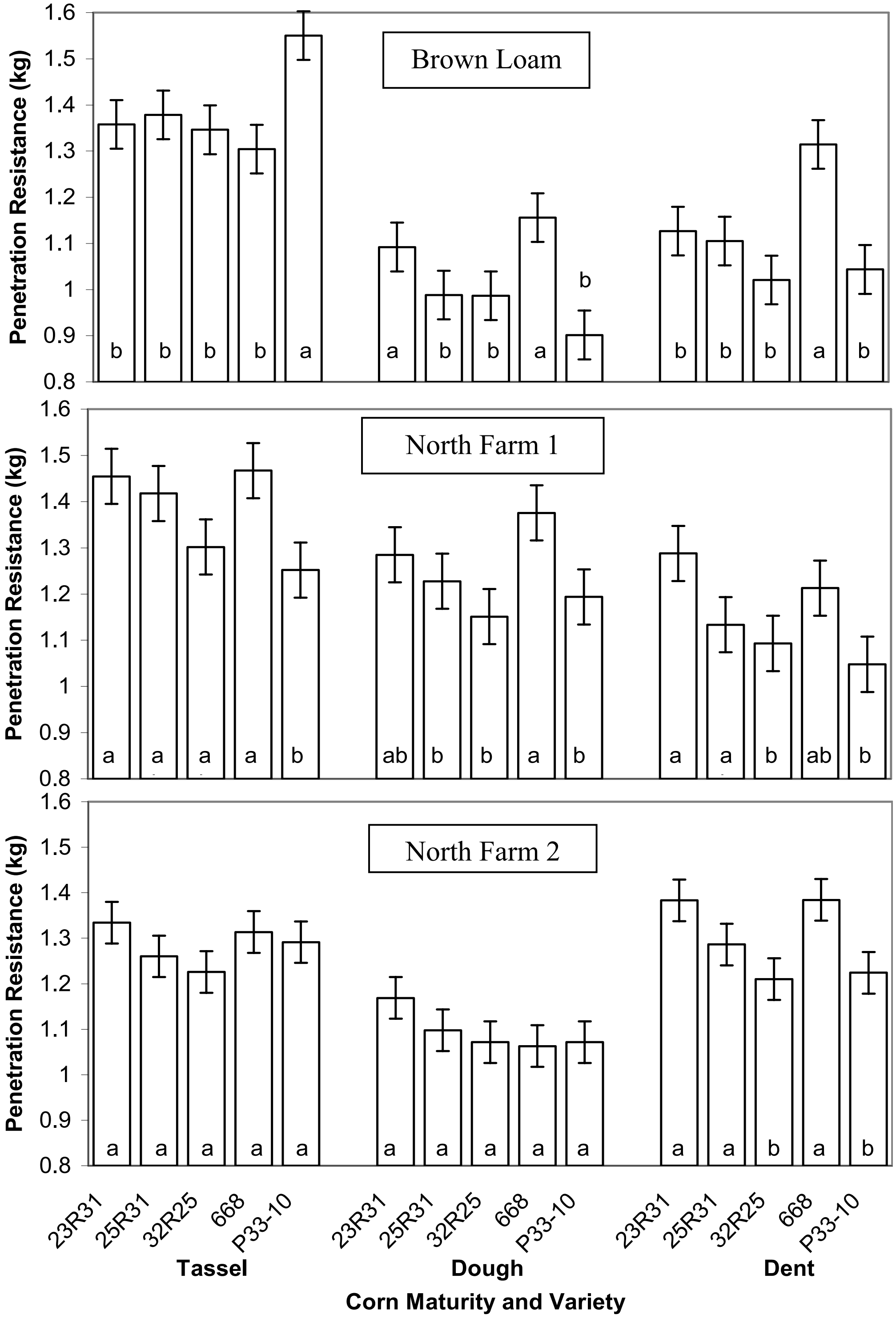

Figure 3 Least square mean penetration resistance (± SEM) of five corn varieties during three growth stages at Brown Loam, North Farm 1 and North Farm 2 locations during 2007. Resistance of varieties with the same letter within a maturity group at a location are not significantly different (α = 0.05).

Table 2 Mean force (±SEM) required to penetrate the corn stalk and successful stalk penetration by the southwestern corn borer (SWCB) over all varieties at three locations in MS during three growth stages in 2007.

Location	Maturity	Penetration Resistance (kg)	% SWCB Survival
Brown Loam	Tassel	1.387 ± 0.016	6.75 ± 1.26
	Dough	1.025 ± 0.013	13.00 ± 1.68
	Dent	1.122 ± 0.015	2.50 ± 0.78
North Farm 1	Tassel	1.379 ± 0.016	47.25 ± 2.50
	Dough	1.247 ± 0.013	24.25 ± 2.15
	Dent	1.155 ± 0.014	14.00 ± 1.74
North Farm 2	Tassel	1.285 ± 0.012	36.50 ± 2.41
	Dough	1.094 ± 0.013	35.75 ± 2.40
	Dent	1.298 ± 0.012	2.00 ± 0.70

location after omitting the Brown Loam data, so the combined larval survival data from the two North farm locations are presented.

Southwestern corn borer survival was significantly impacted by corn maturity, variety, penetration resistance and interactions of these factors (Table 3). Survival was highest during the tassel stage (38.8 ± 2.6%) and lowest during the dent stage (7.3 ± 2.1%) (Figure 4). Survival was impacted by variety during tassel and dough stages, but not during dent stage. However, the varieties with the least survival were not consistent

Table 3 Analysis of variance using type 3 sums of squares for southwestern corn borer survival at North Farm locations in 2007.

Factor	df	F Value	Pr > F
Maturity	2, 95	6.51	0.002
Variety	4, 95	3.32	0.014
Pen. Res.[1]	1, 95	6.67	0.011
Variety * Maturity	8, 95	4.30	<0.001
Pen. Res. * Variety	4, 95	3.32	0.014
Pen. Res. * Maturity	2, 95	9.50	<0.001

[1] Penetration resistance measured by a digital penetrometer

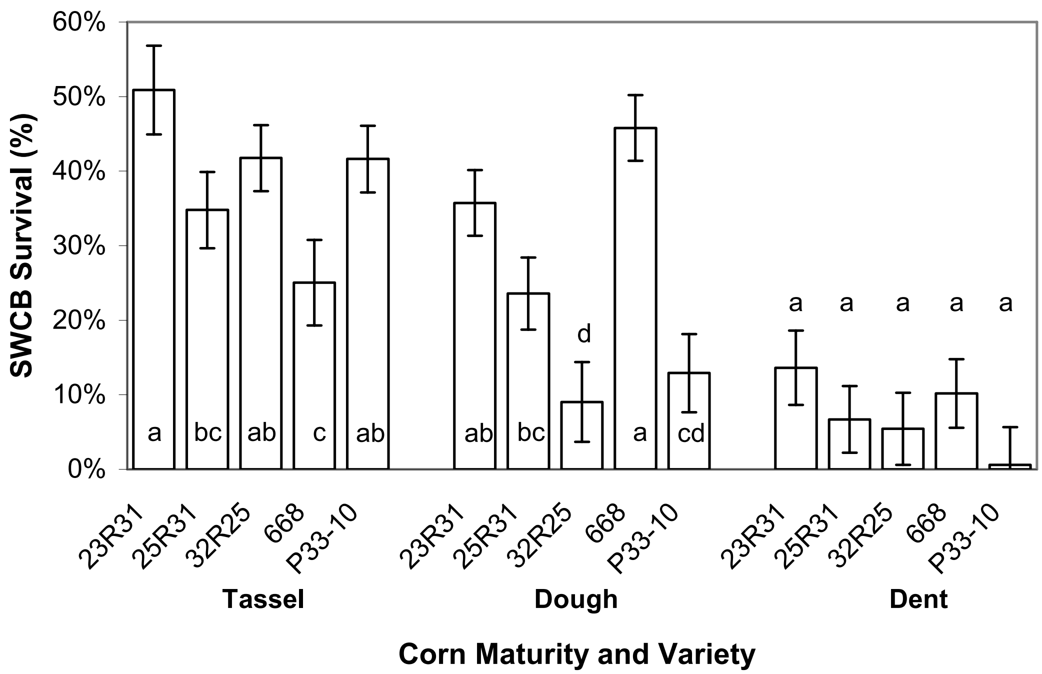

Figure 4 Least square mean southwestern corn borer (SWCB) survival (± SEM) on five corn varieties during three growth stages at two North Farm locations during 2007. Varieties with the same letter within a maturity group do not have significantly different southwestern corn borer survival (α = 0.05).

between tassel and dough stages. Variety 668 had the lowest survival during tassel stage, but the highest survival during dough stage (Figure 4). The reasons for these shifts are not clear, but they are not related to changes in penetration resistance as penetration resistance did not change in this manner (Figure 3).

While penetration resistance is clearly not the only factor influencing southwestern corn borer survival, it was a significant factor, and interactions with maturity and variety were also significant (Table 3). The three-way interaction of penetration resistance with variety and maturity was tested and not found to be significant ($F = 1.51$; $df = 8, 87$; $P = 0.165$). Therefore, the three-way interaction was deleted from the final model. During dough and dent stages an increase in penetration resistance resulted in reduced survival (Table 4). However, penetration resistance had no impact during the tassel stage. For 23R31 and P3310 varieties, an increase in penetration resistance resulted in fewer southwestern corn borer survivors (Table 4). In contrast, penetration resistance was not a significant factor of mortality for any of the other varieties tested.

The initial hypothesis of this experiment was that as the corn stalk physiologically matured, stalk strength would increase and that stalk strength was related to southwestern corn borer survival. It was believed that as the stalks accumulated more lignins and fibers, the stalk would become harder and rigid, causing decreased survival of the southwestern corn borer. The survival of the southwestern corn borer was greatest at tasselling and lowest at dent as expected. However, the stalk was more difficult to penetrate at tasselling than later at dent at two of the three locations (Figure 3). At tasselling, the moisture content of the plant was high, making the cells swollen and turgid. As the plant aged, its cells lost moisture, causing them to lose their turgidity and increase the space between cells. This shrinking of cells could have allowed the probe to penetrate the stalks easier at dent rather than at tasselling.

Table 4 Impact of crop maturity or variety with penetration resistance (PR) interactions on southwestern corn borer (SWCB) survival. North Farm locations during 2007.

Maturity/Variety	**PR impact on SWCB survival (% change / 0.45 kg increase)**	**F-value**	**df**	**Pr > F**
Tassel	17.0 ± 9.6	3.11	1, 31	0.087
Dough	-29.0 ± 9.4	9.60	1, 31	0.004
Dent	-21.7 ± 5.4	16.24	1, 31	<0.001
23R31	-36.7 ± 11.8	9.74	1, 17	0.006
25R31	1.7 ± 13.6	0.01	1, 17	0.904
32R25	-3.1 ± 11.6	0.07	1, 17	0.796
668	-2.1 ± 9.0	0.06	1, 17	0.816
P33-10	-30.1 ± 9.8	9.38	1, 17	0.007

While there were significant relationships between penetration resistance and southwestern corn borer survival, they were highly variable, indicating that stalk strength may not be the key factor, but that it may be correlated with another more important factor in some situations. Differences in plant chemistry is one plausible factor that could be influencing southwestern corn borer survival on different varieties and at different growth stages. Grasses produce all the essential nutrients that insects need for normal growth and development (Bernays and Barbehenn 1987). However, the availability of important nutrients may be limited as the plant ages. One limiting nutrient is nitrogen, which is strongly related to the approximate digestibility of plants to insects. Nitrogen often limits the growth of plants throughout the growing season, so as the corn plant matures and nitrogen levels steadily decline, it becomes increasingly difficult for the insects to obtain enough nitrogen from the plant tissue for normal growth and development. It is also known that lower water content of leaves and vegetation can cause lower growth rates for insects such as caterpillars (Bernays and Barbehenn 1987).

Physical factors also affect the growth and development of insects. As the plant ages, the lignification of plant tissue makes the plant increasingly harder to chew and digest. This lignification deters feeding of chewing insects such as the southwestern corn borer (Bernays and Barbehenn 1987).

This research shows that penetration resistance is a factor impacting the survival of southwestern corn borers, so breeding for a stalk based on the force to penetrate tissue may have some merit. However, because the impact of stalk strength was variable, further research should explore other factors that may be influencing southwestern corn borer survival. If breeding for stalk strength, selections should be for a variety which is strong during tasseling since this was the period of greatest survival. Another factor that may need to be considered for breeding a southwestern corn borer-resistant plant based on mechanical resistance is increased lignification at earlier stages. Breeding for a stalk that contains more lignins and fibers, which would also be expected to be harder to penetrate, could also deter feeding by wearing down the mandibles of chewing insects such as the southwestern corn borer.

The penetrometer used in this experiment proved to be a very useful tool in measuring stalk strength. The modifications such as the stop bar and using a smaller-diameter probe were successful in making the measurements more consistent. One concern with the penetrometer that should be considered is its consistency from operator to operator. This source of variation has been noted for other sampling methods (Musser et al. 2007). For the purpose of this experiment, only one person handled and probed the plant. This was done because the speed with which the operator pierced the stalk varied the readings. Pressing the probe into the corn stalk very slowly resulted in more force required to penetrate. However, when the probe was thrust very quickly into the stalk, the readings were much lower and very erratic. It took practice by the penetrometer operator to exert the probe into each stalk in a consistent manner. This is a concern in that several handlers of the machine may not use a consistent technique to generate comparable data. When planning to use a penetrometer, practice is needed to ensure that different operators will use the tool similarly to get comparable data.

One method that could eliminate the variation in different operators would be by developing a mechanical press system. This would solve the problem of different users. However, it would most likely make the machine large, heavy and cumbersome when transporting it from field to field. Since part of what makes the penetrometer desirable is its quickness, mobility and efficiency, such a method may not be practical. The number of stalks checked with a penetrometer is primarily a function of how quickly the operator moves from plant to plant. A mechanical press system would require the stalks to be cut from the field and moved to wherever the probe was located, making the probe as difficult to use as the stalk crushing techniques (Thompson 1961).

Conclusion

Our research demonstrated that penetration resistance was sometimes a factor in southwestern corn borer survival. However, it appears that other factors may be more critical in determining survival. The explanation for reduced southwestern corn borer survival in more mature corn needs to be further examined. Based on this study, the roles of location, physiological changes and related nutritional changes in stalk strength and southwestern corn borer survival are factors that may be good factors to manipulate in future studies. Clearer insights may also be gained by selecting more diverse genetic lines than the varieties used in this experiment.

Stalk strength and resistance could be used in a variety of crops to deter insect feeding. Just as stalk strength and southwestern corn borer resistance were associated in corn in this experiment, other crops could also have a relationship. Examples of other crops where physical resistance may modify damage could be cotton bolls with relation to bollworm feeding, and sorghum stalks in relation to lepidopteran borers. Because stinkbugs and tarnished plant bugs attack crops by penetrating their needlelike mouthparts into the young stems and fruit, a syringe needle could be attached to the probe and used to measure resistance to piercing-sucking insects. The possibilities using force to penetrate plant tissue as a host plant resistance mechanism are applicable to many different crops and pests and should be explored.

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