EMPOASCA LEAFHOPPER SPECIES RESISTANCE IN COMMON BEAN, PHASEOLUS VULGARIS: FIELD SCREENING AND QTL IDENTIFICATION

By

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A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Plant Breeding, Genetics and Biotechnology Program / Crop and Soil Sciences

2012

ABSTRACT

EMPOASCA LEAFHOPPER SPECIES RESISTANCE IN COMMON BEAN, PHASEOLUS VULGARIS: FIELD SCREENING AND QTL IDENTIFICATION

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Empoasca species leafhoppers are a major insect pest of common bean, *Phaseolus vulgaris* that cause significant economic losses in both tropical (*E. kraemeri)* and temperate (*E. fabae*) regions of the Americas resulting in up to 80% crop yield reductions. Chemical controls are costly, reducing profitability by increasing input costs, and potentially causing damage to the environment and human health. Breeding beans for leafhopper resistance can provide an alternative control of this pest. The current study examined *Empoasca spp.* resistance by evaluating leaf curl and leaf burn damage as well as *Empoasca spp.* nymph counts in an inbred backcross line population (Matterhorn*/EMP507) of beans in temperate and tropical climates. Field screening in Michigan and Puerto Rico in 2009-2011 identified the existence of tolerance, antixenosis and antibiosis mechanisms of resistance to *E. fabae* and *E. kraemeri* in this population. Thirteen QTL associated with resistance to *E. fabae* and *E. kraemeri* were identified on six bean chromosomes that explained from 22.8 % to 61.5 % of the phenotypic variation of individual traits. A major QTL (LH7.1) associated with multiple resistance traits was detected for both leafhopper species in multiple seasons on Pv07. This QTL was tightly linked to the *P* gene that confers the presence of color in the seed coat, validating a similar QTL identified in previous studies. A novel QTL for *E. fabae* nymph counts was identified on Pv02 that may be associated with antibiosis resistance. Resistance to each leafhopper species appear to be controlled by separate genetic mechanisms in common bean.

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CHAPTER 1: Literature Review

I. Common Bean

A. Introduction

Common bean (*Phaseolus vulgaris* L.) is a staple food crop grown world-wide because it is a widely adapted short-season crop that is an excellent and economical source of nutrition. Global annual production in 2010 of both dry (19.6 million MT) and snap beans (1.8 million MT) was greater than 21 million MT, representing more than half of the world's total food legume production (FAOSTAT, 2012). Common bean is the most important grain legume for direct human consumption, with production more than twice that of the next most important grain legume, chickpea (Gepts et al., 2008). Bean production is mainly located in Latin America and eastern Africa, where it is commonly grown under low-input agriculture on small farms for direct consumption by its producers (Broughton et al., 2003). These low-input production systems are more susceptible to disease and insect pest outbreaks and are more likely to suffer from abiotic stresses such as drought and low fertility issues (Miklas et al., 2006).

Beans provide an excellent source of protein and other nutrients for many people in the developing world. In some countries, such as Mexico and Brazil, dry beans are the primary source of dietary protein (Broughton et al., 2003). Protein content makes up 20-25% of seed weight and is predominantly composed of lysine-rich essential amino acids, which are complementary to cereal grain protein content (Gepts, 2008). In addition to protein, beans are an excellent source of many important minerals and vitamins, including Ca, Cu, Fe, Mg, Mn, P, Z, and folate (Welch et al., 2000). Recent studies have also linked regular bean consumption to decreased cholesterol levels and reduction in risk for heart disease as well as certain cancers (Winham et al., 2007; Thompson et al., 2009).

B. Center of Origin

Dry beans fall into two groups based on their geographic centers of origin – the Andean gene pool, which originated in South America, and the Middle American gene pool, which originated in Central America (Gepts et al., 2008). The two gene pools have been defined based on multiple traits including morphological, physiological, and agronomic characteristics. The Andean gene pool is characterized by large seeded beans with determinate or climbing growth habits, such as kidney and cranberry beans. In contrast, the Middle American gene pool is characterized by small and medium seeded beans and both determinate and indeterminate growth habit. In addition to obvious seed size and plant growth habit differences, the gene pools also differ in many physiological traits including resistance to disease and insect pests (Miklas et al., 2006).

The two-gene-pool concept initially was developed based on differences in the phaseolin seed storage protein and the partial reproductive isolation seen between these two groups (Gepts, 2008). The races that make up each gene pool were then identified based on a combination of morphological adaptations and allozyme profiles. Three races were identified within the Andean gene pool: Nueva Granada, Peru, and Chile (Singh et al., 1991). Within the Middle American gene pool, three domesticated races were initially identified: Mesoamerican (blacks and navys), Durango (pinto and great northern), and Jalisco (small red and pink) (Singh et al., 1991). The advent of random amplified polymorphic DNA (RAPD) markers allowed the identification of a fourth race of climbing beans within the Middle American gene pool – race Guatemala (Beebe et al., 2000).

The Mesoamerican gene pool has a significantly higher level of diversity than the Andean based on phaseolin types, allozyme alleles and molecular markers, which suggests that

the Andean gene pool was subject to a severe bottleneck prior to domestication (Kwak and Gepts, 2009). Recently, the two-gene-pool concept was questioned in light of evidence from recent advances in sequencing technology. Bitocchi et al. (2012a) investigated nucleotide diversity at five gene loci within a sample representing the geographical distribution of wild *P. vulgaris*. They found support for a Mesoamerican origin where both gene pools originated from different migration events of Mesoamerican populations from central Mexico (Bitocchi et al., 2012a).

The same group furthered this study and provided evidence that common bean developed as a result of two independent domestication events, one event for each gene pool (Bitocchi et al., 2012b). They also found a reduction in gene diversity following domestication in both gene pools, although the reduction in Mesoamerican gene pool diversity was significantly greater than in the Andean gene pool, further supporting the theory of a pre-domestication bottleneck in the Andean gene pool. They concluded that the origins of the domestication for the Mesoamerican gene pool lie in the Oaxaca valley in Mexico, and for the Andean gene pool, domestication origins trace to southern Bolivia and northern Argentina in South America (Bitocchi et al., 2012b). These findings reflect the adaptability of the original common bean populations, which allowed movement into a wide range of diverse climates, from the lowland tropics of Mesoamerica into the Andean highlands.

Understanding the origin and diversity of common beans is essential for plant breeders. This knowledge can be used to facilitate identification and introgression of diverse traits that are currently lacking in commercial varieties. In addition, this new understanding could facilitate crossing between both gene pools and among races to expand the diversity present in commercial

cultivars to ensure bean production continues to be viable for growers in both developing and developed regions.

C. Production

Beans are produced throughout the world in both developed and developing nations. The largest producers globally in 2010 were Brazil, the US and Mexico (FAOSTAT, 2012), with Brazil also being one of the largest per capita consumers of common beans at rates of approximately 15kg/yr per capita (Morin, 2010). In much of the developing world, most beans are produced on small family farms less than 20 hectares in size and often on marginal lands (Broughton et al., 2003). Bean production is important not only as a source of income for these farm families, but as a source of nutrition. According to a recent report from the International Center for Tropical Agriculture (CIAT), by the end of the last century, an estimated 2 million rural households directly benefitted from improved beans developed through international collaborative programs (CIAT, 2012).

The US is the fifth largest producer of dry beans globally with total US crop value of $826.5 M and 607,000 hectares harvested in 2011 (USDA- NASS, 2012). Michigan is positioned as the second largest producer in the US, representing $153.9 M and 68,000 of the 2.6 M hectares total field crops planted in 2011 (USDA-NASS, 2012). In 2008, organic bean production totaled 6,663 hectares across the US with MI as the largest producer with 1,800 hectares under organic production, providing $3.9 M in income to Michigan growers (ERS, 2012).

The dry bean industry is an important sector of the overall agriculture economy both in Michigan and across the United States. The major bean classes grown in the US are pinto, navy, black and great northern beans, which belong to the Mesoamerican gene pool, and kidney and

cranberry beans, which belong to the Andean gene pool. Michigan is currently the number one producer of black beans and cranberry beans, representing 50% and 85% of national production respectively (NASS, 2012).

II. *Empoasca* Species Leafhoppers

A. Classification

Potato leafhoppers (PLH), *Empoasca fabae* and *E. kraemeri*, belong to the Cicadellidae family in the order Hemiptera. Members of this group are characterized by their mouthparts, which are specially adapted for piercing and sucking, and by the spine-like hairs or setae on the hind legs. Species are identified by the male genitalia, although only experts can decipher the differences (Godfrey and Long, 2007). *Empoasca* leafhoppers are small (0.3 cm long), bright green, wedge-shaped winged insects. The nymphs are similar to adults but lack wings and can move rapidly in all directions, including from side to side, which is unusual. Both adults and nymphs feed primarily on the underside of leaves, but due their high mobility, they can be found on all parts of the plant.

Empoasca kraemeri is the most significant insect pest of common bean production in the tropical environment (Kornegay and Cardona, 1990) and *E. fabae* is a very important cause of economic loss in temperate regions (Schaafsma et al.,1998). Breeding dry beans for resistance to PLH could be a cost effective alternative to the use of systemic and foliar spray insecticides to control this pest (Gonzales et al., 2004). *Empoasca fabae* is found throughout most of North America, east of the Rocky Mountains. However, this species cannot survive year-round in Michigan and only overwinters along the Gulf Coast. It is dispersed northward by wind in the spring and early summer and reaches fields as far north as Michigan and Ontario (Dietrich, 2008). Therefore, weather patterns play a major role in whether or not PLH is a significant

problem in any given season (Murray et al., 2004a). In addition, adults continue to arrive in Michigan in a steady stream from May through June, resulting in overlapping generations, providing an additional challenge to control efforts (Dietrich, 2008). Population densities often peak multiple times following major northward winds that carry additional immigrant leafhoppers to their northern summer habitats (Emmen et al., 2004). In contrast, *E. kraemeri* is found year-round in tropical climates such as Colombia and Puerto Rico. It is considered to be a more serious pests because its populations do not have to rebuild and a greater number of generations are produced each year (Gonzales et al., 2004).

B. Life History

Empoasca leafhoppers begin laying eggs 6 days after maturing to adulthood. Eggs are laid in plant tissues and hatch into wingless nymphs after a 10-day incubation period. Nymphs hatch and molt through five instars over the course of two weeks before reaching maturity. *Empoasca fabae* requires approximately 30 days to develop from egg to adult (Emmen et al., 2004). Females mate within two days following their final molt and begin the cycle again. In the field, adults live up to 30 days, but some have been recorded as living up to 120 days in the lab setting (Baker et al., 2008).

Under ideal conditions, an entire PLH lifecycle can be completed within a single month, but generally up to six generations are produced per year. Two to three generations occur in Michigan and other northern habitats, while three to four generations occur in the overwintering sites (Dietrich, 2008). PLH populations remain relatively constant from June through September until winds begin to carry PLH adults back to their southern overwintering sites (Emmen et al., 2004).

Empoasca spp. attack a number of different host plants, ranging from herbaceous annuals to woody trees (Baker et al., 2008). They are attracted to the sugars in the new leaf tissues and therefore move from host to host throughout the season as the sugars build up in different crops. Some of their hosts include dry bean, alfalfa, potato, soybean, apple, eggplant, peanut and sweet potato (Baker et al., 2008). The broad range of hosts presents a challenge to management of this pest, especially because the insects are very mobile.

C. Feeding Damage

Leafhoppers cause a specific set of symptoms referred to collectively as "hopperburn" (Backus et al., 2005), which is composed of leaf curl (LC) and leaf burn (LB). Damage is caused by several distinct feeding behaviors of both adults and nymphs. Kabrik and Backus (1990) identified "lacerate-and-sip" as the most damaging feeding behavior. Lacerate-and-sip involves brief intracellular probes where insect stylets rapidly puncture multiple columns of stem phloem cells simultaneously, causing cell death and abnormal meristematic development (Backus et al., 2005). This pulsing laceration appears to cause systemic vascular damage to the plant, leading to stunting and chlorosis above the point of feeding and resulting in LB damage (Serrano et al., 2000). *Empoasca kraemeri* feeds by this method more often than *E. fabae*, which may be related to the higher level of damage generally inflicted by the former (Calderon and Backus, 1992). Both *Empoasca* species also use two additional feeding tactics that are hypothesized to cause different symptoms – "lacerate-and-flush" and "lance-and-ingest" (Backus et al., 2005). Lacerate-and-flush, which is thought to lead to LC damage, involves longer intracellular probes that puncture and drain mesophyll and parenchyma cells in the lower surface of the leaf, thereby leading to tissue collapse. Finally, during lance-and-ingest, which may cause stunting but likely causes little damage overall, phloem sieve elements are punctured and leak phloem sap. This sap

is ingested while stylets remain motionless, but as stylets are withdrawn, saliva is released, causing cells on the upper surface of the leaf to expand considerably (Backus et al., 2005). The plant response to these behaviors is believed to be controlled by different genetic traits and is therefore measured separately as quantitative trait loci (QTL) controlling LC and LB reactions (Murray et al., 2004b). Numbers of *E. fabae* adults and nymphs were positively associated with both LC damage and plant height, but this correlation was not seen with *E. kraemeri* (Murray et al., 2004a).

As a result of early season severe infestation, bean plant growth is stunted and delayed (Backus et al., 2005), leading to dramatic yield and subsequent economic losses. Severe attacks during reproductive stages can result in high levels of flower and pod abortion, as well as the development of twisted curved pods, each with few seeds of often poor quality (Kornegay and Cardona, 1990). Crop yield losses will be affected by density, duration and initial timing of leafhopper infestation as well as temperature, plant disease incidence and interactions of any of the above (Lindgren and Coyne, 1995). In Nebraska, *E. fabae* damage resulted in estimated dry bean yield losses of up to 20% or $2M USD (Gonzales et al., 2002). The tropical counterpart, *E. kraemeri*, can be especially devastating. In Latin America, dry bean yield losses are estimated at 64% (Gonzales et al., 2002) and in Colombia, specifically, losses of up to 79% have been recorded (Bullas-Appleton et al., 2005). PLH prefers hot, dry conditions and, as a result, populations flourish and damage is more severe in hot, dry seasons than during cooler, wetter seasons. Currently, PLH is managed using pesticides, which are often prohibitively expensive in many parts of the developing world and pose environmental and health risks (Murray et al., 2004a).

In summary, the challenging characteristics of PLH as a pest of dry beans are threefold: mobility – PLH is highly mobile both through long-distance wind dispersal and local movement throughout the growing season; broad host range – PLH reproduces on many host plants from beans to maple trees; and high reproductive capacity – PLH females lay 3-7 eggs per day for 30 days.

III. Plant Resistance

A. Biotic Stress

Common beans are attacked by numerous diseases and insect pests. Therefore, breeding programs globally have focused on improving resistance to these biotic stresses within cultivated and elite germplasm. Significant progress has been made in breeding for disease resistant cultivars in common bean, as recently reviewed by Singh and Schwartz (2010). Resistance already exists within cultivars to some diseases, such as rust, bean common mosaic virus, and anthracnose. However, many more diseases and insect pests have been identified to which adequate resistance does not currently exist. These include diseases, such as common bacterial blight (CBB), halo blight, bacterial brown spot, ascochyta blight, web blight and white mold, as well as insect pests, such as bean fly, bruchids, as well as potato leafhoppers (Miklas et al., 2006). Much of the global bean production occurs on small family farms under low input agriculture that is subject to both abiotic and biotic stress (Ojwang' et al., 2011b). In these subsistence production systems, limited or no chemical pesticides and fertilizers are available, underscoring the importance and potential impact of developing common bean varieties with resistance to these important insect pests.

B. Mechanisms of Resistance

Plants resist insect pests by a number of different mechanisms. The main types of plant resistance are tolerance, antibiosis and antixenosis (Smith, 2005). A plant is considered tolerant if it has reduced or no damage as a result of insect feeding and oviposition that would cause damage on a non-tolerant (susceptible) plant (Smith, 2005). Antibiosis reduces pest population sizes and plant damage by affecting the biology of the insect. Often antibiosis results in increased mortality or reduced lifespan and reproductive capability of the insect. Antixenosis affects insect behavior in that it is usually associated with plant traits that are less desirable to the insect in comparison to more susceptible plants (Teetes, 1996). Antixenosis is also known as non-preference. Tolerance differs from antibiosis and antixenosis in how it affects the insect-plant relationship. Antibiosis and antixenosis interfere with an insect's behavior or biology, while tolerance has no effect on the insect and depends completely on the plant's ability to withstand insect damage (Teetes, 1996). However, even a tolerant plant may suffer some damage as a result of insect predation if the insect population exceeds a certain threshold (Saxena, 1969).

A recent publication by Singh and Schwartz (2011) reviewed the status of resistance in common bean to many pest species, including bean pod weevil, bruchids, leafhoppers, thrips and nematodes. There has been significant progress in breeding for resistance to some pests such as bruchids, but resistance is still elusive to others, such as nematodes. Resistance to the bean pod weevil, *Apion godmani*, was identified in wild common bean populations and Jalisco landraces in central Mexico, and successfully introgressed into small black and red-seeded beans (Beebe et al., 1993). Resistance to the bean pod weevil has been linked to two epistatic genes: the *Agr* allele, which confers moderate resistance on its own, and the *Agm* allele, which alone has no effect, but increases the level of resistance substantially in the presence of *Agr* (Garza et al.,

1996). The resistance conferred by these epistatic genes is associated with ovipositional non-preference (antixenosis) and antibiosis in the form of hypersensitivity, the only insect-related hypersensitive response reported in common bean (Garza et al., 1996). These genes were recently mapped to the bean genetic map with *Agr* residing on chromosome Pv01 and *Agm* on chromosome Pv07 (Blair et al., 2006c).

Antibiosis was reported to the bruchid pests *Acanthoscelides obtectus* (bean seed weevil) and *Zabrotes subfasciatus* (Mexican bean weevil), linked to the arcelin-phytohemagglutinin-amylase inhibitor (APA) family of seed proteins (Cardona et al., 1989). The resistance demonstrated in common bean involves delayed insect emergence, reduced adult size and weight, as well as reduced number of adults. The APA locus was mapped to Pv04, tightly linked to a QTL for low adult insect emergence (Blair et al., 2006). Mbogo et al. (2009) identified a 33kDa lectin-like protein in tepary bean, *P. acutifolius,* that they believe is responsible for resistance to bruchid pests in tepary bean and which they successfully introgressed into *P. vulgaris*. More recently, Kusolwa and Myers (2011) confirmed the superior resistance to *A. obtectus* seen in common bean backcross lines was a direct result of the presence of the APA proteins introgressed from tepary bean.

Resistance mechanisms are often not isolated and different strategies may be used against the same pest, as is case for *Thrips palmi* resistance. Frei et al. (2003; 2004) reported the presence of tolerance, antixenosis, and antibiosis in resistant common bean germplasm. QTL have also been identified for resistance to this pest on bean chromosomes Pv02, Pv03, Pv06 and Pv08. The QTL on Pv02 ($TP2.1^{BG}$) includes resistance to both *T. palmi* feeding damage and reproductive adaptation (Frei et al., 2005). Physical and chemical attributes are also often credited with contributing to resistance to insect pests. Greathead (1968) found that thickened

hypocotyls may be responsible for tolerance to bean fly, *Ophiomyia sp*. (1968). More recent studies suggest that the volatile compounds released when bean plants are injured by bean flies may be involved in antixenosis (Wei et al., 2006).

C. Techniques for Testing for Insect Resistance

In order to accurately evaluate resistance to an insect pest in a plant population, a number of tests can be conducted. Choice tests enable natural populations of insect pests to infest a field site where subsequent plant damage can be measured. This technique can allow the identification of resistant and susceptible individuals within the plant population as long as sufficient natural insect populations exist to inflict differential damage (Smith, 2005). Kornegay and Temple (1986) used free choice tests to identify resistant germplasm in some of the original studies examining resistance to *Empoasca kraemeri* in common bean. In order to compensate for inadequate natural insect populations, trap crops may be planted around the field study site to attract additional pests, insects may be collected from the surrounding area and re-released at the study site, or environmental conditions such as planting date or irrigation may be manipulated to encourage insect colonization. For example, Ojwang et al. (2011) delayed planting common beans by two weeks from the on-set of rainfall to ensure that optimal bean fly pressure was achieved because delayed planting and drought conditions results in increased bean fly pressure.

Another strategy for ensuring adequate damage to evaluate resistance is the use of cages. The advantages of using cages in choice tests are to limit emigration of the test insect or to protect the pest population from natural predators (Smith, 2005). Caged tests can also mitigate issues arising from natural annual variations in insect populations due to environmental fluctuations. In order to differentiate between the different resistance categories of tolerance, antixenosis and antibiosis described previously, different tests must be conducted and results can

be compared. Using cages in choice tests, often a sub-sample of plants that have been previously identified as resistant in field tests are caged together with a susceptible check and infested with sufficient insects to cause measurable damage. Identifying resistant plants under choice test conditions allows researchers to confirm the presence of antixenosis (Smith, 2005). Cages are also used to conduct no-choice tests where a single plant genotype is inoculated with a damaging level of insect pests for a certain period of time or until a threshold is reached. This allows the identification of resistant individuals possessing antibiosis, as test insects have no choice but to feed or not feed on the specific plant genotype being evaluated. In addition, no-choice tests allow the identification of individuals that appeared resistant under choice conditions but that are susceptible under no-choice conditions. Subsequent measures of insect development, such as nymph or larvae survival, can confirm the presence of antibiosis (Smith, 2005). Cardona et al. (1989) used no-choice testing to determine that bruchid (bean weevil and Mexican bean weevil) resistance in common bean is a result of antibiosis interactions by examining larval survival rates.

The different resistance strategies can also be inferred by comparing plant damage resulting from insect feeding and insect populations. In a free choice study examining common bean resistance to *Thrips palmi*, the authors suggested that certain genotypes maintained lower levels of thrips infestation over multiple tests due to either antixenosis or antibiosis (Cardona et al., 2002). They could not differentiate between these strategies of resistance as only choice tests were conducted. Antixenosis or antibiosis can be potentially differentiated by examining the same genotypes under no-choice conditions and comparing the results (Smith, 2005). If no differences are detected between tests for individuals with low damage and low nymph counts, antibiosis is potentially responsible. If individuals identified as resistant under choice conditions

suffer higher levels of damage under no-choice conditions, the resistance may be the result of non-preference (antixenosis).

D. Leafhopper Resistance in Common Bean

In common bean, resistance to *Empoasca* leafhoppers is believed to be conferred mainly through tolerance and antixenosis, with tolerance being the most common mechanism of resistance towards *E. kraemeri* (Schoonhoven et al., 1978). However, antibiosis is noted in some elite bean germplasm. Kornegay et al. (1986) hypothesized that this may be why some plant lines harbor consistently lower nymph population counts in the field than other tolerant and susceptible lines.

Carmona et al. (2011) conducted a meta-analysis to determine which plant traits can best predict insect resistance or susceptibility. They found that the strongest correlations with plant resistance occurred with life-history traits, such as flowering time and growth rate, morphological traits, such as plant size, and physical resistance traits, such as latex or trichomes. Numerous agronomic traits have been linked to potential antixenosis resistance mechanisms in beans. These include indeterminate growth habit, days to flowering, leaf pubescence and trichome composition (Pillemer and Tingey, 1978). However, since these initial studies, leaf pubescence and trichome composition have not been confirmed to be significant antixenotic mechanisms (Schaafsma et al., 1998; Murray et al., 2001). The *Fin* gene, which is associated with growth habit in dry beans, may be involved in PLH resistance. It controls internode length, lateness of flowering and terminal bud fate. It is believed that when PLH populations are high and then drop off later in the growing season, determinate plants (*fin*) have to subsist on damaged foliage while indeterminate (*Fin*) plants can recover by re-growing following infestation (Murray et al., 2001). Internode distance may influence insect preference, while

delayed onset of flowering might allow the plant to evade peak insect pressure at flowering and pod-filling stages (Murray et al., 2001). Additionally, seed coat color and leaf color have been noted to influence preference (Bullas-Appleton et al., 2004). Together, these traits may discourage PLH feeding or oviposition. Resistance has been noted in a few commercial varieties. Lindgren and Coyne (1995) reported that Sierra, a pinto bean variety from Michigan, and Tacarigua, a black bean cultivar from Costa Rica, showed resistance to PLH injury while Starlight, a great northern bean cultivar from Nebraska, showed susceptibility. This result was later supported by Gonzales et al. (2004).

Beginning in 1976, CIAT undertook extensive breeding efforts to develop resistance to *E. kraemeri* in dry beans, from which a series of resistance lines were developed, denoted by EMP (Schoonhoven et al., 1985; Kornegay and Cardona, 1990). The *Empoasca*-resistant parent (EMP507) of the IBL population examined in this study originated from the CIAT research group. Through this extensive breeding program, a number of criteria were identified that are useful for detecting resistance to PLH in dry beans. Specifically, low PLH nymph populations were noted as indicators of non-preference (antixenosis) and antibiosis (Temple et al., 1982). In addition, low visual damage scores were noted as indicators of potential tolerance in conjunction with nymph counts (Temple et al., 1982). While these EMP lines were originally developed to be resistant to the tropical species *E. kraemeri*, Schaafsma et al. (1998) demonstrated that the resistance is maintained under severe pressure to the temperate congener *E. fabae*. EMP507 represents a line developed for *Empoasca* resistance from the original work at CIAT, but that has gone through further generations of selection than the EMP lines tested by Schaafsma et al. (1998). In addition, EMP507 is poorly adapted to growing in temperate environments based on field tests conducted at the Saginaw Valley Bean and Beet Farm in Saginaw, MI in 2007.

IV. Marker-Assisted Selection and Quantitative Trait Loci Analysis

A. Quantitative Trait Variation

Many important traits in common beans are complex, involving multiple genes, each with often small effects on the actual trait of interest. In addition, these quantitatively inherited traits have measurable phenotypic variation that may be influenced by genetic and/or environmental factors. A QTL is a genetic locus that has an effect on the phenotypic trait variation based on which alleles are present at this locus (Collard et al., 2005). What is detected as a single QTL may sometimes actually be a cluster of closely linked polymorphic genes that are difficult to separate by recombination events (Collard et al., 2005). This can also occur in the case of resistance loci where multiple disease and insect resistance tend to cluster in groups in the common bean genome (Kelly and Vallejo, 2005).

The application of molecular markers linked to trait-associated loci is known as marker-assisted selection (MAS). MAS is commonly used in bean breeding programs currently and is useful for screening for resistance to various disease and insect resistance when phenotypic screening is problematic. This can occur if field conditions do not promote disease or insect infestation, if large-scale screening is not possible or cost prohibitive and/or if complex race or pathogen mixtures make it difficult to identify resistance in the field (Kelly et al., 2003). Using molecular markers that are tightly linked to individual race-specific resistance genes can allow indirect selection of major gene resistance without having to conduct germplasm screening. In addition, breeders can pyramid multiple resistance genes more efficiently, thus enabling more effective and durable resistance. MAS has been particularly successful in screening for resistance to bean golden yellow mosaic virus and CBB (Miklas et al., 2006). In common beans, the use of MAS for CBB resistance has been found to be one-third the cost of more time-consuming

greenhouse testing (Yu et al, 2000). However, in a recent study, Duncan et al. (2012) found that direct disease screening (US$1.55 per plant) was actually less costly than MAS (US$2.03 per plant), underscoring the importance of considering all factors before only using one method in a resistance breeding program. Knapp (1998) found that the frequency of obtaining improved genotypes is higher for MAS than for phenotypic selection, especially for traits of moderate and low heritability.

B. Molecular Markers and Bean Linkage Maps

A core linkage map for common bean was described by Freyre et al. (1998). This map was based on recombinant inbred lines (RILs) derived from a cross between BAT93 and Jalo EEP558 and uses markers from the Florida (Vallejos et al., 1992), Davis (Nodari et al., 1992) and Paris maps (Adam-Blondon et al., 1994). Most markers used to develop core linkage maps in *P. vulgaris* used random fragment length polymorphism (RFLP) and/or random amplified polymorphic DNA (RAPD) markers (Yu et al., 2000). The core bean map is approximated at 1200 cM in size (Gepts, 1999). The relationship between the genetic linkage map and the physical map has been approximated at 500kb/cM (Llaca and Gepts, 1996).

Recently, the core map was expanded using additional molecular markers such as simple sequence repeats (SSR) (Blair et al., 2003). SSRs have a number of advantages over older markers – (1) they are codominant and PCR based; (2) they are multiallelic and hypervariable; (3) they appear to be randomly and uniformly distributed throughout eukaryotic genomes; and (4) they are accessible to other researchers via published primer sequences (Yu et al., 2000).

In 2009, a Common Bean Coordinated Agriculture Project (BeanCAP) was initiated with the goal to "strengthen the bean research, education, and extension communities by focusing on the genetics and genomics aspects of nutrition in this important food crop" (McClean, 2012). The

first research objective of the project was to develop high throughput, market-class-specific breeder-friendly markers. New markers were developed as a result of this project, including ~2,700 insertion-deletion markers (InDels) to date (McClean, 2012). InDels exploit polymorphic genomic regions of base-pair insertions or deletions of various lengths. InDels are numerous and distributed throughout the genome, and are believed to contribute significantly to both intra- and interspecific divergence (Vasemagi et al., 2010). In *Arabidopsis,* it was determined that a substantial proportion of large InDels are the result of transposon insertion or excision. Also, gene structure can be affected by small (2-10bp) InDels (The Arabidopsis Genome Initiative, 2000). These small apparently random DNA insertions-deletions have been found to be amenable for fast and cost-effective genotyping as these polymorphisms can be screened for length differences similar to SSRs (Vasemagi et al., 2010).

These advances in genetics and related technologies have enabled breeders to utilize new tools to decrease breeding time and increase selection specificity. However, it is necessary to link genetic information with agronomic characteristics in order to optimize their use in breeding programs. The variation within a population for a specific trait can differ dramatically. By screening for genetic morphologies associated with the traits in question, genetic loci associated with that trait can be identified. Locating additional markers on the core map increases the usefulness of QTL-marker linkages for other researchers and increases the overall usefulness of the map itself.

C. Mapping *Empoasca* Resistance in Common Bean

Resistance to *E. kraemeri* is quantitatively inherited with low heritability (Gonzales et al., 2006). In addition, selection in the F_4 and F_5 generations generally produces lines with greater unprotected yields than selection in the F_2 and F_3 generations. Gonzales et al. (2004) suggested

that *Empoasca* resistance may be determined by only a few genes (3-4) and found that the heritability of resistance can be explained by a narrow sense heritability model, which quantifies only the portion of the phenotypic variation that is additive (allelic) by nature. The low narrow-sense heritability estimates also indicate large environmental effects on the expression of *Empoasca* injury in dry beans (Gonzales et al., 2004).

While extensive work has been done to identify molecular markers and map resistance to numerous diseases of common bean, comparatively little has been done to identify QTL associated with insect resistance, specifically related to *Empoasca* leafhoppers (Miklas, et al., 2006). Murray et al. (2004b) did identify QTL associated with resistance to both *E. kraemeri* and *E. fabae*. They identified major QTL on Pv01, Pv03 and Pv07. The QTL on Pv01 ($LH1.1^{BE}$) was detected for both *E. kraemeri* and *E. fabae* resistance and was linked to the *fin* locus for determinacy. Resistance to LC and LB damage for both *Empoasca* leafhoppers was controlled by a single QTL on Pv07 ($LH7.1^{BE}$). This QTL was tightly linked to seed coat color at the *P* locus (Murray et al., 2004b). An additional QTL on Pv03 was identified in this study but was only detected for *E. kraemeri* LC damage (Murray et al., 2004b).

V. Objectives

The current study was undertaken in order to further investigate the nature of resistance to *Empoasca* species leafhoppers by examining feeding damage responses in an inbred backcross line population of common beans grown in temperate and tropical climates. The goal of this research was to identify QTL associated with these traits and resistance to both tropical and temperate *Empoasca* species, as well as to verify existing QTL for *Empoasca* resistance. In addition, by utilizing an IBL population with an indeterminate Type II growth habit, the potentially confounding factor of growth habit was eliminated. Identifying molecular markers

associated with these QTL would be used by breeders to incorporate leafhopper resistance into bean germplasm, thereby providing future protection against crop losses due to this damaging pest.

Literature Cited

Literature Cited

Adam-Blondon, A.F., Sevignac, M., Dron, M., and Bannerot, H. 1994. A genetic map of common bean to localize specific resistance genes against anthracnose. Genome. 37: 915-24.

Arabidopsis Genome Initiative, The. 2000. Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. Nature. 408: 796-815.

Backus, E.A., Serano, M.S., and Ranger, C.M. 2005. Mechanisms of hopperburn: An overview of insect taxonomy, behavior, and physiology. Annu. Rev. Entomol. 50:125-151.

Baker, J.R, Beshear, R., Carter, C., Day, E., Grant J., Hamon A.B., Horn, K.F., Liu, T.X., Oetting, R.D., Pollet, D.K., Schultz, P.B., Sparks, B., Staines, C.L., Stephen, D.L., and Williams, M.L., 2008. Insect and Related Pests of Flowers and Foliage Plants: Potato Leafhopper. North Carolina Cooperative Extension Service. Accessed: Oct 2012. [http://ipm.ncsu.edu/AG136/leafhop1.html]

Beebe, S., Skroch, P. W. Tohme, J., Duque, M. C., Pedraza, F., and Nienhuis, J. 2000. Structure of Genetic Diversity among Common Bean Landraces of Middle American Origin Based on Correspondence Analysis of RAPD. Crop Sci. 40: 264–273.

Bitocchi, E., Nanni, L., Bellucci, E., Rossi, M., Giardini, A., Spagnoletti Zeuli, P., Logozzo, G., Stougaard, J., McClean, P., Attene, G., and Papa, R. 2012a. Mesoamerican origin of the common bean (*Phaseolus vulgaris* L.) is revealed by sequence data. PNAS 109: E788-E796.

Bitocchi, E., Bellucci, E., Giardini, A., Rau, D., Rodriguez, M., Biagetti, E., Santilocchi, R., Spagnoletti Zeuli, P., Gioia, T., Logozzo, G., Attene, G., Nanni, L. and Papa, R. 2012b. Molecular analysis of the parallel domestication of the common bean (*Phaseolus vulgaris*) in Mesoamerica and the Andes. New Phytologist. doi: 10.1111/j.1469-8137.2012.04377.x

Blair, M.W., Pedraza, F., Buendia, H.F., Gaitan-Solas, E., Beebe, S.E., Gepts, P., and Tohme, J. 2003. Development of a genome-wide anchored microsatellite map for common bean (Phaseolus vulgaris L.). Theor. Appl. Genet. 107:1362—1374.

Blair, M.W., Giraldo, M.C., Buendía, H.F., Tovar, E., Duque, M.C., and Beebe, S.E. 2006a. Microsatellite marker diversity in common bean (Phaseolus vulgaris L.). Theor. Appl. Genet. 113: 100–109.

Blair, M.W., Iriarte G., and Beebe, S. 2006b. QTL analysis of yield traits in an advanced backcross population derived from a cultivated Andean x wild common bean (Phaseolus vulgaris L.) cross. Theor. Appl. Genet. 112:1149-63.

Blair, M.W., Munoz., C., Garza, R., and Cardona, C. 2006c. Molecular mapping of genes for resistance to the bean pod weevil (*Apion godmani* Wagner) in common bean. Theor. Appl. Genet. 112:913-923.

Broughton, W.J., Hernandez, G., Blair, M., Beebe, S., Gepts, P., and Vanderleyden, J. 2003. Beans (*Phaseolus* spp.) – model food legumes. Plant and Soil. 252: 55-128.

Bullas-Appleton, E.S., Gillard, C., Otis, G., and Schaafsma, A.W. 2004. Potato leafhopper (Homoptera: Cicadellidae) varietal preferences in edible beans in relation to visual and olfactory cues. Enviro. Entomol. 33: 1381–1388.

Bullas-Appleton, E.S., Gillard C., and Schaafsma, A.W. 2005 Aggregation of potato leafhoppers, *Empoasca fabae* (Harris) (Homoptera: Cicadellidae), on a trap crop in an edible bean, *Phaseolus vulgaris* L., intercropping system. Can. J. Plant Sci. 85: 237-242.

Calderon, J.D. and Backus, E.A. 1992. Comparison of the Probing Behaviors of Empoasca fabae and E. kraemeri (Homoptera: Cicadellidae) on Resistant and Susceptible Cultivars of Common Beans. J. Econ. Entomol. 85: 88-99.

Cardona, C. Posso, C.E., Kornegay, J., Valor, J., and Serrano, M. 1989. Antibiosis effects of wild dry bean accessions on the Mexican bean weevil and the bean weevil (Coleoptera: Bruchidae). J. Econ. Entomol. 82: 310-315.

Cardona, C., Frei, A., Bueno, J.M., Diaz, J., Gu, H., and Dorn, S. 2002. Resistance to *Thrips palmi* (Thysanoptera: Thripidae) in beans. J. Econ. Entomol. 95: 1066-1073.

Carmona, D., Lajeunesse, M.J., and Johnson, M.T.J. 2011. Plant traits that predict resistance to herbivores. Funct. Ecol. 25: 358–367.

CIAT (Centro Internacional de Agricultura Tropical). 1987. Standard system for the evaluation of bean germplasm. Schoonhoven, A. van and M.A. Pastor-Corrales (compilers). Cali, Colombia. 54 p.

CIAT (Centro Internacional de Agricultura Tropical). 2012. The Impacts of CIAT's Collaborative Research. Revised: February 2012. Accessed: November 2012. [www.ciat.cgiar.org/Newsroom/Documents/impact_ciat_collaborative_research.pdf]

Collard, B.C.Y., Jahufer, M.Z.Z., Brouwer, J.B. and Pang, E.C.K. 2005. An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. Euphytica 142: 169–196.

Córdoba, J.M., Chavarro, C., Schlueter, J.A., Jackson, S.A., and Blair, M.W. 2010. Integration of physical and genetic maps of common bean through BAC-derived microsatellite markers. BMC Genomics. 11:436.

Dietrich, C.H. 2008. Leafhopper Homepage. Section for Biodiversity, Illinois Natural History Survey, Champaign, IL. Revised: March 2008. Accessed: October 2012. [www.inhs.uiuc.edu/~dietrich/lfhFAQ.html]

Duncan, R.W., Gilbertson, R.L., and Singh, S.P. 2012. Direct and marker-assisted selection for resistance to common bacterial blight in common bean. Crop Sci. 52: 1511- 1521.

ERS. Economic Research Service. 2012. Crops: Vegetables and Pulses. Accessed: November 2012. [http://www.ers.usda.gov/topics/crops/vegetables-pulses/dry-beans.aspx]

Emmen, D.A., Fleischer, S. J., and Hower, A. 2004. Temporal and spatial dynamics of *Empoasca fabae* (Harris) (Homoptera: Cicadellidae) in alfalfa. Enviro. Entomol. 33: 890-899.

FAOSTAT. 2012. About Crops: global dry bean production. In *Food and Agriculture Organization of the United Nations – FAOSTAT.* Retrieved November 2012, from [http://faostat.fao.org]

Garden-Robinson, J., McClean, P., and Osorno, J. 2012. Common Bean Coordinated Agricultural Project Progress Report and Work Plan 1/1/2011 – 12/31/2011. In *Common Bean Coordinated Agricultural Project*. Retrieved October 2012, from [www.beancap.org]

Gepts, P., 1999. Development of an integrated linkage map. In: Singh, S.P. (Ed.), Developments in Plant Breeding. Common Bean Improvement in the Twenty-First Century. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 53–91.

Gepts, P., Aragão, F.J.L., Barros, E., Blair, M.W., Brondani, R., Broughton, W., Galasso, I., Hernández, G., Kami, J., Lariguet, P., McClean, P., Melotto, M., Miklas, P., Pauls, P., Pedrosa-Harand, A., Porch, T., Sánchez, F., Sparvoli, F., and Yu, K. 2008. Genomics of Phaseolus beans, a major source of dietary protein and micronutrients in the Tropics. In: Moore PH, Ming R (eds) Genomics of Tropical Crop Plants. Springer, Berlin, pp 113-143.

Godfrey, L. D., Long R.F., 2007. UC Pest Management Guidelines. Dry Bean – Empoasca Leafhoppers. University of California – Agriculture and Natural Resources. Accessed: October 2012 [http://www.ipm.ucdavis.edu/PMG/r52301611.html]

Gonzales, J.W., Coyne, D.P., Lindgren, and D.T., Jung, G. 2001. Inheritance and heritability of leafhopper resistance in common beans (*Phaseolus vulgaris* L.). Annu. Rep. Bean Improv. Coop. 44: 139-140.

Gonzales, J.W., Coyne, D.P., Lindgren, D.T. 2002. Inheritance and heritability of leafhopper resistance in common beans (*Phaseolus vulgaris* L.). Annu. Rep. Bean Improv. Coop. 45: 80-81.

Gonzales, J.W., Coyne, D.P., Lindgren, D.T., Schaaf, D., and Eskridge, K.M. 2004. Heritability of the resistance to potato leafhopper in dry bean. Hort Science 39: 1578-1580.

Greathead, D.J. 1968. A study in East Africa of the bean flies (Diptera: Agromyzidae) affecting *Phaseolus vulgaris* and their natural enemies, with the description of a new species of Melanagromyza Hend. Bull Entomol Res 59:541–561.

Guzmán-Maldonado, S.H., Martínez, O., Acosta-Gallegos, J.A., Guevara-Lara F., and Paredes-López, O. 2003. Putative quantitative trait loci for physical and chemical components of common bean. Crop Sci. 43:1029–1035.

Hall, R. 1991. The bean plant. P 1-5 *In* R. Hall (ed). Compendium of bean diseases. APS Press. Saint Paul, Minnesota.

Kelly, J.D., Hosfield, G.L., Varner, G.V., Uebersax M.A., and Taylor, J. 1999. Registration of 'Matterhorn' great northern bean. Crop Sci. 39:589-590.

Kelly, J.D, Gepts, P., Miklas, P.N., and Coyne, D.P. 2003. Tagging and mapping of genes and QTL and molecular-marker assisted selection for traits of economic importance in bean and cowpea. Field Crops Res. 82:135-154.

Kelly, J.D., and Vallejo, V.A. 2005. QTL analysis of multigenic disease resistance in plant breeding. p. 21-48. In S. Tuzun and E. Bent (ed.) Multigenic and Induced Systemic Resistance in Plants. Springer, New York.

Kabrick, L.R. and Backus, E.A. 1990. Salivary deposits and plant damage associated with specific probing behaviors of the potato leafhopper, *Empoasca fabae,* on alfalfa stems. Entomol. Exper. Appl.56:287-304.

Knapp, S.J. 1998. Marker-assisted selection as a strategy for increasing the probability of selecting superior genotypes. Crop Sci. 38:1164-1174.

Kornegay, J.L. and Temple, S.R. 1986. Inheritance and combining ability of leafhopper defense mechanisms in common bean. Crop Sci. 26:1153-1158.

Kornegay, J.L., Cardona, C., Van Esch, J., and Alvarado, M. 1989. Identification of common bean lines with ovipositional resistance to *Empoasca kraemeri* (Homoptera: Cicadellidae). J. Econ. Entomol. 82: 649-654.

Kornegay, J.L. and Cardona, C. 1990. Development of an appropriate breeding scheme for tolerance to *Empoasca kraemeri* in common bean. Euphytica 47: 223-231.

Kusolwa, P.M. and Myers, J.R. 2011. Seed storage proteins ARL2 and its variants from the APA locus of wild tepary bean G40199 confers resistance to *Acanthoscellides obtectus* when expressed in common beans. Afri. Crop Sci. J. 19: 255-265.

Kwak, M., and Gepts, P. 2009. Structure of genetic diversity in the two major gene pools of common bean (*Phaseolus vulgaris* L., Fabaceae) Theor. Appl. Genet. 118: 979-992.

Lindgren, D.T. and Coyne, D.P. 1995. Injury and yield of leafhopper-infested dry beans. J. Amer. Soc. Hort. Sci. 120: 839-842.

Llaca, V., and Gepts, P. 1996. Pulsed field gel electrophoresis analysis of the phaseolin locus region in Phaseolus vulgaris. Genome. 39: 722–729.

McClean, P. (Project Director) 2012. Project Summary. In *Common Bean Coordinated Agricultural Project*. Accessed October 2012. [www.beancap.org.]

McClean, P.E., Lee, R.K., Otto, C., Gepts, P., and Bassett, M.J. 2002. Molecular and phenotypic mapping of genes controlling seed coat pattern and color in common bean (*Phaseolus vulgaris* L.). J. Hered. 93: 148-152.

Miklas, P.N., Kelly, J.D., Beebe, S.E., and Blair, M.W. 2006. Common bean breeding for resistance against biotic and abiotic stresses: From classical to MAS breeding. Euphytica 147: 105-131.

Morin, J. 2010. Brazilian dry bean production. In *USDA – Foreign Agriculture Service: Global Agriculture Information System*. Published: 12/8/2010. Accessed: November 2012. [http://gain.fas.usda.gov]

Murray, J.D., Michaels, T.E., Pauls, K.P., and Schaafsma, A.W. 2001. Determination of traits associated with leafhopper (*Empoasca fabae* and *Empoasca kraemeri*) resistance and dissection of leafhopper damage symptoms in common bean (*Phaseolus vulgaris*). Ann. App. Bio. 139: 319-327.

Murray, J.D., Michaels, T.E., Pauls, K.P., Cardona, C., and Schaafsma, A.W. 2004a. Yield and insect injury in leafhopper (*Empoasca fabae* Harris and *Empoasca krameri* Ross and Moore) infested dry beans in Ontario and Colombia. Can. J. Plant Sci. 84: 891-900.

Murray, J.D., Michaels, T.E., Cardona, C., Schaafsma, A.W., and Pauls, K.P. 2004b. Quantitative trait loci for leafhopper (*Empoasca fabae* and *Empoasca kraemeri*) resistance and seed weight in the common bean. Plant Breed. 123: 474-479.

NASS. National Agricultural Statistics Service. 2012. "Quick Stats: Agricultural Statistics Data Base" Revised: October 2011. Accessed: October 2012. [www.nass.usda.gov/QuickStats]

Nodari, R.O., Koinange, E.M.K., Kelly, J.D., and Gepts, P. 1992. Towards an integrated linkage map of common bean. I. Development of genomic DNA probes and levels of restriction fragment length polymorphism. Theor. App. Genet. 84: 186-192.

Ojwang, P.P.O., Melis, R., Githiri, M.S., and Songa, J.M. 2011a. Genetic analysis for resistance to bean fly (*Ophiomyia phaseoli*) and seed yield among common bean genotypes in a semi-arid environment. Field Crop Research. 120: 223-229.

Ojwang, P.P.O., Melis, R., Githiri, M.S., and Songa, J.M. 2011b. Breeding options for improving common bean for resistance against bean fly (*Ophiomyia spp*.): a review of research in eastern and southern Africa. Euphytica 179: 363-371.

Pillemer, E.A. and Tingey, W.M. 1978. Hooked trichomes and resistance of *Phaseolus vulgaris* to *Empoasca fabae* (Harris). Entomol. Exp. Appl. 24: 83-94.

Saxena, K.N. 1969. Patterns of insect-plant relationships determining susceptibility or resistance of different plants to an insect. Entomol. Exp. Appl. 12: 751-766.

Schaafsma, A.W., Cardona, C., Kornegay, J.L., Wylde, A.M., and Michaels, T.E., 1998. Resistance of common bean lines to the potato leafhopper (Homoptera: Cicadellidae). J. Econ. Entomol. 91: 981-986.

Schoonhoven, A. van, Gomez, L.A. and Avalos, F. 1978. The influence of leafhopper (Empoasca kraemeri) attack during various bean (Phaseolus vulgaris) plant growth stages on seed yield. Entomol. Exp. Appl. 23: 115-120.

Schoonhoven, A. van, Hallman, G.J., and Temple, S.R. 1985. Breeding for resistance to *Empoasca kraemeri* Ross & Moore in *Phaseolus vulgaris* L. P: 405-422 *In*: L.R. Nault & J.G. Professor (Eds). Leafhoppers and Planthoppers. John Wiley & Sons Inc.

Serrano, M.S., Backus, E.A., and Cardona, C. 2000. Comparison of AC electronic monitoring and field data for estimating tolerance to *Empoasca kraemeri* (Homoptera: Cicadellidae) in common bean genotypes. J. Econ. Entomol. 93: 1796-1809.

Singh, S.P. 1982. A key for identification of different growth habits of *Phaseolus vulgaris* L. Annu. Rept. Bean Improv. Coop. 25: 92-94.

Singh, S.P., Gepts, P., and Debouck, D.G. 1991. Races of common bean (*Phaseolus vulgaris*, Fabaceae). Econ Bot. 45: 379-396.

Singh, S.P. 1999. *Integrated Genetic Improvement*. In *Common Bean Improvement in the 21st Century*. Kluwer Academic Press, Norwell, MA. p.133-165.

Singh, S.P. and Schwartz, H.F. 2010. Breeding common beans for resistance to diseases: a review. Crop Sci.50: 2199-2223.

Singh, S.P. and Schwartz, H.F. 2011. Breeding common beans for resistance to insect pests and nematodes. Can. J. Plant Sci. 91: 239-250.

Smith, M.C. 2005. *Plant Resistance to Arthropods*. Springer Publishing, Dordrecht, The Netherlands. 423p.

Teetes, G.L., 1996. Plant resistance to insects: a fundamental component of IPM. In *Radcliffe's IPM World Textbook*. Accessed Oct 2012. [http://ipmworld.umn.edu/chapters/teetes.htm]

Temple, S.R., Cardona, C., Van Schoonhoven, A., and Galwey, N. 1982. Advances in breeding for resistance to the leafhopper *Empoasca kraemeri* in dry beans (*Phaseolus vulgaris* L.) Annu. Rept. Bean Improv. Coop. 25: 34-35.

Thompson, M.D., Brick, M.A., McGinley, J.N., and Thompson, H.J. 2009. Chemical composition and mammary cancer inhibitory activity of dry bean. Crop Sci. 49: 179-186.

Vallejos, C.E., Sakiyama, N.S., and Chase, C.D. 1992. A molecular marker based linkage map of *Phaseolus vulgaris* L. Genetics. 131: 733-740.

Vasemägi, A., Gross, R., Palm, D., Paaver, T., and Primmer, C.R. 2010. Discovery and application of insertion-deletion (INDEL) polymorphisms for QTL mapping of early life-history traits in Atlantic salmon. BMC Genomics 11: 156.

Wei, J.N., Zhu, J., and Kang, L. 2006 Volatiles released from bean plants in response to agromyzid flies. Planta 224: 279-287.

Welch, R.M., House, W.A., Beebe, S., and Cheng, Z. 2000. Genetic selection for enhanced bioavailable levels of iron in bean (*Phaseolus vulgaris* L.) seeds. J. Agric. Food Chem. 48: 3576-3580.

Wilde, G., Schoonhoven, A. van, and Gomez-Laverde, L. 1976. The biology of *Empoasca kraemeri* on *Phaseolus vulgaris.* Ann. Entomol. Soc. Amer. 69: 442-444.

Yu, K., Park, S.J., Poysa, V., and Gepts, P. 2000. Integration of simple sequence repeat (SSR) markers into a molecular linkage map of common bean (*Phaeseolus vulgaris* L.). J. Heredity 91: 429-434.

CHAPTER 2: Field Screening of *Empoasca kraemeri* and *Empoasca fabae* resistance in an Inbred Backcross Line Population in Common Bean

Abstract

By

Elizabeth Ilona Brisco

Leafhoppers are a major insect pest of common bean, *Phaseolus vulgaris* L. *Empoasca* species cause significant economic losses for common bean farmers in both tropical (*E. kraemeri)* and temperate (*E. fabae*) regions of the Americas resulting in up to 80% crop yield reductions. Chemical controls are costly, reducing farmer's profits by increasing input costs, and potentially causing damage to the environment and human health. Breeding dry beans for leafhopper resistance can provide an alternative for control of this pest.

The current study examined resistance to *Empoasca* species leafhoppers by measuring leaf curl and leaf burn feeding damage responses as well as *Empoasca spp.* nymph counts in an inbred backcross line population (Matterhorn*/EMP507) of common beans grown in temperate and tropical climates. Field screening in Michigan and Puerto Rico in 2009-2011 identified the existence of tolerance, antixenosis and antibiosis as mechanisms of resistance to *E. fabae* and *E. kraemeri* in this population. Resistance to each species and each damage trait appear to be are controlled by separate genetic mechanisms in common bean.

I. Introduction

Leafhoppers are a major insect pest of common bean. *Empoasca* species feeding cause significant economic losses for bean farmers in both tropical and temperate regions of the Americas resulting in crop yield reductions due to feeding damage. The most important species are *E. kraemeri* in Central and South America (Kornegay and Cardona, 1990) and *E. fabae* in North America (Schaafsma et al., 1998). Chemical controls are costly, reducing farmer's profits by increasing input costs, and potentially causing damage to the environment and human health (Singh and Schwartz, 2011). Breeding dry beans for leafhopper resistance can provide an alternative for control of this pest.

A. Leafhopper Feeding Injury

Empoasca leafhopper feeding causes specific damage symptoms unique to *E. fabae* and *E. kraemeri.* Leaf curl (LC) and leaf burn (LB) damage are caused by both adults and nymphs piercing vascular tissues and using any combination of three feeding strategies: "lacerate-and-sip", "lacerate-and-flush", or "lance-and-ingest" (Backus et al., 2005). Lacerate-and- manifests as leaf burn damage (Serrano and Backus, 1998), as this pulsing laceration causes systemic vascular damage to the plant, leading to stunting and chlorosis above the point of feeding (Serrano et al., 2000). *Empoasca kraemeri* feeds by this method more often than *E. fabae*, which may be related to the higher level of damage generally inflicted by the former (Calderon and Backus, 1992). Lacerate-and-flush is thought to lead to leaf curl damage, since it involves longer intracellular probes that puncture and drain mesophyll and parenchyma cells in the lower surface of the leaf, thereby leading to tissue collapse. Then, as stylets are withdrawn, saliva is released, causing expansion of upper surface cells of the leaf (Serrano and Backus, 1998). Finally, lance-and-ingest may cause stunting but likely causes little damage overall (Backus et al., 2005). The

plant response to these behaviors is believed to be controlled by different genetic traits and is therefore measured separately as LC and LB traits that are inherited as different quantitative trait loci (QTL) (Murray et al., 2004b).

As a result of LB and LC damage, dramatic yield and subsequent economic losses can occur. In Nebraska, it has been estimated that dry bean yield losses of up to 20% or $2M USD are a result of *E. fabae* damage (Gonzales et al., 2002). In Latin America, in general, dry bean yield losses as a result of *E. kraemeri* damage are estimated at 64% (Gonzales et al., 2002) and in Colombia, specifically, losses of up to 79% have been recorded (Bullas-Appleton et al., 2005). While leafhoppers can be controlled using synthetic pesticides either as foliar sprays or seed treatments, the growing organic bean production industry does not have this option. This small but significant sector of the bean industry will benefit greatly from the introduction of commercial quality leafhopper-resistance beans.

B. Host Plant Resistance

Plants resist insect predation by three different mechanisms: tolerance, antibiosis and antixenosis (Smith, 2005). A tolerant plant can withstand a level of insect predation without incurring damage that would cause damage to a susceptible plant. Antibiosis reduces pest population sizes and subsequent plant damage by adversely affecting insect biology such as reducing the reproductive capability of the insect or by direct toxicity to the insect. Antixenosis is also known as non-preference. Antixenosis affects insect behavior in that is it usually associated with plant traits that are less desirable to the insect in comparison to more susceptible plants (Teetes, 1996).

In dry beans, tolerance and antixenosis are the main mechanisms of resistance to *Empoasca* leafhoppers (Kornegay et al., 1986; 1989). Kornegay et al. (1989) hypothesized that

antixenosis may be why some bean lines harbor consistently lower nymph population counts in the field than other tolerant and susceptible lines. Numerous agronomic traits have been linked to potential antixenosis resistance mechanisms in beans. These include indeterminate growth habit, days to flowering, leaf pubescence and trichome density (Pillemer and Tingey, 1976). However, since these initial studies, leaf pubescence and trichome density have not been demonstrated to contribute significantly to resistance in the tropics (Schaafsma et al., 1998). Additionally, seed coat color and leaf color have been noted to influence *Empoasca* preferences (Bullas-Appleton et al., 2004). Together, these traits may discourage feeding or oviposition by both *Empoasca* species.

II. Study Objectives

The objectives of the current study were to examine potato leafhopper (PLH) (*Empoasca fabae, Empoasca kraemeri*) resistance in dry beans through field and greenhouse screenings of an inbred backcross line (IBL) population derived from a cross (Matterhorn*/EMP507) between a resistant germplasm line (EMP507) and a commercial cultivar from Michigan (Matterhorn). Resistance was measured by collecting phenotypic field data from 75 individuals from the IBL population ($BC_1F_{4:8}$) under both choice and no-choice conditions over five growing seasons in two locations (Michigan and Puerto Rico). The IBL population was evaluated against both *E. kraemeri* and *E. fabae* predation.

III. Material and Methods

A. Plant material

The *P. vulgaris* population examined in this study was developed from a single cross Matterhorn/EMP507 followed by a single backcross to Matterhorn to create an inbred backcross line (IBL) population consisting of 75 $BC_1F_{4:8}$ individuals. Matterhorn is a high-yielding

commercially available great northern cultivar developed in Michigan (MI) with quality seed and agronomic characteristics (Kelly et al., 1999). EMP507 is a carioca germplasm line developed at the Centro International de Agricultura Tropical (CIAT) (Kornegay and Cardona, 1990) as part of a long-term recurrent selection program designed to enhance resistance to *E. kraemeri* (Schaafsma et al., 1998). While these EMP lines were originally developed to be resistant to *E. kraemeri,* Schaafsma et al. (1998) demonstrated that the resistance is maintained under severe pressure to the temperate congener *E. fabae.* An IBL population was created in order to generate a higher frequency of lines that would resemble the recurrent parent, Matterhorn, in seed, agronomic and performance traits, as EMP507 lacks adaptive traits for production in a temperate environment. Both parental genotypes have a type II growth habit, as defined by Singh (1982).

The crosses resulting in the IBL population were made by Dr. Tim Porch at the USDA-ARS-Tropical Agriculture Research Station, in Mayaguez, Puerto Rico. The F_1 was made in the greenhouse in 2005 and the BC_1F_1 backcross generation made in 2006 was selfed and advanced using single seed descent with no selection at the same location until the BC_1F_4 generation. BC_1F_4 seed was increased in 2008 and 2009 in the greenhouse and in the field in East Lansing, MI until sufficient quantities were obtained for field screening. Individual IBL were coded with G08 prefix if they possessed white great northern seed type or with P08 prefix if they possessed colored pinto bean seed type.

B. Field Screening

Empoasca-resistance screening was initiated in the summer of 2009. Open choice tests were conducted on the Crop and Soil Science Research Farm at Michigan State University, East Lansing, MI and at USDA-ARS-TARS in Isabela, PR. Three replications were planted each year

in MI from 2009 to 2011 in a randomized complete block design (RCBD) of 5.4 m long single-row plots. Individual plot were spaced 20 cm apart and consisted of up to 80 plants per plot. Five replications were planted in December 2009 and January 2011 in PR in a RCBD of 1.8 m long single-row plots. Individual plots were spaced 90 cm apart and consisted of up to 30 plants per plot. *Empoasca* species were allowed to inoculate each field test naturally.

In each location, *Empoasca* nymphs present on three randomly selected trifoliate leaves on each of three randomly selected plants per plot were counted at the flowering stage. The plants were evaluated for LC and LB at physiological maturity using a damage scale from 0-5 as described in Murray et al. (2001), where 0 = no visible damage and 5 = severe damage. Damage scores were assigned as an average of the overall plot.

No-choice tests were conducted in the field in MI in 2009-2011 with a single replication evaluated each year. In the no-choice tests, 3 x 1 ft (approx. 100 x 30 cm) cages were placed over plots following germination and thinned to five plants per cage. Leafhoppers were raised in growth chambers on fava bean (*Vicia faba*) plants at 25°C and 12 hr D:L and collected via an aspirator into individual 25 mL vials that were deposited into each cage. Additional leafhoppers were collected each season from alfalfa fields (*Medicago*) using a sweep net and aspirator when necessary. Cages were inoculated with *E. fabae* adults at current industry economic threshold rates (one adult leafhopper/trifoliate) at the third trifoliate stage. Cages were removed when plants had achieved physiological maturity and plants were evaluated for LC and LB using a damage scale from 0-5 as described in Murray et al. (2001), where 0 = no visible damage and 5 = severe damage. Damage scores were assigned as an average of the overall plot.

C. Trichome Density

Trichome density was measured on the parent genotypes and a subset of the IBL population representing the nine most resistant and nine most susceptible IBLs based on field screening from 2009-2011. Images of abaxial leaf surfaces were taken using a Leica imaging system and all trichomes were counted within a 1 mm^2 area. Three sample images were taken per genotype and mean trichome densities were analyzed.

D. Analysis

Statistical analysis of damage indices (LC, LB, nymph counts) was conducted using the SAS statistical package 9.3 (SAS Institute, Cary, USA). Analysis of Variance (ANOVA) tests were conducted using PROC MIXED. Correlation testing between traits was conducted using PROC CORR. Comparisons of choice and no-choice tests were conducted using PROC TTEST.

Narrow-sense heritability (h^2) was determined for each trait on a progeny mean basis (Hallauer and Miranda, 1981) as:

$$h^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_{gy}^2}{y} + \frac{\sigma_e^2}{ry}}$$

where σ_g^2 = the variance due to genotypes, σ_y^2 = the variance due to years, σ_{gy}^2 = the variance due to genotype by year interactions, σ_e^2 = experimental error, r = the number of replications and y = the number of years.

Inbred backcross lines were categorized into resistance classes ranging from Very Resistant (VR) to Very Susceptible (VS) based on standard deviations (SD) from the mean for LC and LB in each location as outlined in Table 2.1.

Table 2. 1 Resistance class categories based on leaf curl (LC) and leaf burn (LB) scores.

Resistance Classes	
Very Resistant (VR)	$LC_i \leq (\overline{LC})$ - $1SD_{LC}$ and $LB_i \leq (\overline{LB})$ - $1SD_{LB}$
Resistant (R)	$LC_i \leq (\overline{LC})$ - $1SD_{LC}$ and $(\overline{LB}) > LB_i > (\overline{LB})$ - $1SD_{LB}$ or $LB_i \leq (\overline{LB})$ - $1SD_{LB}$ and $(\overline{LC}) \leq LC_i \leq (\overline{LC})$ - $1SD_{LC}$
Moderately Resistant (MR)	$(\overline{LC}) > LC_i > (\overline{LC})$ - $1SD_{LC}$ and $(\overline{LB}) > LB_i > (\overline{LB})$ - $1SD_{LB}$
Moderately Susceptible (MS)	$(\overline{LC}) \leq LC_i \leq (\overline{LC})$ + $1SD_{LC}$ and $(\overline{LB}) \leq LB_i \leq (\overline{LB})$ + $1SD_{LB}$
Susceptible (S)	$LC_i > (\overline{LC})$ + $1SD_{LC}$ and $(\overline{LB}) \leq LB_i \leq (\overline{LB})$ + $1SD_{LB}$ or $LB_i > (\overline{LB})$ + 1SD and $(\overline{LC}) \leq LC_i \leq (\overline{LC})$ + $1SD_{LC}$
Very Susceptible (VS)	$LC_i > (\overline{LC})$ + $1SD_{LC}$ and $LB_i > (\overline{LB})$ + $1SD_{LB}$
LC Resistant (LCR)	$LC_i \leq (\overline{LC})$ and $LB_i > (\overline{LB})$
LB Resistant (LBR)	$LC_i > (\overline{LC})$ and $LB_i \leq (\overline{LB})$

LC_i = Leaf curl damage of the i^{th} individual, LB_i = Leaf burn damage of the i^{th} individual; $\overline{LC}$ = LC test mean; $\overline{LB}$ = LB test mean

SD = Standard deviation; SD_{LC} = LC standard deviation for test; SD_{LB}= LB standard deviation for test

IV. Results

A. Choice Tests

Mean squares were significant for genotype effects and environment effects for all leafhopper damage traits measured in choice tests in MI and PR ($\alpha < 0.0001$), except for PLH nymph counts in PR which were significant at $\alpha = 0.05$ (Table 2.2). Significant genotype by environment (GxE) effects were evident when traits were combined across locations. However, GxE interactions were not significant for LH nymph counts in either PR or MI.

Table 2. 2 ANOVA table showing mean squares and heritability for *Empoasca* species damage-related traits of leaf curl, leaf burn and nymph counts for 75 inbred backcross lines ($BC_1F_{4:8}$) from a Matterhorn*/EMP507 population combined across 3 environments in Michigan and 2 environments in Puerto Rico (2010-2011).

Trait	Combined (MI and PR)			Michigan (*Empoasca fabae)*			Puerto Rico (*Empoasca kraemeri)*		
	Leaf Curl	Leaf Burn	Nymph Counts	Leaf Curl	Leaf Burn	Nymph Counts	Leaf Curl	Leaf Burn	Nymph Counts
Genotype (G)	4.5***	3.4***	26.8***	3.2***	2.7***	19.8***	4.2***	1.9***	3.8*
Environment (E)	90.0***	67.0***	849.8***	128.5***	67.0***	1076.8***	12.9***	-	395.7***
GxE	0.67***	0.87***	5.6***	0.59***	0.87***	6.2ns	0.98***	-	3.3ns
Heritability	0.82	0.55	0.58	0.82	0.68	0.69	0.77	0.74	0.13
Coefficient of Variation	27.0	63.2	61.0	27.4	91.4	51.7	26.0	33.5	112.6

* significant at $\alpha=0.05$, ** significant at $\alpha=0.01$, *** significant at $\alpha<0.001$, ns= not significant.

Table 2. 3 Spearman Rank Correlations for *Empoasca* species resistance traits (leaf curl, leaf burn and leafhopper nymph counts) from a Matterhorn*/EMP507 inbred backcross line population of 75 $BC_1F_{4:8}$ individuals grown in Michigan (MI) in 2009-2011 and in Puerto Rico (PR) in 2010-2011.

Trait	Combined (MI and PR)		Michigan (*E. fabae*)		Puerto Rico (*E. kraemeri*)	
	Leaf Burn	Nymph Counts	Leaf Burn	Nymph Counts	Leaf Burn	Nymph Counts
Leaf Curl	0.57***	0.22***	0.68***	0.46***	0.49***	0.28***
Leaf Burn		-0.15***		0.48***		0.15*

* significant at α=0.05, ** significant at α=0.01, *** significant at α<0.001.

Correlations between all traits were significant when analyzed across all environments (Table 2.3). LC was positively correlated with both LB and nymph counts across all environments. LB was negatively correlated with nymph counts when analyzed together, but positively correlated in MI and PR. When contrasted by environment, only LC scores were correlated between MI and PR using Spearman rank correlations (Table 2.4). LB and nymph count correlations were not significant (α=0.05). Heritability (h^2) estimates of LC were high across all environments, ranging from 0.77 in PR to 0.82 in MI. LB heritability was higher in PR (h^2 = 0.74) than MI (h^2 = 0.68). PLH nymph counts were more highly heritable in MI (h^2 = 0.69) than in PR (h^2 = 0.13) (Table 2.2).

Significant differences were observed for all resistance traits on an entry mean basis in all years in both locations. However, significant differences were only observed for the parents' mean scores for LC (Table 2.4). Matterhorn and EMP507 LC mean values differed significantly in both MI and PR and under combined analysis. In MI, Matterhorn had a LC rating of twice

Table 2. 4 Spearman rank correlations for *Empoasca* species resistance traits (leaf curl, leaf burn and leafhopper nymph counts) from a Matterhorn*/EMP507 inbred backcross line population of 75 $BC_1F_{4:8}$ individuals between Michigan and Puerto Rico.

	Puerto Rico		
Trait	Leaf Curl	Leaf Burn	Nymph Counts
Michigan	0.48***	0.15*ns*	0.16*ns*

* significant at α=0.05, ** significant at α=0.01, *** significant at α<0.0001. *ns* = not significant

EMP507 (Matterhorn = 2.44, EMP507 = 1.22) and in PR, Matterhorn rated an average LC value of 2.60 compared to LC score of EMP507 of 1.74. LB and nymph counts were not significantly differ between the IBL population parents. Both parents performed significantly better in MI than the susceptible check, Swedish Brown, but had higher damage scores and nymph counts than the resistant check, Sierra. In PR, both parents rated significantly better for LC and LB than the susceptible check Othello, although there was no significant differences between nymph counts. In all locations, EMP507 performed similarly to the resistant check, EMP509.

Transgressive segregation was evident in all locations for LC, LB and leafhopper nymph counts. Trait distributions in PR are seen in Figure 2.1 (LC), Figure 2.2 (LB) and Figure 2.3 (nymph counts) and in MI in Figure 2.4 (LC), Figure 2.5 (LB) and Figure 2.6 (nymph counts). Mean distributions were nearly normal for all traits when analyzed by location. In each year, differences were detected with LB being left-skewed in MI in 2010 and 2011 and nymph counts also being left-skewed in MI in 2010 and in PR in 2011.

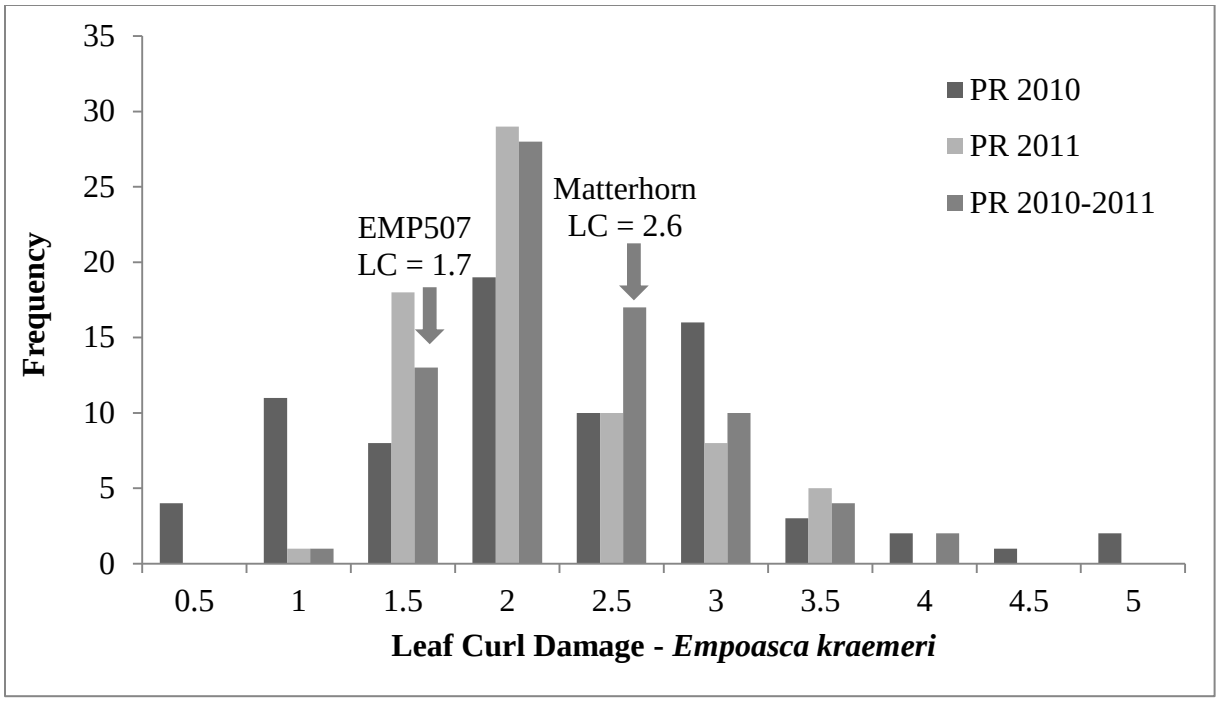

Figure 2. 1 Distribution of *Empoasca kraemeri* leaf curl (LC) damage from a Matterhorn*/EMP507 population of 75 $BC_1F_{4:8}$ individuals grown in Puerto Rico in 2010-2011.

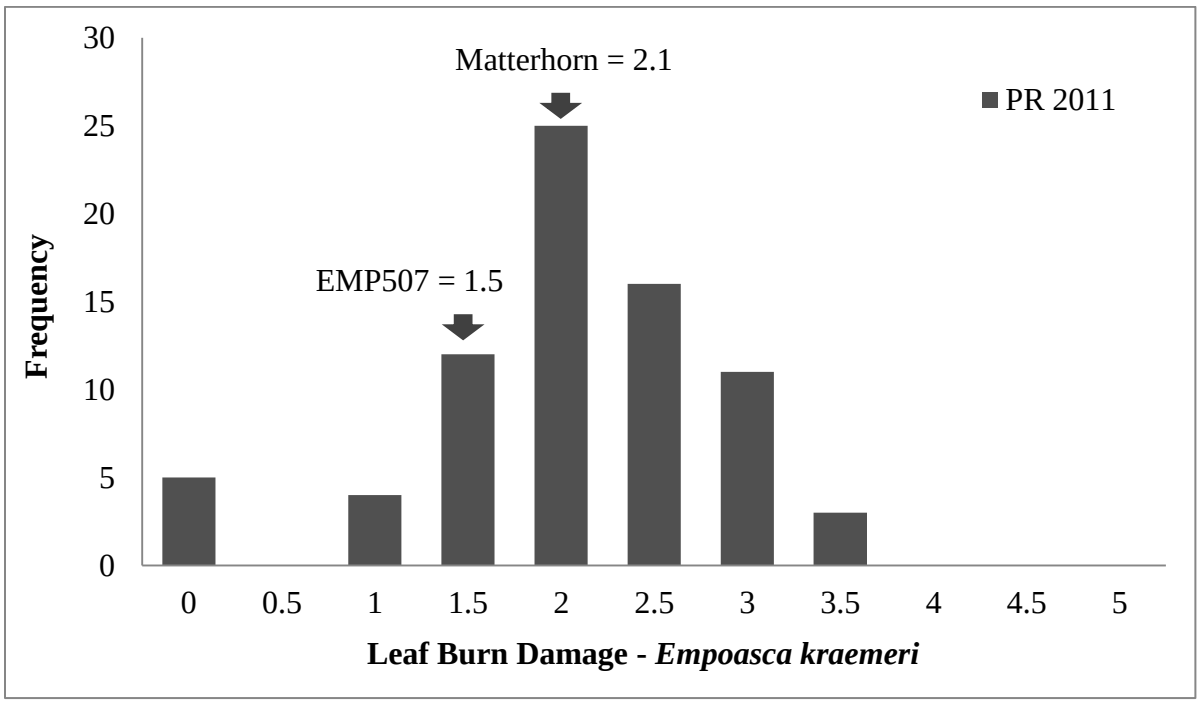

Figure 2. 2 Distribution of *Empoasca kraemeri* leaf burn (LB) damage from a Matterhorn*/EMP507 population of 75 $BC_1F_{4:8}$ individuals grown in Puerto Rico in 2011.

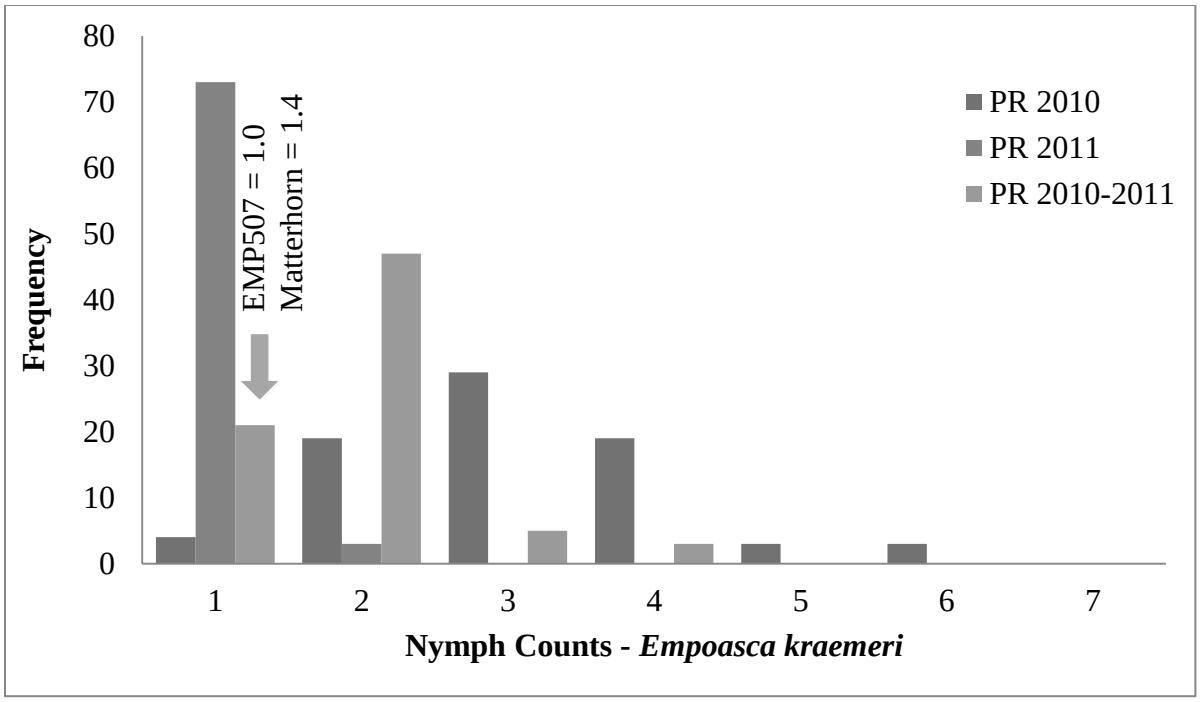

Figure 2. 3 Distribution of *Empoasca kraemeri* nymph counts from three randomly selected trifoliates averaged from three plants per plot from a Matterhorn*/EMP507 population of 75 $BC_1F_{4:8}$ individuals grown in Puerto Rico (PR) in 2010 – 2011.

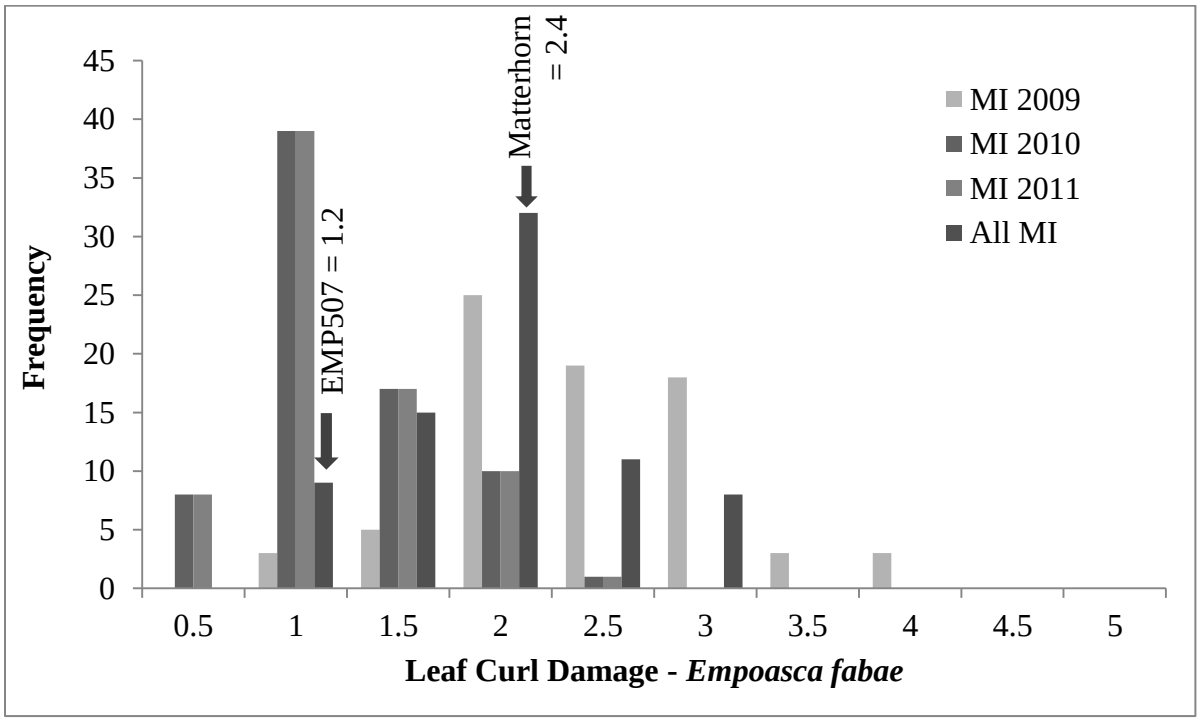

Figure 2. 4 Distribution of *Empoasca fabae* leaf curl (LC) damage from a Matterhorn*/EMP507 population of 75 $BC_1F_{4:8}$ individuals in Michigan in 2009-2011.

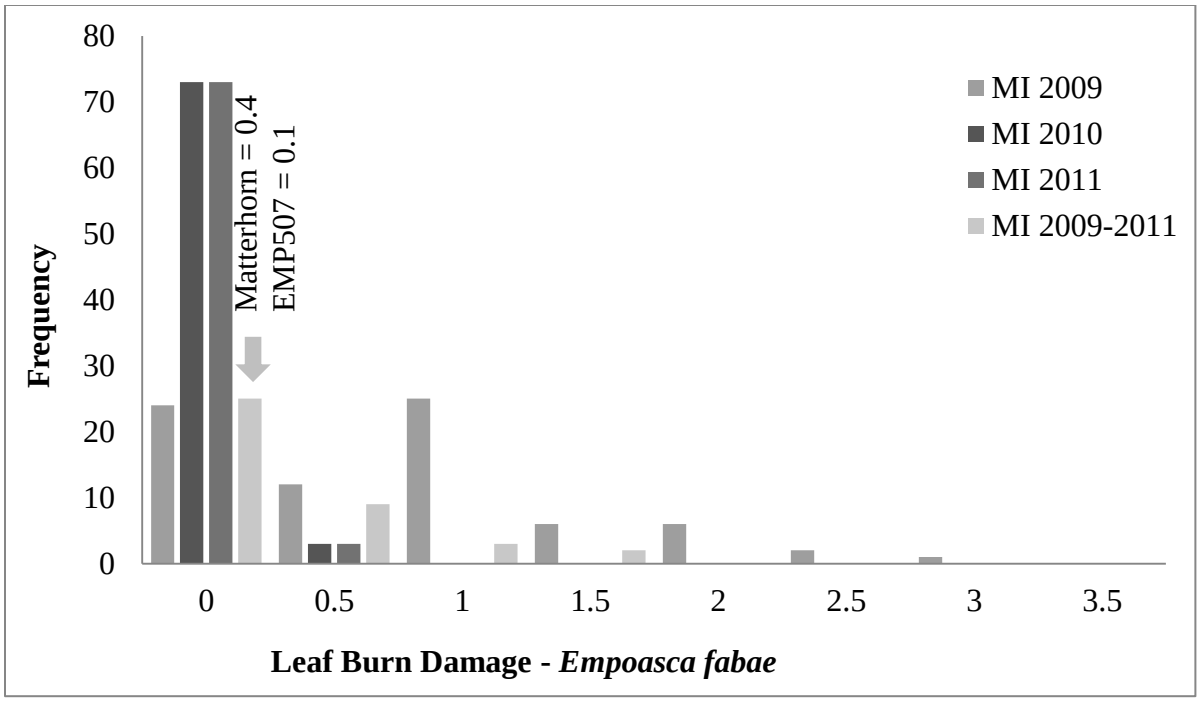

Figure 2. 5 Distribution of *Empoasca fabae* leaf burn (LB) damage from a Matterhorn*/EMP507 population of 75 $BC_1F_{4:8}$ individuals in Michigan (MI) in 2009-2011.

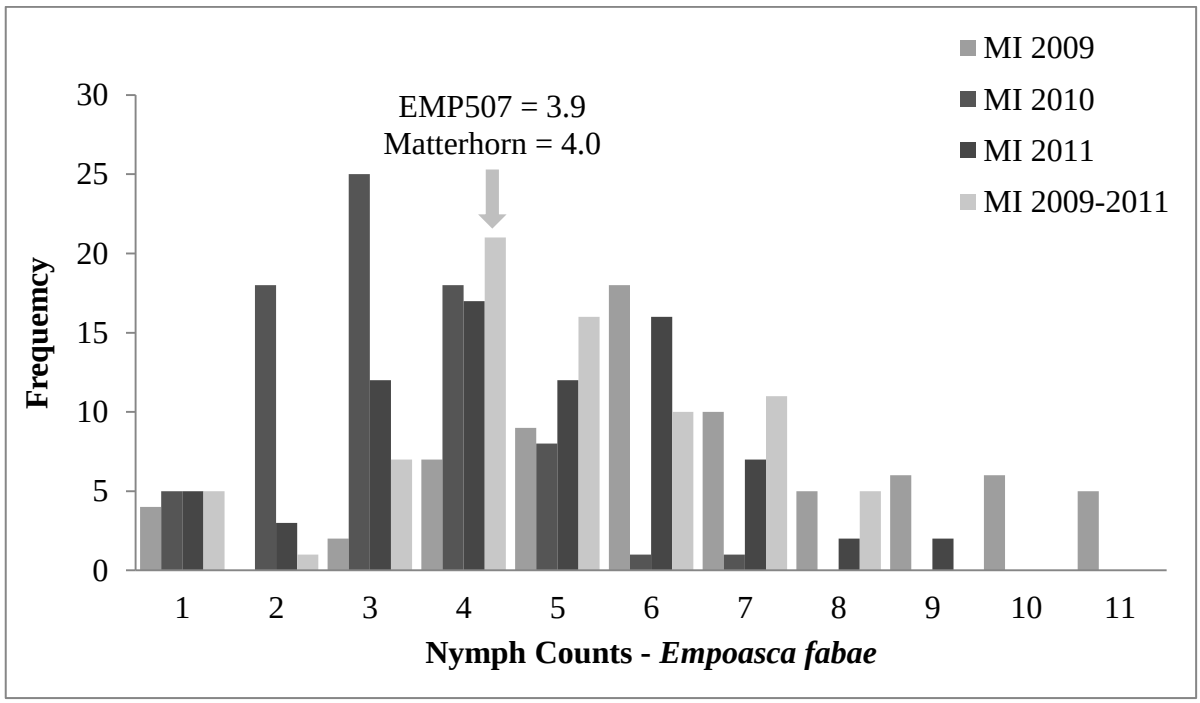

Figure 2. 6 Distribution of *Empoasca fabae* nymph counts from three randomly selected trifoliates averaged from three plants per plot from a Matterhorn*/EMP507 population of 75 $BC_1F_{4:8}$ individuals grown in Michigan (MI) in 2009-2011.

i. Puerto Rico Choice Tests

The combined mean LC score for the 75 IBLs in PR in 2010-2011 was 2.54, ranging from 2.38 in 2011 to 2.71 in 2010 (Table 2.5). Four of the 10 IBLs with the lowest mean LC scores remained in top 10 in both 2010 and 2011: P08125, G08128, G08134 and P08142 (Table 2.6). The 5 lines with the highest mean LC values overall also had the 5 highest LC scores in Puerto Rico in both 2010 and 2011 (Table 2.6).

The IBL population was only evaluated for LB values in 2011 due to infection of common bacterial blight (CBB) in 2010, making it impossible to differentiate CBB disease damage from LB damage as a result of *E. kraemeri* feeding. The mean LB value for the IBL population in 2011 was 2.43 (Table 2.5). LB values for the IBL population ranged from 1.11-1.78 for the 10 most resistant lines and 3.22-3.78 for the 10 most susceptible lines (Table 2.9).

Empoasca kraemeri nymph counts were recorded in 2010 and 2011. The combined mean count for the IBL population was 1.41 and ranged from 0.4 in 2011 to 2.41 in 2010 (Table 2.5). Only a single individual from the 10 IBLs with lowest combine mean nymph counts remained in the top 10 in both years: G08159. Of the 10 individuals with the highest combined mean nymph counts in 2010-2011, four IBLs remained in the bottom 10 in both years: G08109, G08155, G08165, and G08175 (Table 2.8).

Table 2. 5 Phenotypic means and ranges for Empoasca species resistance traits (leaf curl, leaf burn and leafhopper nymph counts) from a Matterhorn*/EMP507 inbred backcross line population of 75 $BC_1F_{4:8}$ individuals combined across 3 years (2009-2011) in Michigan (MI) and 2 years (2010-2011) in Puerto Rico (PR).

Trait	All Locations (PR and MI)									
	Parents		Inbred Backcross Lines			Checks				
	Matt.	EMP507	Mean	Range	LSD (0.05)	EMP509				
Leaf Curl	2.53	1.50	2.36	2.16 – 2.54	0.58	1.42				
Leaf Burn	0.95	0.59	1.29	0.65 – 2.43	0.98	0.54				
Nymph Counts	2.67	2.41	2.99	1.44 – 4.53	2.01	1.97				
	Puerto Rico (*Empoasca kraemeri)*									
	Parents		Inbred Backcross Lines			Checks				
	Matt.	EMP507	Mean	Range	LSD (0.05)	EMP509	Merlot	Othello	Verano	Morales
Leaf Curl	2.60	1.74	2.54	2.38 – 2.71	0.63	1.49	2.02	3.56	1.37	1.73
Leaf Burn	2.09	1.45	2.43	1.11 – 3.78	0.91	1.11	1.45	3.61	1.22	1.45
Nymph Counts	1.43	1.03	1.44	0.41 – 2.48	1.69	0.73	0.93	1.20	0.87	0.80
	Michigan (*Empoasca fabae)*									
	Parents		Inbred Backcross Lines			Checks				
	Matt.	EMP507	Mean	Range	LSD (0.05)	EMP509	Sierra	Swedish Brown	Santa Fe	
Leaf Curl	2.44	1.22	2.16	1.30 – 2.63	0.84	1.33	0.78	4.17	1.33	
Leaf Burn	0.44	0.11	0.65	0.03 – 0.97	0.78	0.22	0.00	2.83	0.67	
Nymph Counts	4.04	3.93	4.53	2.49 – 6.79	2.81	3.34	2.04	8.11	4.90	

Table 2. 6 *Empoasca kraemeri* leaf curl (LC) damage ratings for the inbred backcross lines (IBL) with the ten lowest and five highest LC scores from a Matterhorn*/EMP507 IBL population of 75 $BC_1F_{4:8}$ individuals. IBLs were ranked by average LC rating† across five replication per year in choice tests grown in Puerto Rico from 2010-2011.

Inbred Backcross Lines	LC Mean 2010-2011	2010		2011	
		Mean	Rank	Mean	Rank
P08142	1.58	1.4	3	1.6	4
G08128	1.68	1.8	11	1.6	3
G08130	1.70	1.4	2	2.0	25
P08161	1.70	1.4	5	2.0	32
P08120	1.72	2.0	21	1.4	2
P08125	1.73	1.8	10	1.7	9
G08134	1.73	1.8	12	1.7	7
P08104	1.75	1.6	7	1.9	24
P08151	1.76	1.4	4	2.1	35
G08156	1.83	2.0	22	1.7	8
G08136	3.53	3.4	69	3.7	81
G08158	3.73	3.8	77	3.7	79
G08113	3.83	4.0	79	3.7	80
G08121	4.09	4.4	81	3.8	82
G08149	4.11	5.0	82	3.2	76
Matterhorn	2.60	3.2	66	2.0	27
EMP507	1.74	1.6	8	1.9	17
EMP509	1.49	1.2	1	1.8	13
Morales	1.73	1.8	18	2.4	54
Merlot	2.02	1.6	9	1.7	6
Verano	1.37	1.4	6	3.1	74
Othello	3.56	4.0	80	1.3	1
Test Mean	2.52	2.7		2.4	
LSD (0.05)	0.64	0.91		0.73	

† LC Damage Scale: 0 = no damage; 5 = severe damage (Murray et al., 2001)

Table 2. 7 *Empoasca kraemeri* leaf burn (LB) damage ratings for the inbred backcross lines (IBL) with the ten lowest and five highest LB scores from a Matterhorn*/EMP507 IBL population of 75 $BC_1F_{4:8}$ individuals. IBLs were ranked by average LB rating† across five replications per year in choice tests in Puerto Rico in 2011.

Inbred Backcross Lines	LB Mean (2011)	Rank (N=82)
G08128	1.11	1
P08151	1.22	3
G08156	1.44	5
P08175	1.45	6
P08161	1.56	9
P08104	1.56	10
G08134	1.67	11
G08171	1.67	12
G08130	1.78	14
P08116	1.78	15
G08136	3.33	77
G08164	3.44	78
G08149	3.66	80
G08113	3.67	81
G08118	3.78	82
Matterhorn	2.09	28
EMP507	1.45	7
EMP509	1.11	2
Morales	1.45	8
Othello	3.61	79
Merlot	1.67	13
Verano	1.22	4
Test Mean	2.34	
LSD (0.05)	0.94	

† LB Damage Scale: 0 = no damage; 5 = severe damage (Murray et al., 2001).

Table 2. 8 *Empoasca kraemeri* nymph counts for the inbred backcross lines (IBL) with the ten lowest and five highest nymph counts from a Matterhorn*/EMP507 population of 75 $BC_1F_{4:8}$ individuals. IBLs were ranked by overall average nymph count across five replications per year in choice tests in Puerto Rico in 2010-2011.

Inbred Backcross Lines	Nymph Count Mean 2010 – 2011	2010		2011	
		Mean	Rank (N=82)	Mean	Rank (N=82)
G08159	0.33	0.60	1	0.07	7
G08167	0.57	0.67	2	0.47	61
P08120	0.63	0.87	4	0.40	51
G08154	0.63	0.80	3	0.47	59
G08171	0.73	1.20	10	0.27	27
P08161	0.77	1.06	6	0.47	60
P08175	0.80	1.47	16	0.13	15
P08172	0.83	1.53	19	0.13	13
G08103	0.83	1.27	12	0.40	42
P08151	0.87	1.33	13	0.40	52
G08174	2.53	4.33	78	0.73	72
G08155	2.60	4.20	77	1.00	80
G08165	3.07	5.27	80	0.87	76
G08149	3.07	5.40	81	0.73	70
G08158	3.10	5.80	82	0.40	49
Matterhorn	1.43	2.60	50	0.27	28
EMP507	1.03	2.07	32	0.00	3
EMP509	0.73	1.47	17	0.00	4
Morales	0.80	1.20	9	0.40	50
Merlot	0.93	1.67	21	0.20	19
Verano	0.87	0.87	5	0.87	77
Othello	1.20	2.33	41	0.07	9
Test Mean	1.41	2.41		0.40	
LSD (0.05)	1.69	2.72		0.68	

ii. Michigan Choice Tests

The combined mean LC score for the 75 IBLs in MI from 2009-2011 was 2.16 and ranged from 1.30 in 2010 to 2.63 in 2009 (Table 2.5). Of the top 10 individuals of the IBL population in MI for lowest mean LC rating from 2009-2011, 5 IBLs remained in the top 10 entries in all 3 seasons: P08125, G08128, P08151, G08160, and P08166 (Table 2.9). Of the 10 IBLs with the highest levels of LC damage in MI averaged over 2009-2011 – G08102, G08113, G08170 and G08174 – remained among the bottom 10 entries in all three seasons (Table 2.9).

LB damage scores were much less variable than LC in MI, especially in 2010 where only 5 IBLs had non-zero LB scores (Table 2.10). The combined mean LB score for the 75 IBLs in MI was 0.65 and ranged from 0.03 in 2010 to 0.97 in both 2009 and 2011 (Table 2.5). Of those individuals with the lowest mean LB scores averaged over 2009-2011, 4 IBLs remained in the top 10 across the three seasons in MI: G08143, G08160, P08169, and G08119 (Table 2.10). G08160 is the only IBL that had one of the 10 lowest scores for both LC and LB across all three growing seasons.

Leafhopper nymph counts of *E. fabae* for the 75 IBLs ranged from 2.49 in 2010 to 6.79 in 2009, with a mean count of 4.53 over the 3 growing seasons in MI (Table 2.11). Two individuals from the top 10 IBLs remained in the top 10 in all years: P08153 and P08169 (Table 2.11). Two of the 10 IBLs having the highest mean nymph counts from 2009-2011 remained among the bottom 10 entries in each year: G08165 and G08168.

Table 2. 9 *Empoasca fabae* leaf curl (LC) damage ratings† for the inbred backcross lines (IBL) with the ten lowest and five highest LC scores from a Matterhorn*/EMP507 IBL population of 75 $BC_1F_{4:8}$ individuals. IBLs were ranked by average LC score across three replications per year in choice tests in Michigan from 2009-2011.

Inbred Backcross Lines	LC Mean 2009-2011	2009		2010		2011	
		Mean	Rank (N=80)	Mean	Rank (N=80)	Mean	Rank (N=80)
G08160	1.11	1.00	2	0.67	10	1.67	17
P08125	1.11	1.00	3	1.00	34	1.33	6
P08142	1.22	1.67	8	1.00	28	1.00	1
P08151	1.22	2.00	18	0.67	6	1.00	2
P08166	1.22	1.67	9	0.33	1	1.67	14
P08153	1.33	1.33	6	0.67	7	2.00	21
P08175	1.33	1.67	10	1.00	35	1.33	8
G08128	1.44	2.00	11	0.67	2	1.67	12
P08150	1.44	2.33	34	0.67	11	1.33	5
P08135	1.56	2.33	33	1.00	23	1.33	7
P08144	2.78	3.33	74	2.00	74	3.00	54
G08103	2.89	3.00	53	1.67	60	4.00	76
G08137	3.00	3.00	63	2.00	76	4.00	75
G08157	3.00	3.33	72	1.67	67	4.00	78
G08113	3.11	3.33	69	2.33	79	3.67	69
G08127	3.11	3.00	60	2.33	78	4.00	77
G08102	3.22	3.67	75	2.33	77	3.67	68
G08165	3.22	3.67	77	1.67	62	4.33	79
G08170	3.22	4.00	79	2.00	73	3.67	70
G08174	3.22	4.33	80	1.67	69	3.67	74
Matterhorn	2.44	2.67	52	1.33	41	3.33	64
EMP507	1.22	2.00	15	0.67	5	1.00	3
EMP509	1.33	1.33	4	1.33	45	1.33	9
Santa Fe	1.33	1.33	5	-	-	-	-
Sierra	0.78	0.67	1	0.67	8	1.00	4
Swedish Brown	4.17	-	-	3.33	80	5.00	80
Test Mean	2.14	2.56		1.31		2.55	
LSD (0.05)	0.84	1.04		0.85		0.94	

† LC Damage Scale: 0 = no damage; 5 = severe damage (Murray et al., 2001)

Table 2. 10 *Empoasca fabae* leaf burn (LB) damage ratings† for the inbred backcross lines (IBL) with the ten lowest and five highest LB scores from a Matterhorn*/EMP507 IBL population of 75 $BC_1F_{4:8}$ individuals. IBLs were ranked by average LC rating across three replications per year in choice tests in Michigan from 2009-2011.

Inbred Backcross Lines	LB Mean 2009-2011	2009		2010		2011	
		Mean	Rank (N=80)	Mean	Rank (N=80)	Mean	Rank (N=80)
G08143	0.00	0.0	5	0.0	40	0.0	6
G08160	0.00	0.0	6	0.0	57	0.0	10
P08169	0.00	0.0	8	0.0	64	0.0	16
G08119	0.11	0.3	13	0.0	17	0.0	3
G08124	0.11	0.0	3	0.0	22	0.3	20
P08125	0.11	0.0	4	0.0	23	0.3	27
P08150	0.11	0.3	18	0.0	47	0.0	14
G08152	0.11	0.3	19	0.0	49	0.0	8
G08158	0.11	0.3	22	0.0	55	0.0	9
P08172	0.11	0.0	9	0.0	67	0.3	31
G08137	1.33	1.7	70	0.0	34	2.3	72
G08140	1.33	1.7	71	0.0	37	2.3	73
G08102	1.44	2.3	74	0.0	2	2.0	66
G08163	1.44	2.3	76	0.0	60	2.0	69
G08127	1.44	1.0	46	0.7	79	2.7	75
G08148	1.56	1.3	64	0.0	45	3.3	78
G08174	1.67	2.3	77	0.0	69	2.7	76
G08112	1.78	2.7	78	0.0	11	2.7	74
G08168	2.00	2.7	79	0.3	78	3.0	77
G08165	2.44	3.0	80	0.0	62	4.3	80
Matterhorn	0.44	0.7	39	0.0	73	0.7	41
EMP507	0.11	0.3	24	0.0	71	0.0	11
EMP509	0.22	0.3	25	0.0	72	0.3	26
Sierra	0.00	0.0	10	0.0	74	0.0	18
Santa Fe	0.67	0.7	40	-	-	-	-
Swedish Brown	2.83	-	81	2.0	80	3.7	79
Test Mean	0.65	0.93		0.05		0.97	
LSD (0.05)	0.78	1.18		0.29		1.13	

† LB Damage Scale: 0 = no damage; 5 = severe damage (Murray et al., 2001)

Table 2. 11 *Empoasca fabae* nymph counts for the inbred backcross lines (IBL) with the ten lowest and five highest nymph counts from a Matterhorn*/EMP507 population of 75 $BC_1F_{4:8}$ individuals. IBLs were ranked by overall average nymph count across three replications per year in choice tests in Michigan in 2009-2011.

Inbred Backcross Lines	Nymph Count Mean 2009-2011	2009		2010		2011	
		Mean	Rank (N=80)	Mean	Rank (N=80)	Mean	Rank (N=80)
P08153	1.63	2.43	1	0.90	2	1.56	3
P08172	2.16	3.57	8	1.70	23	1.22	1
P08169	2.40	3.77	9	1.20	8	2.22	6
G08101	2.40	3.10	4	1.33	10	2.78	16
G08147	2.52	3.00	3	1.10	7	3.44	27
G08123	2.63	4.33	13	2.33	38	1.22	2
G08149	2.73	5.00	22	0.87	1	2.33	9
P08135	2.88	4.53	15	1.10	6	3.00	20
P08120	2.96	3.43	6	2.67	46	2.78	17
G08119	3.12	5.10	25	1.47	15	2.78	15
G08102	6.56	11.133	76	3.2	61	5.333	59
G08126	6.57	12.333	78	1.7	24	5.667	61
G08133	6.59	9.767	69	3.333	63	6.667	74
G08174	6.60	8.667	61	3.133	60	8	78
G08127	6.96	10.767	72	4.767	79	5.333	60
G08106	7.11	12.9	79	2.767	51	5.667	63
G08171	7.12	13.033	80	3.1	59	5.222	56
G08111	7.25	10.867	74	6	80	4.889	51
G08168	7.38	10.8	73	4.333	77	7	76
G08165	7.70	11.433	77	4.567	78	7.111	77
Matterhorn	4.04	6.00	41	2.00	27	4.11	39
EMP507	3.93	6.13	44	3.33	64	2.33	7
EMP509	3.34	3.57	7	2.80	52	3.67	34
Sierra	2.04	2.90	2	1.00	5	2.22	5
Santa Fe	4.90	4.90	21	-	-	-	-
Swedish Brown	8.11	-	-	3.77	72	12.44	80
Test Mean	4.50	6.66		2.49		4.34	
LSD (0.05)	2.81	4.78		2.91		3.31	

B. Leaf Curl by Leaf Burn: Resistance Categories

Leaf curl and leaf burn data was analyzed to determine leafhopper resistance categories for individual IBLs. Resistance categories were defined based on standard deviation (SD) from the mean of the test, described in Table 2.1. Based on these values, the IBL population separated out into eight categories ranging from very resistant to very susceptible and including "inverse resistance", which describes those IBLs that were found to be resistant on one scale but susceptible on the other.

In PR, the SD for both LC and LB was 0.6. The mean damage scores for 2010 and 2011 for LC and LB were 2.53 and 2.40 respectively. Figure 2.7 displays the IBL mean LC and LB scores and resistance categories for choice tests in PR from 2010 to 2011. Individual IBLs are listed in each category in Table 2.12. In PR, 28 IBLs fell into the three "Resistant" categories: nine IBLs were very resistant (VR), where both LC and LB values were greater than 1 standard deviation less than the mean; seven IBLs were resistant (R), where both LC and LB values were less than the mean and either LC or LB was greater than 1 SD less than the mean; 12 IBLs were moderately resistant (MR), where both LC and LB were less than the mean but within 1 SD of the mean LC and LB values.

Twenty-five IBLs were found to be "Susceptible" in PR: seven were very susceptible (VS); seven were susceptible (S) and 11 were moderately susceptible (MS). In addition, 22 IBLs were found to have inverse resistance conditions: 14 IBLs were found to be LC resistant (LCR), having LC scores less than the mean but LB scores greater than the mean and eight IBLs were found to be LB resistant (LBR), having LB scores less than the mean, but LC scores greater than the mean.

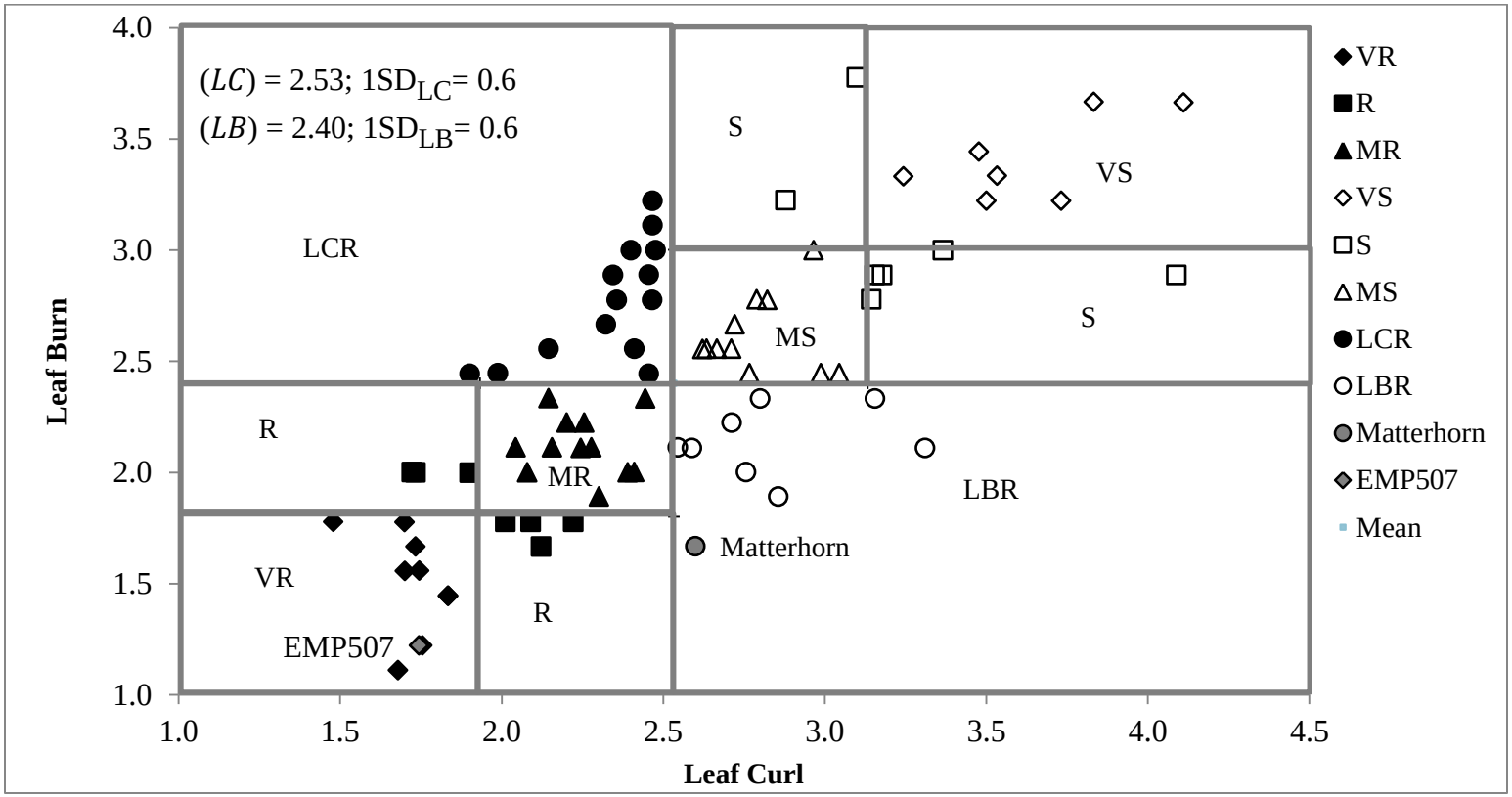

Figure 2. 7Mean leaf curl (LC) scores by mean leaf burn (LB) scores from a Matterhorn*/EMP507 inbred backcross line (IBL) population of 75 BC1F4:8 individuals grown in Puerto Rico under choice test conditions from 2010-2011

VR = Very Resistant; R = Resistant; MR = Moderately Resistant; VS = Very Susceptible; S = Susceptible; MS = Moderately Susceptible; LCR = Leaf Curl Resistant; LBR = Leaf Burn Resistant.

Table 2. 12 Resistance categories for 75 $BC_1F_{4:8}$ inbred backcross lines of a Matterhorn*/EMP507 IBL population based on mean leaf curl (LC) and leaf burn (LB) damage scores from 2010-2011 under choice test conditions in Puerto Rico (PR).

	Very Resistant	Resistant	Moderately Resistant	Moderately Susceptible	Susceptible	Very Susceptible	LB Resistant	LC Resistant
PR	P08104 G08128 G08130 G08134 P08142 P08151 G08156 P08161 P08175	P08116 P08120 P08125 G08146 P08153 P08166 G08171	G08105 G08106 G08108 G08115 G08117 G08123 G08126 G08140 G08147 P08150 G08165 P08169	G08110 G08129 G08131 G08137 G08138 P08144 G08152 G08163 G08174 G08173 G08155	G08102 G08103 G08118 G08119 G08121 G08139 G08145	G08112 G08113 G08136 G08149 G08158 G08164 G08170	G08107 G08109 G08114 G08127 G08132 G08157 G08168 P08162	G08101 G08111 G08122 G08124 G08133 P08135 G08141 G08143 G08148 G08154 G08159 G08160 G08167 P08172
Total	9	7	12	11	7	7	8	14

Mean damage scores: ($\overline{LC}$) = 2.53; ($\overline{LB}$) = 2.40. SD = standard deviation: LC=0.6, LB=0.6.

In MI, the SD for LC was 0.54 and for LB was 0.51. The mean LC score was 2.14 and mean LB score was 0.73 for 2009-2011 under choice test conditions. Mean IBL scores for LB and LC and resistance categories are displayed in Figure 2.6 and listed in Table 2.13. A total of 35 individuals from the Matterhorn*/EMP507 population rated as "resistant" in MI: seven were rated as VR; 13 as R; and 15 as MR. Twenty-six IBLs were rated as "susceptible": nine IBLs were VS, where both mean LC and LB scores were greater than 1 SD above the mean; 10 IBLs were S, where both LC and LB were greater than the mean but only either LC or LB was more than 1 SD greater than the mean; seven IBLs were MS, where both LC and LB were greater than the mean but both were within 1 SD of the mean LC and LB scores. A total of 14 IBLs were shown to have inverse resistance in MI: nine IBLs rated as LC-R, having LC scores less than the mean, but LB scores greater than the mean and five IBLs rated as LB-R, having LB scores below the mean but LC scores above the mean.

Of those IBLs in each category in each location, 11 IBLs had consistent responses to *Empoasca* feeding in both locations. Five IBLs were found to be consistently rated as resistant to both species: P08175 and P08142 were rated as VR in both PR and MI while P08120 rated as R and G08117 and G08147 rated as MR. Four IBLs were consistently susceptible to both leafhopper species. G08117 and G08147 were rated MS to *E. kraemeri* and *E. fabae*, while G08103 rated as S and G08112 rated as VS. Both G08107 and G08109 were rated as LBR to both species, but no IBLs were rated as LCR in both locations.

An additional 11 individuals were identified as being resistant to both leafhopper species by having mean LC and LB scores less than the mean in both locations. P08151 and P08161 were VR in PR and R in MI. P08125, P08153, and P08166 were VR in MI and R in PR. P08104 and G08134 were VR in PR but only MR in MI while P08150 was VR in MI but only MR in PR.

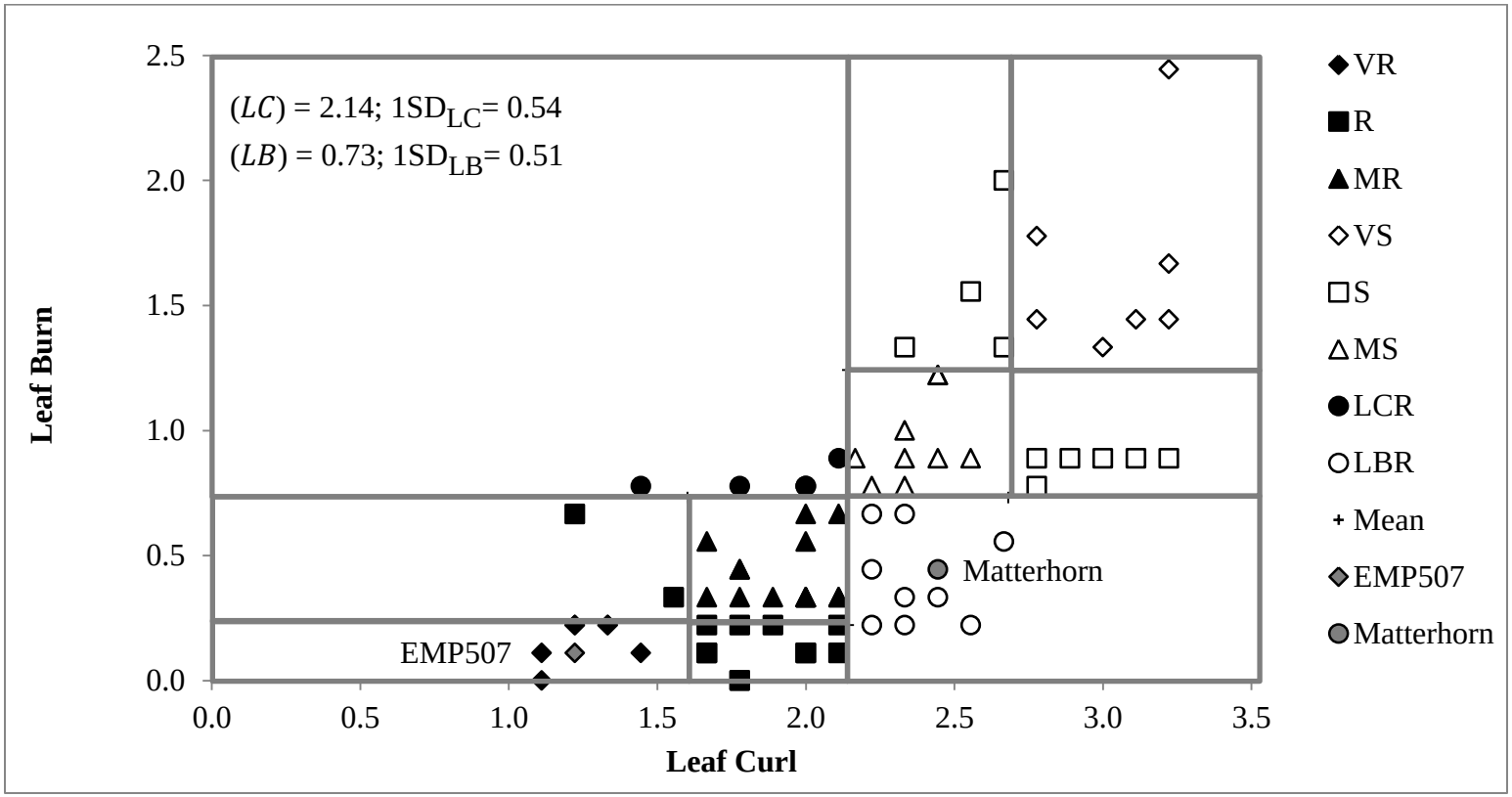

Figure 2. 8 Mean leaf curl (LC) scores by mean leaf burn (LB) scores from a Matterhorn*/EMP507 inbred backcross line (IBL) population of 75 $BC_1F_{4:8}$ individuals grown in Michigan under choice test conditions from 2010-2011.

VR = Very Resistant; R = Resistant; MR = Moderately Resistant; VS = Very Susceptible; S = Susceptible; MS = Moderately Susceptible; LCR = Leaf Curl Resistant; LBR = Leaf Burn Resistant.

Table 2. 13 Resistance categories of 75 $BC_1F_{4:8}$ inbred backcross lines from a Matterhorn*/EMP507 IBL population based on mean leaf curl (LC)† and leaf burn (LB)‡ values from 2009-2011 under choice test conditions in Michigan (MI).

	Very Resistant	Resistant	Moderately Resistant	Moderately Susceptible	Susceptible	Very Susceptible	LB Resistant	LC Resistant
MI	P08125 P08142 P08150 P08153 G08160 P08166 P08175	G08119 P08120 G08124 G08132 P08135 G08143 P08151 G08152 G08158 P08161 G08164 P08169 P08172	G08101 P08104 G08114 P08116 G08117 G08131 G08134 G08139 G08141 G08145 G08147 G08149 G08154 P08162 G08171	G08105 G08106 G08108 G08111 G08118 G08133 G08136 G08155 G08173	G08103 G08113 G08129 G08140 P08144 G08146 G08148 G08157 G08168 G08170	G08102 G08112 G08127 G08137 G08163 G08165 G08174	G08107 G08109 G08115 G08121 G08122 G08123 G08138 G08159 G08167	G08110 G08126 G08128 G08130 G08156
Total	7	13	14	8	10	7	8	5

†LC ($\bar{x}$) = 2.14, ‡LB ($\bar{y}$) = 0.73. SD = standard deviation: LC=0.54, LB=0.51.

P08169 was MR in PR but R in MI while G08171 and P08116 were MR in MI but R in PR. It is also notable that of the 16 individual IBLs having resistant reactions to both species, 12 individuals were pinto seed-type of which there are only a total of 16, representing 21% of the entire population but 75% of the resistant IBLs.

Ten more IBLs were identified as being susceptible to both leafhopper species in addition to the four previously identified. G08136 and G08170 were VS in PR but MS in MI. G08137, G08163 and G08174 were VS in MI but MS in PR. G08113 was VS in PR but S in MI, while G08102 was VS in MI but S in PR. G08129 and P08144 were MS in PR and S in MI and G08118 was MS in MI and S in PR.

Of the six IBLs identified as LB-R in PR, three were identified as resistant in MI and three were identified as susceptible: G08114 and P08162 were MR and G08132 was R; G08157 and G08168 were S while G08127 was VS. Fourteen IBLs were classified as LC-R in PR. In MI, these 14 IBLs were detected in all other categories. Eight were identified as R: G08101, G08141 and G08154 were MR; G08124, P08135, G08143, and P08172 were R; and G08160 was VR. Three were identified as susceptible: G08111 and G08133 were MS; and G08148 was S. Three IBLs were categorized as LB-R in MI: G08122, G08159 and G08167. The other 4 IBLs classified as LB-R in MI were found to be MR (G08115, G08123), MS (G08138) and S (G08121) in PR. Of the five individuals classified as LC-R in MI, two were MS (G08110, G08126) and three were VR (G08128, G08130, and G08156) in PR.

C. Resistance Classes and Nymph Counts

In order to further analyze the relationship between damage scores and *Empoasca* species populations, resistance classes identified in PR in Table 2.12 and in MI in Table 2.13 were further separated by nymph count populations. In PR, six of the nine VR IBLs and five of the seven R IBLs had nymph counts within 1 SD of the test mean ($\bar{y}$ = 1.4), suggesting tolerance may be involved (Table 2.14). Three VR IBLs and two R IBLs had nymph counts of more than 1 SD below the test mean, raising the possibility that these IBLs may have antibiosis resistance. The majority of all IBLs in each resistance category had nymph counts within 1 SD of the test mean in PR. In MI, the majority of IBLs in the R and MR categories had nymph counts of more than 1 SD below the test mean, suggesting that antibiosis may be present in this population and may be more effective against *E. fabae* that *E. kraemeri* (Table 2.15). Only P08120 maintained both its R classification and nymph counts more than 1 SD below the mean in both PR and MI. G08171 was R with *E. kraemeri* nymph counts in the lower threshold in PR but while still maintaining some resistance in MI as MR, this IBL was found to have *E. fabae* nymph counts in the highest threshold.

Table 2. 14 Resistance categories of 75 $BC_1F_{4:8}$ inbred backcross lines from a Matterhorn*/EMP507 IBL population based on mean leaf curl (LC) and leaf burn (LB) values from 2009-2011 under choice test conditions in Puerto Rico, separated by nymph counts (y) of greater than, less than or within one standard deviation (SD) from the mean ($\bar{y}$).

Nymph Counts (y)	Very Resistant	Resistant	Moderately Resistant	Moderately Susceptible	Susceptible	Very Susceptible	LB Resistant	LC Resistant
y> $\bar{y}$ + 1 SD	0	0	G08165	G08137 G08174 G08155	G08145	G08112 G08149 G08158	G08109	0
$\bar{y}$ -1SD <y> $\bar{y}$ + 1SD	P08142 P08104 G08156 G08130 G08128 G08134	P08166 P08153 P08125 P08116 G08146	G08123 P08150 G08117 G08108 G08105 G08106 P08169 G08126 G08147 G08115 G08140	P08144 G08129 G08163 G08131 G08173 G08152 G08110	G08119 G08102 G08121 G08118 G08139	G08164 G08136 G08113 G08170	G08157 G08114 G08107 G08132 P08162 G08127 G08168	G08141 P08135 G08122 G08160 G08133 G08148 G08124 G08111 G08143 G08101
y < $\bar{y}$ - 1 SD	P08161 P08175 P08151	P08120 G08171	0	G08138	G08103	0	0	G08159 G08167 G08154 P08172

Nymph count mean ($\bar{y}$) = 1.41; 1 SD = 0.55

Table 2. 15 Resistance categories of 75 $BC_1F_{4:8}$ inbred backcross lines from a Matterhorn*/EMP507 IBL population based on mean leaf curl (LC) and leaf burn (LB) values from 2009-2011 under choice test conditions in Michigan (MI), separated by nymph counts (y) of greater than, less than or within one standard deviation (SD) from the mean ($\bar{y}$).

Nymph Counts (y)	Very Resistant	Resistant	Moderately Resistant	Moderately Susceptible	Susceptible	Very Susceptible	LB Resistant	LC Resistant
y> $\bar{y}$ + 1 SD	0	0	G08171	G08106 G08111	G08168	G08127 G08165	0	0
$\bar{y}$ -1SD <y> $\bar{y}$ + 1SD	P08150 P08125 G08160 P08142 P08175	P08151 P08161 G08158	G08141 P08116 G08145 G08114 G08117	G08118 G08136 G08108 G08133	G08170 P08144 G08146 G08140 G08129 G08148	G08137 G08163 G08112 G08102 G08174	G08121 G08159 G08138 G08115 G08109	G08110 G08128 G08130 G08126
y< $\bar{y}$ - 1 SD	P08153 P08166	P08172 P08169 P08135 P08120 G08119 G08152 G08124 G08164 G08143 G08132	G08101 G08147 G08149 G08139 G08154 G08131 P08104 P08162 G08134	G08173 G08105 G08155	G08113 G08103 G08157	0	G08123 G08122 G08167 G08107	G08156

Nymph count mean ($\bar{y}$) = 5.39; 1 SD = 1.44

D. No-Choice Tests

Both LC and LB resistance traits were significantly affected by genotypic effects, as seen by the mean square values listed in Table 2.11. LC and LB were also affected by environment (Year) but there were no significant interactions between genotype and year (Table 2.16). Under no-choice conditions, LC had a coefficient of variability of 30.3 % and h^2 of 0.67, while LB had a coefficient of variability of more than double (63.2 %) and an h^2 score of 0.53.

Table 2. 16 ANOVA table showing mean squares and heritability for leaf curl and leaf burn damage scores from a Matterhorn*/EMP507 population of 75 $BC_1F_{4:8}$ individuals under no-choice test conditions averaged across 3 years in Michigan (2009-2011).

No-Choice Test	Trait	
	Leaf Curl	Leaf Burn
Genotype (G)	1.50***	2.94***
Year (Y)	6.31***	19.16***
G x Y	0.50ns	1.37ns
Coefficient of Variability	33.15	65.37
Heritability	0.67	0.53

* significant at α=0.05, ** significant at α=0.01, *** significant at α<0.001.

Table 2. 17 Phenotypic means and ranges for leafhopper resistance traits: leaf curl (LC) and leaf burn (LB) from a Matterhorn*/EMP507 population of 75 $BC_1F_{4:8}$ individuals under no-choice test conditions combined across 3 years (2009-2011) in Michigan.

	No-Choice Test								
	Parents		Inbred Backcross Lines			Checks			
Trait	Matterhorn	EMP507	Mean	Range	LSD (0.05)	EMP 509	Santa Fe	Swedish Brown	Sierra
Leaf Curl	1.67	1.67	2.17	2.16 – 2.54	1.00	1.67	1.00	5.00	1.33
Leaf Burn	1.33	0.33	1.78	1.46 – 2.29	1.79	1.00	2.00	5.00	0.67

Within the IBL population, LC phenotypic means for ranged from 2.00 in 2010 to 2.54 in 2011 (Table 2.17). The LC mean in 2009 for the IBL population was intermediate at 2.16. On average, the IBL LC mean score of 2.17 was higher than the LC score of parent genotypes Matterhorn and EMP507, as both parents averaged LC scores of 1.67 between 2009 and 2011. The LC scores were also higher among the IBLs than among all check genotypes, except highly susceptible Swedish Brown. LB scores averaged 1.78 for the IBL population, higher than both parent and check genotypes except Sierra and Swedish Brown (Table 2.17). IBL LB mean scores ranged from a low of 1.46 in 2010 to a high of 2.29 in 2011. Highly significant positive correlations were seen between LC and LB scores using both Pearson Correlations and Spearman Rank Correlations, indicating that as LC values increase, so do the LB values (Table 2.18). No-choice LC and LB were also significantly correlated with choice test LC and LB scores as well as *E. fabae* nymph counts in MI, but only no-choice LC scores were correlated with choice test LC and LB scores and *E. kraemeri* nymph counts in PR (Table 2.19).

Table 2. 18 Pearson and Spearman Rank Correlation Coefficients for leafhopper resistance traits leaf curl and leaf burn from a Matterhorn*/EMP507 population of 75 $BC_1F_{4:8}$ individuals under no-choice test conditions in Michigan in 2009 – 2011.

Trait	No-Choice Leaf Burn	
	Pearson Correlation Coefficients	Spearman Rank Correlation Coefficients
No-Choice Leaf Curl	0.70**	0.64**

* significant at α=0.05, ** significant at α=0.01, *** significant at α<0.001.

Table 2. 19 Spearman Rank Correlation Coefficients for damage under no-choice test conditions with damage and nymph count results from choice tests in Michigan in 2009 – 2011 and Puerto Rico in 2010 – 2011 from a Matterhorn*/EMP507 IBL population of 75 $BC_1F_{4:8}$ individuals.

Trait	Michigan			Puerto Rico		
	Leaf Curl	Leaf Burn	Nymph Counts	Leaf Curl	Leaf Burn	Nymph Counts
No-choice Leaf Burn	0.45***	0.57***	0.25*	0.12 *ns*	0.20 *ns*	0.08 *ns*
No-Choice Leaf Curl	0.67***	0.57***	0.30**	0.47***	0.43***	0.26*

* significant at α=0.05, ** significant at α=0.01, *** significant at α<0.001, *ns* = not significant

Of the 10 IBLs with the lowest average LC scores, four genotypes ranked in the top 10 in each no-choice field test: P08120, P08142, P08166 and P08169. G08128 and G08149 ranked in the top 10 in 2009 and 2010, while P08172 ranked in the top 10 in 2009 and 2011 (Table 2.20). On the other end of the spectrum, of the 5 IBLs with the highest average LC scores under no-choice conditions, two IBLs consistently had the highest LC scores in each year: G08137 and G08168. Two IB lines ranked in the bottom five in two out of three seasons: G08165 in 2009 and 2011, and G08174 in 2009 and 2010 (Table 2.20).

When the average LB scores for the IBL population were ranked, no individuals remained in the top 10 in all 3 seasons (Table 2.21). G08121, P08169, G08119, and P08142 ranked with the lowest 10 LB scores in 2009 and 2011, while G08131, G08132, G08149 and P08166 ranked with the lowest 10 LB scores in 2010 and 2011 (Table 2.21). Of those that ranked as the most susceptible with respect to LB in no-choice tests, only a single IBL ranked among the bottom five in more than one season: G08165 had the highest overall LB score among the IBLs and had one of the five highest LB scores in both 2009 and 2011.

Table 2. 20 *Empoasca fabae* leaf curl (LC) damage ratings† for the inbred backcross lines (IBL) with the ten lowest and five highest LC scores from a Matterhorn*/EMP507 IBL population of 75 $BC_1F_{4:8}$ individuals. IBLs were ranked by average LC rating per year in no-choice tests in Michigan from 2009-2011.

Inbred Backcross Lines	No-Choice LC 2009-2011	2009		2010		2011	
		Mean	Rank	Mean	Rank	Mean	Rank
P08120	0.67	0.00	1	1.00	1	1.00	1
P08142	1.00	1.00	8	1.00	3	1.00	2
P08166	1.00	1.00	11	1.00	5	1.00	3
P08169	1.29	1.00	12	1.00	6	1.67	11
G08128	1.33	1.00	6	1.00	2	2.00	13
G08149	1.33	1.00	10	1.00	4	2.00	14
P08172	1.43	1.00	13	1.33	22	1.67	12
G08160	1.57	2.00	44	1.00	13	2.00	17
P08125	1.57	1.00	5	1.33	21	2.00	22
G08110	1.67	2.00	22	1.00	7	2.00	15
G08165	3.00	3.00	75	2.33	62	3.67	75
G08174	3.14	3.00	77	3.00	79	3.33	72
G08102	3.17	3.33	78	2.67	63	3.5	73
G08168	3.29	3.00	76	3.00	78	3.67	76
G08137	3.33	3.00	64	3.00	77	4.00	79
Matterhorn	1.67	2.00	53	1.00	17	2.00	21
EMP507	1.67	2.00	51	1.00	15	2.00	19
EMP509	1.67	2.00	52	1.00	16	2.00	20
Sierra	1.33	2.00	54	1.00	18	1.00	4
Santa Fe	1.00	1.00	15	-	-	-	-
Swedish Brown	5.00	-	-	5.00	80	5.00	80
Test Mean	2.18	2.13		1.96		2.43	
LSD (0.05)	1.02	1.93		1.69		1.81	
Coefficient of Variation	33.2	32.0		32.2		27.8	

† LC Damage Scale: 0 = no damage; 5 = severe damage (Murray et al., 2001)

Table 2. 21 *Empoasca fabae* leaf burn (LB) damage ratings† for the inbred backcross lines (IBL) with the ten lowest and five highest LB scores from a Matterhorn*/EMP507 IBL population of 75 $BC_1F_{4:8}$ individuals. IBLs were ranked by average LB rating per year in no-choice tests in Michigan from 2009-2011.

Inbred Backcross Lines	No-Choice LB 2009-2011	2009		2010		2011	
		Mean	Rank (N=80)	Mean	Rank (N=80)	Mean	Rank (N=80)
G08131	0.33	1.00	21	0.00	5	0.00	6
G08121	0.33	0.00	3	1.00	15	0.00	5
P08169	0.57	0.00	12	0.33	11	1.00	14
G08132	0.60	0.67	13	0.00	2	1.00	17
G08114	0.67	1.00	18	1.00	22	0.00	4
G08119	0.67	0.00	2	2.00	52	0.00	1
G08149	0.67	1.00	25	0.00	4	1.00	23
P08142	0.71	0.00	10	0.67	13	1.00	22
P08153	1.00	3.00	72	1.00	32	0.33	8
P08166	1.00	2.00	57	0.00	6	1.00	28
G08102	3.28	3.33	73	2.33	65	4.17	75
G08163	3.29	3.00	68	2.33	67	4.33	77
G08168	3.29	3.00	69	1.67	47	5.00	79
G08137	3.33	3.00	63	3.00	75	4.00	71
G08165	3.57	4.00	78	2.67	68	4.33	76
Matterhorn	1.33	1.00	30	1.00	23	2.00	36
EMP507	0.33	0.00	8	0.00	7	1.00	18
EMP509	1.00	1.00	31	1.00	41	1.00	19
Sierra	0.67	1.00	36	1.00	45	0.00	7
Santa Fe	2.00	2.00	56	-	-	-	-
Swedish Brown	5.00	-	-	5.00	80	5.00	80
Test Mean	1.82	1.71		1.46		2.29	
LSD (0.05)	1.79	2.63		2.54		3.19	
Coefficient of Variation	65.4	54.4		65.0		52.0	

† LB Damage Scale: 0 = no damage; 5 = severe damage (Murray et al., 2001)

E. Comparison of Choice and No-Choice Field Tests

When the mean LC scores from choice and no-choice tests were compared directly, the overall LC mean values were not significantly different among individual IBL genotypes (Figure 2. 9). However, seven individuals had significantly different results (α=0.1) (

Table 2. 22). P08135, G08152, G08168 and P08175 all had significantly higher LC scores under no-choice conditions, while P08120, G08107, and G08123 all had significantly lower LC scores under no-choice conditions. Only P08135 changed from a resistant rating of 1.56 under choice conditions to a susceptible score of 2.67 under no-choice conditions, when susceptibility is defined as an average LC score of 2.5. G08107 dropped from a susceptible score of 2.67 under choice conditions to a resistant score of 1.67 under no-choice conditions.

Table 2. 22 Mean leaf curl values (LC)† for inbred backcross lines (IBL) from a Matterhorn*/EMP507 IBL population of 75 $BC_1F_{4:8}$ individuals with significantly different choice and no-choice LC scores in Michigan in 2009-2011.

Inbred Backcross Lines	Choice Mean	95% CL	No-choice Mean	95% CL	Mean Difference‡
P08135	1.56	1.00 – 2.11	2.67	1.23 – 4.10	-1.11**
G08152	1.67	1.28 – 2.05	2.33	0.90 – 3.77	-0.67*
G08168	2.67	2.12 – 3.21	3.29	2.83 – 3.74	-0.62*
P08175	1.33	0.95 – 1.72	2.00	1.12 – 2.88	-0.67*
P08120	1.78	1.27 – 2.29	0.67	-0.77 – 2.10	1.11**
G08107	2.67	2.12 – 3.21	1.67	0.23 – 3.10	1.00*
G08123	2.33	1.95 – 2.72	1.67	0.23 – 3.10	0.67**
Test	2.14	2.06 – 2.22	2.18	2.08 – 2.27	-0.04*ns*

* significant at α=0.10, ** significant at α=0.05, *** significant at α=0.01, ns= not significant
† LC Damage Scale: 0 = no damage; 5 = severe damage (Murray et al., 2001)
‡ To determine significance, pooled variance used if test variances were equal. Satterthwaite variances were used if test variances were unequal between choice and no-choice tests.

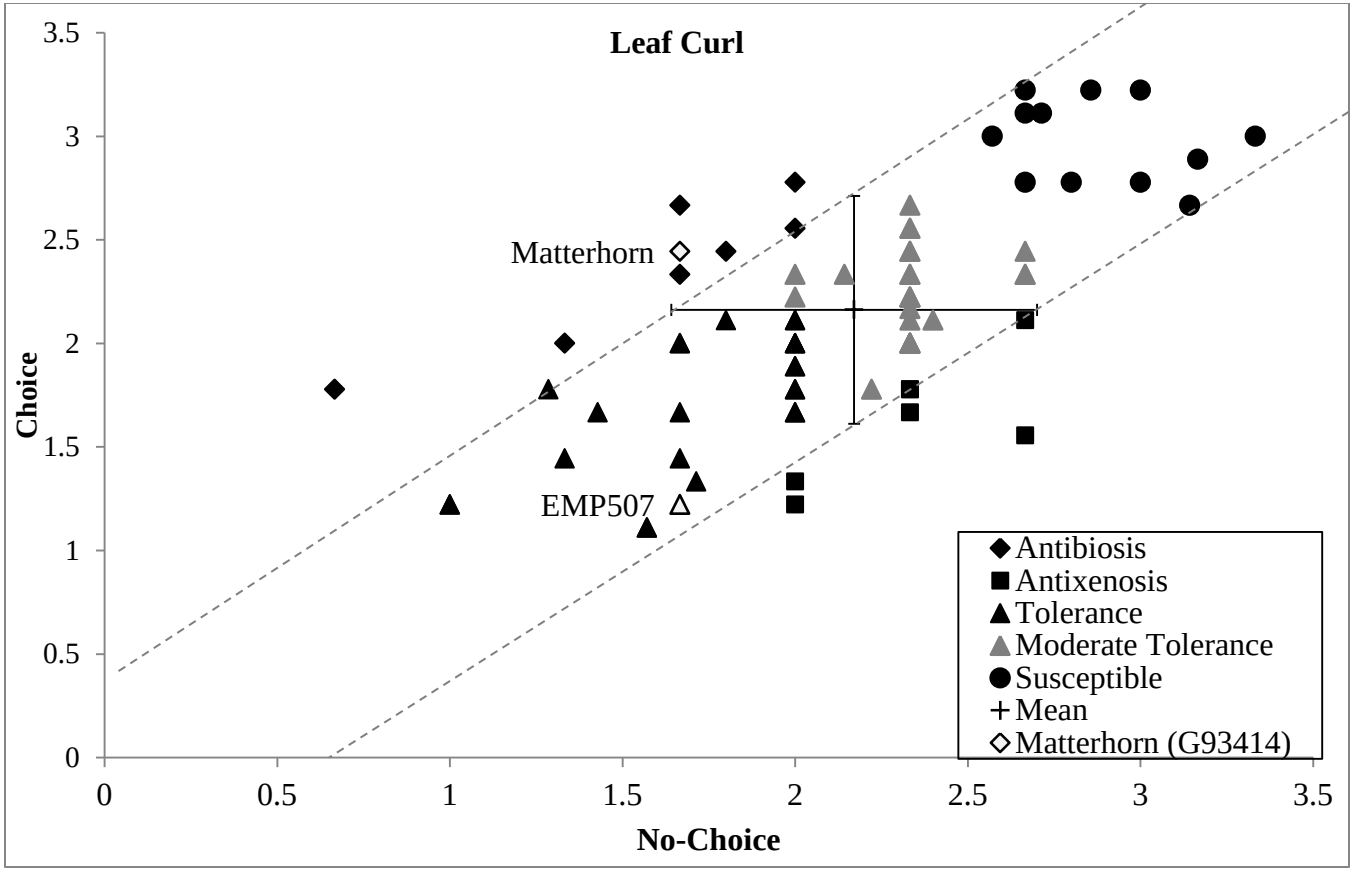

Figure 2. 9 No-choice leaf curl (LC) results plotted against choice test LC scores from a Matterhorn*/EMP507 population of 75 $BC_1F_{4:8}$ individuals grown in Michigan in 2009-2011.

More leaf burn scores varied between choice and no-choice conditions. Overall average LB values were significantly different between choice and no-choice conditions (Table 2.23). Twenty-two IBLs were identified as differing significantly for LB damage between test conditions. All significantly different LB values were higher under no-choice conditions than choice conditions (Figure 2. 10). LB values for G08122, G08157, G08137, G08155, and G08102 increased from resistant choice scores (0.33 – 2.17) to susceptible no-choice scores (2.67 – 3.28).

Table 2. 23 Mean leaf burn (LB) values for inbred backcross lines (IBL) from a Matterhorn*/EMP507 population of 75 $BC_1F_{4:8}$ individuals with significantly different choice and no-choice LB scores in Michigan in 2009-2011.

IBLs	Choice Mean	95% CL	No-Choice Mean	95% CL	Mean Difference‡
G08122	0.33	-0.21 – 0.86	2.67	1.23 – 4.10	-2.33**
P08175	0.33	-0.21 – 0.88	2.40	0.98 – 3.82	-2.07**
G08124	0.17	-0.26 – 0.60	2.00	0.76 – 3.24	-1.83**
G08143	0.00	0.00 – 0.00	1.78	0.85 – 2.70	-1.78**
G08134	0.67	-0.19 – 1.52	2.33	0.90 – 3.77	-1.67*
G08157	1.33	0.79 – 1.88	3.00	1.24 – 4.76	-1.67*
G08101	0.50	-0.07 – 1.07	2.17	-0.42 – 4.75	-1.67**
G08147	0.50	-0.07 – 1.07	2.00	2.00 – 2.00	-1.50**
G08137	2.00	1.34 – 2.66	3.33	1.90 – 4.77	-1.33*
G08145	1.00	0.34 – 1.66	2.33	-0.54 – 5.20	-1.33*
G08155	1.33	0.79 – 1.88	2.67	1.23 – 4.10	-1.33**
G08159	0.67	-0.19 – 1.52	2.00	2.00 – 2.00	-1.33**
G08164	0.33	-0.21 – 0.88	1.67	0.23 – 3.10	-1.33**
P08161	0.33	-0.21 – 0.88	1.67	0.23 – 3.10	-1.33**
P08172	0.17	-0.26 – 0.60	1.43	0.25 – 2.6	-1.26*
G08171	0.50	-0.07 – 1.07	1.67	0.23 – 3.10	-1.17*
P08150	0.17	-0.26 – 0.60	1.33	-0.10 – 2.77	-1.17**
G08102	2.17	1.74 – 2.60	3.28	2.35 – 4.20	-1.11*
G08123	0.33	-0.21 – 0.88	1.33	-0.10 – 2.77	-1.00*
G08160	0.00	0.00 – 0.00	1.00	0.47 – 1.53	-1.00*
P08125	0.17	-0.26 – 0.60	1.14	0.79 – 1.49	-0.98**
P08169	0.00	0.00 – 0.00	0.57	0.08 – 1.07	-0.57*
Test	0.95	0.86 – 1.04	1.83	1.68 – 1.97	-0.88***

* significant at α=0.05, ** significant at α=0.01, *** significant at α<0.0001, ns= not significant.
† LB Damage Scale: 0 = no damage; 5 = severe damage (Murray et al., 2001)
‡ To determine significance, pooled variance used if test variances were equal. Satterthwaite variances were used if test variances were unequal between choice and no-choice tests.

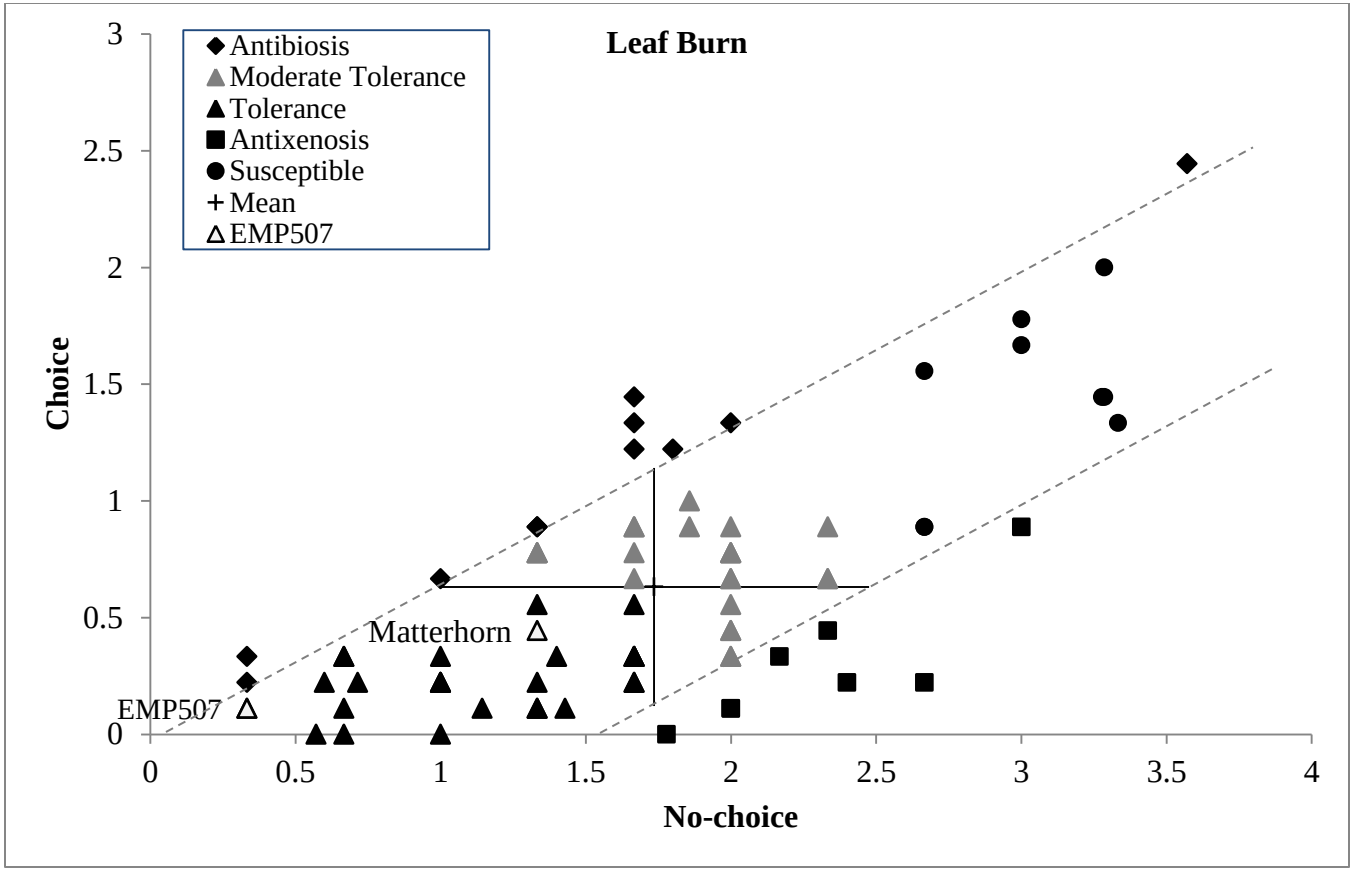

Figure 2. 10 No-choice leaf burn (LB) results plotted against choice LB scores from a Matterhorn*/EMP507 inbred backcross line (IBL) population of 75 $BC_1F_{4:8}$ individuals grown in Michigan in 2009-2011.

F. Agronomic Traits

Hundred seed weight (g) and days to flowering were recorded for the IBL population and parent germplasm (Table 2.24). Matterhorn averaged a seed weight of 46.4g/100 seeds, while EMP507 averaged almost 10g less per 100 seeds at 37.3 g/100 seeds. The IBL population mean was 44.6g/ 100 seeds with a range of 38.7 to 49.9 g/100 seeds. Days to flowering was determined when 50 % of the plot had a single flower ranged from 34.7 to 41.2 days with a mean value of 37.3 days for the IBL population, with Matterhorn flowering 5 days earlier, at 35.2 days, than EMP507 at 40.5 days.

Table 2. 24 Agronomic traits from a Matterhorn*/EMP507 inbred backcross line population of 75 $BC_1F_{4:8}$ individuals grown in Michigan in 2009-2011 and Puerto Rico in 2010-2011.

	Parents		IBL Population		
Trait	Matterhorn	EMP507	Mean	Range	LSD (0.05)
100SDWT (g)	46.4	37.3	44.6	38.7 - 49.9	9.7
FLWR (days)	35.2	40.5	37.3	34.7 - 41.2	2.5

100SDWT = weight of 100 seeds (grams); FLWR = days to flowering.

Seed coat and flower color of the IBL population were recorded (Table 2.25). Matterhorn is a great northern seed type and therefore has a medium-sized white seed, while EMP507 is a carioca seed type and therefore a small-sized colored bean. Of 75 individuals of the Matterhorn*/EMP507 population, 59 had white seeds and 16 had colored seeds, which is in direct contrast to the expected single gene ratio, as white seed color is recessive to colored seed. Likewise, flower color was skewed more than the expected 3:1 towards Matterhorn in that 65 IBLs had white flowers while nine had pink flowers similar to EMP507. One IBL was noted as having purple flowers (P08153).

Trichome density and composition was also examined as it has been linked to *E. fabae* resistance in *Medicago sativa* (Ranger et al., 2004) and suspected of involvement in common bean resistance (Pillemer and Tingey, 1978). However, in this study, no significant differences were evident between the parent genotypes or between resistant and susceptible IBLs; therefore, trichome density was not further analyzed in this study.

Table 2. 25 Seed type and flower color traits from a Matterhorn*/EMP507 inbred backcross line population of 75 $BC_1F_{4:8}$ individuals grown in Michigan in 2009-2011 and Puerto Rico in 2010-2011.

	Parents		Inbred Backcross Lines		
Trait	Matterhorn	EMP507	Matterhorn-type	EMP507-type	Other
Seed Type	Great Northern	Carioca	59	0	16†
Flower Color	White	Pink	65	9	1‡

† 15 IBLs = pinto seed type; 1 IBL = other non-carioca type
‡ 1 IBL = purple flower color

V. Discussion

A. GxE Interactions

By evaluating the inbred backcross line (IBL) population in MI and PR for a combined five seasons, it was possible to evaluate these genotypes under diverse growing conditions as well as test them against two different leafhopper species, *Empoasca kraemeri* and *E. fabae*. Michigan represents a temperate climate with long days and short nights, while PR represents a tropical climate with short days. In addition, the different species presented a challenge in identifying lines with consistent reactions to leafhopper predation in both climates. This challenge presented an opportunity to separate out the different components involved in resistance to each species of *Empoasca* leafhoppers.

While genotypic and environmental effects were significant in both environments for all leafhopper resistance traits, heritability varied largely between traits (Table 2. 2). The low heritability estimate for leafhopper nymph counts in PR suggests this may not be a useful measure of resistance to *E. kraemeri*, but the moderate heritability estimate of nymph counts in MI indicates this is a valid measure of *E. fabae* reaction. Heritability estimates were moderate to high in both locations for LC and LB, confirming their usefulness in evaluating leafhopper damage in this population.

Trait correlations varied between tests (Table 2. 3). When examined in each location, nymph counts were positively correlated with both LC and LB values. This indicates that overall, as leafhopper populations increase, feeding damage also increases as expected. However, when examined on a trait basis, only LC was correlated between MI and PR (Table 2. 4). LB values were significantly lower in MI than PR. An explanation for the lower LB values may be because *E. kraemeri* is known to use lacerate-and-sip feeding more often than *E. fabae*. Lacerate-and-sip

feeding causes chlorosis above the point of feeding and is detectable as LB damage. Another possible explanation is that the presentation of LB damage may be related to environmental factors, such as temperature and humidity.

When the IBL means from each location were compared to the parental means for LC, LB and nymph counts, in all cases the LC mean value for the Matterhorn*/EMP507 population fell between the values of the two parents (Table 2. 5). However, in all cases, the IBL means for LB and nymph counts were greater than the range of the parent values. This suggests that Matterhorn may have some levels of resistance to LB damage and nymph counts but not to LC damage. This is not surprising as Matterhorn's pedigree includes Sierra, a commercial pinto bean variety, which has demonstrated resistance to *E. fabae* (Gonzales et al., 2004).

B. Empoasca Feeding Damage

i. Puerto Rico

Leaf Curl

With a heritability estimate of 0.77, LC damage values were normally distributed in PR in 2010 and 2011 (Figure 2. 1). P08142 and P08104 consistently presented lower or equal LC values in PR than the resistant parent EMP507 in each year (Table 2. 6). When LC means for both years were examined, an additional five IBLs were identified as being more resistant than EMP507 (LC=1.74) under *E. kraemeri* pressure: G08128, G08130, P08161, P08120, P08125 and G08134 (Table 2. 6). In addition to identifying resistant IBLs, a number of individuals were identified that presented significantly higher LC damage than Matterhorn or the test mean in PR. G08158, G08113, G08121, and G08149 ranked the most susceptible IBLs to *E. kraemeri* in 2010-2011 with mean LC values of 3.7 or greater (Table 2. 6).

Leaf Burn

In addition to having LC damage scores outside of the range of the parent genotypes, a number of individuals were identified in PR as having transgressive segregation for LB damage (Figure 2. 2). G08128, which had been already noted for its low LC scores, was also identified as having lower LB scores in PR in 2011 than EMP507 (LB=1.45) (Table 2. 7). Three other IBLs were identified as being equally or more resistant to *E. kraemeri* damage than EMP507 with respect to LB damage: P08151, G08156, P08175 (Table 2. 7). G08149 and G08113 also ranked among the most susceptible IBLs with respect to LB damage in 2011 in PR. G08118 also had a higher LB score than the susceptible check Othello (LB=3.6).

Resistance Classes

When IBLs were analyzed by comparing LC values with LB values, individuals were able to be classified into either resistant or susceptible categories (Figure 2. 7Mean leaf curl (LC) scores by mean leaf burn (LB) scores from a Matterhorn*/EMP507 inbred backcross line (IBL) population of 75 BC1F4:8 individuals grown in Puerto Rico under choice test conditions from 2010-2011). Individuals for which LC damage was significantly correlated with LB damage were identified in all categories but “susceptible” (Table 2. 12). Nine IBLs were classified as VR in PR with respect to both LC and LB values, while seven IBLs were VS with respect to both LC and LB values (Table 2. 12). Overall, as LC values increase, LB values also increase. Resistance and susceptibility were not found to be segregating in a single or duplicate gene ratio indicating more than two genes may be involved in resistance/susceptibility to *E. kraemeri*. The presence of individuals classified in the inverse resistance categories of either LC-R (14 IBLs) or LB-R (8 IBLs) further supports the conclusion that LC and LB reactions are controlled by independent genetic mechanisms.

ii. Michigan

Leaf Curl

In MI, two IBLs had lower mean LC scores than the resistant parent EMP507 (LC=1.22): G08160, P08125 (Figure 2. 4). Three additional IBLs were identified that had mean LC values equal to EMP507: P08142, P08151, and P08166 (Table 2. 9). No IBLs were more resistant to LC damage than EMP507 in every season. The 10 most susceptible IBLs with respect to LC damage in MI all had higher LC values than Matterhorn; however, no individuals ranked higher than the susceptible check Swedish Brown in any year (Table 2. 9).

Leaf Burn

The low LB damage scores in MI provided a challenge in identifying individuals with higher levels of resistance to *E. fabae* (Figure 2. 5). G08143, G08160, and P08169 all had lower mean LB values than EMP507, but this is because EMP507 had a LB score of greater than 0.0 only in 2009 (Table 2. 10). While genotypic effects were found to be significant factors in determining LB scores, all of the 10 most resistant IBLs had LB scores of less than one. In 2010, LB values were even more left-skewed with the test mean being 0.05. In determining the most susceptible IBLs, similar to LC, all of the 10 most susceptible IBLs had higher LB scores than Matterhorn. G08165 was the most susceptible IBL in 2009 and 2011, achieving higher LB scores (LB=4.33) than the susceptible check Swedish Brown (LB=3.67) in 2011 (Table 2. 10).

Resistance Classes

Seven IBLs were classified as VR to *E. fabae* feeding damage and seven IBLs were classified as VS with respect to both LC and LB damage (Table 2. 13). As LC damage increased in MI, so too did LB damage, similar to PR (Figure 2. 8). Segregation ratios did not match

expected ratios for either a single gene or for two dominant genes, indicating that resistance to *E. fabae* may involve multiple genes with possible epistatic effects.

iii. Conclusions

The lack of correlations between locations and the existence of significant environmental effects presented a challenge in identifying individual IBLs that demonstrate resistance to both *E. fabae* and *E. kraemeri*. However, multiple individuals were identified within the population where resistant or susceptible classes overlapped across the different species.

High levels of transgressive segregation were seen in the IBL population for all resistance traits. Transgressive segregation is a rare phenomenon that occurs as a result of a unique recombination of alleles from both parents (Guzmán-Maldonado et al., 2003). These alleles can be additive or complementary in nature or could have epistatic effects between them. The IBLs having lower damage scores than the resistant parent may have also inherited resistant alleles from the recurrent parent which are not normally obvious in the genetic background of Matterhorn. In addition, overdominance caused by heterozygosity at specific loci can also lead to transgressive segregation. Although the IBL population was a $BC_1F_{4:8}$ population, some individuals were observed to be segregating for certain traits, such as flower color, and therefore overdominance could be a contributing factor in the LB and LC scores outside of the range of the parent genotypes.

Only P08125 had lower LC scores than EMP507 in both MI and PR. No individuals were identified that had lower LB scores than EMP507 in both environments. The fact that so few demonstrated extreme resistance to both leafhopper species may be as a result of the small population size. G08160 had both lower LC and lower LB scores than the recurrent parent when tested against *E. fabae*, but not when tested against *E. kraemeri*. In contrast, G08128 had both

lower LC and LB scores than EMP507 when tested against *E. kraemeri*, but not against *E. fabae*. These results suggest that resistance to each species may be controlled by separate mechanisms. Among the susceptible IBLs identified in PR, only G08113 was also identified in the 10 most susceptible in MI.

Overall, in both MI and PR, negative transgressive segregation was more common than positive transgressive segregation. Since the recurrent parent demonstrated moderate tolerance to both *E. kraemeri* and *E. fabae*, this also supports the possibility that Matterhorn and EMP507 each have unique alleles that have some effect on leafhopper feeding damage. This is not surprising as Matterhorn was developed from the cross WM1-85-56/2*Sierra/3/WM1-85-45//Sierra/P86241 (Kelly et al., 1999), and Sierra is a local pinto variety that has previously been documented as having resistance to *E. fabae* (Gonzales, et al., 2004). In addition, although both parents are members of the Middle American gene pool, they have distinct origins, with Matterhorn developed for adaptation to temperate climates and EMP507 developed with tropical germplasm from Brazil and Colombia.

The IBL damage scores were compared between the two environments and species by using SD from the mean, because LB mean values were very low in MI in comparison to PR. P08175 and P08142 were found to be very resistant in both environments, while G08107 and G08109 were very susceptible in both environments. These IBLs may have alleles that confer either susceptibility or resistance to both leafhopper species. In total, 16 IBLs were identified as resistant in both MI and PR, i.e. having LC and LB scores greater than one SD less than the mean, and 14 were identified as susceptible in both locations. The remaining 45 IBLs had inconsistent reactions to leafhopper feeding in each location, supporting the case that LC damage and LB damage are controlled by different genetic mechanisms. This finding also suggests that

resistance to each *Empoasca* species is controlled by different mechanisms. Those IBLs conferring resistance to both damage mechanisms and both species may be the best candidates for introducing broad resistance to *Empoasca* leafhoppers into future bean varieties.

C. Empoasca Nymph Counts

Nymph counts were included in this study because low insect population number can be indicative of antibiosis or antixenosis. Nymphs were counted in the place of adult *Empoasca* leafhoppers due to the lower mobility of nymphs in comparison to the winged adults which could not be counted within the limitations of this research.

Empoasca kraemeri nymph counts were significantly lower than *E. fabae* counts in all years. This is surprising as *E. kraemeri* is a non-migratory pest and it was expected that since the insect population would be present in the environment already, high pressure from large numbers of individuals would occur. One explanation is that *E. kraemeri* has lower fecundity *than E. fabae*, i.e. the tropical leafhopper lays fewer eggs than its temperate counterpart (Wilde et al., 1976). Therefore, it may not be appropriate to compare nymph populations between the different *Empoasca* species. This species difference presented a challenge in identifying resistant individual IBLs with respect to nymph counts alone. In addition, significant environmental variation occurred from year to year within each location. Therefore, resistant individuals were identified as those having mean nymph counts more than one standard deviation below the mean in each location.

i. Puerto Rico

Transgressive segregation was also found to occur within the IBL population for nymph counts for both species. The IBLs with the 10 lowest nymph counts in PR (Table 2. 8) had lower mean nymph counts than EMP507. This was consistent in both 2010 and 2011. In addition, the 5

IBLs with the highest nymph counts had higher mean counts than the recurrent parent in both years. These five IBLs also had higher nymph counts than the susceptible check Othello. From the positive transgressive segregants that were identified for their extreme low LC scores, P08120 and P08161 also fell in the lowest 10 mean nymph counts (Table 2. 14). P08175 and P08151 had been identified as falling below the range of the parents for LB values and were also identified as having low nymph counts outside of the parental genotype range. Those individual with low nymph counts and low damage scores may harbor antibiosis or antixenosis resistance to *E. kraemeri* by negatively affecting *E. kraemeri* biology in the case of antibiosis or by providing an unattractive environment for feeding or oviposition in the case of antixenosis.

ii. Michigan

In MI, the IBLs with the lowest mean nymph counts all fell below the test mean and the mean for EMP507 (Table 2. 11). Five of the ten IBLs also had lower counts than EMP507 in all three years. Of the individuals with the 10 lowest *E. fabae* nymph counts, P08153 and P08135 were also identified in 10 most resistant IBLs for LC, and P08172, P08169, and G08119 were also identified in 10 most resistant IBLs for LB. Four of the 10 IBLs with the highest *E. fabae* nymph counts also had the highest mean LC and LB scores in MI: G08102, G08174, G08127, and G08165 (Table 2. 15). Those individuals that have low damage scores and low nymph counts may be resistant to *E. fabae* through antibiosis or antixenosis mechanisms while those individuals having both high damage scores and high nymph populations may be highly attractive to *E. fabae*, therefore attracting higher numbers for feeding or by providing a superior environment for oviposition and nymph development.

iii. Conclusions

By examining both resistance categories and nymph counts, it may be possible to further test the relationship between the host plant genotype and the pest. When IBLs from each resistance category were separated by their nymph counts, individuals were noted as having nymph counts greater than 1 SD less than the mean in almost all resistant categories in each location. In PR, P08151, P08161, and P08175 were found to be both very resistant with low nymph counts. In MI, P08153 and P08166 were classified as very resistant and had very low nymph counts. While these individuals did not overlap between locations, P08120 was identified in the resistant category and had significantly lower nymph counts than the mean in both locations (Table 2. 14, Table 2. 15). Antibiosis or antixenosis may be involved in the resistance demonstrated in each of these individuals. G08103 also overlapped categories and nymph counts in both locations but this IBL was deemed moderately susceptible even with very low *Empoasca* populations. Of those IBLs having nymph counts more than one SD greater than the mean, G08171 and G08165 were the only IBLs to be retained in the resistant classes in MI and PR respectively. Tolerance may play a role in both of these genotypes. Inverse resistance individuals did not assist in determining what mechanisms may be related to resistance of either LC or LB damage with respect to nymph population numbers. For example, if the IBLs categorized as LC resistant (i.e. LB susceptible) had had consistently lower nymph counts than the LB resistant (i.e. LC susceptible) IBLs, it may have suggested that LB resistance is more likely to involve tolerance while LC resistance may be more likely to involve antibiosis or antixenosis. However, this phenomenon was not evident in the choice test results.

Many more IBLs had lower nymph counts in each category in MI than in PR. In addition, particular, more IBLs had very low nymph counts in each resistant class in MI than in PR. Only

five IBLs in the resistant to very resistant categories had nymph counts greater than one SD below the mean in PR. This lack of correlations between *E. kraemeri* nymph counts and visual injury scores was also previously reported by Kornegay and Cardona (1990). These findings suggest that resistance to *E. fabae* may involve antixenosis or antibiosis mechanisms while resistance to *E. kraemeri* may be more likely to involve tolerance. In addition, Schoonhoven et al. (1978) also found that tolerance to *E. kraemeri* was more common than antixenosis in common bean.

D. No-Choice Tests

Both LC and LB were highly heritable under no-choice conditions but LB was found to have a much higher coefficient of variation than LC. There were also significant environmental effects between years. LB may be more sensitive than LC to environmental differences such as temperature and humidity. The test did not have significant effects on either the mean LC or LB damage scores for population, however individual IBLs did differ significantly under the different test conditions, thus allowing further insight into the mechanisms involved in resistance to *E. fabae* in the Matterhorn*/EMP507 IBL population.

i. Leaf Curl

The IBLs having the ten lowest and five highest LC scores under no-choice conditions are listed in Table 2. 20. Five of the most resistant IBLs under no-choice conditions were also identified as having the lowest LC scores under choice-conditions: P08142, P08166, G08128, G08160 and P08125 (Table 2. 9). Because of the similar IBL responses within each test, it can be suggested that these five IBLs are very tolerant of *E. fabae* feeding with respect to LC damage. Four of the five IBLs with the highest LC damage scores under no-choice testing also had the

highest LC values under choice conditions indicating that the no-choice test was able to accurately identify susceptible individuals.

When individual genotypes were directly compared, seven IBLs were identified that had significantly different responses under no-choice test conditions than under choice test conditions. Four individuals had higher LC scores under no-choice conditions: P08135, G08152, G08168 and P08175. P08175 is particularly interesting as this IBL ranked as one of the most resistant to LC under choice tests with a choice LC mean of 0.33 but a no-choice LC mean of 2.40. Given that under no-choice conditions, leafhoppers cannot make preference decisions whether or not to feed on a particular genotype; it can be assumed that these IBLs have lower LC scores under choice conditions due to antixenosis (non-preference) mechanisms.

The other three IBLs with significantly different responses with respect to LC all had lower LC scores under no-choice conditions versus choice conditions: P08120, G08107 and G08123. Given that each genotype was caged and inoculated with the same number of adult leafhoppers and that no additional leafhoppers should have been able to enter the cage following inoculation, it was assumed that *E. fabae* adults were not able to maintain a sufficient population on these genotypes to continue to cause LC damage. In addition, of these three IBLs, G08120 had a LC score of less than the mean in choice tests but G08107 and G08123 presented LC damage greater than the choice test mean. G08107 and G08123 may also have some antibiotic effects on *E. kraemeri* and *E. fabae* as they also had significantly lower LC damage under no-choice conditions. However, G08107 was considered only LB resistant in MI and PR, and G08123 was moderately resistant in PR but only LB resistant in MI. And while nymph counts were more than 1 SD below the mean in both locations, the resistance present in these IBLs may

not be sufficient to overcome damage as a result of additional incoming leafhoppers which will occur in an open choice system.

In addition, while ideally no *E. fabae* adults could either enter or leave the cages once they were inoculated, the system was not perfect and there exists a possibility of escapes as some cages did open briefly due to storm winds during each field season. These instances could have provided an opportunity for the inoculated leafhoppers to escape or for additional leafhoppers to enter the caged system.

ii. Leaf Burn

Leaf burn damage under no-choice conditions differed significantly from choice test results. Twenty-four IBLs had significantly different LB scores under no-choice test conditions than choice test conditions. In each case, no-choice LB scores were higher than choice test LB values. Seven individual genotypes had LB scores less than the test mean ($\overline{LB}$ = 0.95) under choice conditions but higher than the test mean ($\overline{LB}$ = 1.83) under no-choice conditions: G08101, G08122, G08124, G08134, G08147, G08159 and P08175. P08175 has already been implicated as having antixenosis resistance based on LC scores.

While it is unknown why consistently higher LB damage was seen in the cages, it is suspected that LB may be more susceptible to higher temperatures or humidity as the cages likely maintained higher humidity levels due to reduced air circulation. However, there is no empirical evidence to support this suspicion as temperature and humidity levels within the cages were not recorded.

iii. Conclusions

Despite the small size of the IBL Matterhorn*/EMP507 population, field screening for *Empoasca fabae* and *E. kraemeri* feeding damage supports the finding that all three major

mechanisms of resistance are involved in *P. vulgaris* resistance to these major pest species. Extreme tolerance to both species is demonstrated by P08142 which was classified as very resistant in choice tests in both MI and PR and had very low LC and LB scores in each year under no-choice conditions. In addition, nymph counts for P08142 were within 1 SD of the mean suggesting that this genotype did not have a significant antibiotic impact on *E. fabae.*

Evidence of antixenosis is suggested by P08175 as this IBL ranked as one of the most resistant under choice test conditions with nymph counts more than 1 SD less than the test mean, but P08175 had significantly higher levels of both LC and LB in the no-choice test, especially LB damage, which was considered a susceptible level of damage under no-choice conditions. Together, these findings indicate that *E. fabae* may avoid feeding or laying eggs on P08175, but if there is no alternative, P08175 is an acceptable host for *E. fabae.* In addition, the fact that P08175 is the only IBL that was identified as having both LC and LB damage that was significantly different under the two test conditions further reinforces that LC and LB damage are separate trait responses.

Antibiosis may be involved in the resistance demonstrated by P08120. This IBL ranked as resistant in choice tests in both PR and MI with nymph counts falling more than 1 SD below the mean for both *Empoasca* species. However, P08120 ranked as the most resistant IBL with respect to LC damage under no-choice conditions in every year and the actual level of LC damage was significantly lower under no-choice conditions than under choice conditions. These results when considered together suggest that P08120 may have deleterious effects on *Empoasca* biology, limiting the pest's survival.

E. Agronomic Traits

Agronomic traits measured in the Matterhorn*/EMP507 IBL population had been observed in the field as being skewed towards the donor parent, EMP507, such as the particular loss of the upright type II growth habit of the Matterhorn parent (Kelly et al., 1999) in the IBLs, accompanied by later maturity. However, seed traits appeared skewed towards the recurrent parent, Matterhorn. In the case of seed coat color, which has been co-localized to a QTL for *Empoasca* resistance in previous studies (Murray et al., 2004), 59 IBLs had white seeds and 16 had colored seeds. Because the presence of the *P* allele conditioning color in the seed coat is dominant, this result is contrary to the expected 3:1 ratio of colored seed coat to white seed coat. As mentioned previously, Matterhorn was derived from a predominantly pinto background and, as such, the pinto seed coat pattern is present in Matterhorn but masked by the presence of the epistatic *p* allele. Also noteworthy is that among the 16 IBLs with colored seeds, none displayed the carioca seed coat pattern of EMP507. Fifteen individuals displayed a pinto seed type and a single individual was segregating for multiple seed patterns, none of which were carioca. These findings suggest that there may be a genetic incompatibility which is preventing transmission of the carioca seed pattern into the population despite the clear introgression of agronomic traits of the EMP507 parent in the IBL lines.

Among only 16 IBLs with colored seeds, ten were classified as VR and R in PR and 12 were classified as VR and R in MI. This finding suggests that the presence of seed coat color may either be involved in resistance mechanisms as pigment components can be metabolically active compounds. In addition, leaf color, which is related to seed coat color, may play a role in resistance to *Empoasca* leafhoppers (see Appendix A). Since the presence of color in the seed coat is tightly linked to *Empoasca* resistance, as previously demonstrated by Murray et al.

(2004b), this observation could simply be an artifact of this tight linkage. If such a linkage exists, it could present a challenge in developing highly resistant white seeded cultivars.

F. Conclusions

In summary, the examination of resistance to *Empoasca* species undertaken in this study has found that multiple resistance mechanisms, including tolerance, antixenosis and antibiosis, may be functional in the Matterhorn*/EMP507 population. While previous studies have demonstrated tolerance and antixenosis, this is the first study to suggest antibiosis as a possible resistance mechanism in common bean against *Empoasca* leafhoppers. Individual IBLs identified in this study can be used to introduce resistance to both *E. kraemeri* and *E. fabae* into bean breeding programs. By increasing levels of genetic resistance using multiple mechanisms, bean growers in tropical and temperate regions of the Americas could potentially reduce costly pesticide application without compromising bean yields.

Literature Cited

Literature Cited

Adam-Blondon, A.F., Sevignac, M., Dron, M., and Bannerot, H. 1994. A genetic map of common bean to localize specific resistance genes against anthracnose. Genome. 37: 915-24.

Backus, E.A., Serano, M.S., and Ranger, C.M. 2005. Mechanisms of hopperburn: An overview of insect taxonomy, behavior, and physiology. Annu. Rev. Entomol. 50: 125-151.

Baker, J.R, Beshear, R., Carter, C., Day, E., Grant J., Hamon A.B., Horn, K.F., Liu, T.X., Oetting, R.D., Pollet, D.K., Schultz, P.B., Sparks, B., Staines, C.L., Stephen, and D.L., Williams, M.L., 2008. Insect and Related Pests of Flowers and Foliage Plants: Potato Leafhopper. North Carolina Cooperative Extension Service. Accessed: Oct 2012. [http://ipm.ncsu.edu/AG136/leafhop1.html]

Broughton, W.J., Hernandez, G., Blair, M., Beebe, S., Gepts, P., and Vanderleyden, J. 2003. Beans (*Phaseolus* spp.) – model food legumes. Plant and Soil. 252: 55-128.

Bullas-Appleton, E.S., Gillard, C., Otis, G., and Schaafsma, A.W. 2004. Potato leafhopper (Homoptera: Cicadellidae) varietal preferences in edible beans in relation to visual and olfactory cues. Enviro. Entomol. 33: 1381-1388.

Bullas-Appleton E.S., Gillard C, and Schaafsma A.W. 2005 Aggregation of potato leafhoppers, *Empoasca fabae* (Harris) (Homoptera: Cicadellidae), on a trap crop in an edible bean, *Phaseolus vulgaris* L., intercropping system. Can. J. Plant Sci. 85: 237-242.

Calderon, J.D. and Backus, E.A. 1992. Comparison of the probing behaviors of *Empoasca fabae* and *E. kraemeri* (Homoptera: Cicadellidae) on resistant and susceptible cultivars of common beans. J. Econ. Entomol. 85: 88-99.

Cardona, C. Posso, C.E., Kornegay, J., Valor, and J., Serrano, M. 1989. Antibiosis effects of wild dry bean accessions on the Mexican bean weevil and the bean weevil (Coleoptera: Bruchidae). J. Econ. Entomol. 82: 310-315.

Cardona, C., Frei, A., Bueno, J.M., Diaz, J., Gu, H., and Dorn, S. 2002. Resistance to *Thrips palmi* (Thysanoptera: Thripidae) in beans. J. Econ. Entomol. 95: 1066-1073.

Carmona, D., Lajeunesse, M.J., and Johnson, M.T.J. 2011. Plant traits that predict resistance to herbivores. Funct. Ecol. 25: 358–367.

CIAT (Centro Internacional de Agricultura Tropical). 1987. Standard system for the evaluation of bean germplasm. Schoonhoven, A. van and M.A. Pastor-Corrales (compilers). Cali, Colombia. 54 p.

Dietrich, C. H. 2008. Leafhopper Homepage. Section for Biodiversity, Illinois Natural History Survey, Champaign, IL. Revised: March 2008. Accessed Oct 2012. [http://www.inhs.uiuc.edu/~dietrich/lfhFAQ.html]

Emmen, D.A., Fleischer, S. J., and Hower, A. 2004. Temporal and spatial dynamics of *Empoasca fabae* (Harris) (Homoptera: Cicadellidae) in alfalfa. Enviro. Entomol. 33: 890-899.

Godfrey, L. D. and Long R.F., 2007. UC Pest Management Guidelines. Dry Bean – Empoasca Leafhoppers. University of California – Agriculture and Natural Resources. Retrieved October 2012, from [http://www.ipm.ucdavis.edu/PMG/r52301611.html]

Gonzales, J.W., Coyne, D.P., Lindgren, D.T., and Jung, G. 2001. Inheritance and heritability of leafhopper resistance in common beans (*Phaseolus vulgaris* L.). Annu. Rep. Bean Improv. Coop. 44: 139-140.

Gonzales, J.W., Coyne, D.P., and Lindgren, D.T. 2002. Inheritance and heritability of leafhopper resistance in common beans (*Phaseolus vulgaris* L.). Annu. Rep. Bean Improv. Coop. 45: 80-81.

Gonzales, J.W., Coyne, D.P., Lindgren, D.T., Schaaf, D., and Eskridge, K.M. 2004. Heritability of the resistance to potato leafhopper in dry bean. HortSci. 39: 1578-1580.

Guzmán-Maldonado, S.H., Martínez, O., Acosta-Gallegos, J.A., Guevara-Lara F., and Paredes-López, O. 2003. Putative quantitative trait loci for physical and chemical components of common bean. Crop Sci. 43: 1029-1035.

Hall, R. 1991. The bean plant. P 1-5 *In* R. Hall (ed). Compendium of bean diseases. APS Press. Saint Paul, Minnesota.

Hallauer, A.R. and Miranda Filho, J.B. 1981. Quantitative genetics in maize breeding. Iowa State University Press, South State Ave., Ames, IA 50010. 468 p.

Kelly, J.D., Hosfield, G.L., Varner, G.V., Uebersax M.A., and Taylor, J. 1999. Registration of 'Matterhorn' great northern bean. Crop Sci. 39: 589-590.

Kabrick, L.R. and Backus, E.A. 1990. Salivary deposits and plant damage associated with specific probing behaviors of the potato leafhopper, *Empoasca fabae*, on alfalfa stems. Entomol. Exper. Appl.56: 287-304.

Kornegay, J.L., Cardona, C., Van Esch, J., and Alvarado, M. 1989. Identification of common bean lines with ovipositional resistance to *Empoasca kraemeri* (Homoptera: Cicadellidae). J. Econ. Entomol. 82: 649-654.

Kornegay, J.L. and Cardona, C. 1990. Development of an appropriate breeding scheme for tolerance to *Empoasca kraemeri* in common bean. Euphytica 47: 223-231.

Kornegay, J.L. and Temple, S.R. 1986. Inheritance and combining ability of leafhopper defense mechanisms in common bean. Crop Sci. 26: 1153-1158.

Lindgren, D.T. and Coyne, D.P. 1995. Injury and yield of leafhopper-infested dry beans. J. Amer. Soc. Hort. Sci. 120: 839-842.

Miklas, P.N., Kelly, J.D., Beebe, S.E., and Blair, M.W. 2006. Common bean breeding for resistance against biotic and abiotic stresses: From classical to MAS breeding. Euphytica 147: 105-131.

Murray, J.D., Michaels, T.E., Pauls, K.P., and Schaafsma, A.W. 2001. Determination of traits associated with leafhopper (*Empoasca fabae* and *Empoasca kraemeri*) resistance and dissection of leafhopper damage symptoms in common bean (*Phaseolus vulgaris*). Ann. App. Bio. 139: 319-327.

Murray, J.D., Michaels, T.E., Pauls, K.P., Cardona, C., and Schaafsma, A.W. 2004a. Yield and insect injury in leafhopper (*Empoasca fabae* Harris and *Empoasca krameri* Ross and Moore) infested dry beans in Ontario and Colombia. Can. J. Plant Sci. 84: 891-900.

Murray, J.D., Michaels, T.E., Cardona, C., Schaafsma, A.W., and Pauls, K.P. 2004b. Quantitative trait loci for leafhopper (*Empoasca fabae* and *Empoasca kraemeri*) resistance and seed weight in the common bean. Plant Breed. 123: 474-479.

Nodari, R.O., Koinange, E.M.K., Kelly, J.D., and Gepts, P. 1992. Towards an integrated linkage map of common bean. I. Development of genomic DNA probes and levels of restriction fragment length polymorphism. Theor. App. Genet. 84: 186-192.

Ojwang, P.P.O., Melis, R., Githiri, M.S., and Songa, J.M. 2011. Genetic analysis for resistance to bean fly (*Ophiomyia phaseoli*) and seed yield among common bean genotypes in a semi-arid environment. Field Crop Research. 120: 223-229.

Pillemer, E.A. and Tingey, W.M. 1978. Hooked trichomes and resistance of *Phaseolus vulgaris* to *Empoasca fabae* (Harris). Entomol. Exp. Appl. 24: 83-94.

Ranger, C.M., Backus, E.A., Winter, R.E.K., Rottinghaus, G.E., Ellersieck, M.R., and Johnson, D.W. 2004. Glandular trichome extracts from *Medicago sativa* deter settling by the potato leafhopper *Empoasca fabae*. J. Chem. Ecol. 30: 927-943.

Saxena, K.N. 1969. Patterns of insect-plant relationships determining susceptibility or resistance of different plants to an insect. Entomol. Exp. Appl. 12: 751-766.

Schaafsma, A.W., Cardona, C., Kornegay, J.L., Wylde, A.M., and Michaels, T.E., 1998. Resistance of common bean lines to the potato leafhopper (Homoptera: Cicadellidae). J. Econ. Entomol. 91: 981-986.

Schoonhoven, A. van, Gomez, L.A., and Avalos, F. 1978. The influence of leafhopper (*Empoasca kraemeri*) attack during various bean (*Phaseolus vulgaris*) plant growth stages on seed yield. Entomol. Exp. Appl. 23: 115-120.

Serrano, M.S., and Backus, E. 1998. Differences in cellular abnormalities induced by the probing behaviors of *Empoasca kraemeri* (Homoptera: Cicadellidae) on tolerant and susceptible common beans. J. Econ. Entomol. 91: 1481-1491.

Serrano, M.S., Backus, E.A., and Cardona, C. 2000. Comparison of AC electronic monitoring and field data for estimating tolerance to *Empoasca kraemeri* (Homoptera: Cicadellidae) in common bean genotypes. J. Econ. Entomol. 93: 1796-1809.

Singh, S.P. 1982. A key for identification of different growth habits of *Phaseolus vulgaris* L. Annu. Rept. Bean Improv. Coop. 25: 92-94.

Singh, S.P. 1999. *Integrated Genetic Improvement*. In *Common Bean Improvement in the 21st Century*. Kluwer Academic Press, Norwll, MA. p.133-165.

Singh, S.P. and Schwartz, H.F. 2011. Review: Breeding common bean for resistance to insect pests and nematodes. Can. J. Plant Sci. 91: 239-250.

Smith, M.C. 2005. *Plant Resistance to Arthropods*. Springer Publishing, Dordrecht, The Netherlands. 423 p.

Teetes, G.L., 1996. Plant resistance to insects: a fundamental component of IPM. In *Radcliffe's IPM World Textbook*. Retrieved Oct 2012. [http://ipmworld.umn.edu/chapters/teetes.htm]

Temple, S.R., Cardona, C., Van Schoonhoven, A., and Galwey, N. 1982. Advances in breeding for resistance to the leafhopper *Empoasca kraemeri* in dry beans (*Phaseolus vulgaris* L.) Annu. Rept. Bean Improv. Coop. 25: 34-35.

Vallejos, C.E., Sakiyama, N.S., and Chase, C.D. 1992. A molecular marker based linkage map of *Phaseolus vulgaris* L. Genetics. 131: 733-740.

Vasemägi, A., Gross, R., Palm, D., Paaver, T., and Primmer, C.R. 2010. Discovery and application of insertion-deletion (INDEL) polymorphisms for QTL mapping of early life-history traits in Atlantic salmon. BMC Genomics 11: 156.

Wilde, G., Schoonhoven, A. van, and Gomez-Laverde, L. 1976. The biology of *Empoasca kraemeri* on *Phaseolus vulgaris.* Ann. Entomol. Soc. Amer. 69: 442-444.

Yu, K., Park, S.J., Poysa, V., and Gepts, P. 2000. Integration of simple sequence repeat (SSR) markers into a molecular linkage map of common bean (*Phaeseolus vulgaris* L.). J. Heredity 91: 429-434.

CHAPTER 3: Identification of QTL for Resistance to *Empoasca kraemeri* and *Empoasca fabae* in an Inbred Backcross Line Population in Common Bean

Abstract

By

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A QTL study examining resistance to leafhopper species *Empoasca fabae* and *E. kraemeri* was conducted using a Matterhorn*/EMP507dry bean inbred backcross line (IBL) population, where Matterhorn is the recurrent parent and EMP507 is the resistant donor parent. The IBL population was evaluated for *Empoasca* resistance in Michigan and Puerto Rico in 2009-2011 by examining damage traits and *Empoasca spp.* nymph counts.

The goal of this research was to identify QTL associated with these traits and resistance to both tropical and temperate *Empoasca* species, as well as to verify existing QTL for *Empoasca* resistance. Twelve QTL associated with resistance to *E. fabae* and *E. kraemeri* were identified on Pv02, Pv03, Pv06, Pv07, Pv08 and Pv09 explaining from 22.8 % to 61.5 % of the total phenotypic variation trait. A major QTL (LH7.1$^{BE, ME}$) was associated with multiple traits and was detected for both leafhopper species in multiple seasons. This QTL was tightly linked to the *P* gene that confers the presence of color in the seed coat and may be related to antixenosis. A novel QTL for *E. fabae* nymph counts was identified on Pv02 that may be associated with antibiosis resistance. QTL for each species and each trait co-localized in few regions, suggesting resistance to each species and each trait is controlled by separate genetic mechanisms in common bean.

I. Introduction

The status of resistance in common bean to many insect pests, including bean pod weevil, bruchids, leafhoppers, thrips and nematodes was recently reviewed by Singh and Schwartz (2011). There has been significant progress in breeding for resistance to some pests such as bruchids, but resistance to others, such as nematodes, is still elusive. The development of insect-resistant cultivars can have a significant economic and health impact for common bean growers globally, especially in developing countries where subsistence agriculture is common and growers rely on common bean production for income and sustenance (Ojwang' et al., 2011). In particular, insect-resistant cultivars can minimize yield loss while maintaining crop quality, reduce pesticide use and minimize risks to health and the environment, and lower production costs so profitability and competitiveness are improved (Singh and Schwartz, 2011).

A. Mapping Insect Resistance in Common Bean

The development of resistant cultivars can be greatly enhanced by using molecular markers to locate and map quantitative trait loci (QTL) involved in resistance (Kelly and Vallejo, 2005), as well as by understanding the nature of the resistance involved, such as tolerance, antibiosis, or antixenosis (Smith, 2005). In addition, by locating resistance loci in relation to other QTL for traits controlling physiological processes or plant morphology, the interaction between resistance and these traits has been confirmed (Kelly and Vallejo, 2005).

Resistance mechanisms are often not isolated and plants may use different strategies against the same pest, as is case for *Thrips palmi* resistance. Frei et al. (2003, 2004) reported the presence of tolerance, antixenosis, and antibiosis in resistant common bean germplasm. QTL for *Thrips* resistance have been subsequently been identified on bean chromosomes Pv02, Pv03, Pv06 and Pv08 (Frei et al., 2005).

Resistance to the bean pod weevil, *Apion godmandi*, has been linked to two epistatic genes: the *Agr* allele, which confers moderate resistance on its own, and the *Agm* allele, which alone has no effect, but increases the level of resistance substantially in the presence of *Agr* (Garza et al., 1996). The resistance conferred by these epistatic genes is associated with ovipositional non-preference (antixenosis) and antibiosis in the form of hypersensitivity, the only insect-related hypersensitive response reported in common bean (Garza et al., 1996). These genes were mapped to the genetic bean map with *Agr* residing on Pv01 and *Agm* on Pv07 (Blair et al., 2006c).

Antibiosis has also been reported to be involved in resistance to the bruchid pests *Acanthoscelides obtectus* (bean seed weevil) and *Zabrotes subfasciatus* (Mexican bean weevil) (Cardona et al., 1989). This resistance was first detected in wild bean accessions, associated with the lectin-like protein arcelin (Osborn et al., 1988). Since the initial studies, it has been determined that multiple genes and multiple alleles make up the arcelin, α-amylase inhibitors and phytohemagglutinins (APA) locus, which was mapped to Pv04 (Blair et al., 2003). Recently, a novel arcelin-like protein (*ARL2*) was identified in tepary beans (*P. acutifolius*) and successfully introgressed into common bean providing superior resistance to bruchid pests (Kusolwa and Myers, 2011).

Recent studies have looked at enhancing resistance in common bean to bean fly (*Ophiomyia* spp), an important pest of African common bean production. Physical and chemical attributes are also often credited with contributing to resistance to insect pests. Volatile compounds released when bean plants are injured by bean flies may be involved in antixenosis (Wei et al., 2006). The genetic basis of resistance to this pest is not yet understood and therefore developing resistant cultivars is challenging (Ojwang' et al., 2011).

B. Mapping *Empoasca* Resistance in Common Bean

Empoasca resistance has been reported to be quantitatively inherited with low heritability, making it an excellent candidate for marker-assisted selection (MAS) as expression of *Empoasca* injury in the field is subject to strong environmental effects and other confounding factors (Gonzales et al., 2004). Previous studies have identified QTL associated with resistance in common bean to both *E. kraemeri* and *E. fabae* (Murray et al., 2004b). Major QTL were identified on Pv01, Pv03 and Pv07. The QTL on Pv01 (LH1.1 ^{BE}) was detected for both *E. kraemeri* and *E. fabae* resistance and was linked to the *fin* locus for determinacy. Because the RIL population (Berna/EMP419) used by Murray et al. (2004b) was segregating for growth habit, this QTL may in fact be an artifact of growth habit differences in the population and not related directly to leafhopper resistance. Numerous agronomic traits have been linked to potential antixenosis resistance mechanisms in beans, including indeterminate growth habit, days to flowering, leaf pubescence and trichome density (Pillemer and Tingey, 1976). However, since these initial studies, leaf pubescence and trichome density have not been demonstrated to have a significant effect on resistance to *Empoasca* species (Schaafsma et al., 1998).

Resistance to LC and LB damage for both *Empoasca* leafhoppers was found to be controlled by a major QTL on Pv07 (LH7.1^{BE}). This QTL was found to be tightly linked to seed coat color at the *P* locus (Murray et al., 2004b). Color genes have been linked to other resistance loci. For example, the color-intensifying B allele is linked with the *I* gene for bean common mosaic virus (BCMV) resistance on Pv02, and the V locus on Pv06 is linked to common bacterial blight (CBB) resistance. These findings suggest that genes involved in pigmentation and related pathways may also be involved in plant defense mechanisms. Additionally, seed coat color and leaf color have been noted to influence *Empoasca* preferences (Bullas-Appleton et al.,

2004). Together, these traits may contribute to resistance to both *Empoasca* species seen in common bean. Murray et al. (2004b) identified two other QTL for *Empoasca* resistance. On Pv03, they identified a QTL for *E. kraemeri* LC damage and a fourth QTL for *E. kraemeri* LC damage that could not be located on the common bean genetic linkage map (Murray et al., 2004b).

Multiple clusters of resistance genes and QTL have been located in common bean (Kelly et al., 2003). While the majority of the genes identified in these clusters are involved in disease resistance, it is possible that quantitative insect resistance traits could also cluster. Additionally, QTL for resistance have also co-localized with morphological traits, such as the *fin* and *P* genes mentioned previously. There are generally two kinds of co-localized QTL – those that map with major genes and those that map with defense response genes (Kelly and Vallejo, 2005). Given the polygenic nature of most insect resistance QTL, it is more likely that colocalized QTL for resistance to different insect pests are involved in some kind of general defense mechanism versus species-specific resistance genes.

II. Study Objectives

The goal of this investigation was to identify quantitative trait loci associated with resistance to both tropical and temperate *Empoasca* species, as well as to verify existing QTL for *Empoasca* resistance in an IBL population with the same growth habit. Molecular markers associated with these QTL can then be used by breeders to incorporate leafhopper resistance into bean germplasm, thereby providing protection against crop losses due to these damaging pests.

III. Materials and Methods

A. Plant Material

The *P. vulgaris* population examined in this study was developed from a single cross Matterhorn/EMP507 followed by a single backcross to Matterhorn to create an inbred backcross line (IBL) population consisting of 75 $BC_1F_{4:8}$ individuals. Matterhorn is a high-yielding commercially available great northern cultivar developed in Michigan (Kelly et al., 1999) with quality seed and desirable agronomic characteristics. EMP507 is a carioca germplasm line developed at the Centro International de Agricultura Tropical (CIAT) (Cardona et al., 1989) as part of a long-term recurrent selection program for resistance to *E. kraemeri* (Schaafsma et al., 1998). While these EMP lines were originally developed to be resistant to *E. kraemeri*, Schaafsma et al. (1998) demonstrated that the resistance is maintained under severe pressure to the temperate congener *E. fabae.* Both parental genotypes have a type II growth habit (Singh, 1982).

The crosses resulting in the IBL population were made by Dr. Tim Porch at the USDA-ARS-Tropical Agriculture Research Station, in Mayaguez, Puerto Rico. The F_1 was made in the greenhouse in 2005 and the BC_1F_1 backcross generation made in 2006 was selfed and advanced using single seed descent with no selection at the same location until the BC_1F_4 generation. BC_1F_4 seed was increased in 2008 and 2009 in the greenhouse and in the field in East Lansing, MI until sufficient quantities were obtained for field screening. Individual IBL were coded with G08 prefix if they possessed white great northern seed type or with P08 prefix if they possessed colored pinto bean seed type.

B. DNA Extraction and Isolation

The Matterhorn*/EMP507 IBL population and parents were grown in the greenhouse and young trifoliate leaves from three to four individuals per genotype were collected for DNA extraction. Total genomic DNA was extracted from leaf samples following a modified CTAB protocol (Haley et al., 1994). Isolated DNA was quantified using a flurometer (Hoeffer DyNA Quant 200, San Francisco, CA) and diluted to a working concentration of 40ng μl^{-1}. DNA was stored at -20°C.

C. Molecular Marker Analysis

Parent DNA was screened for polymorphic molecular markers including 369 simple sequence repeats (SSRs) and 152 InDels (Insertion-Deletions) (the author acknowledges receipt of Indel markers developed by the BeanCAP project at North Dakota State University, by S. Mafi Moghaddam and P. McClean). Markers that were identified as being polymorphic between Matterhorn and EMP507 were used to genotype the IBL population. Seed coat color was also included in the analysis as a phenotypic marker (*P* locus).

SSR marker amplification was conducted using the following PCR reaction for each genotype: 1.0 µl DNA [40ng μl^{-1}], 1.0 µl of (2mM) primer, 0.2 µl (1U) of Taq polymerase, 0.6 µl (50mM) $MgCl^2$, 2.0 µl (10x) PCR buffer, 0.8 µl of a 5mM mix of dNTPs, and 14.4 µl sterile distilled water. PCR was performed using a 96-well PTC-100 Programmable Thermal Controller (MJ Research Inc., Waltham, MA) programmed for 1 cycle of 5 minutes at 94°C, followed by 30 cycles of 1 minute at 94°C, 1 minute at 47°C, and 1 minute at 72°C, then final extension was conducted at 72°C for 5 minutes. To each PCR product, 8µl Formaldehyde loading buffer was added. Five µl of each mixed product was loaded onto a 6% acrylamide gel and separated via

electrophoresis at a constant power of 300 W for approximately 3 hours in 0.5% TE buffer. PCR products were visualized using the following staining protocol: fixed in solution of 210 ml 95% ethanol, 10 ml acetic acid and 1780 ml distilled water for 5 minutes, stained in solution of 2 g silver nitrate, 3 ml formaldehyde and 2 L distilled water for 7 minutes; and developed in solution of 3 ml formaldehyde, 30 g sodium hydroxide, and 2 L distilled water until bands appear.

InDel markers were amplified using the following PCR reaction for each genotype: 1 µl DNA [40ng μl^{-1}], 2 µl each forward and reverse primers, 10 µl GoTaq® Master Mix (Promega Corp., Madison, WI), and 5 µl distilled water. PCR was performed using a 96-well PTC-100 Programmable Thermal Controller (MJ Research Inc., Waltham, MA) programmed for 1 cycle at 95˚C for 3 minutes, 45 cycles of 95˚C for 20 seconds, 55˚C for 30 seconds and 72˚C for 1 minute, followed by a final extension cycle at 72˚C for 10 minutes. 5 µl of each PCR product was loaded on a 3% TBE agarose gel and separated by electrophoresis at a constant voltage of 100 V. PCR products were subsequently visualized under UV light after staining with ethidium bromide.

D. Linkage Mapping

Linkage analysis was performed on genotypic data using QTL IciMapping Version 3.2 (Wang, et al., 2012). The Kosambi mapping function was used, which assumes the existence of interference that is negatively related to recombination frequency. Molecular markers were anchored to linkage groups based on previous assignment on the common bean core map (Blair et al., 2003; Galeano et al., 2011). Grouping, ordering and rippling were conducted to divide the 107 markers into linkage groups, determine marker order and calculate the relative map positions. The logarithm of odds (LOD) threshold was set to a minimum of 3.0 and the recombination counting and ordering algorithm (RECORD) was used, which calculates the pair-

wise expected number of recombination events from genotyping data in a mapping population. Rippling was conducted using sum of adjacent recombination frequencies (SARF) with a window size of 5.0. Linkage groups were designated according to Pedrosa-Harand et al. (2012). Segregation distortion of molecular markers was analyzed using the SDL protocol of the QTL IciMapping Version 3.2 (Wang et al., 2012).

E. QTL Analysis

QTL analysis was performed using the mean for each trait in either environment across three seasons in MI and two seasons in PR, and separately for each individual environment using the mean for each IBL in the respective year. QTL IciMapping Software Version 3.2 was used to identify QTL for LC, LB and nymph counts in choice tests, and LC and LB in no-choice tests using the Biparental Population (BIP) protocol. Additive Inclusive Composite Interval Mapping (ICIM-Add) function was set to a window size of 1.0 with a probability in stepwise regression of 0.001. The LOD threshold was determined by 1000 permutations with Type I Error level set at $\alpha = 0.05$. Linkage maps and QTL were visualized using MapChart v2.2 (Voorips, 2002). QTL identified in this study were named according to the guidelines established by the Bean Improvement Cooperative (Miklas and Porch, 2010).

IV. Results

A. Linkage Map

A total of 370 SSRs and 150 InDels were screened for polymorphisms between the parent genotypes Matterhorn and EMP507. SSR and InDel markers were found to have polymorphisms rates of 32% and 27% polymorphic respectively. Four markers were deleted as they could not be mapped despite being polymorphic between the parent genotypes, or all IBLs were found to have only alleles from the recurrent parent. 105 molecular markers were placed on the Matterhorn*/EMP507 linkage map and divided among 12 linkage groups for a total map distance of 1386 cM (Figure 3.1). Each chromosome is represented by a single linkage group with the exception of chromosome Pv04, which is represented by linkage group Pv04 and Pv04b because InDel markers PT059 and PT103 could not be linked to other markers on Pv04. The number of markers on each linkage group ranged from two on Pv04b to 19 on Pv03. Limited coverage was observed of Pv01, Pv05 and Pv11 with the number of mapped loci being 3, 5, and 5 respectively.

B. Segregation Distortion

Segregation distortion was detected on 10 of 12 linkage groups. In total, 34 markers were detected with significant segregation distortion from expected Mendelian ratios of 1:3 for a $BC_1F_{4:8}$ population (Table 3. 1). Markers with distorted segregation towards recurrent parent Matterhorn were detected on chromosomes Pv02, Pv03, Pv04b, Pv05, Pv06, and Pv10. Regions of distortion towards the donor parent EMP507 were detected on chromosomes Pv02, Pv04, Pv07, and Pv11. In particular, all markers on Pv04 exhibited a skewed segregation towards the donor parent EMP507.

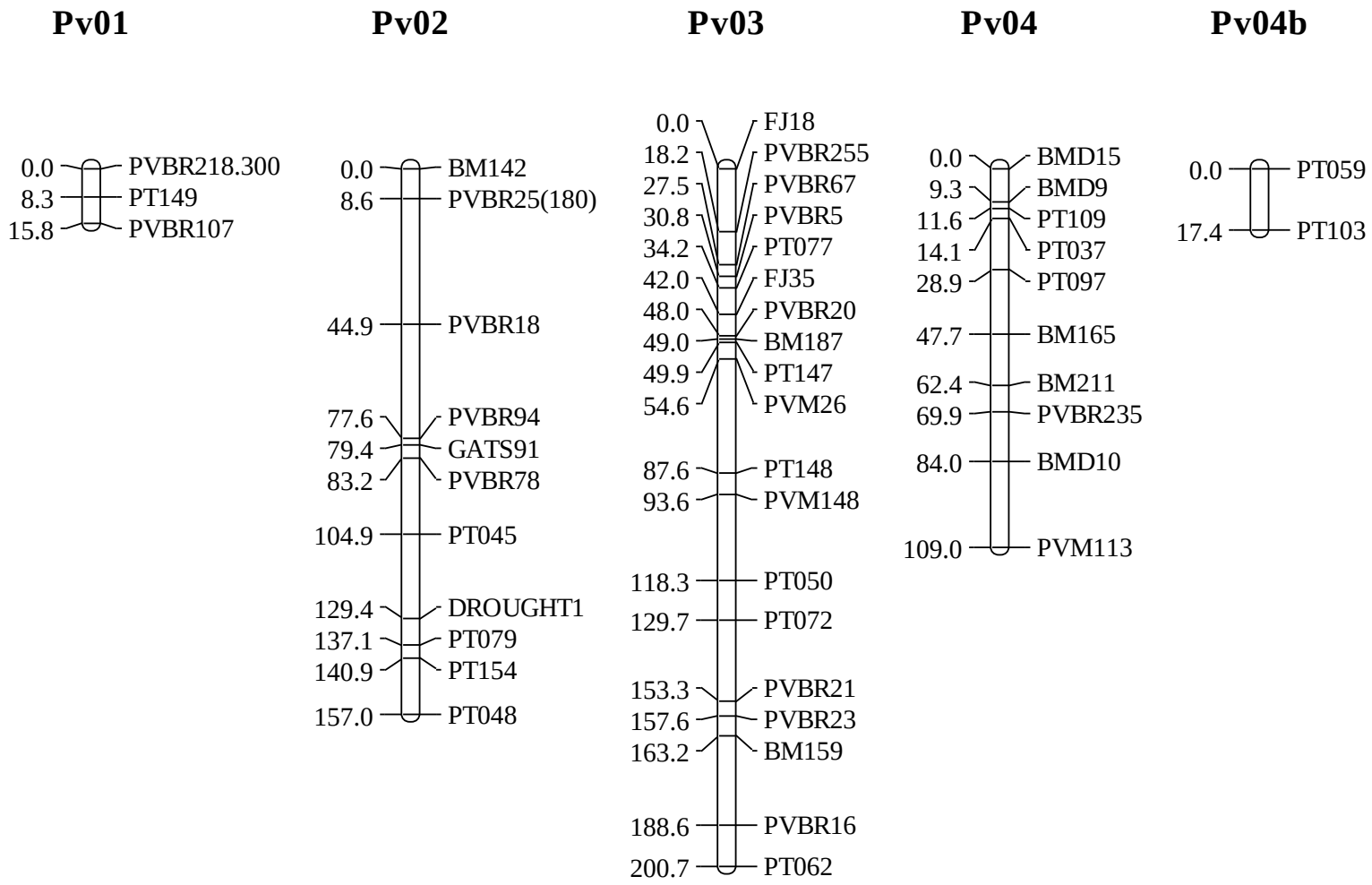

Figure 3. 1 Genetic linkage map of Matterhorn*/EMP507 IBL population. Mapped loci = 103. Linkage groups: 12. Total map distance: 1386 cM.

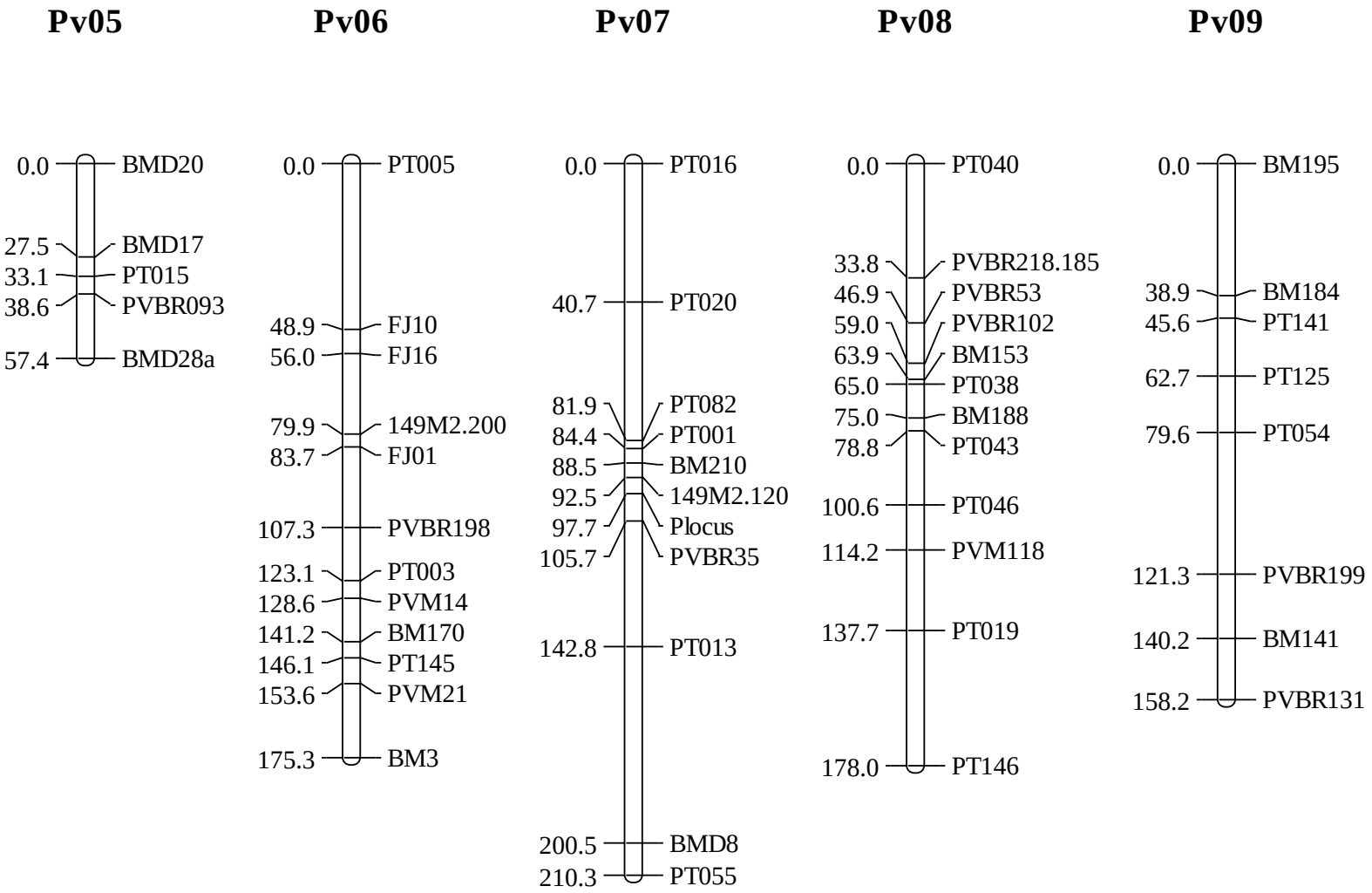

Figure 3.1 (Cont'd).

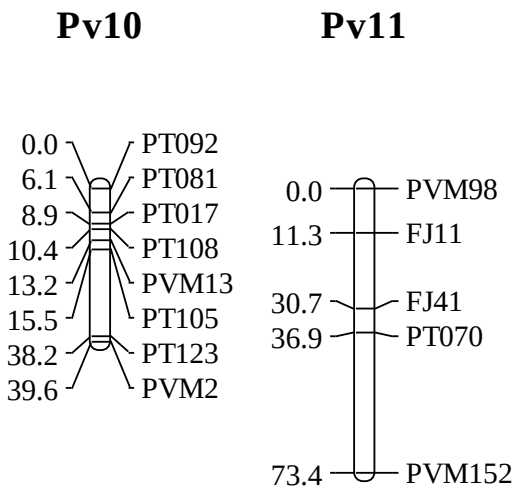

Figure 3.1 (Cont'd).

Inclusive composite interval mapping (ICIM) identified 13 QTL associated with seven traits on six linkage groups in nine marker intervals when data was combined in each environment (Table 3.2). QTL per linkage group ranged from one to four, with clusters of two or more QTL located on four linkage groups. Individual QTL explained from 9.8 % to 62.9 % of the phenotypic variation, and the total phenotypic variation explained for a trait varied from 22.8% for LB under no-choice conditions to 61.5 % for nymph counts in Michigan. Named QTL are listed in Table 3. 5.

Table 3. 1 Chi-square (χ^2) test for segregation distortion of molecular markers in the linkage map of the Matterhorn*/EMP507 IBL population.

Linkage Group	Marker	Position	Matterhorn (A/A)	H (A/B)	EMP507 (B/B)	Chi-Square (χ^2)	Direction of Skew
Pv01	PVBR218	0	70	0	5	13.44**	Matterhorn
Pv02	BM142	0	66	3	3	15.70**	Matterhorn
	PVBR25	8.6	71	2	2	19.29**	Matterhorn
	PT045	104.9	38	0	37	23.68**	EMP507
Pv03	PVBR255	18.2	70	0	5	13.44**	Matterhorn
	PVBR67	27.5	72	0	3	17.64**	Matterhorn
	PT077	34.2	71	1	2	19.29**	Matterhorn
	FJ35	42.0	70	2	3	16.99**	Matterhorn
	BM187	49.0	71	2	0	23.67**	Matterhorn
	PT147	49.9	72	1	0	24.00**	Matterhorn
	PVM26	54.6	65	5	2	17.32**	Matterhorn
Pv04	BMD15	0.0	34	4	30	16.33**	EMP507
	BMD9	9.3	28	0	47	56.75**	EMP507
	PT109	11.6	28	2	44	50.07**	EMP507
	PT037	14.1	27	7	40	43.04**	EMP507
	PT097	28.9	27	0	47	58.54**	EMP507
	BM165	47.7	21	0	54	88.36**	EMP507
	BM211	62.4	11	0	64	145.60**	EMP507
	PVBR235	69.9	3	0	69	192.67**	EMP507
	BMD10	84.0	21	0	48	73.09**	EMP507
	PVM113	109.0	36	0	38	27.41**	EMP507
Pv04b	PT059	0.0	73	0	2	19.95**	Matterhorn
Pv05	BMD28a	57.4	51	0	3	10.89**	Matterhorn
Pv06	PT005	0.0	64	3	5	11.60**	Matterhorn
Pv07	PT055	210.5	36	0	34	20.74**	EMP507
Pv10	PT092	0.0	73	0	1	22.07**	Matterhorn
	PT081	6.1	68	6	1	20.41**	Matterhorn
	PT017	8.9	67	3	2	17.98**	Matterhorn
	PT108	10.3	69	3	1	20.74**	Matterhorn
	PVM13	13.2	70	1	3	16.99**	Matterhorn
	PT105	15.5	71	1	3	17.32**	Matterhorn
Pv11	PVM98	0.0	38	0	37	23.68**	EMP507
	FJ11	11.3	36	0	30	14.73**	EMP507
	FJ41	30.7	42	0	33	14.44**	EMP507

Significance of chi-square tests: * ≤ 0.05, ** ≤ 0.01

Table 3. 2 Putative QTL for *Empoasca* leafhopper resistance traits identified in combined environments from 75 inbred backcross lines developed from a cross Matterhorn*/EMP507 and evaluated in Michigan in 2009-2011 and in Puerto Rico in 2010-2011.

Location	Agronomic Trait	Linkage Group	Position	Flanking Markers†		LOD‡ Score	R^2§	Add¶
				Left	Right			
Puerto Rico	Leaf Burn	Pv06	79	FJ16	149M2.200	7.0	24.5	0.30
		Pv06	142	BM170	PT145	4.4	17.2	0.26
		Pv07	86	PT001	149M2.120	3.7	20.4	0.32
	Leaf Curl	Pv06	170	PVM21	BM3	3.9	17.1	0.25
		Pv07	85	PT001	149M2.120	4.7	25.5	0.36
		Pv08	177	PT019	PT146	3.8	14.5	-0.26
Michigan	Leaf Burn	Pv02	135	PT079	DROUGHT1	4.6	22.3	-0.29
	Leaf Curl	Pv03	88	PT148	PVM148	5.9	19.8	0.30
		Pv07	85	PT001	149M2.120	7.2	36.3	0.38
		Pv09	154	BM141	PVBR131	2.8	10.0	-0.18
	Nymph Counts	Pv02	102	PVBR78	PT045	14.4	61.2	-1.14
No-Choice	Leaf Curl	Pv07	83	PT082	PT001	5.9	33.1	0.38
	Leaf Burn	Pv03	158	PVBR23	BM159	4.8	22.8	0.38

† Primer information for SSR markers available online at http://bic.css.msu.edu/_pdf/Bean_SSR_Primers_2007.pdf.
‡ LOD: Log of odds; log of likelihood ratio statistic at p=0.05.
§ Proportion of the phenotypic variance explained by the QTL at peak LOD using inclusive composite interval mapping (ICIM).
¶Additivity: Effect of substituting a single allele from one parent for another. Positive values indicate allele originates from Matterhorn. Negative values indicate allele originates from EMP507

Table 3. 3 Putative QTL for *Empoasca* leafhopper resistance traits identified in the combined and individual environments from 75 inbred backcross lines developed from a cross Matterhorn*/EMP507 and evaluated in Michigan in 2009-2011.

Agronomic Trait	Linkage Group	Position	Flanking Markers		LOD‡ Score	R^2§	Add¶
			Left	Right			
Leaf Burn – Choice Tests							
Combined	Pv02	137	DROUGHT1	PT079	4.6	22.3	-0.29
2009	Pv02	137	DROUGHT1	PT079	6.1	34.3	-0.50
Leaf Burn – No-Choice Tests							
Combined	Pv03	158	PVBR23	BM159	4.8	22.8	0.38
2009	Pv03	3	FJ18	PVBR255	4.2	30.6	-0.91
Leaf Curl – Choice Tests							
Combined	Pv03	88	PT148	PVM148	5.9	19.9	0.30
	Pv07	85	PT001	149M2.120	7.2	36.3	0.39
	Pv09	154	BM141	PVBR131	2.8	10.0	-0.18
2009	Pv03	88	PT148	PVM148	4.1	12.3	0.28
	Pv07	85	PT001	149M2.120	7.6	38.2	0.48
	Pv09	126	PVBR199	BM141	3.6	12.4	-0.26
2010	Pv03	88	PT148	PVM148	3.4	14.7	0.21
	Pv07	85	PT001	149M2.120	4.4	23.9	0.26
2011	Pv07	85	PT001	149M2.120	3.7	21.0	0.44
Leaf Curl – No-Choice Tests							
Combined	Pv07	83	PT082	PT001	5.9	33.1	0.38
2009	Pv07	75	PT020	PT082	3.8	37.9	0.65
2011	Pv07	98	P-locus	PVBR35	3.3	19.0	0.37
Nymph Counts (*E. fabae*)							
Combined	Pv02	102	PVBR78	PT045	14.4	61.2	-1.14
2009	Pv02	112	PT045	DROUGHT1	10.3	68.4	-2.22
2010	Pv02	110	PT045	DROUGHT1	4.6	33.8	-0.61
2011	Pv02	105	PT045	DROUGHT1	8.4	37.8	-0.98

† Primer information for SSR markers available online at http://bic.css.msu.edu/_pdf/Bean_SSR_Primers_2007.pdf.

‡ LOD: Log of odds; log of likelihood ratio statistic at p=0.05.

§ Proportion of the phenotypic variance explained by the QTL at peak LOD using inclusive composite interval mapping (ICIM).

¶Effect of substituting a single allele from one parent for another. Positive values indicate allele originates from Matterhorn. Negative values indicate allele originates from EMP507

Table 3. 4 Putative QTL for *Empoasca* leafhopper resistance traits identified in combined and individual environments from 75 inbred backcross lines developed from a cross Matterhorn*/EMP507 and evaluated in Puerto Rico in 2010-2011.

Agronomic Trait	Linkage Group	Position	Flanking Markers		LOD‡ Score	R^2§	Add¶
			Left	Right			
Leaf Burn							
2011	Pv06	79	FJ16	149M2.200	7.0	24.5	0.30
	Pv06	142	BM170	PT145	4.4	17.2	0.26
	Pv07	86	PT001	149M2.120	3.7	20.4	0.32
Leaf Curl							
Combined	Pv06	170	PVM21	BM3	3.9	17.1	0.25
	Pv07	85	PT001	149M2.120	4.7	25.5	0.36
	Pv08	177	PT019	PT146	3.8	14.5	-0.26
2010	Pv06	0	PVM21	BM3	5.6	20.7	0.35
	Pv07	85	PT001	149M2.120	5.6	29.3	0.48
2011	Pv03	129	PT050	PT072	5.5	18.8	-0.26
	Pv08	167	PT019	PT146	4.6	27.1	-0.34

† Primer information for SSR markers available online at http://bic.css.msu.edu/_pdf/Bean_SSR_Primers_2007.pdf.
‡ LOD: Log of odds; log of likelihood ratio statistic at p=0.05.
§ Proportion of the phenotypic variance explained by the QTL at peak LOD using inclusive composite interval mapping (ICIM).
¶Additivity: Effect of substituting a single allele from one parent for another. Positive values indicate allele originates from Matterhorn. Negative values indicate allele originates from EMP507

C. Leaf Curl

QTL for LC were detected on Pv03, Pv06, Pv07, Pv08, and Pv09. QTL for LC damage in both *Empoasca species* were detected on Pv03 and Pv07. Separate QTL for *E. fabae* LC damage were detected on Pv09 with QTL for *E. kraemeri* LC occurring on Pv06 and Pv08 (Table 3. 2). The *E. fabae* LC-associated QTL on Pv03 was detected in the combined analysis with an R^2 value of 19.9 and additive effect of 0.3. R^2 values ranging from 12.3 to 14.7 with additive effects of 0.21 to 0.28 were detected in 2009 and 2010 (Table 3. 3). All three QTL were tightly linked to

InDel marker PT148 (87.6 cM) with a map distance of 0.4 cM. The only QTL for LC detected on Pv09 was detected in the combined analysis in MI and in 2009. R^2 values ranged from 10.0 to 12.4 with additive effects from -0.26 to -0.18.

QTL for LC in PR were detected on Pv06 in the combined analysis and in 2010 with R^2 values ranging from 17.1 to 20.7 and additive effects of 0.25 and 0.35 respectively (

Table 3. 4). Another QTL for *E. kraemeri* LC damage was detected on PV08 in 2011 and in the combined analysis (

Table 3. 4). In 2011, this QTL explained 27.5% of the variation for LC with an additive effect of -0.34, but in the combined analysis, this QTL explained only 14.5% of the phenotypic variation with an additive effect of -0.26 (

Table 3. 4). An additional QTL for LC was detected on Pv03 only in PR in 2011 and is distinct from the QTL detected on this linkage group in MI. This QTL has an R^2 value of 18.8 and an additive effect of -0.26.

Despite the lack of overlap between the two species in many of the QTL for LC, a major QTL was identified on Pv07 that was associated with both *E. fabae* and *E. kraemeri* LC damage (Table 3. 2). This QTL was detected in the combined analysis in MI under both choice and no-choice conditions (Table 3. 2) as well as in 2009-2011 in choice tests and in 2009 and 2011 in no-choice tests (Table 3. 3). In PR, this QTL was detected in the combined analysis (Table 3. 2) as well as in 2010 (

Table 3. 4). R^2 values for this QTL in MI ranged from 21.0 to 38.2 with effects ranging from 0.26 to 0.65. In PR, R^2 values ranged from 25.5 to 29.3 with effects ranging from 0.36 to 0.48.

D. Leaf Burn

QTL for LB damage in Michigan were detected on Pv02 and Pv03. A major QTL for LB in the combined MI environment was detected on Pv02 with $R^2 = 22.3$ and additivity of -0.29 and in 2009 with $R^2 = 34.3$ and additivity of -0.5 (Table 3. 2). This QTL was found to be tightly linked to InDel marker PT079 with QTL located 0.1 cM from this marker. Two separate and significant QTL for *E. fabae* LB damage were detected on Pv03 under no-choice conditions. One QTL was

detected in the combined environment and the other was detected only in 2009 (Table 3. 3). R^2 values ranged from 22.8 to 30.6 with additive effects ranging from -0.91 to 0.38. The QTL identified in the combined analysis was found 0.4 cM from SSR marker PVBR23. In 2009, the QTL was found 3.0 cM from marker FJ18. No overlap was detected between QTL for LB damage associated with *E. fabae* and *E. kraemeri* as QTL for LB damage were only detected in PR on Pv06 and Pv07 (Table 3. 2). Two QTL were detected on Pv06 with R^2 values ranging from 17.2 to 24.5 and having additive effects of 0.26 to 0.30 respectively (

Table 3. 4). Another QTL for *E. kraemeri* LB damage was detected on Pv07 co-localized with the major QTL for LC damage. The R^2 value for this QTL was 20.4 with additive effects of 0.32 (

Table 3. 4).

E. Nymph Counts

A very significant QTL was detected on Pv02 for *E. fabae* nymph counts. This QTL was detected in the combined analysis (Table 3. 2) and in each individual year (Table 3. 3). R^2 values ranged from 33.8 in 2010 to 68.4 in 2009 and additive effects ranged from -0.61 to -2.22 nymphs/plant. QTL locations ranged from 2.9 to 7.1 cM from InDel marker PT045. No QTL were detected for *E. kraemeri* nymph counts in the analysis.

F. Co-localized QTL

QTL co-localized at four locations in the genome (Table 3. 5). On Pv02, QTL for *E. fabae* nymph counts were found adjacent to QTL for LB in MI. The QTL for nymph counts was detected in the combined analysis (2009-2011) and in each year. The QTL for LB was detected in MI in the combined analysis and in 2009. QTL for LC in MI co-localized with QTL for LC in PR and LB in MI under no-choice conditions on Pv03. A QTL for *E. kraemeri* LC damage was identified on Pv06, adjacent to a QTL for *E. kraemeri* LB damage. A large cluster of QTL for LC in MI under choice and no-choice conditions, as well as both LC and LB damage in PR co-localized on Pv07.

V. Discussion

A. Linkage Map

A total of 105 SSR and InDel markers were used in the development of a linkage map for the Matterhorn*/EMP507 IBL population. SSR and InDel marker polymorphism rates of 32% and 27% were found to be similar to the 31.7% polymorphism rate identified in other Mesoamerican intra-gene pool populations, although lower than the average rate 37.5% for race Mesoamerica by race Durango parental combinations (Blair et al., 2006a). EMP507 has a carioca seed type and therefore a member of race Mesoamerica, while Matterhorn has a great northern seed type and is a member of race Durango.

The total map distance in this population was found to be 1386 cM (Figure 3.1). This is larger than the bean core map which is estimated at 1200 cM (Freyre et al., 1998). Marker density was less than the recommended one marker per 10 cM and marker intervals ranged up to 57 cM. On Pv07, three marker intervals of greater than 40 cM were identified. These

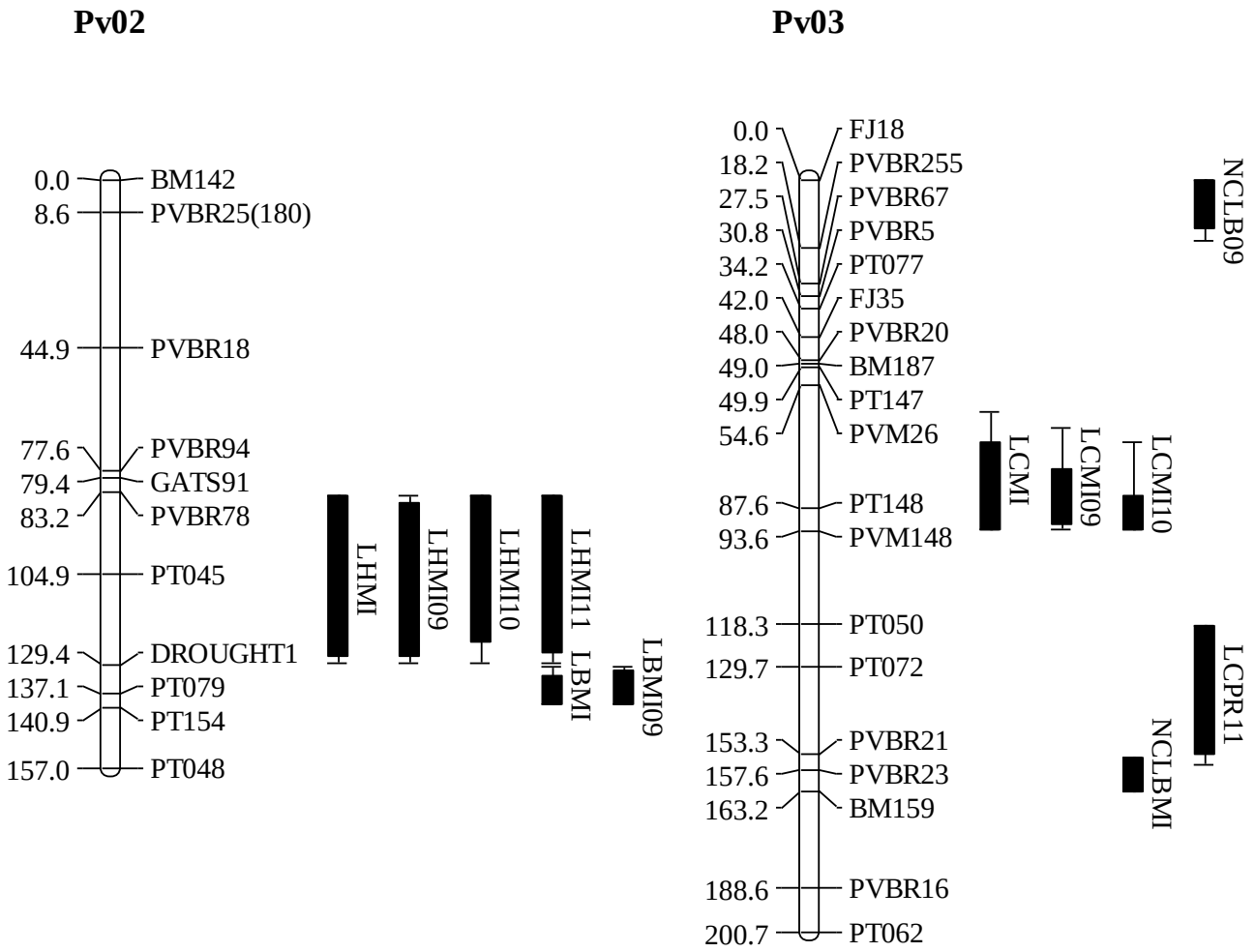

Figure 3. 2 QTL locations for leaf burn (LB), leaf curl (LC) and nymph counts (LH). QTL are further identified by location (Michigan=MI, Puerto Rico=PR) and by the last two digits of the year in which they were detected (09-11). QTL with no year specified were identified in the combined environment for each location (MI = 2009-2011; PR = 2010-2011)

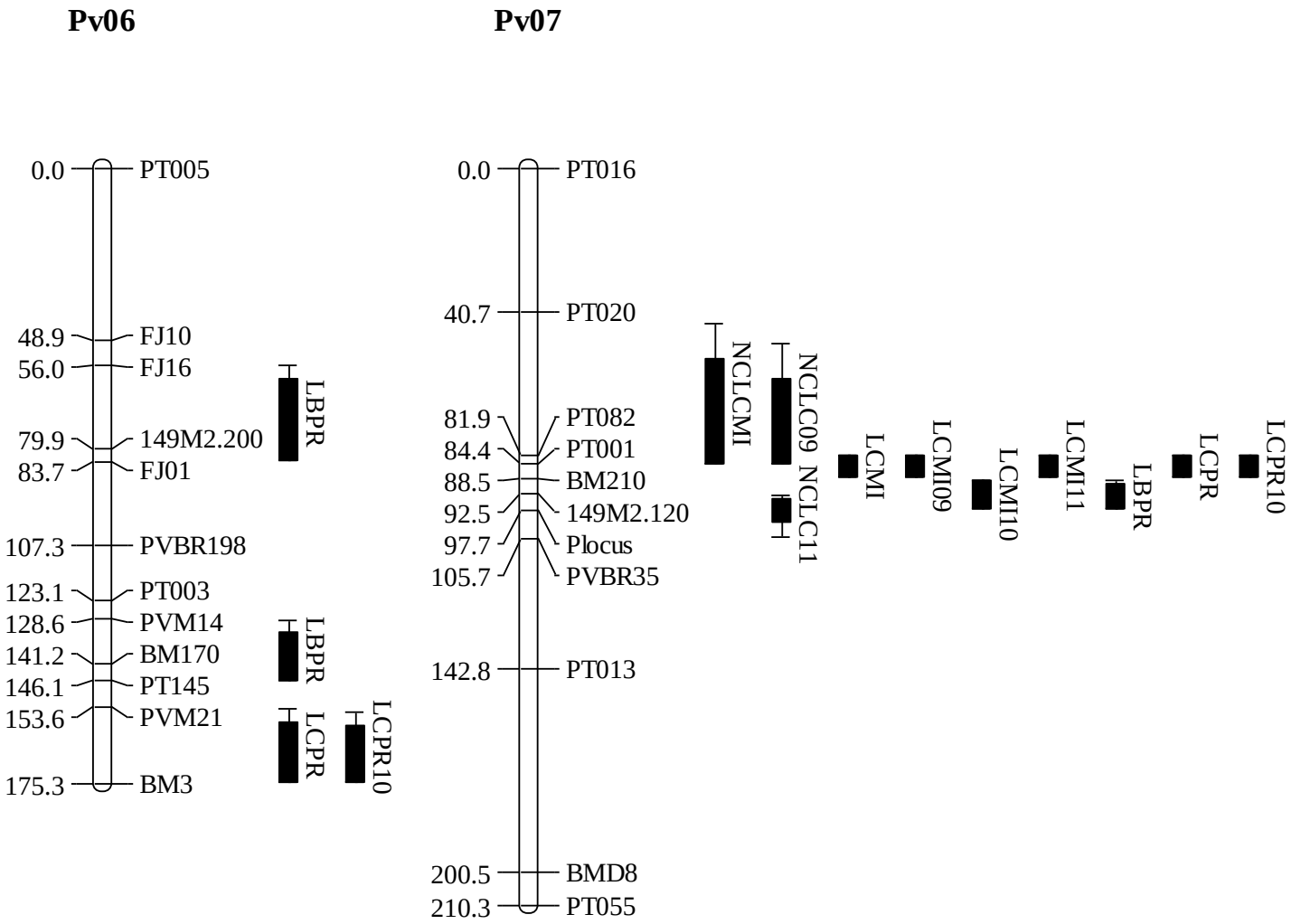

Figure 3.2 cont'd

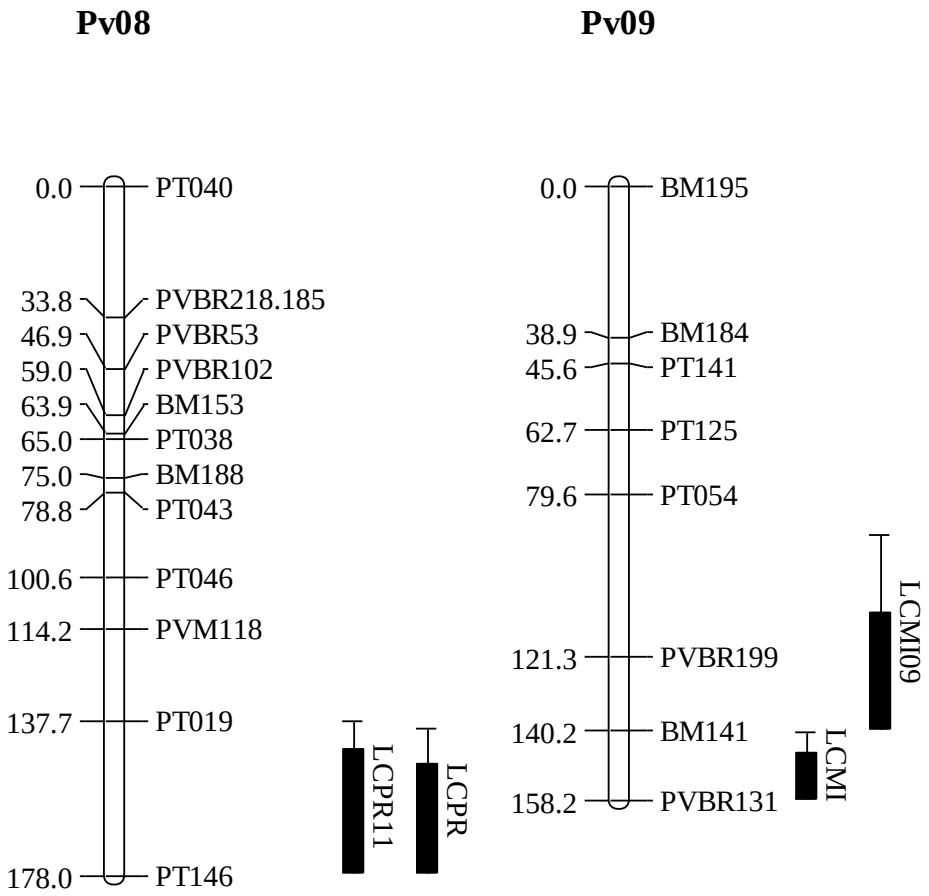

Figure 3.2 cont'd

Table 3. 5 Location and description of named QTL identified in multiple environments from 75 inbred backcross lines developed from a cross Matterhorn*/EMP507 and evaluated in Michigan and Puerto Rico in 2009-2011.

Assigned QTL†	Trait	LG	Flanking Markers	LOD‡	R^2§	Add¶
LH2.1 ME	Nymph counts - *E. fabae*	Pv02	PVBR78-PT045	14.4	61.2	-1.14
LH2.2 ME	Leaf Burn - *E. fabae*	Pv02	PT079-Drought1	4.6	22.3	-0.29
LH3.1 BE,ME	Leaf Curl - *E. kraemeri*	Pv03	PT050-PT072	5.5	18.8	-0.26
LH3.2 ME	Leaf Curl - *E. fabae*	Pv03	PVM148-PT148	5.9	19.9	0.3
LH3.3 ME	Leaf Burn - No-Choice	Pv03	PVBR23-BM159	4.8	22.8	0.38
LH3.4 ME	Leaf Burn - No-Choice	Pv03	FJ18-PVBR255	4.2	30.6	-0.91
LH6.1 ME	Leaf Burn - *E. kraemeri*	Pv06	FJ16-149M2.200	7	24.5	0.3
LH6.2 ME	Leaf Burn - *E. kraemeri*	Pv06	BM170-PT145	4.4	17.2	0.26
LH6.3 ME	Leaf Curl - *E. kraemeri*	Pv06	PVM21-BM3	3.9	17.1	0.25
LH7.1 BE,ME	Leaf Burn - *E. kraemeri*	Pv07	PT001-149M2.120	3.7	20.4	0.32
	Leaf Curl - *E. fabae*	Pv07	PT001-149M2.120	7.2	36.3	0.39
	Leaf Curl - *E. kraemeri*	Pv07	PT001-149M2.120	4.7	25.5	0.36
	Leaf Curl - No-choice	Pv07	PT082-PT001	5.9	33.1	0.38
LH8.1 ME	Leaf Curl - *E. kraemeri*	Pv08	PT019-PT146	3.8	14.5	-0.26
LH9.1 ME	Leaf Curl - *E. fabae*	Pv09	BM141-PVBR131	2.8	10	-0.18

†LH: Leafhopper, § R^2: Proportion of phenotypic variance explained by QTL at peak LOD; ¶ Additivity: Effect of substituting a single allele from one parent for another. Positive values indicate allele originates from Matterhorn. Negative values indicate allele originates from EMP507

overestimations may be a result of high levels of recombination in this population. Increasing marker density in these and other regions could help reduce the size marker intervals and thereby reduce the size of the linkage map. However, it can be assumed that the Matterhorn*/EMP507 linkage map does represent approximately 100% coverage of the bean genome. Limited coverage was seen with three, five and five mapped loci on Pv01, Pv05 and Pv11 (Figure 3.1). This could be a result of large linkage blocks that did not recombine easily in the Matterhorn*/EMP507 mapping population.

B. Segregation Distortion

Significant segregation distortion of molecular markers was detected in the Matterhorn*/EMP507 population. In a $BC_1F_{4:8}$ population, the expected Mendelian ratio is 3:1 towards the recurrent parent. However, 34 markers (32.4 %) were detected with significant segregation distortion outside of this expectation (Table 3.1). Ochoa et al. (2006) found similar levels of segregation distortion which they explained to be a result of significant preferential transmission of maternal alleles. Specifically in the Matterhorn*/EMP507 IBL population, 55 % of the distorted loci were skewed towards Matterhorn alleles leaving 45 % skewed towards EMP507. These distortions are supported by field observations as seed coat pattern was predominantly Matterhorn-type in that all population lines were either great northern or pinto seed types. No carioca seed types were observed in the population. However, agronomic traits such as flowering and maturity tended towards EMP507 (see Chapter 2).

Large regions of distortion towards recurrent parent Matterhorn were detected on Pv03, and Pv10. Regions of distortion towards the donor parent EMP507 were detected on Pv04 and Pv11. In particular, all markers on Pv04 exhibited a skewed segregation towards donor parent EMP507. Deviations from expected segregation ratios can be a result of linkage to factors

involved in the transmission of genes (Paredes and Gepts, 1995). Segregation distortion of molecular markers has been found in many crops, including maize, barley, rice and common bean, and is often attributed to gametophytic or sporophytic selection, the presence of genes for incompatibility, as well as directed selection (Gonzalez et al., 2009). Overall, Matterhorn may have improved fitness over EMP507 in terms of transmission of alleles; however, EMP507 preferentially donated alleles for all markers on Pv04. This finding suggests that Matterhorn may contain deleterious alleles on Pv04 that are selected against during meiosis.

C. QTL Analysis

Inclusive composite interval mapping (ICIM) identified 12 QTL associated with seven traits on six linkage groups in 9 marker intervals when data was combined in each environment (Table 3.2). QTL per linkage group ranged from one to four, with clusters of two or more QTLs occurring on four linkage groups, as is often the case with resistance genes clustering in the genome (Kelly et al., 2003). Specifically, two QTL were identified on Pv02, three QTL were identified on Pv03, two QTL were identified on Pv06, and one QTL were identified on Pv07.

i. Linkage Group Pv02

QTL for both *E. fabae* nymph counts and LB damage in Michigan were detected on Pv02. In the case of nymph counts, this QTL was detected in the combined analysis (2009-2011) and in each individual year, indicating the stability and heritability of this QTL (Figure 3.2). This QTL is designated LH2.1^{ME}, using the standard nomenclature as described by Miklas and Porch (2010), as this is the first QTL for leafhopper (LH) resistance on linkage group Pv02 detected in the Matterhorn*/EMP507 (ME) population. In the combined analysis, LH2.1 explained 63% of the variation for *E. fabae* nymph counts and was responsible for decreasing nymph counts by more than one nymph per trifoliate (Table 3.2). The LH2.1 QTL spans a region including InDel

marker PT045 (Figure 3.2), which was noted as being one of only five markers on the Matterhorn*/EMP507 linkage map with distorted segregation towards the EMP507 allele, not including those on Pv04 (Table 3.1). This region may contain alleles donated from EMP507 that are particularly advantageous or with better fitness than Matterhorn alleles.

The LB QTL on this linkage group was detected 35 cM distant from the LH2.1 and was detected in the combined analysis as well as in 2009 and explained up to 34% of the trait variation. This QTL is designated LH2.2^{ME} and it decreased LB damage by 0.5 out of the damage scale of zero to five. LH2.2 was the only QTL for *E. fabae* LB damage detected under choice conditions, suggesting that this trait may be difficult to detect under field conditions and/or may be subject to strong environmental interactions.

Previous studies have identified QTL on Pv02 for resistance to multiple diseases (Kelly et al., 2003), as well as to another insect pest, *Thrips palmi* (Frei et al., 2005). Because the same markers used to map the *Thrips*-resistance QTL did not map in the Matterhorn*/EMP507 population, it is unknown how close these QTL may be located. However, they are believed to be clustered because similar to LH2.1, the TP2.1^{BG} loci included resistance to both *Thrips* feeding damage and reproductive adaptation. Since many disease-resistance loci have been known to cluster in the bean genome (Kelly et al., 2003), it is possible that these insect-resistance QTL may also cluster in the same genomic region. Frei et al. (2003, 2004) reported evidence of tolerance, antibiosis and antixenosis to *Thrips palmi* in common bean, demonstrating that while antibiosis is an uncommon mechanism of insect resistance in common bean; it has been known to occur in some genotypes. The LH2.1 locus is the first major QTL for nymph counts found in common bean and was specifically associated with reductions in *E. fabae* leafhopper nymph populations. Therefore, if antibiosis resistance is present in this population as field data suggests

(see Chapter 2), LH2.1 QTL is a likely candidate. Previous studies have also identified agronomic traits linked to the same markers on Pv02 as LH2.1 and LH2.2, including seed weight, yield and days to flowering (Blair, et al., 2006b). Agronomic traits such as flowering and days to maturity have been noted as potentially linked to leafhopper resistance (Galwey, 1983), possibly involved antixenosis mechanisms.

ii. Linkage Group Pv03

Four QTL for *Empoasca*-related damage were detected on Pv03 in the Matterhorn*/EMP507 IBL population. A previous study had identified QTL for resistance to *E. kraemeri* on Pv03 (Murray et al., 2004a). A QTL was identified for resistance to *E. kraemeri* and associated with LC damage in PR in this study between flanking markers PT050 and PT072. This QTL was only evident in PR in 2011 and may be the same as previously identified by Murray et al. (2004a), because both of these QTL were only found to be associated with *E. kraemeri* LC damage. Therefore, this QTL is designated LH3.1$^{BE, ME}$. A separate QTL for *E. fabae* LC damage was detected between flanking markers PVM148 and PT148, designated LH3.2 ^{ME}, as it is distinct from the previously described QTL. It was detected in the combined analysis as well as separately in 2009 and 2010, and explained 12.3% to 19.9% of the variation for LC in the field. Also, this QTL may have utility in MAS as it is tightly linked to both flanking markers. Three clusters of QTL for disease resistance have also been located on Pv03 (Kelly et al., 2003). Unfortunately, none of the markers mapped in this study have been linked to any previously identified QTL on this linkage group. Therefore, it is unknown where LH3.2 may be located in relation to these previously mapped QTL that condition disease resistance.

Two additional QTL were detected on Pv03 for resistance to *E. fabae* LB damage. Both were evident only under no-choice test conditions suggesting involvement in antixenosis. LH3.3^{ME} was detected in the combined analysis between PVBR23 and BM159 explaining 22.8% of the LB variation. The second LB-associated QTL (LH3.4^{ME}) was detected only in 2009 under no-choice conditions and while it explains 30.1% of the no-choice LB variation, the fact that it was only detected in a single year and because no-choice phenotypic data was only replicated by a small proportion of the IBL population in each year, it is unlikely this QTL is very meaningful. In addition, because its additive effects are negative, thereby reducing LB damage by 0.9 out of the 0-5 scale, it likely would be involved in either tolerance or antibiosis and should have been evident in open-choice tests as well.

iii. Linkage Group Pv06

Three separate QTL were identified on Pv06 for resistance to *E. kraemeri*. Two QTL were identified for LB damage and one QTL was identified for LC damage. The first LB-associated QTL explained 24.5 % of the trait variation in 2011 with a positive additive effect of 0.30. This QTL is designated LH6.1^{ME}. The second QTL, designated LH6.2^{ME}, was responsible for 17.2 % of the trait variation, with additive effects on LB scores of 0.26. LH6.2 QTL was closely linked to marker BM170 (0.8 cM distant), which has also been linked to many agronomic traits, including days to end of flowering (DE6.1), days to maturity (DF6.1), and seed width (WI6.1) (Pérez-Vega et al., 2010). Previous studies have shown resistance to *E. kraemeri* to be associated with late maturity (Galwey, 1983).

The LC-associated QTL on Pv06 was detected in both the combined analysis as well as individually in 2010. This QTL is designated LH6.3^{ME} and explained 20.7% of the LC variation

in 2010 and 17.1% of the LC variation overall. Additive effects were found to be 0.25 in the combined analysis and 0.35 in 2010. If these QTL could be pyramided and incorporated into germplasm using MAS, significant reductions to the impact of *E. kraemeri* predation on common bean could be made. When combined with LC and LB field data from PR (Tables 2.5 and 2.6), those IBLs with the five lowest LB values all had EMP507 alleles at BM170 and four of the five had EMP507 alleles at 149M2 indicating that individuals with donor parent alleles at both loci do have lower LB damage. The picture for LC is slightly less clear. Four of the five IBLs with the lowest LC scores in PR have EMP507 alleles at BM3 with three of the five having Matterhorn alleles at PVM21. This suggests that there are possibly beneficial effects originating from both parents that interact to reduce LC damage as a result of *E. kraemeri* feeding. Also, increasing marker density in this map interval may help pinpoint the source of the LC resistance associated with this QTL.

iv. Linkage Group Pv07

A major QTL for resistance to both species of *Empoasca* leafhoppers was detected on Pv07. This QTL is designated LH7.1 $^{BE, ME}$ as Murray et al. (2004a) previously identified a QTL for leafhopper resistance on Pv07. In the current study, this QTL was detected for LC damage in both PR and MI and was also detected for LB damage in PR between flanking markers PT001 and 149M2. In MI, the LC-associated QTL was detected in choice tests in both the combined analysis and in every individual year as well as under no-choice conditions in the combined analysis and in 2009. Furthermore, LH7.1 was also detected for *E. kraemeri* LC damage in PR in both the combined analysis and in 2010. R^2 values for the LC-associated QTL detected in MI choice tests ranged from 0.21 to 0.38 with effects ranging from 0.26 to 0.48. Under no-choice conditions, this QTL explained 19.0 – 37.9 % of the trait variation with additive

effects of 0.37 – 0.65. In PR, LC damage R^2 values ranged from 0.26 to 0.29 with effects on LC ranging from 0.36 to 0.48 respectively. LH7.1 also explained 20.4 % of the trait variation for *E. kraemeri* LB damage with additive effects of 0.32.

LH7.1 was also found to be closely linked to the *P* gene, which is responsible for determining the presence or absence of pigments in the seed coat (McClean et al., 2002). Similar results were found by Murray et al. (2004a), when they identified a QTL on Pv07 for both *E. fabae* and *E. kraemeri* damage symptoms located 7.2 cM and 39.3 cM from the *P* locus respectively. The co-localization of LH7.1 with the *P* gene for seed coat color and the similar association with resistance traits for both species assists in confirmation of these QTL as occurring at the same locus. Having many QTL co-localized to the same position with the linkage group could indicate multiple tightly linked loci or a single locus with pleiotropic effects (Hittalmani et al., 2002). The *P* gene may be involved in resistance mechanisms as it has been linked to other disease resistance QTL on Pv07, including a recent study that linked the *P* gene to resistance to multiple strains of white mold *Sclerotinia sclerotiorum* (Perez-Vega et al., 2012). In addition, other color alleles have been linked to disease resistance including tight linkage of the B allele with the *I* gene to confer bean common mosaic virus resistance on Pv02 and the V locus on Pv06 is associated with CBB resistance (Kelly et al., 2003).

Because additive effects were positive under no-choice conditions and because of the tight linkage to the *P* gene, LH7.1 is believed to be involved in antixenosis, which was evident in field studies (see Chapter 2) and may be related to leaf color *Empoasca spp.* preferences (see Appendix A). Of the IBLs with the five lowest LC scores under choice conditions in MI, all individuals carry the EMP507 allele for markers PT082 and PT001 and four of the five carry the EMP507 allele for marker 149M2. In PR, only three of the five IBLs with the lowest LC scores

had EMP507 alleles for PT082, PT001 and 149M2, suggesting other QTL may be more applicable for MAS for resistance to the tropical *E. kraemeri.*

In addition to identifying QTL for *Empoasca* resistance, previous studies have also identified a QTL for bean pod weevil resistance on Pv07. However, this locus is associated with the *Agm* gene which only has an effect on *A. godmani* resistance in the presence of *Agr*, which is located on Pv01 (Blair et al., 2006c). It is unknown whether this gene is present in the Matterhorn*/EMP507 IBL population or whether it has implications in resistance to *Empoasca* leafhopper species.

v. Linkage Group Pv08

A QTL for *E. kraemeri* LC damage was detected on Pv08 in the combined analysis and in 2011 (Table 3.3). Designated as LH8.1^{ME}, this QTL explained 34.8% of the variation for LC with an additive effect of -0.39 in 2011, and in the combined analysis, this QTL explained 16.5% of the phenotypic variation with an additive effect of -0.28 (Table 3.3). As additive effects were found to be negative for this QTL, LH8.1 is likely involved in tolerance or antixenosis.

vi. Linkage Group Pv09

The only QTL identified on Pv09 was detected for LC damage in the combined analysis in MI as well as in 2009. R^2 values for QTL LH9.1^{ME} ranged from 10 % to 24 % with additive effects of -0.18 to -0.26. Although the QTL regions do not overlap, it is believed to be a single QTL located between SSR markers PVBR199 and PVBR131. Increasing marker density in this region may assist in determining the specific location of LH9.1. Since the additive effects are negative for LC damage and this QTL was not evident under no-choice test conditions, LH9.1 is believed to be involved in tolerance to *E. fabae* predation.

D. Empoasca species x QTL Interactions

This study did not find strong correlations between the two species of *Empoasca* leafhoppers, despite the fact that previous studies have demonstrated that resistance to *E. fabae* is consistent with resistance to *E. kraemeri* (Schaafsma et al., 1998). Out of 10 genomic regions where QTL associated with resistance to *Empoasca* species were identified, only LH7.1 was evident for both species in multiple seasons. In addition, only LH7.1 was expressed for more than one damage symptom. These findings support the hypothesis that LC damage and LB damage are results of distinct plant responses. In addition, it is known that *E. kraemeri* and *E. fabae* feeding behaviors differ on common bean (Backus et al., 2005). The lack of overlap between QTL for *E. kraemeri* and *E. fabae* may be due to these distinct insect feeding behaviors. These differences could also be due to QTLxEnvironment interactions as a result of the significantly different environmental conditions of photoperiod, temperature and moisture in MI and PR. These findings also raise the question of whether the EMP lines developed by CIAT to be resistant to *E. kraemeri* are an effective source of resistance to *E. fabae*. The recurrent parent, Matterhorn, was found to have moderate tolerance to *E. fabae* in the field (see Chapter 2) and was originally developed from Sierra, a commercially released pinto bean that has demonstrated resistance to *E. fabae* (Gonzales et al., 2004). This evidence suggests that there may be more effective sources of resistance to *E. fabae* already existing in the temperate germplasm, possibly as a result of indirect selection under annual *E. fabae* pressure in the Midwest region of the USA.

The QTL identified in this study for resistance to *Empoasca* leafhopper species may have utility for MAS. In particular, LH2.1 is a strong candidate for reducing *E. fabae* nymph populations, possibly as a result of antibiosis mechanisms, because it was seen in all seasons indicating stability of the locus and it was responsible for a large proportion of the phenotypic

variation observed in the Matterhorn*/EMP507 IBL population. LH3.1 could also be used for MAS of *E. fabae* resistant material as it also was apparent in multiple seasons with markers flanking a small map distance of only 6 cM. The QTL on Pv06 may be most useful if pyramided to provide resistance to *E. kraemeri* leafhoppers in tropical common bean germplasm. The LH7.1 QTL on Pv07 may have the broadest application for MAS to incorporate *Empoasca* leafhopper resistance as it was evident in both MI and PR in multiple seasons. LH7.1 might be most useful if it is pyramided with other QTL utilizing different mechanisms for leafhopper resistance, as it is potentially involved in antixenosis resistance and because R^2 values in MI are relatively low.

Previous field and QTL studies have suggested that LC and LB are distinct genetic plant responses to *Empoasca* leafhopper feeding (Murray et al., 2001, 2004a, 2004b). The lack of overlap in this study of QTL for LC and LB damage supports this hypothesis. Only two significant QTL for *E. fabae* LB damage were detected in this study – LH2.2 under choice conditions and LH3.2 under no-choice conditions. In contrast, four QTL were detected for *E. fabae* LC damage with choice and no-choice LC QTL co-localized on Pv07. The fact that *E. fabae* LB-associated QTL for each test condition were detected on different linkage groups while *E. fabae* LC-associated QTL overlapped on the same linkage group suggests that LB damage may be more significantly influenced by QTLxEnvironment interactions than LC damage. Damage responses from *E. kraemeri* may be more closely linked as QTL for both LC and LB were detected on Pv06 and Pv07. This may be a result of *E. kraemeri* feeding behavior as it is known that *E. kraemeri* uses lacerate-and-sip feeding tactic more commonly than *E. fabae* (Backus et al., 2005). Lacerate-and-sip feeding is believed to be responsible for causing LB damage (Serrano et al., 2000).

E. Conclusions

In summary, this study has identified multiple QTL for resistance to both *Empoasca* leafhopper species. These QTL can be utilized by bean breeders in both temperate and tropical climates seeking to enhance levels of resistance in their germplasm. In addition, by increasing the level of resistance to these important pest species in commercial bean cultivars, bean growers throughout the Americas can mitigate potential yield losses from pest damage without costly insecticide treatments.

Literature Cited

Literature Cited

Backus, E.A., Serano, M.S., and Ranger, C.M. 2005. Mechanisms of Hopperburn: An overview of insect taxonomy, behavior, and physiology. Annu. Rev. Entomol. 50: 125-151.

Blair, M.W., Pedraza, F., Buendia, H.F., Gaitan-Solas, E., Beebe, S.E., Gepts, P., and Tohme, J. 2003. Development of a genome-wide anchored microsatellite map for common bean (*Phaseolus vulgaris* L.). Theor. Appl. Genet. 107: 1362-1374.

Blair, M.W., Giraldo, M.C., Buendía, H.F., Tovar, E., Duque, M.C., and Beebe, S.E. 2006a. Microsatellite marker diversity in common bean (*Phaseolus vulgaris* L.). Theor. Appl. Genet. 113: 100-109.

Blair M.W., Iriarte G., and Beebe, S. 2006b. QTL analysis of yield traits in an advanced backcross population derived from a cultivated Andean x wild common bean (*Phaseolus vulgaris* L.) cross. Theor. Appl. Genet. 112: 1149-63.

Blair, M.W., Munoz, C., Garza, R., and Cardona, C. 2006c. Molecular mapping of genes for resistance to the bean pod weevil (*Apion godmani* Wagner) in common bean. Theor. Appl. Genet. 112: 913-923.

Cardona, C., Posso, C.E., Kornegay, J., Valor, J., and Serrano, M. 1989. Antibiosis effects of wild dry bean accessions on the Mexican bean weevil and the bean weevil. (Coleoptera: Bruchidae). J. Econ. Entomol. 82: 310-315.

Frei, A., Gu, H., Bueno J.M., Cardona, C., and Dorn, S. 2003. Antixenosis and Antibiosis of Common Beans to *Thrips palmi* Karny (Thysanoptera: Thripidae). J. Econ. Entomol. 96: 1577-1584.

Frei, A., Bueno, J.M., Diaz-Montano, J., Gu, H., Cardona, C., and Dorn, S. 2004. Tolerance as a mechanism of resistance to *Thrips palmi* in common beans. Entomol. Exp. Appl. 112: 73-80.

Frei, A., Blair, M.W., Cardona, C., Beebe, S.E., Gu, H., and Dorn, S. 2005. QTL mapping of resistance to *Thrips palmi* Karny in common bean. Crop Sci. 45: 379-387.

Freyre, R., Skroch, P., Geffroy, V., Adam-Blondon, A.F., Shirmohamadali, A., Johnson, W., Llaca, V., Nodari, R., Pereira, P., Tsai, S.M., Tohme, J., Dron, M., Nienhuis, J., Vallejos, C. and Gepts, P. 1998. Towards an integrated linkage map of common bean. 4. Development of a core linkage map and alignment of RFLP maps. Theor. Appl. Genet. 97: 847-856.

Galeano, C.H., Fernandez, A.C., Franco-Herrera, N., Cichy, K.A., and McClean, P.E. 2011. Saturation of an Intra-Gene Pool Linkage Map: Towards a Unified Consensus Linkage Map for Fine Mapping and Synteny Analysis in Common Bean. PLoS ONE 6: 1-10.

Galwey, N.W. and Evans, A.M. 1982. The inheritance of resistance to *Empoasca kraemeri* Ross & Moore in the common bean, *Phaseolus vulgaris* L. Euphytica 31: 933-952.

Galwey, N.W. 1983. Characteristics of the common bean, *Phaseolus vulgaris*, associated with resistance to the leafhopper *Empoasca kraemeri*. Ann. App. Bio. 102: 161-175.

Gonzalez, A.M., Rodin, A.P., Santalla, M., and De Ron, A.M. 2009. Genetics of intra-gene pool and inter-gene pool hybridization for seed traits in common bean (Phaseolus vulgaris L.) germplasm from Europe. Field Crops Research 112: 66-76.

Gonzales, J.W., Coyne, D.P., Lindgren, D.T., Schaaf, D., and Eskridge, K.M. 2004. Heritability of the resistance to potato leafhopper in dry bean. HortSci. 39: 1578-1580.

Haley, S.D., Miklas, P.N., Afanador, L., and Kelly, J.D. 1994. Random amplified polymorphic DNA (RAPD) marker variability between and within gene pools of common bean. J. Amer. Soc. Hort. Sci. 119: 122-125.

Kelly, J.D., Hosfield, G.L., Varner, G.V., Uebersax M.A., and Taylor, J. 1999. Registration of 'Matterhorn' great northern bean. Crop Sci. 39:589-590.

Kelly, J.D, Gepts, P., Miklas, P.N., and Coyne, D.P. 2003. Tagging and mapping of genes and QTL and molecular-marker assisted selection for traits of economic importance in bean and cowpea. Field Crops Res 82: 135-154.

Kelly, J.D., and Vallejo, V.A. 2004. A comprehensive review of the major genes conditioning resistance to anthracnose in common bean. HortScience 39: 1196-1207.

Kelly, J.D., and V.A. Vallejo. 2005. QTL analysis of multigenic disease resistance in plant breeding. p. 21-48. In S. Tuzun and E. Bent (ed.) Multigenic and Induced Systemic Resistance in Plants. Springer, New York.

Miklas, P.N., and Porch, T. 2010. Guidelines for common bean QTL nomenclature. Annu. Rep. Bean Improv. Coop. 53: 202-204.

McClean, P.E., Lee, R.K., Otto, C., Gepts, P., and Bassett, M.J. 2002. Molecular and Phenotypic Mapping of Genes Controlling Seed Coat Pattern and Color in Common Bean (Phaseolus vulgaris L.) J Hered. 93: 148-152.

Murray, J.D., Michaels, T.E., Pauls, K.P., and Schaafsma, A.W. 2001. Determination of traits associated with leafhopper (Empoasca fabae and Empoasca kraemeri) resistance and dissection of leafhopper damage symptoms in common bean (Phaseolus vulgaris). Ann. App. Bio. 139: 319-327.

Murray, J.D., Michaels, T.E., Cardona, C., Schaafsma, A.W., and Pauls, K.P. 2004a. Quantitative trait loci for leafhopper (Empoasca fabae and Empoasca kraemeri) resistance and seed weight in the common bean. Plant Breed. 123: 474-479.

Murray, J.D., Michaels, T.E., Pauls, K.P., Cardona, C., and Schaafsma, A.W. 2004b. Yield and insect injury in leafhopper (*Empoasca fabae* Harris and *Empoasca krameri* Ross and Moore) infested dry beans in Ontario and Colombia. Can. J. Plant Sci. 84: 891-900.

Ochoa, I.E., Checa, O.E., Beebe, S.E., Lynch, J.P., and Blair, M.W. 2006. Mapping and segregation distortion analysis in the G2333 X G19839 recombinant inbred line population of common bean. Annu. Rep. Bean Improv. Coop. 49: 271-273.

Ojwang, P.P.O., Melis, R., Githiri, M.S., and Songa, J.M. 2011b. Breeding options for improving common bean for resistance against bean fly (*Ophiomyia spp*.): a review of research in eastern and southern Africa. Euphytica 179: 363-371.

Osborn, T.C., Alexander, D.C., Sun, S.S., Cardona, C., and Bliss, F.A. 1988. Insecticidal activity and lectin homology of arcelin seed protein. Science. 240: 207-210.

Paredes, O.M. and Gepts, P. 1995. Segregation and Recombination in Inter-Gene Pool Crosses of *Phaseolus vulgaris* L. J. Heredity 86: 98-106.

Pedrosa-Harand, A., Porch, T., and Gepts, P. 2008. Standard nomenclature for common bean chromosomes and linkage groups. Annu Rep Bean Improv Coop. 51: 106-107.

Perez-Vega, E., Paneda, A., Rodriguez-Suarez, C., Campa, A., Giraldez, R., and Ferreira, J.J. 2010. Mapping of QTLs for morpho-agronomic and seed quality traits in a RIL population of common bean (Phaseolus vulgaris L.) Theor Appl Genet 120: 1367-1380.

Perez-Vega, E., Pascual, A., Campa, A., Giraldez, R., Miklas, P.N., and Ferreira, J.J. 2012. Mapping quantitative trait loci conferring partial physiological resistance to white mold in the common bean RIL population Xana 3 Cornell 49242. Mol. Breeding. 29: 31-41.

Serrano, M.S., Backus, E.A., and Cardona, C. 2000. Comparison of AC electronic monitoring and field data for estimating tolerance to Empoasca kraemeri (Homoptera: Cicadellidae) in common bean genotypes. J. Econ. Entomol. 93: 1796-1809.

Schaafsma, A.W., Cardona, C., Kornegay, J.L., Wylde, A.M., and Michaels, T.E., 1998. Resistance of common bean lines to the potato leafhopper (Homoptera: Cicadellidae). J. Econ. Entomol. 91: 981-986.

Singh, S.P. 1982. A key for identification of different growth habits of Phaseolus vulgaris L. Annu. Rept. Bean Improv. Coop. 25: 92-94.

Singh, S.P. and Schwartz, H.F. 2011. Review: Breeding common bean for resistance to insect pests and nematodes. Can. J. Plant Sci. 91: 239-250.

Smith, M.C. 2005. Plant Resistance to Arthropods. Springer Publishing, Dordrecht, The Netherlands. 423p.

Voorrips, R.E., 2002. MapChart: Software for the graphical presentation of linkage maps and QTLs. J. Heredity 93: 77-78.

Wang, J., H. Li, L. Zhang, and L. Meng. 2012. Users' Manual of QTL IciMapping Version 3.2. The Quantitative Genetics Group, Institute of Crop Science, Chinese Academy of Agricultural Sciences (CAAS), Beijing 100081, China, and Genetic Resources Program,

International Maize and Wheat Improvement Center (CIMMYT), Apdo. Postal 6-641, 06600 Mexico, D.F., Mexico.

Wei, J.N., Zhu, J., and Kang, L. 2006. Volatiles released from bean plants in response to agromyzid flies. Planta 224: 279-287.

Appendix A: Influence of leaf color on leafhopper populations (*Empoasca fabae and Empoasca kraemeri*) and host plant resistance in common bean (*Phaseolus vulgaris*)

I. Introduction

Many insects use visual cues as a determining factor when selecting host plants for feeding and oviposition. Historically, insect vision has been ranked as less important than olfaction in selecting host plants. However, in a recent review by Reeves (2011), he reports that visual cues may be the first line of host plant recognition, especially for highly polyphagous insects such as *Empoasca fabae* (Harris) and *E. kraemeri* (Ross & Moore), which have a broad range of potential host plants. Common beans, *Phaseolus vulgaris,* are among their preferred host plants and are an important staple food crop in much of the world. Temperate *E. fabae* is a pest of North American common bean production east of the Rocky Mountains, while tropical *E. kraemeri* limits common bean yields throughout South and Central America. *Empoasca* feeding can cause serious damage by reducing bean seed yield and quality, while causing distinct damage symptoms – leaf curl and leaf burn.

Previous studies have shown that *P. vulgaris* leaf color influences *E. fabae* preferences, more so than host odor, suggesting that *Empoasca* leafhoppers may also avoid certain plants based on the color of the leaves (Bullas-Appleton et al., 2004). *Empoasca* preferences for egg-laying and feeding (antixenosis interactions) can be assessed by evaluating nymph incidence (Schaafsma et al., 1998). The importance of visual traits, such as leaf color, in determining insect preferences can be inferred by correlating nymph incidence with such traits. When insect preferences are better understood, developing resistant plant material can become more achievable. If leaf color preferences and aversions are limited to specific spectra, this data can be included in routine breeding program screening. Therefore, having a simple objective method for measuring leaf color would be very useful for breeders who are looking to include this method of resistance. The Hunter *L*a*b** color space system is just such a method. Hunter *L*a*b** values

characterize specific color spectra as described in Table A.1, and can represent all perceivable colors. *L*a*b** coordinates can only represent an absolute color if the white point (the XYZ data they were converted from) is also specified. The objectives of this study were to compare *L*a*b** color spectrum of bean genotypes that differ in reaction to *Empoasca* feeding damage.

Table A. 1. The Hunter *L*, a*, b** scale values and associated maximum and minimum color spectrum. The *L*, a*, b** values provide an objective measurement of color that correlates with human eye perceptions.

Hunter Coordinate	Scale	Minimum	Maximum
*L**	0 to 100	Dark	Light
*a**	-100 to +100	Green	Red
*b**	-100 to +100	Blue	Yellow

II. Materials and Methods

F. Plant Material

In order to determine the role that leaf color may play in *Empoasca* species preferences of *P. vulgaris*, an inbred backcross line (IBL) population (Matterhorn*/EMP507) that is segregating for leaf color was examined. The IBL population examined in this study was originally developed from a single cross Matterhorn/EMP507 followed by a single backcross to Matterhorn to create an IBL population, consisting of 75 $BC_1F_{4:8}$ individuals. Matterhorn is a commercially available great northern cultivar developed in Michigan (Kelly et al., 1999) with high yield and quality seed and agronomic characteristics. EMP507 is a carioca germplasm line developed at CIAT (Cardona et al., 1989) as part of a long-term recurrent selection program for resistance to *E. kraemeri* (Schaafsma et al., 1998). While these EMP lines were originally developed to be resistant to the tropical species of PLH, *E. kraemeri*, Schaafsma et al. (1998) demonstrated that the resistance is sustained under severe pressure to the temperate congener *E.*

fabae. The population was developed with the USDA dry bean breeding program in Puerto Rico, led by Dr. Tim Porch.

G. Field Screening

Field screening of leaf color traits was conducted on the Crop and Soil Science Research Farm at Michigan State University, East Lansing, Michigan (MI) and at USDA-ARS-TARS in Isabela, Puerto Rico (PR). Three replications were planted in MI in 2011 in a randomized complete block design (RCBD) of 5.4 m long single-row plots. Individual plot were spaced 20 cm apart and consisted of up to 80 plants per plot. Five replications were planted in December 2009 and January 2011 in PR in a RCBD of 1.8 m long single-row plots. Individual plots were spaced 90 cm apart and consisted of up to 30 plants per plot. *Empoasca* species were allowed to inoculate each field test naturally.

Plant leaf color was measured using a Kodak Chromameter CR-400. Measurements were taken by placing the chromameter directly on the leaf surface and recording the *L*a*b** values. A randomly selected leaf from the upper canopy of three plants per plot was analyzed in each replication. Nymphs were counted at pod set by counting nymphs present on three trifoliate leaves on each of three randomly selected plants per plot. Plants were evaluated for leaf curl and leaf burn at 78 and 79 days after planting (DAP) using the damage scale from 0-5 as described in Murray et al. (2001).

H. Greenhouse Screening

Twenty-three IBLs were selected from the population from the extreme ends of each *L**, *a**, *b** value based on field screening results and inoculated with 5 *E. fabae* adults/trifoliate at 23 DAP in a caged greenhouse choice test. *L**, *a**, *b** scores were recorded at 35 DAP and nymph

counts were taken at 42 DAP. All data were analyzed using SAS Statistical software (SAS Institute, Cary, USA).

III. Results and Discussion

I. Color Analysis

L, a*, b** color space coordinates were normally distributed in each field test. Significant correlations between color scale values were determined for each color value both within and across field trials and greenhouse tests (α=0.05). *L** and *b** were positively correlated, indicating as *L** increases towards lightness, *b** increases towards yellow. *a** and *b** were negatively correlated, indicating as *b** increases towards yellow, *a** decreases towards green. *L** and *a** were not significantly correlated in this study. IBL population means and 95% confidence intervals for each Hunter *L*, a*, b** color value are listed in Table A.2.

Table A. 2 *L*, a*, b** color means and 95% confident intervals for 75 inbred backcross lines ($BC_1F_{4:8}$) from a Matterhorn*/EMP507 population evaluated in Michigan in 2011 and in Puerto Rico in 2010-2011.

Color Coordinate	Location	Year	Min	Mean ± SE 95% Confidence Interval	Max
*L**	PR	2011	47.1	51.1 ± 0.10	55.1
		2010	49.1	53.5 ± 0.11	57.9
	MI	2011	35.3	39.31 ± 0.13	43.3
*a**	PR	2011	-22.3	-14.3 ± 0.20	-6.3
		2010	-15.9	-13.2 ± 0.07	-10.5
	MI	2011	-14.8	-12.8 ± 0.06	-10.8
*b**	PR	2011	23.9	28.5 ± 0.17	33.1
		2010	21.2	28.8 ± 0.11	35.8
	MI	2011	15.2	20.4 ± 0.17	25.6

J. Empoasca – Color Correlations

Nymph counts were found to be normally distributed in each field trial. Mean nymph count values were 4.3±0.16 (MI11), 2.4±0.11 (PR10), and 0.4±0.02 (PR11) with an overall mean of 2.1±0.07. When color scores were analyzed with both *Empoasca sp*. nymph scores using Spearman rank correlation coefficients, significant correlations were seen with all color spectra across all years and locations (α=0.05) (Table A.3). *L** (light-dark) was negatively correlated with nymph counts. Nymph counts were also negatively correlated with *b** (yellow-blue), while *a** values (green-red) were positively correlated with nymph counts across years and locations. Environment was found to play a significant role in leaf color as location was a significant factor for all *L*, a*, b** values (α=0.05).

Table A. 3 Spearman rank correlations between *Empoasca spp.* nymph counts and *L*, a*, b** color values for inbred backcross lines ($BC_1F_{4:8}$) achieving *Empoasca spp.* economic threshold levels (>1 nymph/trifoliate) or higher from a Matterhorn*/EMP507 population evaluated in Michigan in 2011 and in Puerto Rico in 2010-2011.

Color Coordinate	Spearman Rank Correlation (r)
*L**	-0.15*
a*	0.11*
b*	-0.04*

K. Damage Score - Color Correlations

L, a*, b** values were sub-sampled for those where *E. sp.* nymph counts achieved economic threshold levels or greater (N ≥ 1 nymph/trifoliate) and analyzed both by location (Figure A.1a-c) and species (Table A.4). Those individuals included in each range were selected for analysis with LC and LB damage scores. Significant correlations were identified between LB and all *L*, a*, b** color values (α = 0.05). LC was only found to correlate with *L** values. This

result could be due to the fact that LB damage itself directly affects the color of the leaves, and damage symptoms may already have been present at the time of evaluation.

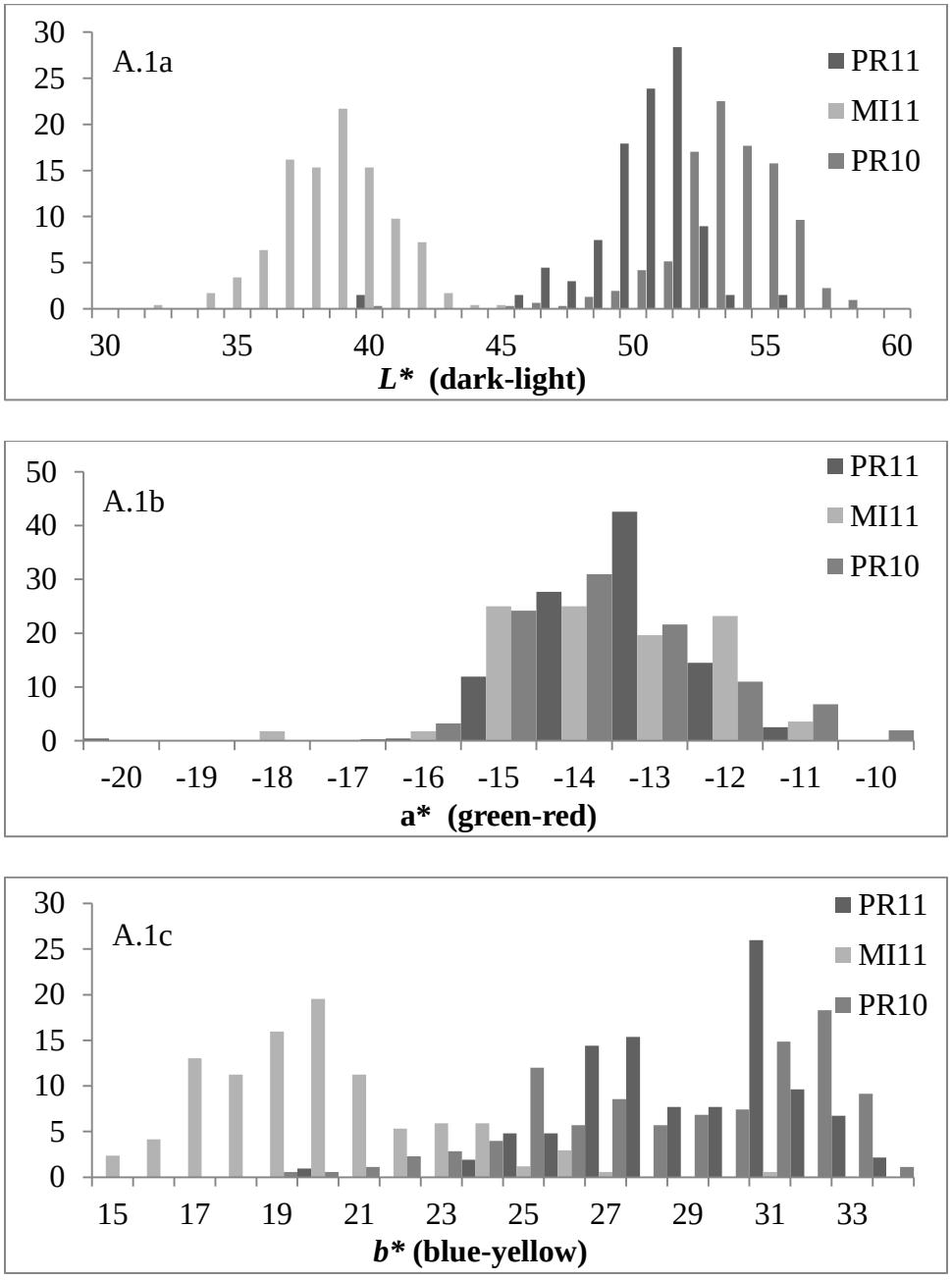

Figure A. 1 Distribution of IBLs ($BC_1F_{4:8}$) achieving *Empoasca spp.* economic threshold levels (>1 nymph/trifoliate) or higher for each measurement of *L** (A.1a), *a** (A.1b), *b** (A.1c) from a Matterhorn*/EMP507 population evaluated in Michigan in 2011 and in Puerto Rico in 2010-2011.

L. Greenhouse Screening

Nymph counts were not found to be significantly correlated with any *L**, *a**, *b** color values under greenhouse conditions; however, correlations between all *L**, *a**, *b** values were significant (α=0.05). Genotype was found to be significantly associated with all *L**, *a**, *b** values ($p<0.001$). Heritability values (h^2) were calculated for all variables – L: $h^2 = 0.65$, a: $h^2 = 0.67$, b: $h^2 = 0.66$ – indicating that under uniform conditions, 65-67% of the variation in plant leaf color is under genetic control.

IV. Conclusions

The IBL population displayed significant segregation for leaf color as indicated by *L**, *a**, *b** values. *Empoasca* species appear to have leaf color preferences as previously indicated by Bullas-Appleton et al. (2004). Each species was found to have distinct preference ranges. Preferences for host plant leaf color ranges for *E. fabae* and *E. kraemeri* are shown in Table A.4. The strong association of plant leaf color with location may be a result of differences in plant growth habit due to the different environments, such as differences in fertility.

Table A. 4 *L**, *a**, *b** coordinates encompassing 90% of the IBLs from a Matterhorn*/EMP507 population evaluated in Michigan in 2011 and in Puerto Rico in 2010-2011 achieving *Empoasca spp.* economic threshold levels (>1 nymph/trifoliate) or higher.

Species	Hunter Color Scale	Min (5% Quartile)	Max (95% Quartile)
E. fabae	*L**	+35.97	+42.82
	*a**	-11.40	-14.40
	*b**	+16.60	+24.89
E. kraemeri	*L**	+48.82	56.29
	*a**	-16.19	-10.79
	*b**	23.99	33.62

Given that *Empoasca sp.* appear to have distinct preference ranges, it can also be inferred that there are ranges of leaf colors that they avoid. This may be involved in the antixenosis resistance demonstrated in choice tests in Chapter 2. By selecting against those plants that fall within the insect's preferred ranges, breeders could retain germplasm that may be more resistant to *Empoasca sp.* predation and subsequent damage and yield reductions.

Literature Cited

Literature Cited

Bullas-Appleton, E.S., Otis, G., Gillard, C., and Schaafsma, A.W. 2004. Potato leafhopper (Homoptera: Cicadellidae) varietal preferences in edible beans in relation to visual and olfactory cues. Enviro. Ent. 33: 1381-1388.

Cardona, C. Posso, C.E., Kornegay, J., Valor, and J., Serrano, M. 1989. Antibiosis effects of wild dry bean accessions on the Mexican bean weevil and the bean weevil (Coleoptera: Bruchidae). J. Econ. Entomol. 82: 310-315.

Kelly, J.D., Hosfield, G.L., Varner, G.V., Uebersax M.A., and Taylor, J. 1999. Registration of 'Matterhorn' great northern bean. Crop Sci. 39:589-590.

Murray, J.D, Michaels, T.E., Pauls, K.P., and Schaafsma, A.W. 2001. Determination of traits associated with leafhopper (*Empoasca fabae* and *Empoasca kraemeri*) resistance and dissection of leafhopper damage symptoms in common bean (*Phaseolus vulgaris*). Ann. Appl. Bio. 139:319-327.

Reeves. J.L. 2011. Vision should not be overlooked as an important sensory modality for finding host plants. Enviro. Ent. 40:855-863.

Schaafsma, A.W., Cardona, C., Kornegay, J.L., Wylde, A.M., and Michaels, T.E. 1998. Resistance of common bean lines to the potato leafhopper (Homoptera: Cicadellidae). J. Econ. Ent. 91:981-986.