EFFECTS OF LIFE HISTORY, DOMESTICATION, AND BREEDING OF *ZEA* ON

THE SPECIALIST HERBIVORE *DALBULUS MAIDIS* (HEMIPTERA:

CICADELLIDAE)

A Thesis

by

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ABSTRACT

A suite of plants from the maize genus *Zea* L. (Poaceae) and the specialist herbivore *Dalbulus maidis* (DeLong and Wolcott, 1923) (Hemiptera: Cicadellidae) were used to test the hypotheses that anti-herbivore defenses are affected by plant life-history evolution and human intervention through domestication and breeding for high yield. The suite of plants included a commercial hybrid maize (*Zea mays* ssp. *mays* L.), a landrace maize, two populations of annual Balsas teosinte (*Z. mays* ssp. *parviglumis* Iltis & Doebley), and perennial teosinte (*Z. diploperennis* Iltis, Doebley & Guzman). Leaf toughness and pubescence, oviposition preference, and feeding and oviposition acceptance parameters were compared among the suite of host plants looking for effects of transitions in life history (perennial to annual teosinte), domestication (annual teosinte to landrace maize), and breeding (landrace maize to hybrid maize) on defenses against *D. maidis*. Observations on leaf toughness suggested that the life history and domestication transitions weakened the plant's resistance to penetration by the herbivore's mouthparts and ovipositor, as expected, while observations on pubescence suggested that the breeding transition led to stronger defense in hybrid maize compared to landrace maize, contrary to expectation. Observations on oviposition preference of *D. maidis* coincided with the expectations that life history and domestication transitions would lead to preference for Balsas teosinte over perennial teosinte, and of landrace maize over Balsas teosinte. A negative correlation suggested that oviposition preference is significantly influenced by leaf toughness. Observations on host plant feeding and

oviposition acceptance under no-choice conditions suggested that *D. maidis* equally accepts all host plants considered in this study, thus these observations did not support the hypotheses associated with the life history, domestication, and breeding transitions evident in the herbivore's host genus. Overall, the results of this study suggested that plant defenses against specialist herbivores are variably affected by plant life history evolution, domestication, and breeding. Additionally, the study's results suggested that chemical defenses may play a role in *Zea* antiherbivore defense because the two physical defenses that were evaluated (i.e. leaf toughness and pubescence) only partially explained host preference of *D. maidis*.

ACKNOWLEDGEMENTS

moments together. Special thanks to Catherine Bravo for making my days brighter during the last part of my research.

My affection to my parents, Encarnacion & Lucila, and my sister Lucy; also to my Aunt Paulina and my cousins Gaddy, Sahida and Rafhael.

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CHAPTER I

INTRODUCTION

This project is based on the premise that anti-herbivore defenses are affected by

plant life-history evolution and human intervention through domestication and breeding

for high yield. I used a suite of plants from the genus *Zea* L. (Poaceae) and the specialist

herbivore *Dalbulus maidis* (DeLong and Wolcott, 1923) (Hemiptera: Cicadellidae) to

test this premise.

Plant anti-herbivore defenses can be classified by their nature into physical (e.g.,

leaf toughness, spinescence, pubescence, epicuticular waxes) (e.g., Bergvinson et al.,

1994; Lucas et al., 2000; Fordyce & Agrawal, 2001; Lill et al., 2006; Hanley et al., 2007;

Kaplan et al., 2009) or chemical (e.g., allelochemicals, volatiles) (e.g., Turlings et al.,

1998; Gouinguené et al., 2001; Tamiru et al., 2011), and by their mode of action into

direct (if they directly target the herbivore, e.g., allelochemicals) or indirect (not

targeting the herbivore, but enhancing the effectiveness of its natural enemies, e.g., ant-

plant mutualisms). The maintenance of these defenses is usually a trade off with

productivity (vegetative growth and reproduction), so defenses typically are selected

against during domestication and subsequent breeding. Due to different priorities in

growth and reproduction, plant anti-herbivore defense strategies in perennial plants are

likely to be significantly different relative to those of related annual plants. For example,

perennials are expected to allocate more resources to vegetative growth and defense,

while annuals are expected to dedicate more resources to reproduction because they have

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only one season to produce offspring (Mutikainen & Walls, 1995; Rosenthal & Dirzo, 1997) . Thus, while plants living in the wild are selected to develop and maintain defenses against herbivores in order to survive and reproduce, crop plants are selected for productivity (yield) and their defense is aided by human interventions. Due to a trade-off in resource allocation and productivity (growth and reproduction) versus defense (Coley et al., 1985), it is very likely that through domestication and breeding, crop plants would lose defensive traits in order to invest more in the production of resources of human interest (Gepts, 2010). For example, domestication seemingly compromised chemical defense in cranberries resulting in better performance of gypsy moth caterpillars (Rodriguez-Saona et al., 2011); domestication led to larger fruits in olive, which provide a refuge for olive fruit fly larvae beyond the reach of its parasitoid's ovipositor (Wang et al., 2009), and; domestication altered tritrophic interactions between sunflower, sunflower moth, and the moth's larval parasitoids so that parasitism of sunflower moth is higher in wild compared to cultivated sunflowers (Chen & Welter, 2005).

In order to test my premise I used maize (*Zea mays* ssp. *mays* L.) and two of its wild relatives. With more than 160 million hectares harvested in 2010 (FAO, 2011), maize is one of the most important crops worldwide, and an important component of the American diet (Jahren & Kraft, 2008). Due to its economic importance, maize is subject to extensive research, including research on its origins and domestication (Buckler, 2006). As the origin and phylogeny of maize are well known, a suite of *Zea* wild taxa and domesticated cultivars were assembled to represent the evolutionary, domestication,

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and breeding processes leading to modern, high-yielding maize varieties, and this suite was used to test my study's basic premise. The suite of *Zea* host plants considered in my study included: *Zea diploperennis* Iltis, Doebley & Guzman (perennial teosinte), a wild perennial species); two "geographic populations" of *Z. mays* ssp. *parviglumis* Iltis & Doebley (Balsas teosinte), a wild annual subspecies, ancestral to maize); a Mexican landrace of maize, and; a Mexican, commercial hybrid maize. Similar suites have been used in other studies (e.g., Rosenthal & Dirzo, 1997; Gouinguené et al., 2001; Van Esbroeck et al., 2008). An important feature of the suite is that it includes three transitions within *Zea* leading to modern, high-yielding maize: (i) a life history transition, from perennial to annual life cycle (perennial teosinte to Balsas teosinte); (ii) a domestication transition, from wild annual to cultivated annual (Balsas teosinte to landrace maize), and; (iii) a breeding transition, from a genetically diverse, open-pollinated cultivar to a highly inbred cultivar bred for maximum yield (landrace to hybrid maize).

Rosenthal & Dirzo (1997) evaluated a suite of *Zea* taxa and cultivars similar to mine in terms of resource allocation (production of biomass) and anti-herbivore defense (injury by herbivores). Their results showed a clear pattern in which the wild relatives of maize suffered less injury by herbivores, mainly the stem borer *Diatraea grandiosella* Dyar, while a modern maize cultivar produced more biomass (seed and foliage). However, Rosenthal & Dirzo (op. cit.) did not identify the actual defensive traits that influenced herbivory in their study. In this study, I compared two putative physical defense traits, leaf toughness and pubescence, and assessed host preference and

acceptance by *D. maidis* among the plants of my suite to address whether differences in the relevance of those traits and the preference and acceptance of *D. maidis* were correlated with a trend of stronger to weaker defense between perennial teosinte and hybrid maize.

The genus *Dalbulus* is specialized on host plants in the genera *Zea* and *Tripsacum* (Poaceae) (Nault & Delong, 1980; Dietrich et al., 1998). *Dalbulus maidis* feeds mostly on phloem (Alivizatos, 1982; Carpane et al., 2011), and is specialized on the genus *Zea*, particularly on maize, and occurs throughout the American continent, from southern California and Texas southward to northern Argentina (Nault, 1990; Summers et al., 2004; Palomera et al., 2012). Additionally, *D. maidis* vectors three important corn diseases: Corn Stunt Spiroplasma (CSS), Maize Rayado Fino Virus (MRFV), and Corn Bushy Phytoplasma, which cause significant economic damage in the tropical Americas (Bradfute et al., 1981; Virla, 2004; Vasquez & Mora, 2007). Because the host range of *D. maidis* is restricted to *Zea*, it was hypothesized that it expanded its host range from the teosintes (wild species of *Zea*) to the recent domesticate, maize (Nault & Delong, 1980). Moreover, *D. maidis* seemingly successfully coevolved with maize, on which it is the dominant species of *Dalbulus*, and followed the crop as it was moved by humans throughout the American continent (Nault, 1990; Medina et al. 2012). Thus, *D. maidis* and *Zea* are an ideal herbivore-plant system for testing my study's premise that wild crop relatives will be better defended against herbivory in comparison to their domesticated relatives.

This study is divided into two parts. First, I compared among the suite of *Zea* taxa and cultivars two putative physical defenses of maize: leaf toughness, in terms of work for stylet and ovipositor penetration, and pubescence, in terms of trichome density. Additionally, I compared host preference by corn leafhopper among the suite of *Zea* plants, and sought correlations between host preference and leaf toughness and/or pubescence. Second, I compared the feeding and oviposition acceptance of *D. maidis* females in a no-choice scenario among the suite of *Zea* host plants. Feeding acceptance was assessed using indices based on honeydew excretion, while oviposition acceptance was assessed using indices related to number of eggs laid and time spent on oviposition. In both cases, comparisons were made in the contexts of the three transitional steps evident within the suite of *Zea* taxa and cultivars: (i) transition from annual to perennial life history (perennial teosinte to Balsas teosinte), (ii) transition from wild to domesticated taxon (Balsas teosinte to landrace maize), and (iii) transition from genetically diverse, outbred cultivar to genetically narrow, inbred variety (landrace maize to hybrid maize).

CHAPTER II

LEAF TOUGHNESS AND PUBESCENCE ALONG A SUITE OF *Zea* WILD TAXA
AND MAIZE CULTIVARS, AND ITS EFFECT ON OVIPOSITION PREFERENCE
BY THE CORN LEAFHOPPER (*Dalbulus maidis*)

Introduction

Domestication and plant breeding are processes of directed selection that

typically affect plant anti-herbivore defenses (Gepts, 2002; Doebley et al., 2006; Gepts,

2010). Because metabolic resources allocated to productivity (growth and reproduction)

and defense are limited, plant domestication and subsequent breeding for greater

productivity frequently result in weakened plant defenses (Massei & Hartley, 2000;

Chen & Welter, 2005; Wang et al., 2009; Macfadyen & Bohan, 2010; Rodriguez-Saona

et al., 2011). In the case of the grass genus *Zea*, previous studies showed that different

strategies in life history (perennial versus annual) and metabolic resource allocation to

defense versus productivity resulted in greater injury by stem-boring and leaf-chewing

herbivores, and consequently greater yield loss in maize (*Zea mays* ssp. *mays* L.)

compared to its wild relatives, the teosintes (Rosenthal & Welter, 1995; Rosenthal &

Dirzo, 1997). However, those studies did not identify particular defensive traits

associated with differences in herbivory damage.

Several authors hypothesized that once maize was domesticated in present-day

Mexico, around 9,000 years ago (Matsuoka et al., 2002; Buckler, 2006), the corn

leafhopper *Dalbulus maidis* (DeLong and Wolcott 1923) (Hemiptera: Cicadellidae)

expanded its host range from its ancestral hosts, the teosintes (probably Balsas teosinte,

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Zea mays ssp. *parviglumis* Iltis & Doebley) to include the recently available domesticate, maize (Nault & Delong, 1980; Nault, 1990; Medina et al., 2012). *Dalbulus maidis* is a mostly-phloem feeding herbivore, specialized on the genus *Zea* (Alivizatos, 1982; Nault, 1990; Wayadande & Nault, 1996), with a geographical distribution encompassing much of the American continent, from southern California and Texas southwards to northern Argentina (Nault, 1990; Summers et al., 2004; Palomera et al., 2012). *D. maidis* vectors three important maize pathogens, Corn Stunt Spiroplasma (CSS), Maize Rayado Fino Virus (MRFV), and Corn Bushy Phytoplasma, which cause significant economic damage in the tropical Americas (Bradfute et al., 1981; Virla, 2004; Vasquez & Mora, 2007).

Leaf toughness is a physical defensive trait (Schoonhoven et al., 2005; Hanley et al., 2007), as reported, for example, in studies aimed to explain herbivory in tropical forests (Coley, 1983), or caterpillar resistance in maize (Bergvinson et al., 1994). Although most studies concerning leaf toughness have focused on its effects on leaf-chewing insects (e.g., caterpillars), some studies have addressed its effects on piercing-sucking insects, such as hemipterans, or insects that insert their eggs into host tissues. For example, one study showed that leaf toughness was negatively correlated with the number of eggs inserted in leaves by an hemipteran predator (Constant et al., 1996), while another showed that leaf biomechanical properties were correlated with the distributions of herbivores, including cicadellid leafhoppers, in a community of plants (Peeters et al., 2007). In another study, leaf toughness and pubescence increased in

Lantana camara (L.) (Verbenaceae) in response to injury by an hemipteran, and led to failed establishment by the hemipteran herbivore (Heshula & Hill, 2011).

Trichomes or plant hairs (collectively known as pubescence), are a means of plant defense (Levin, 1973), whether by interfering with foraging, and/or deterring oviposition (Fordyce & Agrawal, 2001; Lill et al., 2006; Horgan et al., 2007; Matos et al., 2009). Although effects of trichomes on leafhoppers have been reported in some studies (e.g., Ranger & Hower, 2002; Dellinger et al., 2006; Kaplan et al., 2009), such studies frequently involved glandular trichomes, which are not purely a means of physical defense. However, some studies showed a clear deterrent action of non-glandular trichomes on leafhopper distribution (e.g., Broersma et al., 1972; Butler Jr. et al., 1991; Lokesh & Singh, 2005). Maize plants may have three types of hairs or trichomes, macrohairs, prickle hairs, and bicellular microhairs (Moose et al., 2004), and macrohairs have been shown to deter oviposition of a pyralid moth (Kumar, 1997).

In this study I assessed leaf toughness and pubescence among a suite of *Zea* host plants representative of the life history, domestication, and breeding transitions of *Zea* leading to modern, high-yielding maize varieties. In particular, the goal of the study was to assess whether life history (perennial to annual life cycle), domestication (wild, annual teosinte to landrace maize), and breeding (landrace maize to hybrid maize) transitions in the host genus affected these two putative, physical defense against the specialist herbivore *D. maidis*. In addition, I assessed whether these transitions affected host plant preference of *D. maidis*. The suite of *Zea* host plants I included in this study were: (i) perennial teosinte (*Zea diploperennis* Iltis, Doebley & Guzman), a perennial,

wild species basal to the genus *Zea*; (ii) and (iii) two "geographic populations" of Balsas teosinte (*Z. mays* ssp. *parviglumis*), an annual subspecies, ancestral to maize, and; (iv) and (v) two maize cultivars, a Mexican landrace and a Mexican hybrid. I expected to find regressive trends from greater to lesser leaf toughness and pubescence among the suite of host plants, and that host plant preference of *D. maidis* would be correlated with those trends.

Materials and methods

    Insects: The *D. maidis* used in this study came from a colony initiated with individuals collected from maize near the city of El Grullo (Jalisco state, Mexico; 19°48' N, 104°13' W) in the summer of 2008. The colony was kept in a plastic frame mesh cage (BugDorm-44545F, Megaview Science Co., Ltd., Taichung 40762, Taiwan) on seedlings of a Mexican landrace of maize (Elotes Occidentales), usually in their 4-6 leaf stage, in a growth room with a photoperiod of 12:12 (L:D) and a temperature of 24-28 °C.

    The *D. maidis* used in the experiment described below were 4 or 5 d-old adult individuals, which were obtained by placing potted maize seedlings (4th or 5th-leaf stage) inside the cage holding the *D. maidis* colony (described above) so they would receive eggs. After 3 d, the seedlings were removed from the colony cage, and all nymph or adult *D. maidis* were removed, leaving only the newly-laid eggs on the seedlings. The seedlings were then placed in a cage as described above free of *D. maidis* where they were held until adult *D. maidis* adults began emerging (within ~3-4 weeks). *D. maidis* adults were then collected daily for use in the experiment, and females were assumed to

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have previously mated. All *D. maidis* were isolated in vials for ~6 h before they were used in the experiment.

Plants: Seeds of perennial teosinte (hereafter "wild perennial") were collected in the location of Corralitos, within the Sierra de Manantlán Biosphere Reserve (Jalisco state, Mexico; 19°36'48.78" N, 104°18'23.49" W) (UNESCO, 2011). Those of Balsas teosinte were collected from two different locations: San Lorenzo (Ejutla, Jalisco, Mexico; 19°56'60" N, 103°59'0" W) (hereafter "wild annual 1") and El Cuyotomate (Ejutla, Jalisco, Mexico; 19°58'10.39" N, 104°4'3.00" W) (hereafter "wild annual 2"). In the case of maize, seed of the Mexican landrace Tuxpeño (hereafter "landrace") were obtained from USDA NPGS (GRIN accession PI 511649, collected at El Palmar de Susana, Tecolutla, Veracruz state; 20°20'35.32" N, 96°53'25.42" W), and of a Mexican hybrid (hereafter "hybrid") were purchased from a commercial seed provider (accession NB2, Hibridos NOVASEM, S.A., Zapopan, Jalisco, Mexico).

In order to improve germination, teosinte seeds were removed from their fruitcases with the aid of nail clippers, then placed between two moist filter papers within a Petri dish until they germinated, when they were transferred to pots filled with soil (Sunshine mix 1, Sun Gro Horticulture, Seba Beach, AB TOE 2 BO Canada). Plants were kept in a greenhouse. The plants used to compare trichome density were planted in January 2011, those used to compare leaf toughness in February 2011, and those used to assess host plant preference between August and September 2011.

Leaf toughness: Work for penetration was assessed as an index of leaf toughness. This was done employing a texture analyzer (TA-XT2i Texture Analyzer,

Texture Technologies Corp., Scarsdale, NY/Stable Micro Systems, Godalming, Surrey, UK), to which an insect pin #3 (Bioquip products Inc., Rancho Dominguez, CA 90220, USA) was adapted as a proxy probe for *D. maidis* mouthparts and ovipositor. The texture analyzer was configured to provide a reading every 0.005 seconds, and to move the probe at a constant speed of 0.5 mm sec^{-1} to a depth of 2 mm upon contact with the leaf surface. Thus, for every assay plant the analyzer yielded at least 800 readings, each consisting of three parameters: time (sec), force (g) and calculated distance (mm), as the speed was constant.

The 4th collared leaf of each plant (counting upward from the bottom leaf) was assayed in the texture analyzer, and the plants were in the 5th or 6th leaf stage. The leaves were excised from the plant, and immediately assayed in the texture analyzer. These leaves were fixed to the texture analyzer's base with the aid of two Plexiglas plates (15 cm × 15 cm). The plates held the leaf, sandwich-style, and had three matching 5 mm-diam holes through which the probe (insect pin) punctured each leaf. Each leaf was assayed three times in the midrib (adaxial side). We focused on the midrib because prior observations showed that the majority (>99%) of *D. maidis* eggs are inserted in the midrib, and that detection of stylet (feeding) scars is least ambiguous on the midrib (EBV pers. obsrv.). The puncture site was between 25 and 40 mm from the base of the leaf. Leaf toughness was evaluated in 91 plants: 19 wild perennial, 12 wild annual 1, 26 wild annual 2, 18 landrace maize, and 15 hybrid maize.

Each assay (leaf) yielded at least 800 readings (data points indicating time, force and distance), as noted above. All readings recorded prior to the probe's (insect pin)

contact with a leaf, were excluded from analysis so that each assay included 800

readings. Our comparisons among host plants considered two penetration depths by the

probe: 0.3 mm (or 0.6 sec), as a proxy for penetration by *D. maidis* mouthparts, and 0.8

mm (or 1.6 sec) as a proxy for penetration by the ovipositor: previous observations

showed that mean mouthpart (stylet) length was 0.293 (SE = 0.006 mm, n = 20), and

mean ovipositor length was 0.802 (SE = 0.006 mm, n = 20). The amount of work (g sec^{-1}) necessary for penetration to 0.3 mm and 0.8 mm was calculated as the sum of the

product of the forces (g) per every 0.025 sec time interval. Only the intermediate value

of estimated work among the three assays per each leaf was considered for statistical

analyses.

Analysis of variance (ANOVA) was used to determine whether mean work for

penetration varied among the suit of *Zea* host plants. Data corresponding to work for

penetration to 0.3 mm were normalized by transforming to their natural log values. *A priori* contrasts were used to assess effects of life history (wild perennial vs. wild

annuals 1 and 2), domestication (wild annuals 1 and 2 vs. landrace maize), or breeding

(landrace maize vs. hybrid maize) transitions on mean work for penetration to 0.3 and

0.8 mm, as warranted by a significant ANOVA. The critical P-value used for the

(multiple) contrast comparisons was 0.02, as corrected with the Sidak equation (Abdi &

Williams, 2010).

Trichome density: Trichomes (macrohairs) were counted on the 6th or 7th leaf of

each of 70 plants on its 7th or 8th leaf developmental stage: 17 wild perennial, 8 wild

annual 1, 15 wild annual 2, 14 maize landrace, and 16 maize hybrid. The length of these

leaves was measured with a ruler, and then a 5 mm disk was cut with a cork cutter at the basal ⅓ of the leaf's length. Two disks were cut per leaf, one to count adaxial trichomes and the other to count abaxial trichomes. The counting was done with the aid of a dissecting microscope.

Analysis of variance (ANOVA) was used to determine whether trichome density varied among the suite of *Zea* host plants. When warranted by a significant ANOVA, *a priori* contrasts were used to assess effects of life history, domestication, and breeding transitions on trichome density, as described above for work for penetration.

Host preference: Ten female and five male *D. maidis* were released into a cage (BugDorm-44545F) holding one pot of each *Zea* host plant in the 4th- or 5th-leaf stage. The cages were maintained in a greenhouse. After 60 h, the plants were recovered from each cage, and their leaves were excised then stained using the McBride staining technique (Backus et al., 1988) in order to count the eggs laid in them. Once the leaves were stained, each leaf was digitally scanned to determine leaf length, and the eggs found on the leaves were counted, noting whether these were laid on the adaxial or abaxial side of the leaf. Leaf midrib length rather than leaf surface area was measured because a preliminary analysis of our results showed that 99.8% of all eggs laid by *D. maidis* females were inserted in the leaf midrib (EBV, unpubl.). This procedure was replicated thrice, once every other week. Overall, 75 plants and 225 *D. maidis* (150 females) were used in the experiment.

Data consisting of eggs per centimeter of leaf (midrib) length could not be normalized, so were converted to their rank values, and subjected to randomized

complete block ANOVA by ranks. In addition, the proportion of rejected plants (= plants not receiving eggs) per replicate trial was estimated by dividing the number of each *Zea* host plant without eggs by the corresponding number of plants (5) per trial. These proportions were transformed to their arc-sine $\sqrt{x}$ values and subjected to randomized complete block ANOVA. As described above, *a priori* contrasts were used to assess the effects of life history, domestication, and breeding transitions on the mean number of eggs laid and proportion of rejections per host plant, as warranted by a significant ANOVA.

Correlation between oviposition preference and leaf toughness or pubescence: Pearson's correlations were used to generate a correlation matrix indicating the degrees of linear association between oviposition preference, leaf toughness, and pubescence. The data included in these correlations were the mean values of work for penetration (leaf toughness), trichome density (pubescence), and number of eggs per centimeter of leaf (oviposition preference).

Results

Leaf toughness: Leaf toughness, assessed as work for penetration to 0.3 mm (stylet reach) varied among the suite of *Zea* host plants (P = 0.012, F = 3.73, df = 4, 85) (Figure 2.1A). Further contrast analyses showed a significant effect of the transition from perennial to annual life cycle (P = 0.008, t = 2.74, df = 85) (Figure 2.1B). Similarly, leaf toughness, assessed as work for penetration to 0.8 mm (ovipositor length) varied among the *Zea* host plants (P < 0.001, F = 9.17, df = 4, 85) (Figure 2.2A). Contrast analyses showed significant effects of the transition from perennial to annual

life cycle (P = 0.012, t = 2.56, df = 85) (Figure 2.2B), and from wild annual to landrace

maize (P = 0.001, t = 3.36) (Figure2.2C).
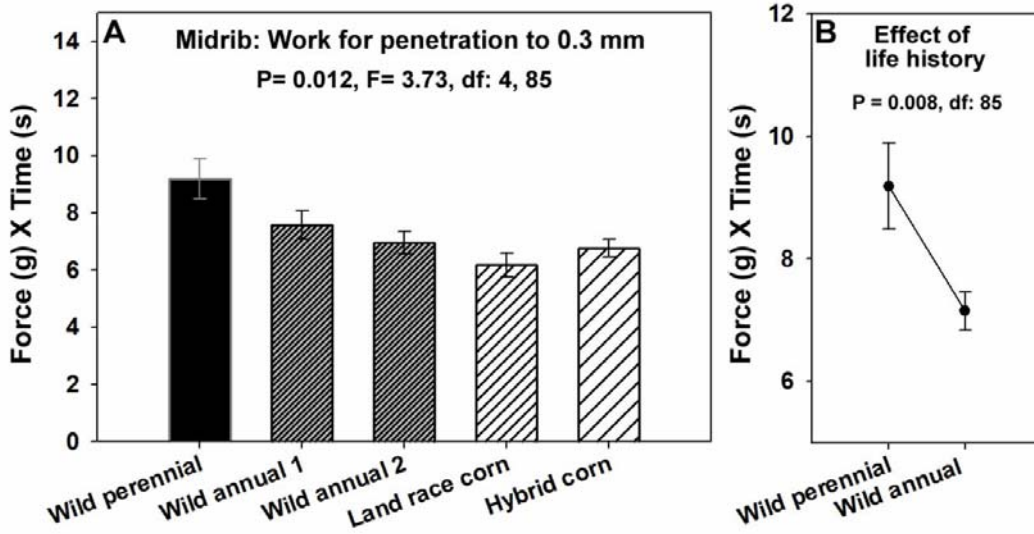
Figure 2.1: A): Values of work for penetration to stylet reach (0.3 mm) among a suite of *Zea* host plants. **B)**: Effect of life history transition (wild perennial *Zea* vs wild annual *Zea*) on the work required to penetrate the leaf to 0.3 mm.
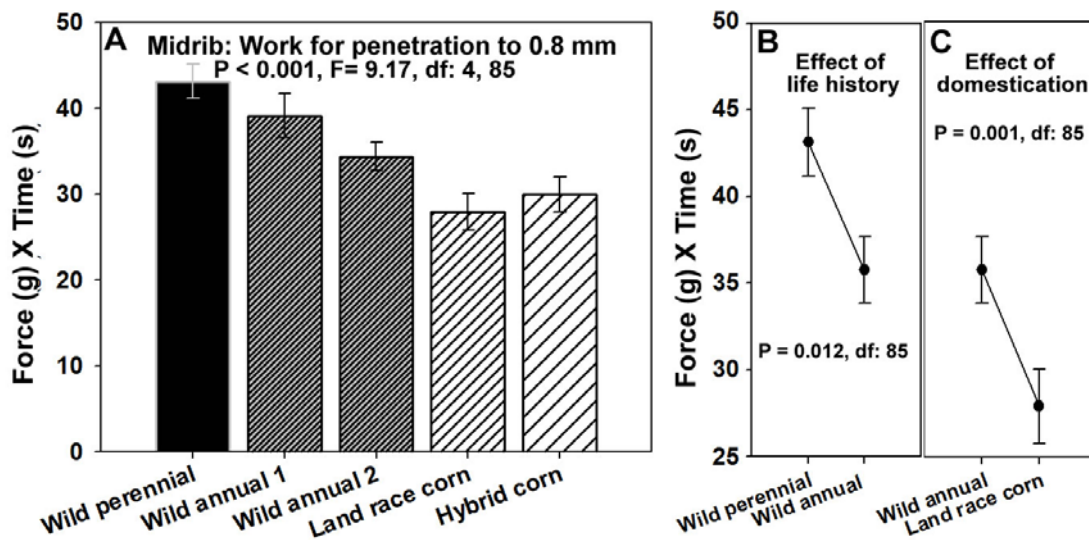
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Trichome density: With the exception of a few cases (4 of 17) in the wild perennial, trichomes were not found on the abaxial side of the leaf. In contrast, trichomes were always present on the adaxial side of the leaf, and their density varied among the suite of *Zea* host plants (P = 0.008, F = 3.79, df = 4, 65). The most pubescent host was the hybrid with 28.6 trichomes $\times$ cm^{-2}, and the least pubescent was the wild annual 2 (San Lorenzo population) with 13.4 trichomes $\times$ cm^{-2} (Figure 2.3A). Only the breeding contrast was significant (P = 0.007, t = 2.80, df = 65) (Figure 2.3B).
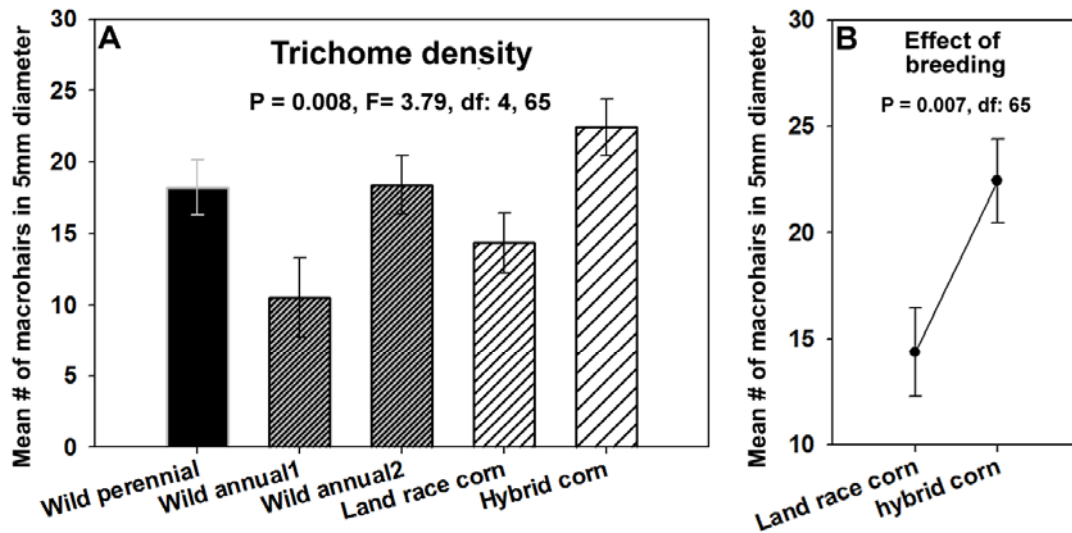
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Host preference: A total of 2,706 eggs were counted in the three replicate trials, of which 1,440 were inserted in the adaxial side, and 1,266 in the abaxial side. Only five eggs were found off the midrib. The density of eggs laid varied among the suite of *Zea* host plants (P = 0.002, F = 4.63, df = 4, 68) (Figure 2.4A). The contrast comparisons revealed only a domestication effect (P = 0.02, t = 2.37, df = 68) (Figure 2.4B). The proportion of rejected plants per cage, varied along the suite of *Zea* host plants (P = 0.001, F =13.74, df = 4, 8) (Figure 2.5A). The contrast comparisons revealed an effect of life history transition (P = 0.009, t = 3.44, df = 8) (Figure 2.5B).
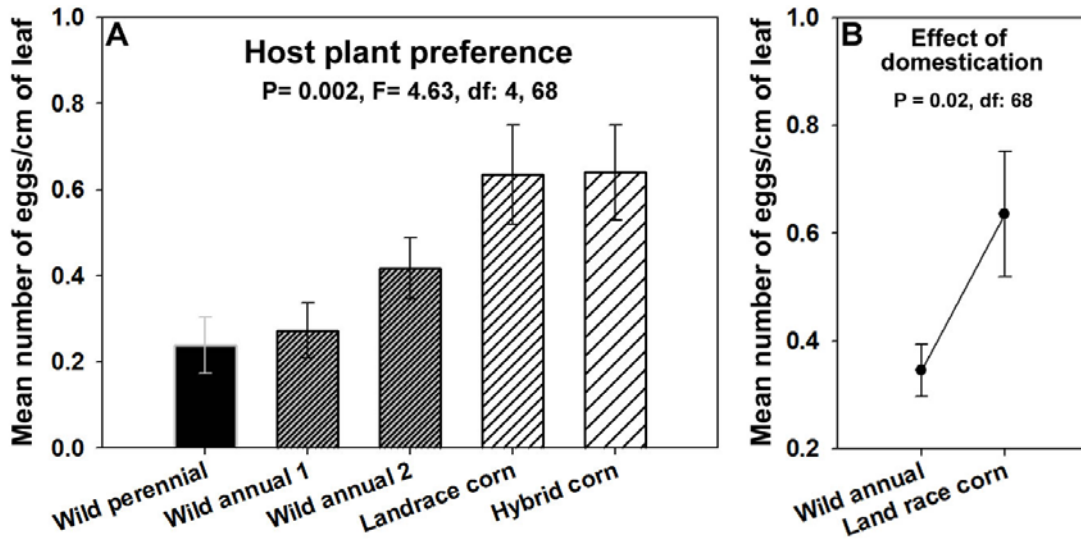
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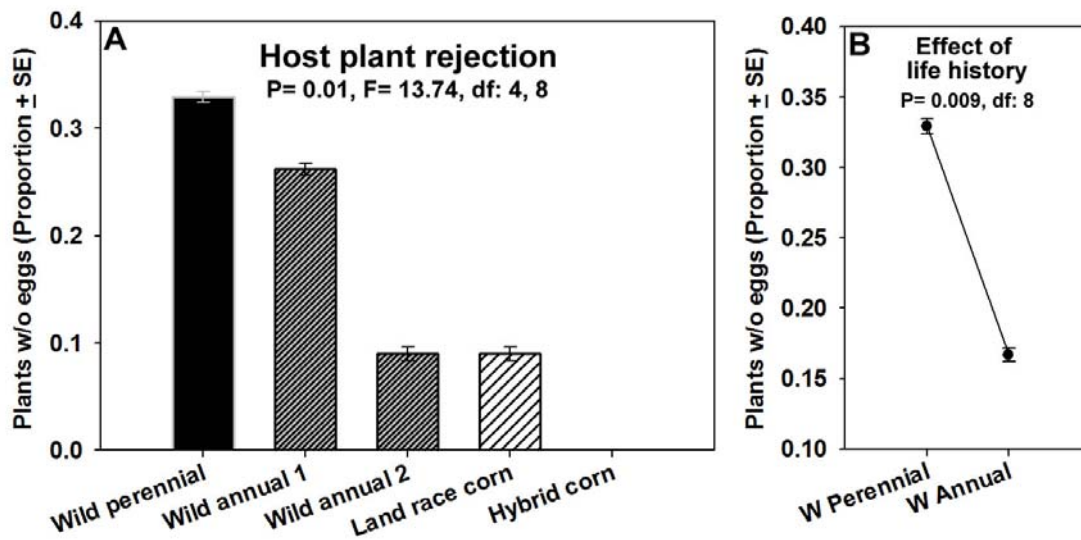
Figure 2.5: *Dalbulus maidis* host plant rejection. **A):** Proportion of plants rejected for oviposition by *D. maidis* (plants with no eggs laid on them). **B):** Effect of life history transition on the proportion of plants rejected for oviposition.

Correlation between oviposition preference and leaf toughness or

pubescence: There was no correlation between pubescence (trichome density) and

oviposition preference (Pearson's r = 0.401, P = 0.504). However, a negative correlation

was evident between leaf toughness and oviposition (Pearson's r = -0.971,

P = 0.006) (Figure 2.6)
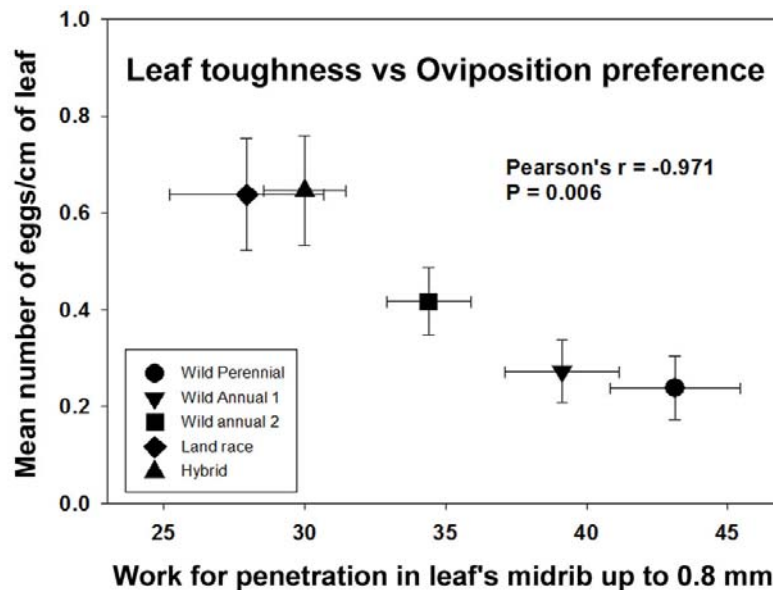
Figure 2.6: Negative correlation between leaf toughness and oviposition preference of *Dalbulus maidis* among the suite of *Zea* host plants. Vertical and horizontal bars around the different host plant values show the corresponding standard errors on oviposition preference and leaf toughness respectively.

Discussion

Transitions in life history, from perennial to annual life cycle, and in

domestication, from wild, annual teosinte to landrace maize, had a clear effect on leaf

toughness. This corresponds with the expectation that leaves on perennial plants are tougher because they live longer (Herms & Mattson, 1992; Coley & Barone, 1996). However, our assessment of leaf toughness may have been influenced the probe's diameter (insect pin #3, = 0.5 mm diam), which was considerably greater than the stylet's diameter (0.004 mm; Alivizatos, 1982). My attempts at using a finer probe, closer in size to the leafhopper's stylet, were unsuccessful because texture analyzer readings became increasingly variable with decreasing probe diameters. Therefore, while our results showed differences in mean work for penetration among the host plants, it remains unclear whether and how strongly *D. maidis* feeding and oviposition behaviors are correlated with those differences. Leaf toughness can also be affected by environmental conditions (Onoda et al., 2008), so even though the plants I used were grown under common conditions, future studies should address whether and how strongly environmental conditions, such as light or water stress may influence leaf toughness in maize and its putative defensive value against *D. maidis*. Moreover, future studies should more closely examine feeding and oviposition by *D. maidis* on the host plants considered herein, including whether actual resistance to oviposition and feeding is correlated with leaf toughness as measured in this study.

The density of trichomes did not significantly change with the life history or domestication transitions. The contrast analysis indicated that only modern breeding, understood as the use of scientific techniques beyond mere farmer selection to obtain high yielding varieties, positively affected trichome density, thus suggesting greater protection by trichomes in hybrid maize compared to landrace maize, although this

doesn't correspond to the expectation of weaker defenses in modern crop cultivars. Typically macrohairs, the type of trichomes I counted, mechanically interfere with herbivore locomotory, feeding, and oviposition activities. At the highest trichome density in my study, 1 trichome every 1.9 mm (28.6 trichomes $\times$ cm^{-2}) in the hybrid, a *D. maidis* individual with a body dimensions of ~3 length $\times$ 1 wide mm may still have sufficient space to maneuver unimpeded over the leaf surface, between trichomes, to feed and lay eggs. Also, considering that macrohairs only develop on the adaxial side of the leaf, and very rarely on the midrib, where practically all the eggs are laid indistinctly on the adaxial or abaxial surface, my results suggest that trichomes pose very weak, if any, interference for *D. maidis* oviposition. Moreover, hybrid maize is expected to produce more biomass than the land race (e.g., Rosenthal & Dirzo, 1997), which implies that, higher trichome density is compatible with higher productivity, contrary to the prediction of the resource allocation hypothesis. Therefore, at least in the case of trichomes, there does not seem to be a trade-off between productivity and putative plant protection provided by pubescence against *D. maidis*, as shown for the leafhopper *Empoasca fabae* on potato, where greater trichome density was not correlated with productivity, but was correlated with lower feeding injury by these leafhoppers (Kaplan et al., 2009). My results, however, do not address whether maize macrohairs act as defensive structures against other herbivores, such as caterpillars (i.e. *C. partellus*), nor whether they are relevant in the context of tritrophic interactions, particularly in reference to predation (e.g., Styrsky et al., 2006, Loughner et al., 2008)

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Having the option to choose a host for oviposition, *D. maidis* seemingly preferred maize cultivars to wild host plants. In particular, the host with the seemingly toughest midribs (per *a posteriori* contrast between perennial teosinte and maize cultivars, $P < 0.0001$, Scheffe's $F = 8.29$, $df = 50$), perennial teosinte, received the fewest eggs (per *a posteriori* contrast, $P = 0.001$, Scheffe's $F = 5.55$, $df = 68$) and was rejected with the greatest frequency (per *a posteriori* contrast, $P = 0.004$, Scheffe's $F = 9.79$, $df = 8$). Additionally, I found that leaf toughness was (negatively) correlated with host preference. However, confirmation that *D. maidis* preferred maize cultivars because their leaves are less tough than those of wild plants, requires deliberate evaluation , especially with knowledge that when not given a choice *D. maidis* equally oviposits on any of the hosts of our suite (see chapter III), and that it may oviposit on up to 20 other plant species (Pitre, 1967).

My results concerning the numbers of eggs laid in the host preference experiment suggest that *D. maidis* can easily penetrate the midrib of any of the host plants with their mouthparts (stylets) because a parallel study showed a significant, positive correlation between feeding (stylet) scars and eggs inserted in the midrib (see chapter III). Moreover, the work for penetration to stylet length (0.3 mm) is substantially greater (1.5 × fold) on the midrib compared to the leaf blade (EBV, unpubl.), so that blade toughness is an unlikely barrier for *D. maidis* feeding. However, my study included only adult *D. maidis*, while much of the feeding is done by juveniles, which are smaller and have plausibly weaker and shorter stylets. Earlier studies showed that while *D. maidis* was capable of feeding and laying eggs on a variety of grass and non-grass plants, complete

development occurred only on *Zea* hosts (Pitre, 1967; Nault & Madden, 1985; Nault, 1990). Though, the inability to successfully develop may have been due a combination of factors, such as physical incapability of the nymphs to feed from a host with tough leaves and thick epidermis (Walker, 1985), or inability to overcome the chemical defenses posed by host plants other than *Zea*. Thus, leaf toughness cannot be discounted as an effective defense against *D. maidis*.

It is important to note that the individuals of *D. maidis* used in this study were from a colony originated from individuals collected from maize in Mexico. Recently, it was shown that *D. maidis* consists of at least two reproductively discrete subpopulations, one from maize and Balsas teosinte, and the other from perennial teosinte (Medina et al., 2012). Thus, our results concerning host preference may have been biased against perennial teosinte. However, the finding that a subpopulation of *D. maidis* seemingly specializes on perennial teosinte, presumably the best-defended host among my suite per the resource allocation hypothesis, suggests that despite differences in life history and resource allocation priorities within *Zea*, some *D. maidis* genotypes can overcome the defenses posed by its hosts in the genus *Zea*.

Agricultural fields are managed environments where soil fertility, plant defense, and generally plant growth and reproduction are strongly influenced by human interventions. Thus, domesticated plants typically benefit more than their wild relatives from external resources for growth, survival, and reproduction. In the absence of human intervention, however, crop plant genotypes whose defenses were diminished through domestication and breeding are likely penalized more by herbivory than crop relatives.

For example, maize suffered greater herbivory and yield loss compared to teosintes in common garden experiments in both agricultural and natural environments (Rosenthal & Dirzo, 1997). Similarly, under common conditions crop plants and wild relatives follow different growth strategies, as shown for Balsas teosinte and maize in nutritionally poor substrate (Gaudin et al., 2011). While my results concerning leaf toughness and host preference are consistent with a trend from stronger to weaker defenses between the wild perennial and the maize hybrid, it is relevant that they were obtained under greenhouse conditions, in exclusion of much environmental variability, and did not address effects on plant growth and reproduction, nor effects on the herbivore, *D. maidis*. Future studies should address whether and how strongly (i) the prediction of weaker defense in maize versus teosintes is subject to environmental variability, (ii) herbivory affects growth and reproduction in maize relative to teosinte, and (iii) the performance of *D. maidis* is affected by the transitions I evaluated in this study.

Conclusion

Overall, my results suggested that *Zea* life history evolution and maize domestication and breeding may have affected leaf pubescence less than leaf toughness, and that leaf toughness may play a role in host plant preference by *D. maidis*. Moreover, my results showed a preference for maize over its wild relatives. Because host preference is tied to host selection, my results suggest that maize is a better host than the teosintes for *D. maidis*, so indirectly point to weaker defenses in maize compared to teosintes.

CHAPTER III

HOST PLANT FEEDING AND OVIPOSITION ACCEPTANCE BY THE CORN
LEAFHOPPER (*Dalbulus maidis*) ALONG A SUITE OF *Zea* WILD TAXA AND
MAIZE CULTIVARS

Introduction

Domestication is a directed selection process that typically affects a plant's antiherbivore defenses (Doebley et al., 2006; Gepts, 2010). As the maintenance of these defenses is frequently a trade-off with higher yield, plant defenses are typically selected against during domestication and subsequent breeding (Massei & Hartley, 2000; Chen & Welter, 2005; Wang et al., 2009; Macfadyen & Bohan, 2010; Rodriguez-Saona et al., 2011). In the case of the genus *Zea* L. (Poaceae), prior studies showed that different life histories and strategies for metabolic resource allocation to defense versus productivity (growth and reproduction) translated into greater herbivore damage by stem boring and leaf chewing insects, and consequently greater yield loss in maize (*Zea mays* ssp. *mays* L.) compared to its wild relatives (Rosenthal & Welter, 1995; Rosenthal & Dirzo, 1997). However, those studies did not identify particular defensive traits that determined the differences in herbivore damage.

The corn leafhopper *Dalbulus maidis* (DeLong and Wolcott 1923) (Hemiptera: Cicadellidae) is a mostly-phloem feeding specialist on the genus *Zea* (Alivizatos, 1982, Nault, 1990; Wayadande & Nault, 1996). Its distribution ranges throughout the American continent from southern California and Texas southward to northern

Argentina (Nault, 1990; Summers et al., 2004; Palomera et al., 2012). *Dalbulus maidis* is a vector of three important corn diseases: Corn Stunt Spiroplasma (CSS), Maize Rayado Fino Virus (MRFV), and Corn Bushy Phytoplasma, which cause significant economic damage in the tropical Americas (Bradfute et al., 1981, Gamez, 1982; Virla, 2004; Vasquez & Mora, 2007). It's been hypothesized that following maize domestication in Mexico, around 9,000 years ago (Matsuoka et al., 2002; Buckler, 2006), *D. maidis* expanded its host range from the teosintes (probably *Zea mays* ssp. *parviglumis* Iltis & Doebley) and colonized the newly available domesticate, maize (Nault & Delong, 1980; Nault, 1990), as well as perennial teosinte (*Zea diploperennis* Iltis, Doebley & Guzmán) (Medina et al., 2012).

Because *D. maidis* seemingly coevolved with maize and became an important pest (Nault, 1990), it is likely that defense-related changes experienced by the crop plant through its domestication process can be reflected in parameters relevant to this herbivore's host acceptance, in relation to both oviposition and feeding. In order to test this hypothesis, I used a suite of *Zea* host plants representative of the life history, domestication and breeding transitions evident in *Zea* that led to modern, high-yielding maize varieties. Using this suite we compared indices of oviposition and feeding acceptance by *D. maidis* among the host plants through a series of observations under no-choice conditions. The plants composing the suite of *Zea* hosts were: (i) perennial teosinte (*Z. diploperennis*), a perennial, wild species basal to the genus *Zea*; (ii) and (iii) two "geographic populations" of Balsas teosinte (*Z. mays* ssp. *parviglumis*), an annual subspecies, ancestral to maize, and; (iv) and (v) two maize cultivars, a Mexican landrace

and a Mexican hybrid. In particular, the goal of the study was to assess whether life history strategy (perennial to annual life cycle), domestication (wild annual to domesticated annual), and breeding (landrace to modern hybrid) transitions within the suite of host plants affected their defenses against the specialist herbivore *D. maidis*. This study will shed light on the influence of changes in plant physical defenses, brought about through life history evolution, and domestication and breeding processes on oviposition and feeding by sap-sucking herbivores. Moreover, studies such the present may aid efforts toward developing plant cultivars resistant to sap-sucking pests, and improve our understanding of plant resistance to diseases vectored by those insects.

Materials and methods

Insects: All *D. maidis* came from a colony initiated with individuals collected from maize fields near El Grullo (Jalisco state, Mexico; 19°48'N, 104°13'W) in the summer of 2008. The colony was kept in a plastic frame mesh cage (BugDorm-44545F) on seedlings of a Mexican maize landrace (Elotes Occidentales), usually in their 4-6 leaf stage, in a growth room with a photoperiod of 12:12 (L:D) and a temperature of 24-28 °C. All *D. maidis* were isolated in vials at least 19 h before observations in order to promote feeding and oviposition during the 60-min duration of observations (see below) (cf. Wayadande & Nault, 1996; Carpane et al., 2011).

The *D. maidis* used in the trials were 4 to 10 d-old adult females. Uniformly aged females were obtained by placing a potted maize seedling (4- or 5-leaf stage) inside the cage holding the *D. maidis* colony (described above) so it would receive eggs. The

seedlings were removed from the colony cage after 3 d, and all nymph or adult *D. maidis* were removed, leaving only the newly-laid eggs on the seedlings. The seedlings were then placed in a clean cage (type and dimensions as above) until adult *D. maidis* began emerging (within ~3-4 weeks). *D. maidis* adult females were then collected daily for use in observations, and were assumed to have mated by the time they were collected.

Plants: Seeds of perennial teosinte (hereafter "wild perennial") were collected in the location of Corralitos (19°36'48.78"N, 104°18'23.49"W), within the Sierra de Manantlán Biosphere Reserve (Jalisco state, Mexico) (UNESCO, 2011). Those of Balsas teosinte were collected from two different locations: San Lorenzo (Ejutla, Jalisco, Mexico; 19°56'60"N, 103°59'0" W) (hereafter "wild annual 1) and El Cuyotomate (Ejutla, Jalisco, Mexico; 19°58'10.39"N, 104°4'3.00" W) (hereafter "wild annual 2"). In the case of maize, seed of the Mexican landrace Tuxpeño (hereafter "landrace") were obtained from USDA NPGS (Ames, IA; GRIN accession PI 511649, collected at El Palmar de Susana, Tecolutla, Veracruz state; 20°20'35.32" N, 96°53'25.42" W), and of a Mexican hybrid (hereafter "hybrid") were purchased from a Mexican commercial seed provider (accession NB2, Hibridos NOVASEM S.A., Zapopan, Jalisco, Mexico).

Seedlings were grown in a greenhouse, and were at 5-leaf stage when used in observations; the fourth leaf was used as a substrate for all observations (see below). In order to enhance germination, the seeds of the wild plants were removed from their fruitcases with the aid of a pair of nail clippers, wrapped into a wet piece of filter paper and then placed in a Petri dish for ~ 72 h in the growth room; seeds were transplanted

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into pots filled with potting soil (Sunshine mix 1, Sun Gro Horticulture, Seba Beach, AB TOE 2 BO Canada) once the radicle and the cotyledon were clearly emerged.

Observation of oviposition and feeding activities: Observations were done using a video camera focusing on a cage attached to the 4th leaf of a plant (Figure. 3.1a, details below). Fifteen plants of each *Zea* host plant were used, except in the case of wild annual 2 of which 16 plants were used. The cage for observing *D. maidis* oviposition and feeding was made from plastic transparency film (all sides except bottom), glass microscope slide (bottom), and black foam-board as a visual background for contrast. The internal dimensions of the cage were 7 mm × 11 mm × 25 mm (height × width × depth) (Figure 3.1a). Observations were made for 60 min using a digital video camera (AM413ZT, Dino-Lite, Torrance, CA, 90501) connected to a computer; the observer took note of every movement of the insect as seen on the computer's monitor.

The cage enclosed a portion of a seedling's 4th leaf, and the leaf was positioned so that it covered the bottom of the cage and exposed its adaxial side to the insect, including an ~11 mm length of midrib, which is the leaf tissue preferred for oviposition (Chapter II). On average, the portion of leaf offered to the *D. maidis* female and enclosed in the cage was ~50 mm distant from the seedling's stem, the leaf portion preferred for oviposition (Heady et al., 1985; EBV unpubl.). The cage was attached to a microscope arm at a fixed height of 30 cm, and was distant ~1cm from the camera. The plant, the cage, and the camera were enclosed in a Plexiglas box (60 long × 45 high × 45 wide cm) open on both ends, and with all sides covered with white paper in order to

minimize visual disturbance of the *D. maidis* during the 60 min observation period. Two white-light lamps, placed outside the Plexiglas box, provided diffuse lighting.

The ovipositional and feeding activity of a single *D. maidis* female was observed during 60 min on leaves of each of the individual host plant. Each female was placed in a freezer at 2 °C for 2 min immediately prior to beginning an observation in order to facilitate its placement inside the cage, which was done with the aid of an aspirator. Once inside the cage, the *D. maidis* females became active within a few seconds, and then would walk on the cage walls before settling on the leaf. During that time, the camera was manually re-focused until the leafhopper settled to initiate oviposition or feeding. The 60 min observation period initiated upon the female's first contact with the leaf.

Feeding: Because honeydew excretion is unequivocally correlated with probing activity and actual feeding, the following parameters were recorded as comparative indices of feeding acceptance: (i) the time elapsed between the insect's first contact with the leaf until the excretion of the first honeydew drop; (ii) the proportion of *D. maidis* females excreting honeydew during the 60min observation period; (iii) the number of honeydew drops excreted by the leafhopper during the observation period, and; (iv) the proportion of leafhoppers with observed, "continuous honeydew excretion" (= leafhoppers excreting at least 5 honeydew droplets within 20 minutes), which was taken as an indication of successful feeding (cf. Carpane et al. 2011). Univariate analysis of variance (ANOVA) was used to assess any difference among host plants in (i) and (iii), and Chi-square tests were used to determine any difference among plants in the

proportions of indices (ii) and (iv). Data from parameter (i), viz. time to excretion of the first honeydew drop, was log-transformed in order to normalize prior to conducting ANOVA.

Oviposition: Observations recorded the following comparative indices of ovipositional acceptance: (i) time elapsed between the insect's first contact with the leaf and the first oviposition attempt; (ii) proportion of *D. maidis* females attempting oviposition; (iii) number of eggs laid by each *D. maidis* female, and; (iv) time invested per completed oviposition attempt. A completed oviposition attempt was recorded when the *D. maidis* female proceeded through the three steps of oviposition: deployment, insertion, and retraction (Figure 3.1 b-d, see Results), otherwise it was considered simply an oviposition attempt. Univariate analysis of variance (ANOVA) was used to determine any differences in indices (i), (iii), and (iv), and Chi-square tests were used to determine any difference among the proportions of index (ii). Three outlying oviposition events were excluded in estimating the mean time spent on every completed oviposition event because the number of (observed) oviposition events (n = 266) was slightly higher than the number of eggs found after staining (n = 262) (see below); the outlying events were identified as those outside the range between three standard deviations (i.e. >99% of all normally distributed observations) (Kriegel et al., 2009) below and above the mean time for completed oviposition event. When warranted by a significant ANOVA, *a priori* planned contrasts were used to separate means reflecting the life history (perennial teosinte vs. annual teosintes), domestication (annual teosinte vs. maize landrace), and

breeding (maize landrace vs. maize hybrid) transitions within our suite of *Zea* host

plants.

In order to count eggs and stylet scars, the leaf section exposed to each *D. maidis*

female during observations was stained using an optimized McBride technique (Backus

et al., 1988). Briefly, the leaflet was immersed in a solution of acetic acid, ethanol and

acid fuchsin (20:20:1) for ~48 h. After this, the leaves were dried with a paper towel,

immersed in a second solution made of lactic acid and glycerol (1:1) before placing them

in an oven at 75 °C for ~3 h. Regression analysis was applied to address whether a

relationship existed between the number of stylet scars on the midrib and the number of

oviposition attempts observed; moreover, only females that did not appear to feed

because they did not excrete honeydew were included in this analysis in order to address

whether stylet probing was correlated with oviposition. Additionally a regression

analysis was to address the relationship between completed oviposition attempts

observed and actual oviposition (i.e. number of eggs laid) by the leafhopper, in order to

confirm the veracity of my observations with the camera.
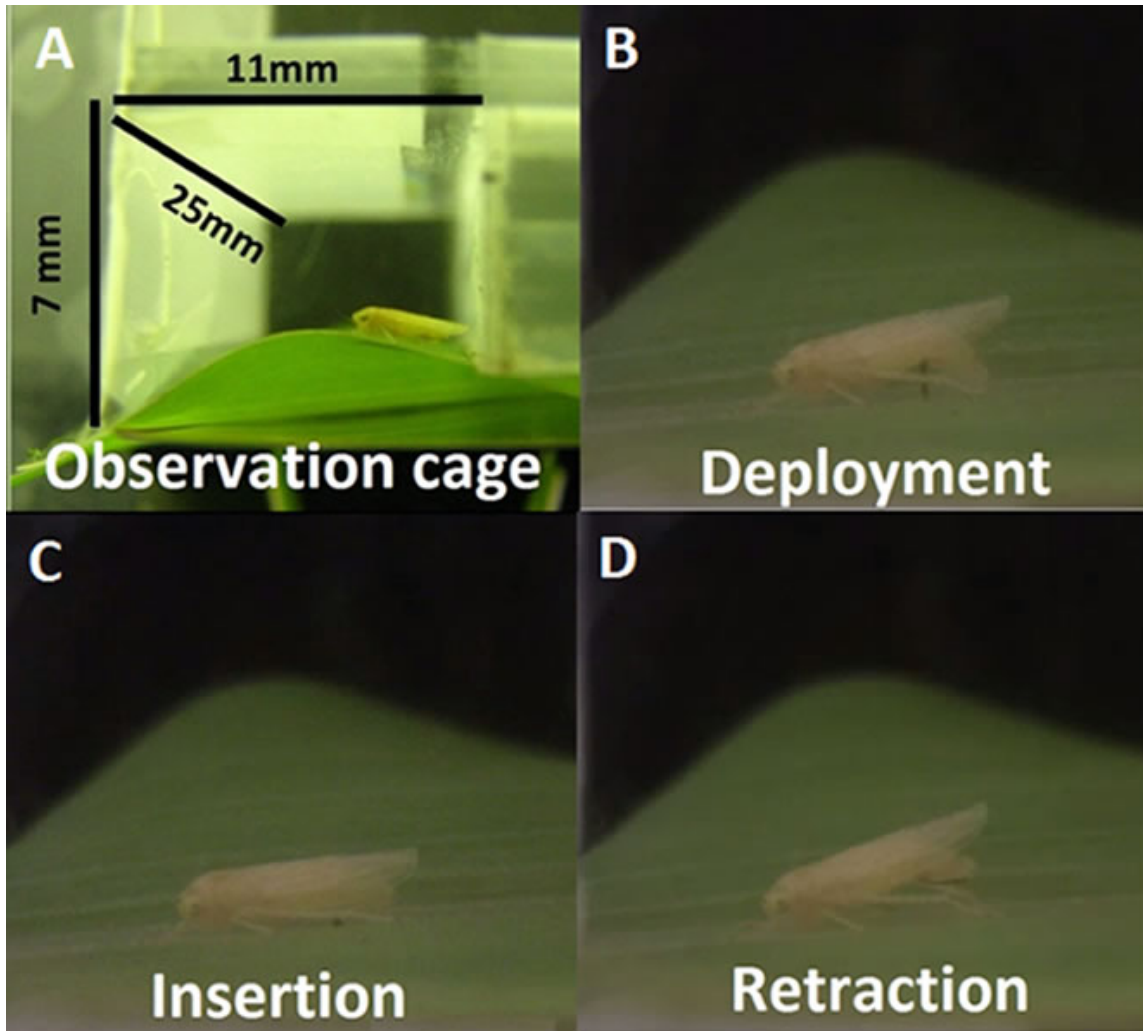
Figure 3.1: A): *Dalbulus maidis* inside the observation cage, with cage dimensions. **B):** First stage of oviposition, the *D. maidis* female bends her abdomen and positions the ovipositor perpendicular to the leaf (deployment). **C):** Second stage of oviposition, the *D. maidis* female inserts her ovipositor into the leaf and relaxes the abdomen, presumably indicating actual oviposition (insertion) **D):** Final stage of oviposition, *D. maidis* female retracts its ovipositor from the leaf (retraction).

Results

Feeding: The time between first contact of a *D. maidis* female with a leaf and excretion of the first honeydew droplet varied between 2.2 and 51 min (Table 3.1). However, no significant difference was found among the mean times corresponding to the host plants of my suite ($P = 0.829$, $F = 0.37$, df = 4, 18) (Table 3.1). Among all *D. maidis* that produced honeydew droplets, the mean time to excretion of the first droplet was 14.1 ± 2.3 min (mean ± standard error). The proportion of *D. maidis* females in which honeydew excretion was observed varied between 0.31 in the wild annual 2 to 0.67 in the hybrid (Table 3.1), though no significant difference was found among the suite of host plants ($P = 0.345$, $Chi^2 = 4.48$, df = 4). The overall proportion of leafhoppers excreting at least 1 droplet of honeydew was 0.38. The mean number of honeydew droplets excreted per *D. maidis* female varied between 6.3 droplets in the land race to 10 droplets in the wild annual 1 (Table 3.1), though no significant difference was found among the host plants of my suite ($P = 0.996$, $F = 0.04$, df = 4, 28) (Table 3.1). The mean number of droplets excreted by females was 7.4 ± 2.9. Finally, the proportion of *D. maidis* female leafhoppers in which continuous honeydew excretion was observed varied between 0.07 to 0.27, with an overall proportion of 0.14, but the differences among the host plants were not significant ($P = 0.614$, $Chi^2 = 2.67$, df = 4) (Table 3.1).

Table 3.1: Indices of feeding host acceptance by *Dalbulus maidis females* along a suite of *Zea* host plants (60 min, no-choice trials)

Host plant	Time (sec) between contact and excretion of the first honeydew drop[1] (mean ± SE)	Proportion with observed honeydew excretion[2] (proportion ± SE)	Honeydew droplets (leafhoppers producing at least 1 droplet)[3] (mean ± SE)	Proportion with observed, continuous honeydew excretion (feeding on the phloem)[4] (proportion ± SE)
Wild perennial	353.67 ± 160.81	0.40 ± 0.13	6.67 ± 3.70	0.07 ± 0.07
Wild annual 1	409.17 ± 170.10	0.40 ± 0.13	10.0 ± 3.70	0.13 ± 0.09
Wild annual 2	568.85 ± 184.67	0.31 ± 0.12	6.40 ± 4.05	0.13 ± 0.09
Land race	498.88 ± 160.81	0.40 ± 0.13	6.33 ± 3.70	0.20 ± 0.11
Hybrid	727.61 ± 154.31	0.67 ± 0.13	7.40 ± 2.87	0.27 ± 0.12
Overall	805.78 ± 175.40	0.38 ± 0.04	7.40 ± 1.49	0.14 ± 0.03

[1]: differences among means are not significant, $P = 0.83$, $F = 0.37$, df = 4, 18
[2]: differences among proportions are not significant, $P = 0.35$, $Chi^2 = 4.48$, df = 4.
[3]: differences among means are not significant, $P = 0.99$, $F = 0.04$, df = 4, 28
[4]: differences among proportions are not significant, $P = 0.61$, $Chi^2 = 2.67$, df = 4

Oviposition: Once a *D. maidis* female is on the midrib, she begins the process of oviposition, which I divided into three steps (Figure 3.1). In the first step, "deployment," the female bends her abdomen and displays the ovipositor perpendicularly to the leaf surface (Figure. 3.1b). In the next step, "insertion," the leafhopper female inserts the ovipositor with successive drilling movements until the ovipositor is completely within leaf tissue (typically midrib), followed by relaxation of the abdomen and, presumably, actual oviposition (Figure. 3.1c). The final step, "retraction," involves the sudden withdrawal of the ovipositor from the leaf (Figure. 2.1d).

Table 3.2: Indices of oviposition host acceptance by *D. maidis* females along a suite of *Zea* host plants (60 min, no-choice trials)

Host plant	Time (min) between contact and first oviposition attempt[1] (mean ± SE)	Proportion of leafhoppers which attempted oviposition[2] (mean ± SE)	Mean number of eggs laid by females[3] (mean ± SE)
Wild perennial	10.42 ± 1.96	0.47 ± 0.13	9.33 ± 2.04
Wild annual 1	14.06 ± 4.12	0.53 ± 0.13	9.00 ± 1.77
Wild annual 2	10.31 ± 2.83	0.25 ± 0.11	5.75 ± 2.50
Land race	8.47 ± 1.49	0.60 ± 0.13	8.33 ± 1.66
Hybrid	6.27 ± 1.47	0.33 ± 0.13	7.20 ± 2.23
Overall	10.35 ± 1.19	0.43 ± 0.06	8.19 ± 0.85

[1]: Difference among means are not significant $P = 0.41$, $F = 1.02$, $df = 4, 31$
[2]: There was no significance among proportions $Chi^2 = 6.58$, $P = 0.16$, $df = 4$
[3]: Difference among means are not significant $P = 0.79$, $F = 0.42$, $df = 4, 27$

The time spent between a female's first contact with the leaf and the first oviposition attempt was highly variable among the *Zea* host plants, varying between 2 and 36 minutes. However, no significant difference was found among mean times to first oviposition attempt ($P = 0.41$, $F = 1.02$, $df = 4, 31$). Overall, the mean time between a female's contact with a leaf and the first attempt to oviposit was $10.0 ± 1.2$ min (mean ± SE). Of the 76 *D. maidis* females observed, 36 of these attempted oviposition (47%), 32 of which were recorded performing at least one completed oviposition attempt. Overall, 266 completed oviposition attempts were observed across the suite of *Zea* host plants. There was no significant differences among the proportion of females attempting to oviposit on the different host plants ($P = 0.16$, $Chi^2 = 6.58$, $df = 4$) (Table 3.2) The proportions varied between 0.25 in the wild annual 2 to 0.60 in the landrace, with an

overall proportion of 0.43. The number of eggs laid per *D. maidis* female on each of the

host plants varied between 1 and 13, but there was no significant difference among the

host plants (P = 0.79, F = 0.42, df = 4, 27) (Table 3.2). The mean number of eggs laid by

D. maidis females was 8.2 ± 0.9. Overall the time spent per *D. maidis* female on a single

completed oviposition event varied between 0.6 (wild perennial) and 4.9 min (wild

annual 2). The mean times varied significantly among the suite of *Zea* host plants (P =

0.003, F = 4.15, df = 4, 258) (Figure. 3.2a). Contrast comparisons showed that the mean

time for oviposition increased significantly in the transition from perennial to annual life

history (t = 3.24, P = 0.001, df = 145) (Figure 3.2b).
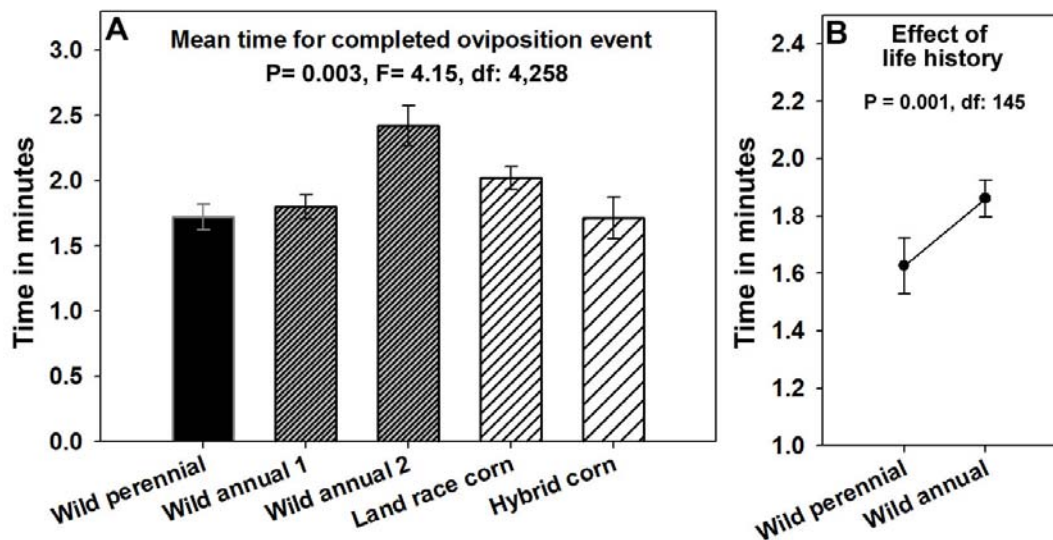
Figure 3.2: A): Mean time (min) spent by a *D. maidis* female in laying one egg along a suite of *Zea* host plants. This is starting from the moment the female displays the ovipositor (deployment) to the moment it completely remove it from the leaf (retraction). **B):** A positive effect of change in life history (wild perennial *Zea* vs wild annual *Zea*) was evident on the time spent by *D. maidis* females on laying a single egg.

A significantly positive correlation was found between number of oviposition attempts, and the number of stylet scars produced by *D. maidis* females (P < 0.001, r^2 = 0.638, df = 1, 20) (Figure. 3.3a). Additionally, there was a significantly positive correlation between the number of observed completed oviposition events and the number of eggs laid by the *D. maidis* females during the observation time (P < 0.001, r^2 = 0.952, df = 1, 33) (Figure 3.3b).
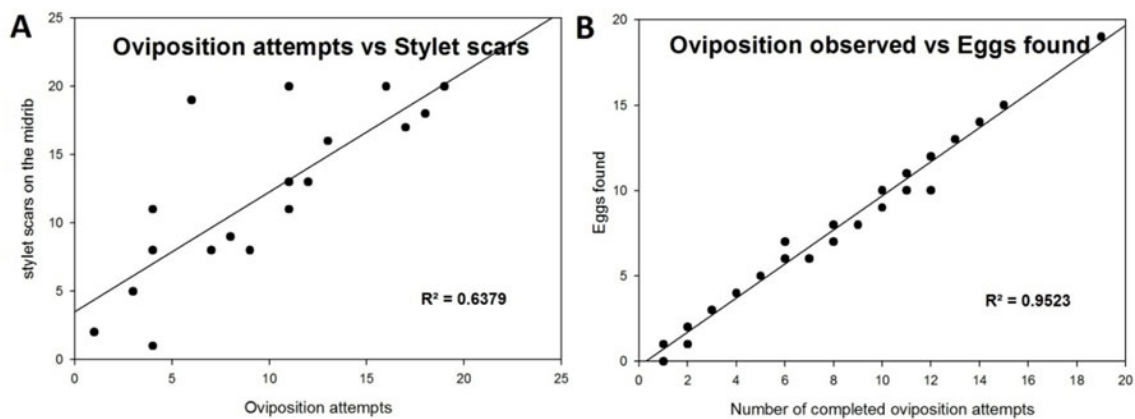
Figure 3.3: A): Correlation between the number of stylet scars found on the midrib and the number of oviposition attempts observed; only the leaves where no honeydew excretion was observed were used in the analysis. **B):** correlation between the number of completed oviposition attempts observed vs the actual number of eggs laid by *D. maidis* female.

Discussion

Host acceptance (feeding): I found that, the excretion of a single honeydew droplet is a sudden event requiring a fraction of a second. Once the leafhopper is on the leaf it was difficult to anticipate the excretion of a droplet because the insect is constantly probing the leaf (Carpane, 2007), and excretion is not preceded by any particular behavior. Once the droplet was expelled, it would land on the leaf or on a wall

of the cage, where it would evaporate within ~2 min after its excretion. Therefore, the only way to accurately record excretion was to uninterruptedly observe the leafhopper. Furthermore, honeydew excretion could only be observed if the apex of the leafhopper's abdomen was visible, e.g., in lateral view. Thus, I was unable to confidently record honeydew excretion when the *D. maidis* was facing the camera.

Despite being previously starved for ca. 20 h, only between 1/3 and 2/3 of *D. maidis* females excreted honeydew droplets, a definitive indication of feeding activity. In particular, honeydew excretion within the 60 min observation period was recorded in <50% of all *D. maidis* females. Seemingly, the period of starvation was insufficiently long to compel the majority of females to initiate feeding, indicating that a longer time of observation might be needed as it was in a previous study (Carpane et al 2007). The proportion of females excreting honeydew droplets did not vary across the suite of host plants offered to them, which is consistent with a previous study showing that *D. maidis* could feed from up to 30 different plants (Pitre, 1967).

Most of what is known about hemipteran feeding behavior comes from studies relying on electric penetration graph technology (EPG) (e.g., Buduca et al., 1996; Kimmins & Bosque-Perez, 1996; Almeida & Backus, 2004), where several probing behaviors are correlated with different electric waveforms (e.g., stylet pathway, active ingestion, putative stylet work, salivation, passive ingestion in Lett et al., 2001). In the case of *D. maidis*, which mostly feeds on phloem contents, honeydew excretion is correlated with various EPG waveforms (probing behaviors), though honeydew is

steadily excreted only in case of actual feeding, whether feeding from non-phloem cells or phloem sieve elements. If continuous honeydew excretion of honeydew indicates actual feeding, then only 12 of 76 females (16%) fed successfully on the different host plants. However, while our results suggested a lower frequency of feeding on the wild perennial host compared to the wild annual and domesticated hosts, the difference was not significant. The proportion of *D. maidis* females that successfully fed (leafhoppers with continuous honeydew excretion) when given access to hybrid maize was similar in this study to that reported in a prior study using the EPG technique (Wayadande & Nault, 1996). The rate of excretion I observed (honeydew drops per minute) was likely higher than in previous studies, such as Carpane (2007) who made observations of leafhoppers with wire attachments required for EPG techniques, however, this behavior was observed too infrequently in this study to warrant statistical comparisons.   .

In conclusion, despite known differences in leaf toughness (e.g., work to penetrate a leaf, Chapter II), I found no consistent evidence that in a no-choice scenario acceptance for feeding in *D. maidis* varied among the suite of *Zea* host plants. This suggests that all *Zea* host plants are similarly acceptable for feeding, and that resistance to stylet penetration may not be an effective defense of *Zea* against *D. maidis*, at least when the insect is in adult stage. This coincides with previous findings that *D. maidis* can feed, but not develop, on various grasses other than *Zea* (Pitre, 1967), and with studies of overwintering biology where adults of *D. maidis* were observed at the beginning of the season when maize was not available, an indication that they may feed on non-maize hosts (Moya-Raygoza et al., 2007). It is unknown whether *D. maidis* feeds

more efficiently on maize compared to other *Zea* hosts, though the rate of phloem consumption (number of honeydew drops excreted in a set amount of time), may be less an indicator of feeding efficiency than a reflection of the balance between phloem nutritional quality and the insect's ability to overcome any defensive compounds to achieve complete development. Addressing this question was beyond the scope of this study but should be considered for future studies.

Host acceptance (oviposition): *D. maidis* females constantly probe upon host plants (Carpane et al., 2011), and insects in general are constantly assessing the quality of their hosts for oviposition (Hilker & Meiners, 2011). In this study I found a positive correlation between the numbers of stylet scars and oviposition attempts by *D. maidis* females that seemingly did not feed. Moreover, stylet scars were consistently found close to the egg's operculum at a distance that roughly matched the length of the leafhopper (data not shown). This suggests that *D. maidis* females assess host quality with their mouthparts prior to, and during, oviposition. However, females showed no differences in oviposition acceptance (time passed before the female initiates oviposition) among host plants, which may indicate that any potential differences in suitability among host plants were insufficient to deter oviposition by *D. maidis*. This is consistent with previous studies, which found that in a no-choice scenario, *D. maidis* was able to oviposit in up to 20 different plants species (Pitre, 1967), although it could only achieve complete development on maize.

My results on time spent by *D. maidis* per completed oviposition event (an index of egg laying efficiency) suggested that the transition in host plant life history had an

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effect on *D. maidis*, although the effect I found was opposite of expected because the time spent per completed oviposition event was shorter on the wild perennial, contrary to the results of a prior study suggesting that the wild perennial was harder to penetrate with the ovipositor (Chapter II). The discrepancy may be due mainly to the great variation among "geographic populations" (San Lorenzo and El Cuyotomate) of the wild annual plants used in this study. It is noteworthy that in a previous study on host preference of *D. maidis* females the wild perennial was the host that was most frequently rejected (see Chapter II), while this study showed that *D. maidis* needs less time to lay an egg on the wild perennial. According to prior studies *D. maidis* females on average lay ~9 eggs per day (Davis, 1966; Pitre, 1970; Marin, 1987; Carpane, 2007), while the results of my study showed that *D. maidis* can lay that number of eggs within 60 min. Such a difference may indicate that once a female encounters an acceptable host she is not constrained by time available for oviposition. Therefore, oviposition efficiency (time spent on every completed oviposition event) as assessed herein, may not be an effective parameter to discriminate among *Zea* host plants in the context of plant defenses against *D. maidis.*

Conclusion

The results of my study suggest that in the contexts of initial oviposition and feeding interactions, life history, domestication, and breeding transitions in *Zea* may not be relevant to defense against the specialist herbivore *D. maidis*, in a no-choice scenario. However, the results of a prior study conducted under choice condition, suggested that *D. maidis* females preferred to oviposit on maize compared to wild *Zea* (Chapter II),

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supporting predictions that antiherbivore defenses are weaker in crop plants compared to their wild relatives. Effective *Zea* antiherbivore defenses (e.g., inducible defensive compounds) against *D. maidis* may be more relevant to other performance components, such as egg fertility (Pitre, 1967), and development time, survivorship and reproduction (Nault & Madden, 1985), among others. Evaluation of such performance components was beyond the scope of this study, but should be considered in future ones. Additionally, it is important to note that the individuals of *D. maidis* used in this study originated from a colony established from founder individuals collected from maize in Mexico, while recently it was shown that *D. maidis* consists of at least two reproductively discrete subpopulations, one from maize and Balsas teosinte and the other from perennial teosinte (Medina et al., 2012). Thus, my results may be influenced by host adaptation to wild and domesticated annual *Zea* over perennial *Zea* in the *D. maidis* used in this study. Moreover, the fact that a subpopulation of *D. maidis* seems to be adapted to the wild perennial (*Z. diploperennis*) as a host, presumably the better defended plant of our suite, confirms the conclusion of this study, that despite the differences in life history and resource allocation priorities along the suite of *Zea* host plants, *D. maidis* is equally capable of accepting all the plants of the suite.

Although life history evolution, domestication, and modern breeding in *Zea* appear to have differentially affected two putative, physical defenses, leaf toughness and pubescence (see Chapter II), the results of this study did not suggest significant host plant effects in host acceptance indices. The lack of significant effects suggests that in the absence of a choice, e.g., when dispersing *D. maidis* colonize a patch of hosts of a

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single species, females equally accept *Zea* host plants for oviposition and feeding. This result may be a reflection of the degree of specialization of *D. maidis* and coevolution with its host plants, as suggested earlier (Nault, 1990). Moreover, given the ubiquity of maize and the insect's capacity for dispersal (Power, 1992), *D. maidis* may have opportunities for switching hosts under natural conditions. Finally, it is worth noting that it is unlikely that *Zea* antiherbivore defenses target a single, specialized herbivore, such as *D. maidis*, instead targeting a guild of herbivores capable of colonizing *Zea* (Howe & Jander, 2008)

CHAPTER IV

CONCLUSION

Overall, my study suggested that life history evolution, domestication, and breeding affected two putative, physical defenses in *Zea*, leaf toughness and pubescence, though the direction of the effects did not generally follow the prediction of weaker defense in domesticated relative to wild cultivars (e.g., trichome density). My study also indicated that the specialist herbivore *D. maidis* has a clear preference for maize over the teosintes, which suggests a lower level of antiherbivore defense in the former, as suggested by others (Rosenthal & Dirzo1997; Szczepaniec et al., 2012; Takahashi et al., 2012). However, leaf toughness (assessed in terms of work for penetration) did not completely explain host preference in *D. maidis* because direct observations under no-choice conditions suggested that leaf toughness may not be an effective barrier for feeding and oviposition, on any of the host plants, including the predicted, better-defended plant of my suite, the wild perennial. Indeed a subpopulation of *D. maidis* appears to live exclusively on this host (Medina et al., 2012).

The results presented in Chapter II showed that transitions in life history, from perennial to annual life cycle, and in domestication, from annual teosinte to landrace maize, affected leaf toughness (Table 4.1). On the other hand, trichome density was affected only by the breeding transition, from maize landrace to hybrid (Table 4.1), though the direction of this difference was opposite the prediction that the hybrid would be less defended than the landrace. Finally, the host preference experiment showed a clear female preference for the domesticated hosts over the wild hosts (Table 4.1), which

supported the prediction of weaker defense in the agricultural hosts of my suite, if host

preference by *D. maidis* is correlated with the strength of its host's defenses. Overall,

however, it was unclear whether and how much *D. maidis* feeding and oviposition may

be affected by the differences I found in leaf toughness and trichome density, although

effects of these two traits on hemipteran activity have been shown in other studies

(Peeters et al., 2007; Kaplan et al., 2009; Heshula & Hill, 2011).

It is interesting that the results presented in Chapter III showed that despite

differences in leaf toughness and trichome density (Chapter II), *D. maidis* showed no

differences in feeding or oviposition acceptance indices among the *Zea* host plants of my

suite when these were offered in a no-choice context (Table 4.1), which is similar to

what was found by Carpane (2007), using *D. maidis* against a suite of diverse maize

cultivars with different pathogen-resistance levels. Thus, it seems that at least from the

physical dimension of the insect-plant interaction, adult *D. maidis* females are not

strongly, nor differentially challenged by the hosts of my suite. This may be explained

by the recentness of maize domestication (Matsuoka et al., 2002; Buckler, 2006) and the

specialization of *D. maidis* on better defended, wild hosts, the teosintes, so that its host

range expansion from wild, better-defended hosts to a putatively, lesser-defended host,

maize (Nault, 1990), may have not implicated substantial adaptations at least in the

scope of overcoming physical defenses.

Leaf toughness and pubescence seem to be mainly structural traits, with little

additional defensive value, i.e. defense is not their raison d'etre. Therefore, the level of

their expression and the cost of their maintenance are not exclusively correlated to

defense against herbivory, but to a wide range of environmental variables, such as light (Onoda et al., 2008) water stress or soil quality. Moreover, plant defense is not easily explained in the light of one or a few traits, but in terms of "defensive syndromes" (Agrawal & Fishbein, 2006) in which many traits contribute to antiherbivore defense. Thus, defenses in *Zea* are likely tuned to an optimal combination of defenses against a guild of herbivores that includes *D. maidis*, rather than to *D. maidis* alone. It is also worth to note that under normal population densities *D. maidis* is only a threat to the *Zea* when it vectors diseases (e.g., Maize Rayado fino virus, Corn Stunt Spiroplasma), therefore host plant defenses may be more focused on specialist pathogens rather than on deterring injury by one specialist insect among many other insects that feed on plant.

In conclusion, the differences I found in leaf toughness and pubescence among the *Zea* host plants did not closely follow the prediction of stronger defense in crop wild relatives compared to crops, so they did not completely explain host preference and acceptance by *D. maidis*. Overall, two putative physical defenses in *Zea*, leaf toughness and pubescence, seemed unimportant against *D. maidis*, thus it may be warranted to seek differences in chemical defenses among wild and domesticated *Zea* that could explain the clear host preferences I found in *D. maidis*.

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Table 4.1: Overall results showing the effects of transitions in life history, domestication, and breeding in *Zea* on leaf toughness and pubescence, and on host preference and acceptance by *D. maidis.*

Test / Contrast	Life history (wild perennial to wild annual)	Domestication (wild annual to landrace maize)	Breeding (Landrace maize to hybrid maize)
Leaf toughness: Effects on work for penetration to hypothetical stylet reach of 0.3 mm	P = 0.008	ns	ns
Effects on work for penetration to ovipositor reach of 0.8 mm	P = 0.012	P = 0.001	ns
Pubescence: Effects on trichome density	ns	ns	P = 0.007
D. maidis: Effects on oviposition preference	ns	P = 0.008	ns
D. maidis: Effects on host plant rejection (for oviposition)	ns	ns	P = 0.009
D. maidis: Effects on feeding acceptance (no choice scenario)	ns	ns	ns
D. maidis: Effects on oviposition acceptance (no choice scenario)	ns	ns	ns

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