Lima beans on defense: studies of cyanogenesis and the complex relationship of crop plants and insect herbivores

By

KIMBERLY GIBSON
DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Horticulture and agronomy

in the

OFFICE OF GRADUATE STUDIES

of the

UNIVERSITY OF CALIFORNIA

DAVIS

Approved:

Paul Gepts, Chair

Li Tian

Jay Rosenheim

Committee in Charge

2023

To the first farmers, the future farmers, and the many hands that have connected them.

Abstract

Lima beans (*Phaseolus lunatus*) are a globally important crop with great potential to contribute to future food security through their high nutritional value, vigor, drought tolerance, and ability to fix nitrogen. A major limiting factor for the contribution of Lima beans to the global food supply is their susceptibility to herbivory by insects like *Lygus hesperus.* The goal of the research presented in this dissertation is to understand the domestication history, current variability, and breeding potential of the anti-herbivore defense traits in Lima bean with a special emphasis on cultivars adapted to the Central Valley of California, and on cyanogenesis as a mechanism of defense against *L. hesperus*. Chapter 1 is a comprehensive review of the existing research on the effect of domestication on anti-herbivore defense traits. In chapter 2, a genome-wide association study is used to explore the inheritance of cyanogenesis in Lima beans within the Mesoamerican gene pool. This study identified highly significant SNPs for the release of cyanide immediately following tissue disruption that could contribute to a deterrence defense strategy. Significant SNPs were also identified for a more sustained release of cyanide that could contribute to a defense strategy of herbivore intoxication. In chapter 3, the trait of cyanogenesis is characterized and studied for its plasticity in the presence of *L. hesperus* and its effect on the survival and growth of *L. hesperus* populations. This study found that cyanogenesis was not induced by the presence of *L. hesperus* and the survival and reproduction of *L. hesperus* was negatively correlated with the cyanogenic capacity of their Lima bean host. These results advance the study of anti-herbivore defense mechanisms and the complex relationships between crop plants and their insect pests. They will contribute to efforts of crop improvement for reduced pesticide use and increased food security.

Acknowledgements

Table of Contents

Introduction

Domestication has altered the interactions among crop plants, herbivorous insects, and higher trophic levels of agroecosystems (Chen et al. 2018). While selection has resulted in enhanced agronomic traits like yield, defensive traits such as toxic compounds have been reduced or removed (Rodriguez-Saona et al. 2011). A consistent pattern of reduced defense has not been established across species, but crop plants tend to be more vulnerable to herbivory than their wild relatives (Chen et al. 2015). Domesticated beans (*Phaseolus* spp.) may be more attractive to insect herbivores than their wild relatives, but they are also more effective at recruiting parasitoids (Benrey et al. 1998). This indicates that despite the drastic reduction in defense, some mechanisms have remained in domesticated types or have been recently introgressed by geneflow from wild populations. Identifying and amplifying these anti-herbivore defense mechanisms in modern cultivars, as well as introducing new mechanisms from wild relatives and landraces, could greatly improve productivity and profitability, while limiting the use of toxic agrochemicals.

Like other crops, Lima bean (*Phaseolus lunatus*) has lost many of its mechanisms of defense against insect herbivores during the bottlenecks of domestication and modern breeding programs. This is illustrated by the finding that wild *P. lunatus* seedlings have greater chemical diversity than their domesticated relatives (Lindig-Cisneros et al. 1997). Lima beans have been used as an experimental model in numerous studies of herbivore-induced direct and indirect defenses (Ballhorn et al. 2008). Previous studies have examined the role of cyanogenesis, volatile organic compounds, and extrafloral nectaries as plant defense mechanisms against herbivores (Moreira et al. 2015; Kost and Heil 2008; Ballhorn

et al. 2008; Heil 2004). Due to the high metabolic cost to the plant of producing these defensive compounds, *P. lunatus* makes tradeoffs between direct and indirect defense mechanisms (Ballhorn et al. 2008).

The goal of the research presented In this dissertation Is to understand the domestication history, current variability, and breeding potential of the antiherbivore defense traits in Lima bean with a special emphasis on cultivars adapted to the Central Valley of California and cyanogenesis as a mechanism of defense against *Lygus hesperus*.

Lima Beans (Phaseolus lunatus) as the Predominant Grain Legume in California

Lima beans are one of five domesticated species in the genus *Phaseolus.* They were independently domesticated first in Central Mexico and again on the western slope of the Andes Mountains of Ecuador and northern Peru (Gutiérrez-Salgado et al. 1995; Andueza-Noh et al. 2013). Dating of starch grains on human dental remains, indicates that beans were domesticated in Northern Peru approximately 8000 years ago (Piperno and Dillehay 2008). Estimates for the date of domestication of Lima beans in Mexico are less certain but range from 2300-3400 years ago (Kaplan and Lynch 1999; Brown 2006). The domesticated Andean gene pool is characterized by large flat seeds while the Mesoamerican gene pool has smaller round (potato type) or flat (sieva type) seeds (Mackie 1943; Chacón-Sánchez et al. 2012). Lima beans are multi-annual or semi-perennial, with crops typically needing 115-135 days to reach maturity (Long et al. 2014). They are adapted to a range of climates but are especially suited to warm and humid environments (Delgado and Gama López 2015).

Lima beans are grown in many regions of the world, including Africa, Asia, and Central and South America (Heuzé et al. 2015). In the United States, California is the

primary growing region for mature dry Lima beans, where they are an important crop for the agricultural systems of the Central Valley. As a nitrogen-fixing, highly vigorous rotation crop for tomatoes and other high-value crops, Lima beans provide an essential service of sustaining soil fertility and breaking pest and weed lifecycles (Long et al. 2014). Additionally, Lima beans are very drought tolerant, making them ideal for the perennial water shortages experienced by California in recent decades (Martínez-Nieto et al. 2022).

Two market classes – small (baby) white and large white – are grown in the state. Baby Limas are grown mostly in the region around Sutter and Colusa Counties. Large Limas, which need cooler nighttime temperatures, are grown mostly in Stanislaus County (Lazicki et al. 2016; Long et al. 2014). Approximately 20,200 acres of Lima beans were grown in 2018, representing nearly half (44%) of the dry bean production in the state (USDA 2018). Improved cultivars of Lima bean yield approximately 2,500-3,500 pounds per acre (Long et al. 2014; Wootten 1992). Production of Lima bean in California is limited by its vulnerability to *L. hesperus.* Regular treatment in the field with pesticides, specifically pyrethroids, is the only known effective method of control (Long 2014). This practice is costly for a low-value crop like Lima beans and is unsuitable for organic production.

Lygus hesperus as a Pest of Lima beans in California

Herbivory by the polyphagous, native Californian insect *Lygus hesperus* Knight (Hemiptera: Miridae), negatively affects the yields of several important crops, including alfalfa, strawberries, safflower, peaches, almonds, and dry beans (Metcalf and Flint 1951). Current methods of controlling *L. hesperus* are costly, environmentally toxic, and only moderately effective (Long 2014). In sensitive crops like alfalfa and Lima bean, *L. hesperus*

can cause up to 70% yield loss as measured in sprayed versus unsprayed plots (Bushing et al. 1974; S. Dohle, A. Palkovic, and P. Gepts, unpubl. results).

There are typically four or five generations of *L. hesperus* each summer, with variability due to climate (Beards and Strong 1966). The rate of development is dependent on temperature, but it takes on average 27 days from egg to reproductive adult at 20°C (Butler and Wardecker 1971). Each generation develops from egg to adult with five nymphal instars going through incomplete metamorphosis (Schull 1932). All nymphs are flightless, but adults are highly mobile (Butler 1972; Goodell and Bentley 2003).

L. hesperus are omnivorous but feed mostly on plant tissue (Hagler et al. 2010). Their style of feeding is known as rupture feeding or "lacerate and flush." Feeding starts with the insect probing the food tissue with its straw-like stylet, causing cells to rupture. Saliva secreted from the stylet has enzymes like polygalacturonase and α-amylase, which further break down the tissue, creating a slurry that the insect can ingest through their stylet tube (Strong 1970). *L. hesperus* feeding on Lima beans results in the abscission of flowers or young pods and consequently, yield loss. When feeding occurs on mature pods seed viability may be reduced and scarring can occur on seeds, thereby lowering market value (Strong 1970; Long et al. 2014). Adults spend about 20% of their time, and nymphs about 30% of their time, probing plants with their stylets. Actual ingestion represents only 3% of the probing time (Cervantes et al. 2016). The mechanical and chemical damage caused by this frequent probing and feeding behavior contributes to the heavy impact that *L. hesperus* have on crop yields.

Research Objectives

Understanding the mechanisms and inheritance patterns of *L. hesperus* tolerance or resistance in Lima bean will aid efforts to breed new varieties that require fewer pesticides and are suitable for organic production. To start, chapter 1 presents a comprehensive review of the literature on how domestication has affected the genetics of insect defense traits. Chapter 2 details the results of a genome-wide association study of cyanogenesis in Lima bean. The goal of this study was to explain how cyanogenesis has been affected by domestication in the Mesoamerican gene pool of Lima beans, with special consideration of the cyanogenic capacity of California cultivars. Chapter 3 will explore the variation and heritability of the tolerance or resistance to *L. hesperus* in cultivars of Lima bean adapted to the Central Valley of California. This will include a study of how cyanogenesis in Lima bean is affected by the presence of *L. hesperus*, specifically to determine the extent to which this trait is constitutive or induced. Analysis of the survival and reproduction of *L. hesperus* on varieties of Lima bean with variable expression of cyanogenesis will also be presented.

References

Andueza-Noh, R. H., Serrano-Serrano, M. L., Sánchez, M. C., ... & Martínez-Castillo, J. (2013). Multiple domestications of the Mesoamerican gene pool of Lima bean (*Phaseolus lunatus* L.): evidence from chloroplast DNA sequences. *Genetic Resources and Crop Evolution*, *60*(3), 1069-1086. doi: 10.1007/s10722-012-9904-9

Ballhorn, D. J., Kautz, S., Lion, U., & Heil, M. (2008). Trade-offs between direct and indirect defenses of Lima bean (*Phaseolus lunatus*). *Journal of Ecology*, *96*(5), 971-980. doi: 10.1111/j.1365-2745.2008.01404.x

Beards, G. W. and F. E. Strong. (1966). Photoperiod in relation to diapause in *Lygus hesperus* Knight. *Hilgardia* 37(10): 345-362. doi: 10.3733/hilg.v37n10p345

Benrey B., Callejas A., Ríos L., Oyama K., Denno R. (1998). The effects of domestication of *Brassica* and *Phaseolus* on the interaction between phytophagous insects and parasitoids. *Biological Control* 11:130-140. doi: 10.1006/bcon.1997.0590

Brown, C. H. (2006). Prehistoric chronology of the common bean in the New World: The linguistic evidence. *American Anthropologist*, *108*(3), 507-516. doi:10.1525/aa.2006.108.3.507

Bushing, R. W., & Burton, V. E. (1974). Partial pest management programs on dry large Lima beans in California: regulation of *L. hesperus*. *Journal of Economic Entomology*, *67*(2), 259-261. doi: 10.1093/jee/67.2.259

Butler G. D. and A. L. Wardecker. (1971). Temperature and the development of eggs and nymphs of *Lygus hesperus*. *Annals of the Entomological Society of America* 64(1): 144-145. doi: 10.1093/aesa/64.1.144

Cervantes, F. A., Backus, E. A., Godfrey, L., Akbar, W., & Clark, T. L. (2016). Characterization of an EPG waveform library for adult *Lygus lineolaris* and *Lygus hesperus* (Hemiptera: Miridae) feeding on cotton squares. *Annals of the Entomological Society of America*, *109*(5), 684-697. doi: 10.1093/aesa/saw039

Chacón-Sánchez M., Motta-Aldana J., Serrano-Serrano M., Debouck D. (2012). Domestication of Lima beans: a new look at an old problem. In: Gepts P, Famula T, Bettinger R, Brush S, Damania A, McGuire P, Qualset C (eds) Biodiversity in agriculture – Domestication, evolution, and sustainability. Cambridge University Press, Cambridge, United Kingdom, pp 330-343. doi:10.1017/CBO9781139019514.019

Chen Y.H., Gols R., Benrey B. (2015). Crop domestication and its impact on naturally selected trophic interactions. *Annual Review of Entomology* 60:35-58. doi: 10.1146/annurev-ento-010814-020601

Chen, Y. H., Ruiz-Arocho, J., & von Wettberg, E. J. (2018). Crop domestication: anthropogenic effects on insect–plant interactions in agroecosystems. *Current Opinion in Insect Science*, 29, 56-63. doi: 10.1016/j.cois.2018.06.004

Delgado A, Gama López, S. (2015). Diversidad y distribución de los frijoles silvestres en México. Universidad Nacional Autónoma de México Revista Digital Universitaria 16: http://www.revista.unam.mx/vol.16/num12/art10/

Goodell P. and W. Bentley. 2003. Lygus bugs. In L.E. Ferguson, ed., Pistachio Production Manual. Oakland, USA: University California Agriculture and Natural Resources 182-185pp.

Gutiérrez-Salgado, A., Gepts, P., & Debouck, D. G. (1995). Evidence for two gene pools of the Lima bean, *Phaseolus lunatus* L., in the Americas. *Genetic Resources and Crop Evolution*, *42*(1), 15-28. doi: 10.1007/BF02310680

Hagler, J. R., Jackson, C. G., and Blackmer, J. L. (2010). Diet selection exhibited by juvenile and adult life stages of the omnivores western tarnished plant bug, *Lygus hesperus* and tarnished plant bug, *Lygus lineolaris*. *Journal of Insect Science* 10(1): 127. doi: 10.1673/031.010.12701

Heil, M. (2004). Induction of two indirect defenses benefits Lima bean (*Phaseolus lunatus*, Fabaceae) in nature. *Journal of Ecology*, *92*(3), 527-536. doi: 10.1111/j.0022-0477.2004.00890.x

Heuzé V., Tran G., Sauvant D., Bastianelli D., Lebas F. (2015). Lima bean (*Phaseolus lunatus*). Feedipedia, by INRA, CIRAD, AFZ and FAO. https://www.feedipedia.org/node/267 Last updated on May 11, 2015, 14:31. doi: 10.1016/B978-0-12-821450-3.00001-9.

Kaplan, L., & Lynch, T. F. (1999). *Phaseolus* (fabaceae) in archaeology: AMS radiocarbon dates and their significance for pre-Columbian agriculture. *Economic Botany*, *53*(3), 261-272. doi: 10.1007/BF02866636

Kost, C., & Heil, M. (2008). The defensive role of volatile emission and extrafloral nectar secretion for Lima bean in nature. *Journal of Chemical Ecology*, *34*(1), 2-13. doi: 10.1007/s10886-007-9404-0.

Lazicki P., Geisseler, D., Horwath, W. R. (2016). Dry Bean Production in California. California Department of Agriculture Fertilizer Research and Education Program.

Lindig-Cisneros, R., Benrey, B., Espinosa-García, F. (1997). Phytoalexins, resistance traits, and domestication status in *Phaseolus coccineus* and *Phaseolus lunatus*. *Journal of Chemical Ecology* 23:1997-2011 doi: 10.1023/B:JOEC.0000006485.38713.8c

Long, R., Temple, S., Meyer, R., Schwankl, L., Godfrey, L., Canevari, M., & Roberts, P. (2014). Lima Bean Production in California. ANR Publication 8505. https://escholarship.org/uc/item/4cb5376v

Mackie, W. W. (1943). Origin, dispersal and variability of the lima bean, *Phaseolus lunatus*. *Hilgardia* 15:1-29 doi: 10.3733/hilg.v15n01p001

Martínez-Nieto, M. I., González-Orenga, S., Soriano, P., Prieto-Mossi, J., Larrea, E., Doménech-Carbó, A., Tofei, A. M., Vicente, O., & Mayoral, O. (2022). Are traditional Lima bean (*Phaseolus lunatus* L.) landraces valuable to cope with climate change? Effects of drought on growth and biochemical stress markers. *Agronomy*, *12*(7), 1715. doi: 10.3390/agronomy12071715

Metcalf, C. L. & Flint, W. P. (1951). Destructive and useful insects (3rd edition). New York, McGraw-Hill Book Co. Inc., 1071 pp. doi: 10.1086/334020

Moreira, X., Abdala-Roberts L., Hernández-Cumplido J., Cuny, M. A. C., Glauser G., & Benrey B. (2015). Specificity of induced defenses, growth, and reproduction in Lima bean

(*Phaseolus lunatus*) in response to multispecies herbivory. *American Journal of Botany* 102:13001308. doi: 10.3732/ajb.1500255

Piperno, D. R., & Dillehay, T. D. (2008). Starch grains on human teeth reveal early broad crop diet in northern Peru. *Proceedings of the National Academy of Sciences*, *105*(50), 19622-19627. Doi: 10.1073/pnas.0808752105

Rodriguez-Saona, C., Vorsa, N., Singh, A. P., Johnson-Cicalese, J., Szendrei, Z., Mescher, M. C., & Frost, C. J. (2011). Tracing the history of plant traits under domestication in cranberries: potential consequences on anti-herbivore defenses. *Journal of Experimental Botany*, *62*(8), 2633-2644. doi:10.1093/jxb/erq466

Schull, W. E. 1932. An investigation of the *Lygus* species which are pests of beans (Hemiptera, Miridae). *Iowa State University Digital Repository of retrospective Theses and Dissertations.*

Strong, F. E. (1970). Physiology of injury caused by *Lygus hesperus*. *Journal of Economic Entomology* 63(3): 808-814. doi: 10.1093/jee/63.3.808

United States Department of Agriculture (USDA) (2018). Quick Stats 2.0. U.S. Department of Agriculture, National Agricultural Statistics Service, Washington DC. https://quickstats.nass.usda.gov/. doi: 10.15585/mmwr.mm6433a11.

Wootten, T. (1992). The search for improved Lima beans yields: A starting point. *HortScience*, *27*(11), 1161-1161. Doi: 10.21273/HORTSCI.27.11.1161a

Chapter 1: The Effect of Domestication on Crop Plant Anti-Herbivore Defense Traits

Introduction

The processes of cultivation and domestication have transformed wild species into crop plants that are an invaluable food source for the human population. A shrinking number of these crop species provide a growing share of global calories (Khoury et al. 2014). These same processes have also made crop plants more vulnerable to damage by insect pests than their wild relatives (Whitehead et al. 2017). Globally, insect herbivory accounts for an estimated 18-20% of yield loss during crop production (Sharma et al. 2017). It is expected that these losses will increase if current trends in climate change continue (Deutsch et al. 2018). Recovering the defensive abilities of crop wild relatives in domesticated plants would result in reduced need for pesticides – which are harmful to human and environmental health – as well as an increase in global food security.

Plant defenses against insect herbivores typically consist of complex suites of traits (Agrawal and Fishbein 2006; Barbour et al. 2015; Schuman and Baldwin 2016). These may include resistance traits like chemical deterrents, physical barriers, and reduced palatability, or tolerance traits like increased vigor and delayed phenology (Mitchell et al. 2016). Defensive traits can also involve attraction or resource benefits for beneficial organisms such as parasitoids and predators of insect herbivores (Chen et al. 2015).

Defense traits may be constitutively expressed and may also be induced to a higher level of expression by factors such as the presence of the target herbivore or volatile signals from a neighboring plant (Stout 2014). Induction of a defense trait may occur immediately or slowly over time (Bruce 2015; Frost et al. 2008). The response can also be localized to

the area immediately surrounding the site of damage or be widespread throughout the plant (Karban and Baldwin 1997). Several environmental factors can affect the expression of these traits including light intensity (Roberts and Paul 2006), the interspecific and intraspecific diversity of neighboring plants (Solís-Montero et al. 2020; Abdala-Roberts et al. 2016), photoperiod, temperature, and climate (Jamieson et al. 2012). Control of these traits typically involve the expression of multiple loci, which may be linked to other useful or unfavorable agronomic traits (Ordas et al. 2010; Boerma and Walker 2005).

The process of domestication involves selecting wild plants with desirable agronomic traits. The resulting crop plants typically exhibited some or all a suite of traits known as the domestication syndrome. This can include increased palatability, loss of dormancy, seed retention, increased seed size, and seed number (Gepts 2004, 2014; Hufford et al. 2019). Selection intensity varied across crops and domestication events and there is serious academic debate about the duration and intentionality of this process (Hillman and Davies 1990; Abbo et al. 2011; Purugganan and Fuller 2011).

Identifying the genes that control agronomically important traits and understanding the way in which they have been affected by domestication is foundational to conserving, amplifying, and increasing their utility. This review will focus on the ways in which the process of domestication has altered the genes underlying plant defenses against insect herbivores. Prior reviews have focused on the mechanisms and physiology of plant defense against insect pests as well as the ways in which domestication has affected the interactions between crop plants, insect herbivores, and higher trophic levels (Chen et al. 2015; Mitchell et al. 2016). To my knowledge this is the first review on the subject of how

domestication has affected plant and insect herbivore interactions with a specific focus on crop genetics.

Reductions in genetic diversity within crop species

Domesticated gene pools have significantly less genetic diversity than wild gene pools (Hufford et al. 2019; Khoury et al. 2021). During the transition from wild plant to domesticated crop, diversity is lost during the actual domestication process as well as during cultivation, dispersal outside the center of origin, and later improvement through modern breeding (Meyer and Purugganan 2013; Gepts 2002). This loss of diversity within crop species has contributed to a loss of insect herbivore resistance traits (Johnson 2008; Tooker and Frank 2012; Dar et al. 2021). This is illustrated in maize through a comparison of teosinte, maize landraces from within the center of origin, maize landraces from outside the center of origin, and modern maize cultivars. Each stage of transition resulted in changes to the anti-herbivore defense strategies as well as changes in gene expression (Köllner et al. 2008; Fontes-Puebla et al. 2021). A similar pattern can be seen in an analysis of the *GsRbohA1* locus in soybean of which the *A* haplotype confers resistance against common cutworm (*Spodoptera litura* Fabricius). This allele was only present in 2.2% of modern cultivars compared with 23.5% of landraces and 95.6% of wild accessions (Du et al. 2022).

In the case of some anti-herbivore defense mechanisms, the selection against certain phenotypes had clear benefit to human consumers. For example, selections against genotypes which produce toxic compounds like cyanogenic glycosides, or distasteful physical defenses like trichomes, improved the safety and palatability of food (Benrey et al.

1998; Wittkop et al. 2009; Whitehead et al. 2017). However, several important defense traits such as herbivore-induced volatile organic compounds cannot be perceived by casual observation and have only recently been revealed by careful scientific study (Turlings et al. 1990; Turlings et al. 1995). Despite the impossibility of direct selection for or against these traits prior to their discovery, several studies have identified differences in the expression between wild and domesticated crop plants. For example, in several studies of phytophagous insects and their parasitoids, parasitism was higher for hosts on domesticated rather than cultivated plants (Benrey et al. 1998; Garvey et al. 2020). In other studies, reduced predation or parasitism was attributed to the loss of chemical diversity or volatile signals (Chen and Welter 2002; Souna et al. 2019; Jaccard et al. 2022). The loss or amplification of indirect anti-herbivore defense traits may be due to genetic drift, linkage, pleiotropic effects, or selection for alternate resource allocation. However, these were not intentionally selected against as their function has only recently been discovered (Rodriguez-Saona et al. 2011). Breeding programs with little or no insecticide protection may help maintain insect defense traits compared to programs in which insecticides protect plants and mask susceptibility (Hagel 1978).

Two early steps in the process of domestication were cultivation and storage, the intentional planting of future crop species and the saving of seeds between planting seasons (Wilcox 2012; Fuller and Allaby 2018). Both cultivation and storage created novel selection pressures on crop plants and their insect herbivores. Traits changed in frequency from the wild population when under the selection pressures of cultivation and storage (Chen et al. 2018). For example, in common bean, *Phaseolus vulgaris*, resistance to Mexican bean weevil *Zabrotes subfasciatus* (Coleoptera: Bruchidae) is most likely due to a protein of

the APA family, arcelin (Osborn et al. 1988; Cardona et al. 1990; Acosta-Gallegos et al. 1998). Arcelin is only found in some accessions of wild *Phaseolus vulgaris* from Mesoamerica (Osborn et al. 1986) and is controlled by a single mendelian gene for which arcelin production alleles are dominant over alleles for its absence (Romero et al. 1986; Osborn et al 1986). Wild collections are diverse across geographic regions and highly heterogeneous within populations, including the presence or absence of arcelin (Bennett 1970). The great variation in their expression of arcelin production, suggests that this seed protein may have a limited effect on fitness. Alternatively, the cost of acrelin production may outweigh the benefits in some settings. This raises the possibility that domestication did not occur in the limited region where arcelin producing accessions are found or by chance during domestication non-arcelin individuals were selected (Gepts 1984; Gepts 1988; Debouck and Tohme 1989; Cardona et al. 1990).

Insect herbivores have exerted selective pressures on both wild and domesticated plants (Campbell 2014). For self-pollinating lineages to evolve and be maintained, the fitness cost of inbreeding depression – the difference in fitness of inbred and outcrossed progeny - cannot be too high (Jarne and Charlesworth 1993; Campbell 2014). Herbivory can increase inbreeding depression, though the interaction between pest and mating system can be complex and varied (Carr and Eubanks 2001; Muola et al. 2011; Schrieber et al. 2021). Autogamy, or self-pollination, and asexual reproduction have been favored over allogamy, commonly known as out-crossing, during domestication (Rick 1988; Allard 1999; Gepts 2004). The combination of selection for inbreeding and inbreeding depression as a result of herbivore induced selection has contributed to the trend of increased susceptibility to insect pests of crop plants as compared to their wild relatives.

Reductions of genetic diversity in agricultural landscapes

The accelerating fragmentation or total clearing of wild ecosystems to make way for farmland has resulted in an incalculable loss of genetic diversity (Tilman 1999; Norris 2008; Raven and Wagner 2021). This loss of biodiversity has increased the pressure from insect herbivores on crop plants by several mechanisms, including the loss of predators and parasitoids, as well as genetic erosion and reduced gene flow between crops and their wild relatives.

Close correlations between plant and insect diversity have been found in wild and agricultural ecosystems (Murdoch et al. 1972; Strong et al. 1984; Zhang et al. 2016). The habitat needs of specialist herbivores as well as predators and parasitoids may not be met by simplified agricultural landscapes. As a result, the ecosystem services they provide in more diverse landscapes will be lost (Root 1973; Keesing and Wratten 1997; Woltz 2012). Overall, crop domestication and the ecological disturbance of agriculture has exerted a r-selection pressure on insect herbivores resulting in the evolution of more serious pests (Horn 2009).

Within the center of domestication, landscape diversity is a reserve of crop diversity and insect diversity (Keesing and Wratten 1997). Loss of genetic diversity within these ecosystems can have several consequences for the future evolution of crops and insect herbivores. Genetic diversity in a population is a prerequisite for evolution by natural and artificial selection (Darwin 1859). This applies to both crop plants and their insect herbivores (Tooker and Frank 2012). Gene flow from wild relatives is an important source of anti-herbivore traits in crop plants. Hence, genetic erosion of crop gene pools is occurring rapidly due the loss of habitat for wild relatives (Maxted et al. 2007). While

modern breeding efforts have sought to identify and incorporate defensive traits from wild relatives, this is slow work and cannot proceed without conservation (Rick and Chetelat 1995; Hodgkin and Hajjar 2007). Additionally, the loss of spatial diversity across agricultural ecosystems counters the ability of balancing selection to maintaining non-neutral genetic variation in crop populations including landraces (Mercer and Perales 2010; Gloss et al. 2013).

In addition to trait maintenance and ease of adaptation to novel circumstances, both intraspecific and interspecific diversity make agricultural landscapes more difficult for herbivores to navigate (Root 1973; Agrawal 2006). In polycultures, insects are less likely to find and stay on their host plants (Strong 1984). Additionally, increased niche partitioning in diverse landscapes leads to more efficient capture of available resources. This increases plant productivity in diverse landscapes as compared to monocultures (Crutsinger et al. 2012; Tooker and Frank 2012)

Changes in Ploidy

Polyploidy is common in plants and is especially prevalent in domesticated species (Renny-Byfield and Wendel 2014). Autopolyploidy is when polyploidy arises from whole genome duplication. Allopolyploidy is when polyploidy arises from the hybridization of two or more whole genomes. Adding copies of the genome can result in new expression and interactions between genes and alleles, thereby affecting anti-herbivore defense traits (Albuzio et al. 1978; Nusimer and Thompson 2001; Wendel et al. 2014; Osborn 2004).

Whether a change in ploidy comes through autopolyploidy or allopolyploidy may affect the outcome for anti-herbivore defense traits. In allopolyploids, some evidence

indicates that there is expression of novel phenolic compounds and enzymatic diversity (Dhawan and Lavania 1996). This greater chemical diversity may help combat the overall loss of chemical diversity that results from the initial domestication event and as a result amplify some anti-herbivore traits (Benrey et al. 1998; Renny-Byfield and Wendel 2014; Salman-Minkov 2016; Dar et al. 2021). However, more research is needed on this subject. By contrast, autopolyploidy has been found to have varied effects on chemical phenotypes including the expression of novel compounds, the absence of compounds found in diploids, and changes in the regulation of tissue-specific chemical production (Levy 1976).

There is evidence to support two main scenarios of insect herbivore response to polyploidization. In the first, polyploidy may create new defenses that allow a host plant to escape from herbivory and coevolutionary relationships with its insect pests (Ehrlich and Raven 1964; Segraves and Anneberg 2016). An example of this is found in the order Brassicales, which includes many domesticated species. The successful evolution of increasingly complex glucosinolate defense chemicals for protection against the cabbage moth (Lepidoptera: Peridae) has been driven by gene and genome duplication (Edger et al. 2015). Duplicated genes in the glucosinolate pathway were retained at a rate of over 95% after whole genome duplication compared to an average rate of 45% maintenance in duplication overall in protein coding genes (Hofberger et al. 2013). This indicates that polyploidization during domestication may have improved antiherbivore defense mechanisms in some cases (Renny-Byfield and Wendel 2014).

In the second scenario, polyploidy may expand the range of hostplants and their herbivores resulting in greater diversification and ecological opportunities for the pest (Segraves and Anneberg 2016). This can be seen in the example of gall midges (*Asphondylia*

spp.), which have species-specific preferences for – and therefore habitat limitation to –

cytotypes in creosote bush*, Larrea tridentata* (O'Connor et al. 2019) and the perennial

brassica, *Cardamine pratensis* (Avantis et al 2010). While no examples for this scenario can

be found in research on domesticated crops, it is likely that polyploidization during

domestication may have altered crop plant and insect herbivore interactions by expanding

the ecological range of both crops and insect herbivores.

Given the syndrome-like nature of plant defense, the multi-locus control of many

individual plant defense traits, the higher load of deleterious mutations, and the gene loss

known to occur after polyploidization, it might seem that polyploid crops would be

especially sensitive to changes in ploidy and genetic drift during domestication (Browers et

al. 2003; Paterson et al. 2003; Agrawal and Fishbein 2006; Otto 2007). However, there is

strong evidence to suggest that the genes controlling specialized metabolic pathways for

certain defense traits are clustered (Dar et al. 2021). As a result, polyploidy is, in many

cases, an asset to plants for maintaining and evolving anti-herbivore defense mechanisms

while undergoing selection during domestication (Renny-Byfield and Wendel 2014; Dar et

al. 2021). Additionally, polyploid plants are often clonally propagated – a practice which

may maintain advantageous combinations of alleles. Asexual reproduction has been found

especially effective in maintaining defenses against specialist herbivores, but the associated

loss of chemical diversity may come at a cost of adaptations that defend against generalist

herbivores (Johnson et al. 2009; Johnson et al. 2014; Campbell et al. 2014).

Differential expression of defense trait expression in tissue of reproductive and harvested organs

Selection during domestication can have varied effects on the characteristics of crop plants depending on the harvested organ, mating system, and life history of the plant (Zhoray et al. 2004). Humans have found diverse ways of consuming a wide variety of plant organs, though reproductive organs, like fruits and seeds, are favored staples given their high caloric and nutritional value (Klein et al. 2007; Turner et al. 2011). The modular nature of plants and organ-specific genetic control of defense trait expression has allowed for the selection of crop plants with defenses that are differentiated in reproductive and harvested organs (Sánchez-Pérez et al. 2007; McCall and Fordyce 2010; Shang et al. 2014; Whitehead et al. 2017).

Reproductive structures, including flowers, fruits, and seeds, are usually photosynthetic sinks that require nutrient and energy investments from other parts of the plant to build and defend (Herms and Mattson 1992; Whitehead et al. 2017). For crops in which a reproductive organ is also the harvested organ – such as fruits, grains, pulses, and oil seeds – selection for or against herbivore defense traits may have been imposed by consumer preferences as discussed below. However, for crops in which the harvested organ is not a reproductive organ – such as leafy greens, tubers, and biomass crops – the harvested organ may have lost defenses when resources were allocated to yield rather than defense (Herms and Mattson 1992; Huot et al. 2014).

The harvested organs of crops have undergone especially intensive selection during domestication (Darwin 1859). Damage to the harvested organs by insect herbivores can directly affect both the quality and yield of a crop. As a result, selection during

domestication may have been more intense for these organs. For edible crops however, selection may have acted against defense traits, as palatability or safety for human consumers was prioritized. This can be seen in the example of cyanogenic glycosides in almonds. Presence of these compounds in the kernels is an effective defense against some insect herbivores (Zagrobelny et al. 2004), but is also toxic to human consumers (Thodberg et al. 2018). As a result, the trait has been strongly selected against in domesticated almonds (Thodberg et al. 2018). Studies have shown that the pre-cyanogenic compound prunasin is produced outside the kernel and then transported in and stored as amygdalin (Thodberg et al. 2018). While control of prunasin production is multigenic, its presence in kernels is controlled by a single gene (Sánchez-Pérez et al. 2007). This is like other classic domestication syndrome traits which are controlled by single genes or large effect quantitative trait loci (Poncet et al. 2004; Meyer and Purugganan 2013; Olson and Wendel 2013; Parker and Gepts 2021).

A similar genetic effect of domestication on defense compound biosynthesis can be seen in the example of cucurbitacin in the *Cucurbitaceae* family. Cucurbitacin confers a bitter flavor to plant tissue and if consumed in large enough doses it can be fatally toxic to human consumers (Kaushik et al. 2014). While some insects have adapted to tolerate and even benefit from cucurbitacin, it is a strong deterrent to feeding and oviposition to other insect species (Tallamy et al. 1997). Presence of cucurbitacin in the *Cucurbitaceae* is a recessive trait controlled by the *Bi* locus (**Bi**tterness). However, just as amygdalin storage in almond kernels is controlled by a single gene, a transcription factor *Bt* (**Bit**ter fruit) controls biosynthesis of cucurbitacin from the *Bi* locus in fruits and has undergone strong selection during domestication (Shang et al. 2014). This demonstrates strong and

independent selection during domestication that reduced defense traits in the harvested organ of a crop.

In instances in which the antiherbivore trait is not toxic or unpalatable to human consumers, there is evidence that selection during domestication has maintained defensive traits in harvested organs. This is the hypothesis of Rodriguez-Saona et al. (2011) for the case of cranberries and selection for anthocyanins. Anthocyanins are a class of compounds known to provide defense against insect herbivores in some cases and are also understood to be beneficial for human consumers (de Pascual-Teresa and Sanchez-Ballesta 2008). It was found that anthocyanins have not been maintained as an effective defense in North American highbush blueberries (Rodriguez-Saona et a. 2019). Both highbush blueberries and cranberries were recently domesticated so they are likely still undergoing significant selection in cultivated environments (Mainland 2012; Vorsa and Zalapa 2019).

In some cases, the harvested organs of a crop may have become more susceptible to an insect pest because selection during domestication made them more attractive hosts rather than eliminating defenses. This can be seen in the example of chili peppers, which were domesticated for both ornamental and culinary uses. Culinary peppers are much more susceptible to pepper weevils than their ornamental or wild relatives (Chabaane et al. 2021). This is despite having been selected for both higher and lower levels of the defense compound capsaicin as compared to their wild relatives. The greater susceptibility could instead be due to factors like flower size and pericarp thickness. Similarly, in Lima bean, the larger seed size of domesticated forms has been found to reduce competition among seed beetle larvae, thereby making them more destructive pests (Cuny et al. 2017).

Coevolution – how insect population genetics have been affected by agriculture

Domestication is primarily thought of as affecting the genes of the domesticated species (Gross and Olsen 2010). However, there is substantial evidence that the genetics of insect herbivores have also been affected by crop evolution prior to, during, and after domestication (Vialatte et al. 2011; Midamegbe et al. 2011; Bourguet et al. 2014). This is akin to the situation described for some host-pathogen interactions in wild and domesticated common bean (Guzmán et al. 1995; Geffroy et al. 1997; Parker and Gepts 2021).

Insect herbivores feeding on crop plants can form distinct populations from other members of the same species feeding on their wild host plants (Vialatte et al. 2012) and may even diverge into distinct species (Bourguet et al. 2014). For example, generalist herbivores that feed on multiple crop plants can form distinctive strains associated with a given crop. This was demonstrated in the case of Fall Army Worm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae) (Acevedo et al. 2018). In this study, it was shown that when strains preferring maize or Bermuda grass were moved to the other crop, the plants expressed different induced defenses than when they were attacked by the strain adapted to the same crop. Since these induced defenses can compromise caterpillar growth, strong selection pressure is being exerted on the composition of their saliva to minimize plant defense trait selection (Acevedo et al. 2018).

Experimental evidence has also demonstrated that insect herbivores evolve into pests that are more difficult to control when a genetically variable population undergoes selection in the novel pressures of an agricultural environment (Via 1990). For example, strains of the polyphagous spider mite *Tetranychus urticae* (Trombidiformes: Arachnida)

moved from high-quality common bean host plants to low-quality tomato host plants showed a progressive increase in expression of 7.5% of all genes over five generations (Dermauw et al. 2012). This finding indicates that creating more adverse conditions for pests through the development of resistant varieties and pesticide applications could lead to better adaptation among pests in the future.

The coevolutionary relationship between crop wild relatives and insect pests in the center of origin predates domestication (Delgado Salinas et a. 1988). In some cases, coevolutionary relationships between wild plants and insects were disrupted by domestication (Chen and Welter 2002) but in other cases there is strong evidence of continued coevolution. For example, in apples, it has been found that autumn color has been maintained by coevolution with overwintering aphids (Archetti 2009). Red leaf color in autumn is achieved through the active production of anthocyanins. While aphids lay their eggs in the bark rather than on the leaves, red leaf color is an honest signal of the defenses of the tree and its quality as a host. Aphids have lower fitness on trees with red leaf color in the fall, and overall, these trees attract fewer aphids looking for egg-laying sites in the autumn (Archetti 2009; Holopainen et al. 2009).

The selective pressures of cultivation and storage can also create significant problems for pest management. In the example of bean weevils (Coleoptera: Bruchidae) which feed on both the wild and domesticated seeds of common bean, research has shown that the *Horismenus* parasitoids that control this pest in the wild have been unable to adapt well to conditions in which large quantities of seeds are stored between planting seasons (Laurin-Lemay et al 2013). Seed storage creates an ideal environment for the bean weevil population to quickly amplify through successive generations (Tuda et al. 2006) while the

parasitoids require alternate sources of nutrition such as nectar and pollen to complete
their lifecycle (Laurin-Lemay et al 2013).

Recommendations for future research

Domestication has greatly altered the interactions of insect pests and their host crop
plants. While this topic has been well studied, it is complex and highly specific to the
evolutionary history of each crop and insect pest. Understanding the ways in which
domestication has altered the genetic control of anti-herbivore defense traits may help
with breeding for recovery of these traits (Mammadov et al. 2018). More research is
needed to improve understanding of these relationships in agricultural systems, especially
on indigenous crops, which have been understudied but may play an important role in food
security as climate change creates adverse conditions for current staple crops (Dawson et
al. 2019; Dwyer et al. 2022).

While additional research may provide critical insight into the function and
evolution of unitary anti-herbivore defense traits, more consideration should also be given
to the interaction of multiple defense traits and what, if any, tradeoffs and synergies may
occur between them. Integrating the concept of defense syndrome and domestication
syndrome could yield interesting insight into overarching patterns of change within crop
and insect pest interactions (Poncet et al. 2004; Agrawal and Fishbein 2006; Meyer and
Purugganan 2013; Olson and Wendel 2013; Parker and Gepts 2021). Meta-analyses have
also provided helpful insight into the ways in which anti-herbivore defense traits have
been altered by domestication (Whitehead et al. 2017; Fernandez et al. 2021). With newly

developed software and machine learning methods improving the ease and accuracy of

such studies, more broad patterns may be revealed (Borenstein 2022).

References

Abbo S., Lev-Yadun S., Gopher A. (2011). Origin of Near Eastern plant domestication: homage to Claude Levi-Strauss and "La Pensée Sauvage". *Genetic Resources and Crop Evolution,* 58:175-179 doi: 10.1007/s10722-010-9630-0

Abdala-Roberts, L., Hernández-Cumplido, J., Chel-Guerrero, L., Betancur-Ancona, D., Benrey, B., & Moreira, X. (2016). Effects of plant intraspecific diversity across three trophic levels: Underlying mechanisms and plant traits. *American Journal of Botany*, 103(10), 1810–1818. doi: 10.3732/ajb.1600234

Acevedo, F. E., Peiffer, M., Ray, S., Meagher, R., Luthe, D. S., & Felton, G. W. (2018). Intraspecific differences in plant defense induction by fall armyworm strains. *New Phytologist*, 218(1), 310–321. doi: 10.1111/nph.14981

Acosta-Gallegos, J. A., Quintero, C., Vargas, J., Toro, O., Tohme, J., & Cardona, C. (1998). A new variant of arcelin in wild common bean, *Phaseolus vulgaris* L., from southern Mexico. *Genetic Resources and Crop Evolution*, 45(3), 235–242. doi: 10.1023/A:1008636132108

Agrawal, A. A., & Fishbein, M. (2006). Plant defense syndromes. *Ecology*, *87*(sp7), S132-S149. doi: 10.1890/0012-9658(2006)87[132:PDS]2.0.CO;2

Albuzio, A., Spettoli, P., & Cacco, G. (1978). Changes in gene expression from diploid to autotetraploid status of *Lycopersicon esculentum*. *Physiologia plantarum*, *44*(2), 77-80. doi: 10.1111/j.1399-3054.1978.tb01617.x

Allard, R. W. (1999). History of Plant Population Genetics. *Annual Review of Genetics*, 33(1), 1–27. doi: 10.1146/annurev.genet.33.1.1

Archetti, M. (2009). Evidence from the domestication of apple for the maintenance of autumn colours by coevolution. *Proceedings of the Royal Society B: Biological Sciences*, 276(1667), 2575–2580. doi: 10.1098/rspb.2009.0355

Arvanitis, L., Wiklund, C., Münzbergova, Z., Dahlgren, J. P., & Ehrlén, J. (2010). Novel antagonistic interactions associated with plant polyploidization influence trait selection and habitat preference. *Ecology Letters*, 13(3), 330–337. doi: 10.1111/j.1461-0248.2009.01429.x

Barbour, M. A., Rodriguez-Cabal, M. A., Wu, E. T., Julkunen-Tiitto, R., Ritland, C. E., Miscampbell, A. E., Jules, E. S., & Crutsinger, G. M. (2015). Multiple plant traits shape the genetic basis of herbivore community assembly. *Functional Ecology*, *29*(8), 995-1006. doi: 10.1111/1365-2435.12409

Bennett, E. (1970). Adaptation in wild and cultivated plant populations. In *Genetic resources in plants: their exploration and conservation;* Distributed by Blackwell Scientific.

Benrey, B., Callejas, A., Rios, L., Oyama, K. and Denno, R.F., 1998. The effects of domestication of *Brassica* and *Phaseolus* on the interaction between phytophagous insects and parasitoids. *Biological Control*, *11*(2), pp.130-140. doi:10.1006/bcon.1997.0590

Boerma, H. R., & Walker, D. R. (2005). Discovery and utilization of QTLs for insect resistance in soybean. *Genetica*, *123*(1), 181-189. doi: 10.1007/s10709-004-2741-9

Borenstein, M. (2022). Comprehensive meta-analysis software. *Systematic Reviews in Health Research: Meta-Analysis in Context*, 535-548. doi: 10.1002/9781119099369.ch27

Bourguet, D., Ponsard, S., Streiff, R., Meusnier, S., Audiot, P., et al. (2014). Becoming a species by becoming a pest' or how two maize pests of the genus *Ostrinia* possibly evolved through parallel ecological speciation events. *Molecular Ecology* 23: 325–342. doi: 10.1111/mec.12608.

Bowers, J. E., Chapman, B. A., Rong, J., & Paterson, A. H. (2003). Unravelling angiosperm genome evolution by phylogenetic analysis of chromosomal duplication events. *Nature*, *422*(6930), 433-438. doi: 10.1038/nature01521

Bruce, T. J. (2015). Interplay between insects and plants: dynamic and complex interactions that have coevolved over millions of years but act in milliseconds. *Journal of Experimental Botany*, *66*(2), 455-465. doi:10.1093/jxb/eru391

Campbell, S. A. (2015). Ecological mechanisms for the coevolution of mating systems and defence. *New Phytologist*, 205(3), 1047–1053. doi: 10.1111/nph.13212

Cardona, C., Posso, C. E., Kornegay, J., Valor, J., & Serrano, M. (1989). Antibiosis effects of wild dry bean accessions on the Mexican bean weevil and the bean weevil (Coleoptera: Bruchidae). *Journal of Economic Entomology*, *82*(1), 310-315. doi: 10.1093/jee/82.1.310

Cardona, C., Kornegay, J., Posso, C. E., Morales, F., & Ramirez, H. (1990). Comparative value of four arcelin variants in the development of dry bean lines resistant to the Mexican bean weevil. *Entomologia experimentalis et applicata*, *56*(2), 197-206. doi: 10.1111/j.1570-7458.1990.tb01397.x

Carr, D. E. & Eubanks, M. D. (2002). Inbreeding alters resistance to insect herbivory and host plant quality in *Mimulus guttatus* (Scrophulariaceae). *Evolution*, 56(1), 22–30. doi: 10.1111/j.0014-3820.2002.tb00846.x

Chabaane, Y., Haseeb, M. & Benrey, B. (2021). Domestication of chili pepper has altered fruit traits affecting the oviposition and feeding behavior of the pepper weevil. *Insects*, 12, doi: 10.3390/insects12070630

Chen, Y. H. (2016). Crop domestication, global human-mediated migration, and the unresolved role of geography in pest control: The role of geography in pest control. *Elementa: Science of the Anthropocene*, *4*. doi: 10.12952/journal.elementa.000106

Chen, Y. H., Gols, R., & Benrey, B. (2015). Crop domestication and its impact on naturally selected trophic interactions. *Annual Review of Entomology, 60*, 35-58. doi: 10.1146/annurev-ento-010814-020601

Chen, Y. H., Ruiz-Arocho, J., & von Wettberg, E. J. (2018). Crop domestication: anthropogenic effects on insect–plant interactions in agroecosystems. *Current Opinion in Insect Science*, *29*, 56-63. doi:10.1016/j.cois.2018.06.004

Chen, Y. H., & Welter, S. C. (2002). Abundance of a native moth *Homoeosoma electellum* (Lepidoptera: Pyralidae) and activity of indigenous parasitoids in native and agricultural sunflower habitats. *Environmental Entomology*, *31*(4), 626-636. doi: 10.1603/0046-225X-31.4.626

Crutsinger, G. M., Collins, M. D., Fordyce, J. A., Gompert, Z., Nice, C. C., & Sanders, N. J. (2006). Plant genotypic diversity predicts community structure and governs an ecosystem process. *Science*, *313*(5789), 966-968. doi: 10.1126/science.1128326

Cuny, M. A., Shlichta, G. J., & Benrey, B. (2017). The large seed size of domesticated Lima beans mitigates intraspecific competition among seed beetle larvae. *Frontiers in Ecology and Evolution*, *5*, 145. doi:10.3389/fevo.2017.00145

Dar, M. S., Dholakia, B. B., Kulkarni, A. P., Oak, P. S., Shanmugam, D., Gupta, V. S., & Giri, A. P. (2021). Influence of domestication on specialized metabolic pathways in fruit crops. *Planta*, 253(2), 61–. doi10.1007/s00425-020-03554-4

Darwin, C. (1859). On the origin of species by means of natural selection. J. Murray, London doi: 10.4324/9780203509104.

Dawson, I. K., Powell, W., Hendre, P., Bančič, J., Hickey, J. M., Kindt, R., Hoad, S., Hale, I., & Jamnadass, R. (2019). The role of genetics in mainstreaming the production of new and orphan crops to diversify food systems and support human nutrition. *New Phytologist*, 224(1), 37–54. doi; 10.1111/nph.15895

Debouck, D. G., & Tohme, J. M. (1989). Implications for bean breeders of studies on the origins of common beans, *Phaseolus vulgaris* L. https://hdl.handle.net/10568/88245

Delgado Salinas, A., Bonet, A., & Gepts, P. (1988). The Wild Relative of *Phaseolus vulgaris* in Middle America. In *Genetic resources of* Phaseolus *beans* (pp. 163-184). Springer, Dordrecht.

de Pascual-Teresa, S., & Sanchez-Ballesta, M. T. (2008). Anthocyanins: from plant to health. *Phytochemistry Reviews*, *7*(2), 281-299. doi:10.1007/s11101-007-9074-0

Dermauw, W., Wybouw, N., Rombauts, S., Menten, B., Vontas, J., Grbić, M., Clark, R., Feyereisen, R., & Van Leeuwen, T. (2013). A link between host plant adaptation and pesticide resistance in the polyphagous spider mite *Tetranychus urticae*. Proceedings of the National Academy of Sciences - PNAS, 110(2), E113–E122. doi: 10.1073/pnas.1213214110.

Deutsch, C. A., Tewksbury, J. J., Tigchelaar, M., Battisti, D. S., Merrill, S. C., Huey, R. B., & Naylor, R. L. (2018). Increase in crop losses to insect pests in a warming climate. *Science*, *361*(6405), 916-91. doi: 10.1126/science.aat3466

Dhawan, O. P., & Lavania, U.C. (1996). Enhancing the productivity of secondary metabolites via induced polyploidy: a review. *Euphytica*, *87*(2), 81–89. doi: 10.1007/BF00021879

Du, H., Qin, R., Li, H., Du, Q., Li, X., Yang, H., Kong, F., Liu, B., Yu, D., & Wang, H. (2022). Genome-Wide Association Studies Reveal Novel Loci for Herbivore Resistance in Wild Soybean (*Glycine soja*). *International Journal of Molecular Sciences*, *23*(14), 8016. Doi: 10.1007/s11655-014-1957-5

Dwyer, W., Ibe, C. N., & Rhee, S. Y. (2022). Renaming Indigenous crops and addressing colonial bias in scientific language. *Trends in Plant Science*. doi: 10.1016/j.tplants.2022.08.022

Edger, P., Heidel-Fischer, H. M., Bekaert, M., Rota, J., Glöckner, G., Platts, A. E., Heckel, D. G., Der, J. P., Wafula, E. K., Tang, M., Hofberger, J. A., Smithson, A., Hall, J. C., Blanchette, M., Bureau, T. E., Wright, S. I., de Pamphilis, C. W., Schranz, M. E., Barker, M. S., Conant, G. C., Wahlberg, N., Vogel, H., Pires, J. C., & Wheat, C. W. (2015). The butterfly plant arms-race escalated by gene and genome duplications. *Proceedings of the National Academy of Sciences - PNAS*, 112(27), 8362–8366. doi: 10.1073/pnas.1503926112

Ehrlich, P. R., & Raven, P. H. (1964). Butterflies and plants: a study in coevolution. *Evolution*, 586-608. doi: 10.1111/j.1558-5646.1964.tb01674.x

Fernandez, A. R., Saez, A., Quintero, C., Gleiser, G. & Aizen, M. A. (2021). Intentional and unintentional selection during plant domestication: herbivore damage, plant defensive traits and nutritional quality of fruit and seed crops. *New Phytologist*, 231, 1586-1598. doi: 10.1111/nph.17452

Ferrero, V., Baeten, L., Blanco-Sanchez, L., Planello, R., Diaz-Pendon, J. A., Rodriguez-Echeverria, S., et al (2020). Complex patterns in tolerance and resistance to pests and diseases underpin the domestication of tomato. *New Phytologist*, 226, 254-266. doi: 10.1111/nph.16353

Fontes-Puebla, A. A., Borrego, E. J., Kolomiets, M. V. & Bernal, J. S. (2021). Maize biochemistry in response to root herbivory was mediated by domestication, spread, and breeding. *Planta*, 254, doi: 10.1007/s00425-021-03720-2

Frost, C. J., Mescher, M. C., Carlson, J. E., & De Moraes, C. M. (2008). Plant defense priming against herbivores: getting ready for a different battle. *Plant Physiology*, *146*(3), 818-824. doi: 10.1104/pp.107.113027

Fuller, D. Q., & Allaby, R. (2018). Seed dispersal and crop domestication: shattering, germination and seasonality in evolution under cultivation. *Annual Plant Reviews online*, 238-295. doi: 10.1002/9781119312994.apr0414

Garvey, M., Creighton, C. & Kaplan, I. (2020). Pepper domestication enhances parasitoid recruitment to herbivore-damaged plants. *Arthropod - Plant Interactions*, 14, 695-703. doi: 10.1007/s11829-020-09788-z

Geffroy, V., Sicard, D., de Oliveira, J., Sévignac, M., Cohen, S., Gepts, P., Neema, C., Dron, M. (1999). Identification of an ancestral resistance gene cluster involved in the coevolution process between *Phaseolus vulgaris* and its fungal pathogen *Colletotrichum lindemuthianum*. *Molecular Plant-Microbe Interactions* 12:774-784 doi: 10.1094/MPMI.1999.12.9.774

Gepts, P. (1988). Phaseolin as an evolutionary marker. In *Genetic resources of Phaseolus beans* (pp. 215-241). Springer, Dordrecht. doi: 10.1007/978-94-009-2786-5_11

Gepts, P. (2004). Crop domestication as a long-term selection experiment. *Plant Breeding Reviews* 24 (Part 2):1-44. doi: 10.1002/9780470650288.ch1

Gepts, P. (2014). The contribution of genetic and genomic approaches to plant domestication studies. *Current Opinion Plant Biology* 18:51-59 doi: 10.1016/j.pbi.2014.02.001

Gepts P. (2002). Evolution during domestication. Encyclopedia of Life Sciences http://www.els.net/. Nature Publishing Group, London doi: 10.1038/npg.els.0003071

Gloss, A. D., Dittrich, A. C. N., Goldman-Huertas, B., & Whiteman, N. K. (2013). Maintenance of genetic diversity through plant–herbivore interactions. *Current Opinion in Plant Biology*, 16(4), 443–450. doi: 10.1016/j.pbi.2013.06.002

Gross, B. L., & Olsen, K. M. (2010). Genetic perspectives on crop domestication. *Trends in Plant Science*, *15*(9), 529-537. doi: 10.1016/j.tplants.2010.05.008

Guzmán, P., Gilbertson, R. L., Nodari, R., Johnson, W. C., Temple, S. R., Mandala, D., Mkandawire, A. B. C., Gepts, P. (1995). Characterization of variability in the fungus *Phaeoisariopsis griseola* suggests coevolution with the common bean (*Phaseolus vulgaris*). *Phytopathology* 85:600-607 doi: 10.1094/Phyto-85-600

Hagel, G. T. (1978). *Lygus spp.*: damage to beans by reducing yields, seed pitting, and control by varietal resistance and chemical sprays. *Journal of Economic Entomology* 71.4, 613–15. doi:10.1093/JEE/71.4.613.

Hajjar, R., & Hodgkin, T. (2007). The use of wild relatives in crop improvement: a survey of developments over the last 20 years. *Euphytica*, *156*(1), 1-13. doi: 10.1007/s10681-007-9363-0

Harlan, J. R. (1976). Genetic resources in wild relatives of crops 1. *Crop science*, *16*(3), 329-333. doi: 10.2135/cropsci1976.0011183X001600030004x

Hillman, G. C., & Davies, M. S. (1990). Measured domestication rates in wild wheats and barley under primitive cultivation, and their archaeological implications. *Journal of World Prehistory*, *4*(2), 157-222. doi: 10.1007/BF00974763

Hodgkin T., & Hajjar, R. (2007). Using crop wild relatives for crop improvement: trends and perspectives. In *Crop wild relative conservation and use* (pp. 535–548). CABI. doi: 10.1079/9781845930998.0535

Hofberger, J. A., Lyons, E., Edger, P. P., Chris Pires, J., & Eric Schranz, M. (2013). Whole genome and tandem duplicate retention facilitated glucosinolate pathway diversification in the mustard family. *Genome biology and evolution*, *5*(11), 2155-2173. doi: 10.1093/gbe/evt162

Holopainen, J. K., Semiz, G., & Blande, J. D. (2009). Life-history strategies affect aphid preference for yellowing leaves. *Biology letters*, *5*(5), 603-605. doi: 10.1098/rsbl.2009.0372

Horn, D. J. (2009). IPM as applied ecology: the biological precepts. *Integrated pest management: concepts, tactics, strategies and case studies* (Radcliffe, W. D. Hutchison, & R. E. Cancelado, Eds.). Cambridge University Press. pg 51

Hufford M. B., Berny Mier y Teran J. C., Gepts P. (2019). Crop biodiversity: an unfinished magnum opus of nature. *Annual Reviews in Plant Biology* 70: 727-751 doi: 10.1146/annurev-arplant-042817-040240

Huot, B., Yao, J., Montgomery, B. L., & He, S. Y. (2014). Growth-defense tradeoffs in plants: a balancing act to optimize fitness. *Molecular Plant*, 7(8), 1267–1287. doi: 10.1093/mp/ssu049

Jaccard, C., Marguier, N. T., Arce, C. C. M., Bruno, P., Glauser, G., Turlings, T. C. J., et al (2022). The effect of squash domestication on a belowground tritrophic interaction. *Plant-Environment Interactions,* 3, 28-39. doi: 10.1002/pei3.10071

Jamieson, M. A., Trowbridge, A. M., Raffa, K. F., & Lindroth, R. L. (2012). Consequences of climate warming and altered precipitation patterns for plant-insect and multitrophic interactions. *Plant physiology*, *160*(4), 1719-1727. doi: 10.1104/pp.112.206524

Jarne, P. & Charlesworth, D. (1993). The Evolution of the Selfing Rate in Functionally Hermaphrodite Plants and Animals. *Annual Review of Ecology and Systematics*, 24(1), 441–466. doi: 10.1146/annurev.es.24.110193.002301

Johnson, M. T. J. (2008). Bottom-up effects of plant genotype on aphids, ants, and predators. *Ecology*, *89*(1), 145-154. doi: 10.1890/07-0395.1

Johnson M. T. J., Ives, A. R., Ahern, J., & Salminen, J. (2014). Macroevolution of plant defenses against herbivores in the evening primroses. *New Phytologist*, 203(1), 267–279. doi: 10.1111/nph.12763

Johnson M. T. J., Smith, S. D., & Rausher, M. D. (2009). Plant sex and the evolution of plant defenses against herbivores. *Proceedings of the National Academy of Sciences - PNAS*, 106(43), 18079–18084. doi: 10.1073/pnas.0904695106

Karban, R. & Baldwin, I. T. (1997). *Induced responses to herbivory*. University of Chicago Press. doi: 10.1017/S0007485300042279

Kaushik, U., Aeri, V., & Mir, S. R. (2015). Cucurbitacins – an insight into medicinal leads from nature. *Pharmacognosy Reviews*, *9*(17), 12. doi: 10.4103/0973-7847.156314

Keesing, V., & Wratten, S. D. (1997). Integrating plant and insect conservation. In *Plant Genetic Conservation* (pp. 220-235). Springer, Dordrecht.

Khoury, C. K., Bjorkman, A. D., Dempewolf, H., Ramirez-Villegas, J., Guarino, L., Jarvis, A., ... & Struik, P. C. (2014). Increasing homogeneity in global food supplies and the implications for food security. *Proceedings of the National Academy of Sciences - PNAS*, *111*(11), 4001-4006. doi: 10.1073/pnas.131349011

Khoury, C. K., Brush S., Costich, D. E., Curry, H. A., De Haan, S., Engels, J. M. M., Guarino, L., Hoban, S., Mercer, K. L., Miller, A.J., Nabhan, G.P., Perales, H.R., Richards, C., Riggins, C., Thormann, I. (2021). Crop genetic erosion: understanding and responding to loss of crop diversity. *New Phytologist* 233:84-118 doi: 10.1111/nph.17733

Klein, A. M., Vaissière, B. E., Cane, J. H., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C., & Tscharntke, T. (2007). Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society B: biological sciences*, *274*(1608), 303-313. doi: 10.1098/rspb.2006.3721

Köllner, T. G., Held, M., Lenk, C., Hiltpold, I., Turlings, T. C., Gershenzon, J., & Degenhardt, J. (2008). A maize (E)-β-caryophyllene synthase implicated in indirect defense responses against herbivores is not expressed in most American maize varieties. *The Plant Cell*, *20*(2), 482-494. doi: 10.1105/tpc.107.051672

Laurin-Lemay, S., Angers, B., Benrey, B., & Brodeur, J. (2013). Inconsistent genetic structure among members of a multitrophic system: did bruchid parasitoids (*Horismenus spp.)* escape the effects of bean domestication? *Bulletin of Entomological Research*, *103*(2), 182–192. doi: 10.1017/S000748531200051X

Levy, M. (1976). Altered glycoflavone expression in induced autotetraploids of *Phlox drummondii. Biochemical Systematics and Ecology*, 4(4), 249–254. doi: 10.1016/0305-1978(76)90047-8

Mainland, C. M. M. (2012). Frederick V. Coville and the history of North American highbush blueberry culture. *International Journal of Fruit Science*, *12*(1-3), 4-13. doi: 10.1080/15538362.2011.619117

Mammadov, J., Buyyarapu, R., Guttikonda, S. K., Parliament, K., Abdurakhmonov, I. Y., & Kumpatla, S. P. (2018). Wild relatives of maize, rice, cotton, and soybean: treasure troves for tolerance to biotic and abiotic stresses. *Frontiers in Plant Science*, *9*, 886. doi: 10.3389/fpls.2018.00886

Maxted, N., Kell, S. P., & Ford-Lloyd, B. V. (2007). Crop wild relative conservation and use: establishing the context. In *Crop Wild Relative Conservation and Use* (pp. 3-30). Wallingford UK: CABI. doi: 10.1079/9781845930998.0000

Maxted, N., Kell, S., & Brehm, J. M. (2014). Crop wild relatives and climate change. In *Plant genetic resources and climate change* (pp. 114-136). Wallingford UK: CABI. doi: 10.1079/9781780641973.0114

McCall, A. C., & Fordyce, J. A. (2010). Can optimal defence theory be used to predict the distribution of plant chemical defences. *The Journal of Ecology*, 98(5), 985–992. doi: 10.1111/j.1365-2745.2010.01693.x

Mercer, K. L., & Perales, H. R. (2010). Evolutionary response of landraces to climate change in centers of crop diversity. *Evolutionary Applications*, *3*(5-6), 480-493. doi: 10.1111/j.1752-4571.2010.00137.x

Meyer, R. S., & Purugganan, M. D. (2013). Evolution of crop species: genetics of domestication and diversification. *Nature Reviews Genetics*, *14*(12), 840-852. doi: 10.1038/nrg3605

Midamegbe, A., Vitalis, R., Malausa, T., Delava, É., Cros-Arteil, S., et al. (2011). Scanning the European corn borer (*Ostrinia spp.*) genome for adaptive divergence between host-affiliated sibling species. *Molecular Ecology* **20**: 1414–1430. doi: 10.1111/j.1365-294X.2011.05035.x.

Mitchell, C., Brennan, R. M., Graham, J., & Karley, A. J. (2016). Plant defense against herbivorous pests: exploiting resistance and tolerance traits for sustainable crop protection. *Frontiers in Plant Science*, *7*, 1132. doi: 10.3389/fpls.2016.01132

Mundt, C. C. (2002). Use of multiline cultivars and cultivar mixtures for disease management. *Annual Review of Phytopathology*, *40*(1), 381-410. doi: 10.1146/annurev.phyto.40.011402.113723

Muola, A., Mutikainen, P., Laukkanen, L., Lilley, M., & Leimu, R. (2011). The role of inbreeding and outbreeding in herbivore resistance and tolerance in *Vincetoxicum hirundinaria*. *Annals of Botany*, 108(3), 547–555. doi: 10.1093/aob/mcr174

Murdoch, W. W., Evans, F. C., & Peterson, C. H. (1972). Diversity and pattern in plants and insects. *Ecology*, *53*(5), 819-829. doi: 10.2307/1934297

Myers, J. R., Kusolwa, P. M., Beaver, J. S. (2021). Breeding the common bean for weevil resistance. *Chronica Horticulturae* 61:16-20 doi: 10.3198/jpr2015.10.0064crg

Norris, K. (2008). Agriculture and biodiversity conservation: opportunity knocks. *Conservation letters*, *1*(1), 2-11. doi: 10.1111/j.1755-263X.2008.00007.x

Nuismer, S. L., & Thompson, J. N. (2001). Plant polyploidy and non-uniform effects on insect herbivores. *Proceedings of the Royal Society. B, Biological Sciences*, *268*(1479), 1937–1940. doi: 10.1098/rspb.2001.1760

O'Connor, T. K., Laport, R. G., & Whiteman, N. K. (2019). Polyploidy in creosote bush (*Larrea tridentata*) shapes the biogeography of specialist herbivores. *Journal of Biogeography*, *46*(3), 597-610. doi: 10.1111/jbi.13490

Olsen, K. M., Wendel, J. F. (2013). A bountiful harvest: Genomic insights into crop domestication phenotypes. *Annual Reviews in Plant Biology* 64:47-70 doi: 10.1146/annurev-arplant-050312-120048

Osborn, T. C. (2004). The contribution of polyploidy to variation in Brassica species. *Physiologia Plantarum*, *121*(4), 531-536 doi: 10.1111/j.1399-3054.2004.00360.x

Osborn, T. C., Alexander, D. C., Sun, S. S. M., Cardona, C., Bliss, F. A. (1988). Insecticidal activity and lectin homology of arcelin seed protein. *Science* 240:207-210 doi: 10.1126/science.240.4849.207

Osborn, T. C., Blake, T., Gepts, P., Bliss, F. A. (1986) Bean arcelin. 2. Genetic variation, inheritance and linkage relationships of a novel seed protein of *Phaseolus vulgaris*. *Theoretical and Applied Genetics* 71:847-855 doi: 10.1007/BF02859659

Ordas, B., Malvar, R. A., Santiago, R., & Butron, A. (2010). QTL mapping for Mediterranean corn borer resistance in European flint germplasm using recombinant inbred lines. *BMC Genomics*, *11*(1), 1-10. doi: 10.1186/1471-2164-11-174

Otto, S. P. (2007). The evolutionary consequences of polyploidy. *Cell*, *131*(3), 452–462. doi: 10.1016/j.cell.2007.10.022

Parker, T.A., & Gepts, P. (2021). Population genomics of *Phaseolus spp.*: a domestication hotspot. In: Population Genomics. Springer, Cham. doi: 10.1007/13836_2021_89

Paterson, A. H., Bowers, J. E., Peterson, D. G., Estill, J. C., & Chapman, B. A. (2003). Structure and evolution of cereal genomes. *Current Opinion in Genetics & Development*, *13*(6), 644-650. doi: 10.1016/j.gde.2003.10.002

Poncet, V., Robert, T., Sarr, A., & Gepts, P. (2004). Quantitative trait locus analyses of the domestication syndrome and domestication process. *Encyclopedia of Plant and Crop Science*, *1069*, 1074. doi: 10.1081/E-EPCS 120017088

Purugganan, M. D., & Fuller, D. Q. (2011). Archaeological data reveal slow rates of evolution during plant domestication. *Evolution: International Journal of Organic Evolution*, *65*(1), 171-183. doi: 10.1111/j.1558-5646.2010.01093.x

Ram, T., Deen, R., Gautam, S., Ramesh, K., Rao, Y., and Brar, D. (2010). Identification of new genes for brown planthopper resistance in rice introgressed from *O. glaberrima* and *O. minuta*. *Rice* 25, 67–69. doi: 10.1186/s12284-017-0178-x

Raven, P. H. & Wagner, D. L. (2021). Agricultural intensification and climate change are rapidly decreasing insect biodiversity. *Proceedings of the National Academy of Sciences - PNAS*, *118*(2). doi: 10.1073/pnas.2002548117

Renny-Byfield, S., & Wendel, J. F. (2014). Doubling down on genomes: polyploidy and crop plants. *American Journal of Botany*, *101*(10), 1711-1725. doi: 10.3732/ajb.1400119

Rick, C. M. (1988). Evolution of mating systems in cultivated plants. In *Plant evolutionary Biology* (pp. 133-147). Springer, Dordrecht. doi: 10.1007/978-94-009-1207-6_6

Roberts, M. R., & Paul, N. D. (2006). Seduced by the dark side: integrating molecular and ecological perspectives on the influence of light on plant defence against pests and pathogens. *New Phytologist*, *170*(4), 677-699. doi: 10.1111/j.1469-8137.2006.01707.x

Rodriguez-Saona, C., Vorsa, N., Singh, A. P., Johnson-Cicalese, J., Szendrei, Z., Mescher, M. C., & Frost, C. J. (2011). Tracing the history of plant traits under domestication in cranberries: potential consequences on anti-herbivore defences. *Journal of Experimental Botany*, *62*(8), 2633-2644. doi: 10.1093/jxb/erq466

Rodriguez-Saona, C., Cloonan, K. R., Sanchez-Pedraza, F., Zhou, Y., Giusti, M. M., & Benrey, B. (2019). Differential susceptibility of wild and cultivated blueberries to an invasive frugivorous pest. *Journal of Chemical Ecology*, *45*(3), 286-297. doi: 10.1007/s10886-018-1042-1

Romero-Andreas, J., Yandell, B. S., Bliss, F. A. (1986). Bean arcelin. 1. Inheritance of a novel seed protein of *Phaseolus vulgaris* L. and its effect on seed composition. *Theoretical and Applied Genetics* 72:123-128 doi: 10.1007/BF00261467

Root, R. B. (1973). Organization of a plant-arthropod association in simple and diverse habitats: the fauna of collards (*Brassica oleracea*). *Ecological Monographs*, 43(1), 95-124. doi: 10.2307/1942161

Salman-Minkov, A., Sabath, N., & Mayrose, I. (2016). Whole-genome duplication as a key factor in crop domestication. *Nature Plants*, 2(8), 16115–16115. doi: 10.1038/nplants.2016.115

Sánchez-Pérez, R., Ortega, E., Duval, H., Martinez-Gomez, P., & Dicenta, F. Inheritance and relationships of important agronomic traits in almond. *Euphytica* 155, 381–391 (2007). doi: 10.1007/s10681-006-9339-5

Schrieber, Schweiger, R., Kröner, L., Müller, C., & Campbell, H. (2019). Inbreeding diminishes herbivore-induced metabolic responses in native and invasive plant populations. *The Journal of Ecology*, 107(2), 923–936. doi: 10.1111/1365-2745.13068

Schuman, M. C., & Baldwin, I. T. (2016). The layers of plant responses to insect herbivores. *Annual Review of Entomology*, *61*(1), 373-394. doi: 10.1146/annurev-ento-010715-023851

Segraves, K. A., & Anneberg, T. J. (2016). Species interactions and plant polyploidy. *American Journal of Botany*, *103*(7), 1326-1335. doi: 10.3732/ajb.1500529

Shang, Y., Ma, Y., Zhou, Y., Zhang, H., Duan, L., Chen, H., Zeng, J., Zhou, Q., Wang, S., Gu, W., Liu, M., Ren, J., Gu, X., Zhang, S., Wang, Y., Yasukawa, K., Bouwmeester, H. J., Qi, Z., Zhang, Z., Lucas, W. J., & Huang, S. (2014). Biosynthesis, regulation, and domestication of bitterness in cucumber. *Science*, *346*(6213), 1084-1088. doi: 10.1126/science.1259215

Sharma, S., Kooner, R., & Arora, R. (2017). Insect pests and crop losses. In *Breeding Insect Resistant Crops for Sustainable Agriculture* (pp. 45-66). Springer, Singapore. doi: 10.1007/978-981-10-6056-4_2

Shoji, T., Umemoto, N., & Saito, K. (2021). Genetic divergence in transcriptional regulators of defense metabolism: insight into plant domestication and improvement. *Plant Molecular Biology*, 1-11. doi: 10.1007/s11103-021-01159-3

Solís-Montero, V., Martínez-Natarén, D. A., Parra-Tabla, V., Ibarra-Cerdeña, C., & Munguía-Rosas, M. A. (2020). Herbivory and anti-herbivore defences in wild and cultivated *Cnidoscolus aconitifolius*: disentangling domestication and environmental effects. *AoB Plants*, *12*(3), plaa023. doi: 10.1093/aobpla/plaa023

Souna, D. A., Bokonon-Ganta, A. H., Dannon, E. A., Imorou, N., Agui, B., Cusumano, A., Srinivasan, R., Pittendrigh, B. R., Volkoff, A., & Tamò, M. (2019). Volatiles from *Maruca vitrata* (Lepidoptera, Crambidae) host plants influence olfactory responses of the parasitoid *Therophilus javanus* (Hymenoptera, Braconidae, Agathidinae). *Biological Control*, 130, 104-109. doi: 10.1016/j.biocontrol.2018.11.002

Stout, M. J. (2014). Types and mechanisms of rapidly induced plant resistance to herbivorous arthropods. *Induced Resistance for Plant Defense*, 81-105. doi: 10.1002/9781118371848.ch5

Tallamy, D. W., Stull, J., Ehresman, N. P., Gorski, P. M., & Mason, C. E. (1997). Cucurbitacins as feeding and oviposition deterrents to insects. *Environmental Entomology*, *26*(3), 678-683. doi: 10.1093/ee/26.3.678

Thodberg, S., Del Cueto, J., Mazzeo, R., Pavan, S., Lotti, C., Dicenta, F., Jakobsen Neilson, E. H., Møller, B. L., & Sánchez-Pérez, R. (2018). Elucidation of the amygdalin pathway reveals the metabolic basis of bitter and sweet almonds (*Prunus dulcis*). *Plant physiology*, *178*(3), 1096-1111. doi: 10.1104/pp.18.00922

Tigist, S. G., Raatz, B., Assefa, A., Melis, R., Sibiya, J., Keneni, G., Mukankusi, C., Fenta, B., Ketema, S., & Tsegaye, D. (2021). Introgression of bruchid (*Zabrotes subfasciatus*) resistance into small red common bean (*Phaseolus vulgaris*) background and validation of the BRU_00261 (snpPV0007) resistance marker. *Plant Breeding*, *140*(6), 1081-1089. doi: 10.1111/pbr.12969

Tilman, D. (1999). Global environmental impacts of agricultural expansion: the need for sustainable and efficient practices. *Proceedings of the National Academy of Sciences – PNAS*, *96*(11), 5995-6000. doi: 10.1073/pnas.96.11.5995

Tooker, J. F., & Frank, S. D. (2012). Genotypically diverse cultivar mixtures for insect pest management and increased crop yields. *Journal of Applied Ecology*, *49*(5), 974-985. doi: 10.1111/j.1365-2664.2012.02173.x

Tuda, M, Rönn, J., Buranapanichpan, S., Wasano, N., & Arnqvist, G. (2006). Evolutionary diversification of the bean beetle genus *Callosobruchus* (Coleoptera: Bruchidae): traits associated with stored-product pest status. *Molecular Ecology*, 15(12), 3541–3551. doi: 10.1111/j.1365-294X.2006.03030.x

Turlings, T. C. J., Loughrin, J. H., McCall, P. J., Röse, U. S. R., Lewis, W. J., Tumlinson, J. H. (1995). How caterpillar-damaged plants protect themselves by attracting parasitic wasps. *Proceedings of the National Academy of Sciences – PNAS*, USA 92, 4169–4174. doi: 10.1073/pnas.92.10.4169

Turlings, T. C. J., Tumlinson, J. H., Lewis, W. J. (1990). Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. *Science* 250, 1251–1253. doi: 10.1126/science.250.4985.1251

Turner, N., Łuczaj, Ł. J., Migliorini, P., Pieroni, A., Dreon, A. L., Sacchetti, L. E., & Paoletti, M. G. (2011). Edible and tended wild plants, traditional ecological knowledge and agroecology. *Critical Reviews in Plant Sciences*, *30*(1-3), 198–225. doi: 10.1080/07352689.2011.554492

Via, S. (1990). Ecological genetics and host adaptation in herbivorous insects: the experimental study of evolution in natural and agricultural systems. *Annual Review of Entomology*, 35(1), 421–446. doi: 10.1146/annurev.en.35.010190.002225

Vialatte, A., Dedryver, C. A., Simon, J. C., Galman, M., Plantegenest, M. (2005). Limited genetic exchanges between populations of an insect pest living on uncultivated and related cultivated host plants. *Proceedings of the Royal Society B-Biological Sciences p*272: 1075–1082. doi: 10.1098/rspb.2004.3033

Vorsa, N., & Zalapa, J. (2019). Domestication, genetics, and genomics of the American cranberry. *Plant Breeding Reviews*, *43*, 279-315. doi: 10.1002/9781119616801.ch8

Wendel, J. F., Flagel, L. E., & Adams, K. L. (2012). Jeans, genes, and genomes: cotton as a model for studying polyploidy. In *Polyploidy and genome evolution* (pp. 181-207). Springer, Berlin, Heidelberg. doi: 10.1007/978-3-642-31442-1_10

Whitehead, S. R., Turcotte, M. M., & Poveda, K. (2017). Domestication impacts on plant–herbivore interactions: a meta-analysis. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *372*(1712), 20160034. doi: 10.1098/rstb.2016.0034

Willcox, G. (2012). Pre-domestic cultivation during the late Pleistocene and early Holocene in the northern Levant. *Biodiversity in Agriculture: Domestication, Evolution, and Sustainability*, 92-109. doi: 10.1017/CBO9781139019514.007

Wittkop, B., Snowdon, R. J., & Friedt, W. (2009). Status and perspectives of breeding for enhanced yield and quality of oilseed crops for Europe. *Euphytica*, *170*(1), 131-140. doi: 10.1007/s10681-009-9940-5

Woltz, J. M., Werling, B. P., & Landis, D. A. (2012). Natural enemies and insect outbreaks in agriculture: a landscape perspective. *Insect outbreaks revisited, Wiley-Blackwell, Hoboken*, 355-371. doi: 10.1002/9781118295205.ch17

Zagrobelny, M., Bak, S., Rasmussen, A. V., Jørgensen, B., Naumann, C. M., & Møller, B. L. (2004). Cyanogenic glucosides and plant–insect interactions. *Phytochemistry*, *65*(3), 293-306. doi: 10.1016/j.phytochem.2003.10.016

Zhang, K., Lin, S., Ji, Y., Yang, C., Wang, X., Yang, C., Wang, H., Jiang, H., Harrison, R. D., & Yu, D. W. (2016). Plant diversity accurately predicts insect diversity in two tropical landscapes. *Molecular ecology*, *25*(17), 4407-4419. doi: 10.1101/040105

Zohary, D. (2004). Unconscious selection and the evolution of domesticated plants. *Economic botany*, *58*(1), 5-10. doi: 10.1663/0013-0001(2004)058[0005:USATEO]2.0.CO;2

Chapter 2: Genome-Wide Association Study of Cyanogenesis in Lima Beans (*Phaseolus lunatus*)

Abstract

Cyanogenesis is a common trait in crop plants due to its function in repelling herbivores (Bolarinwa et al. 2016; Jones 1998). However, the production of cyanide, from precursor cyanogenic glycosides, has been selected against in edible tissues during and after crop domestication due to human toxicity (Shlichta et al., 2018; Meyer and Purugganan, 2013). The goal of this study is to better understand the effect of domestication on cyanogenesis in Lima beans (*Phaseolus lunatus*). A genome-wide association study of volatile cyanide production in the first 15 minutes after tissue disruption in both flowers and young pods of a diversity panel of wild and domesticated lines from the Mesoamerican gene pool revealed several highly significant SNPs. Many of these were closely located to matches of the *Li/li* gene from white clover (*trifolium repens*) which controls β-glucosidase production. Additional SNPs were found from the phenotype of cyanide released 15-30 minutes after tissue disruption. Many of these were closely located to matches of the *Ac/ac* gene sequence from white clover. Additionally, a significant SNP on chromosome 5 in the 15-30 minute exposure window was in close proximity to QTL found previously in a biparental recombinant inbred line (RIL) population for cyanogenesis in flowers, immature pods, and leaves. It was also near a match of the white clover gene *Li/li* sequence. These intriguing results will be the basis of future studies designed to identify the genes controlling cyanogenesis in Lima bean and utilize this trait in breeding

Introduction

Cyanogenesis is a trait that has evolved multiple times across diverse plant families (Pichersky and Lewinsohn 2011). The percentage of edible crop plants that are cyanogenic exceeds with statistical significance the percentage of wild plants that are cyanogenic (Jones 1998). This fact has given rise to the theory that cyanogenesis was an advantageous trait selected for by early farmers as it protected crops from herbivores but could be removed by post-harvest processing (Jones 1998; Wilson and Dufour 2002; Ballhorn et al. 2009). Knowledge of detoxification methods such as fermenting, leaching, and cooking predate agriculture and may even predate the evolution of modern humans (Ragir 2000; Wrangham and Conklin 2003). Cyanogenesis in harvested organs was selected against during domestication in some species like cassava, almond, and Lima bean despite the knowledge of detoxification methods and the trait's utility as an anti-herbivore defense (Ballhorn et al. 2009; Shlichta et al. 2018; Sánchez-Pérez et al. 2019; Ospina et al. 2020). This may contribute to higher vulnerability of these and other crops to insect herbivores (Shlichta et al. 2018; Cuny et al. 2019). Analysis of the genes underlying cyanogenesis using a genome-wide association study of a Mesoamerican diversity panel that includes wild and domesticated accessions could elucidate the mechanism by which this trait has been so reduced or eliminated in the domesticated gene pool. This in turn could improve understanding of the domestication history and aid conservation and plant breeding efforts.

Lima beans are one of only a few pulse crops that produce cyanide and are the only species in the genus *Phaseolus* to do so (Jones 1988; National Research Council 2002; Shlichta 2014). Hydrogen cyanide (HCN) has been found in wild Lima beans at levels lethal

to humans, but it is consistently less abundant or undetectable in the seeds of domesticated lines (Vanderborght 1979; Smartt 1988). Cooking eliminates the cyanide content of mature beans, rendering them safe for human consumption (Adeparusi 2001). Cyanide production in the leaves of domesticated Lima beans has been found to be equal to or greater than concentrations in wild Lima beans (Shlichta et al. 2018). Floral tissue was not tested in the previously referenced study, but expression of cyanogenesis in flowers may be important as they are especially vulnerable to economic damage by hemipteran pests like *Lygus hesperus* (Alvarado-Rodriquez et al. 1986)

The two main cyanogenic glucosides produced by Lima bean are linamarin and lotaustralin (Frehner et al. 1990). Linamarin is synthesized from valine and lotaustralin from isoleucine (Hughes and Conn 1976). Cyanogenic glucosides are stored in the vacuole of the plant cell and released when the tissue is ruptured (Hartmann 2007). At that point, they are hydrolyzed by linamarase which produces glucose and acetone cyanohydrin. The acetone cyanohydrin then spontaneously or with hydroxynitrile lyase splits into acetone and hydrogen cyanide.

Prior research has identified several QTL for volatile cyanide produced in the floral buds, immature pods, and leaves of a biparental recombinant inbred line (RIL) population of Lima beans derived from an Andean and a Mesoamerican domesticated parents (Dohle 2017; Zullo 2021). Additional research has identified the sequence of both the gene that controls production of cyanogenic glucosides and the hydrolyzing enzyme in white clover (*Trifolium repens*), a legume relative of Lima bean (Olsen et al. 2008). During the process of domestication, one or both genes may have been lost – or their expression reduced – resulting in lower cyanogenic capacity of domesticated lines compared to wild lines. While

these data provide clues to the control of cyanogenesis in Lima bean, data from more diverse germplasm would strengthen these results. This study examines the relationship between these previously discovered QTL and the findings of a genome-wide association study in a Mesoamerican diversity panel of wild and domesticated Lima beans. This research aims to elucidate how cyanogenesis has been affected by domestication in the Mesoamerican gene pool of Lima beans with special consideration of the cyanogenic capacity of California cultivars.

Materials and Methods

Plant Materials

A diversity panel of 363 lines of wild and domesticated Lima beans was selected for the study. Of these, 270 lines were drawn from the diversity panel used for the study of Lima bean domestication (Chacón-Sánchez and Martinez-Castillo 2017), 76 lines were from a diversity panel of Lima beans adapted to growth in the Central Valley of California, and an additional 20 wild lines were collected in Mexico and grown for the study. Due to issues with germination, photoperiod sensitivity, and disruptions from the COVID-19 pandemic, flower phenotypes were collected for only 207 of these lines and pod phenotypes were collected for only 164 of these lines.

Plants were grown in a greenhouse setting over the course of three plantings initiated in January 2020, September 2020, and January 2021. Plants were grown under natural lighting conditions with a vertical black plastic curtain blocking artificial light from a neighboring greenhouse. Flowers were collected on the first day they opened as judged by their color: white or purple as opposed to the yellow of the second day. Young pods were

collected at approximately 2cm in length. Samples were stored in 96-well plates. Immediately after collection, tissue samples were taken to a -80 °C freezer and stored until processing.

Colorimetric Assay for Measuring HCN

Cyanogenesis was measured using a colorimetric assay with Feigl-Anger paper (Feigl and Anger, 1966). Samples were removed from the freezer and their caps were removed and replaced with a prepared piece of Feigl-Anger paper. The freezing and subsequent thawing of the samples disrupted the cells, freeing the cyanogenic glucosides from the vacuole and allowing the hydrolyzing enzymes to cleave cyanide from the sugar. The liberated cyanide volatilized and rose to meet the Feigl-Anger paper. Once in contact with the Feigl-Anger paper, the cyanide interacted with the chemical treatments to turn the paper blue. The Feigl-Anger paper was changed after 15, 30, 60 and 90 minutes from the start time of the assay to create four distinct exposure windows. This timing was selected to maximize HCN capture and avoid paper saturation based on the results of a time trial that was conducted prior to analysis of actual samples. The Feigl-Anger paper was scanned and analyzed using the readplate2 plugin on ImageJ 1.52q to measure the intensity of blue colored caused by the volatile cyanide (Schneider et al. 2012; Angelani et al. 2018).

Early observations suggested that the position of a sample on the plate appeared to affect the results. Samples on the outside of the 96-well plate appeared to defrost more quickly than samples in the interior of the 96-well plate. During the time trial, this concern did not arise because fewer samples were included and so the thermal mass of the plate was lower. To correct for a possible influence of plate position, the 'emmeans' package in R version 4.2.1 was used to create a linear mixed effects model with variety as a fixed effect

42

and plate, row within plate, and column within plate as random effects (Lenth 2022; R Core Team 2022). The phenotypes tested in the GWAS were the estimated means of each tissue type measured in each exposure window for each variety.

Sequencing

The sequence data for 157 of the lines were downloaded from the NCBI Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra/). Genetic data were acquired for the other 93 lines by first extracting DNA from embryonic radical tissue using a DNeasy 96 Plant Kit (Qiagen). The DNA was then sequenced with genotyping-by-sequencing (GBS) using the restriction enzyme *Ape*K1. This restriction enzyme was selected to ensure compatibility between this dataset and the preexisting dataset (Chacón-Sánchez and Martinez-Castillo 2017). It should be noted for future studies, however, that the *Cvi*AII restriction enzyme produces more evenly spaced markers when conducting GBS on the genus *Phaseolus* (Ariani et al. 2016).

Sequence data were aligned to the *Phaseolus lunatus* reference genome (Garcia et al. 2021), annotated, and filtered using the Next Generation Sequencing Eclipse Plugin version 4.2.0 (Tello et al. 2022; Cruz et al. 2014). Data used for masking SNPs in repetitive regions of the genome were provided by Jorge Duitama (unpublished). The data was then moved to TASSLE, where it was imputed with the LD-kNNi method (Money et al. 2015). In a test with 1% of the data masked, the error rate of this imputation was 0.023.

STRUCTURE analysis

A STRUCTURE analysis was performed to identify population structure that could cause misleading results in the GWAS. This was done in the command line version of

STRUCTURE software 2.3.4 (Pritchard et al. 2000). The results were analyzed with the Evanno method in R version 4.2.1 (Evanno et al. 2005; R Core Team 2022). Plots were generated using the evannoMethodStructure function of the 'pophelper' package in R version 4.2.1 (Francis 2017; R Core Team 2022).

Initially, the parameters were set to run three times per K for K = 1-10, Burnin = 10,000, and Reps = 10,000 with all samples from Chacón-Sánchez and Martinez-Castillo (2017) as well as the California diversity panel included in the analysis (n = 363). These parameters are quite low but were selected to optimize available computing power. This analysis indicated that the optimal K was five. With this result, the analysis was rerun nine times per K for K=1-6, burning = 100,000, and Reps = 100,000. Here again the limiting factor was computer processing time. For future publications, this analysis will be expanded to include ten runs of K for K=1-10.

All samples from the Andean I and Andean II gene pools, as categorized by the STRUCTURE analysis, were removed. Admixed samples with less than 50% of their genome categorized within a single group would have been excluded, but no samples fell within this category. This left 219 Mesoamerican lines for the GWAS analysis. Of these, 162 had cyanogenesis phenotypes for flowers and 119 had cyanogenesis phenotypes for pods. For publication, an admixture threshold of 70% may be considered and tested.

Genome-wide Association Study

The genome-wide association study was conducted using the Bayesian-information and Linkage-disequilibrium Iteratively Nested Keyway (BLINK) Model in the Genomic Association and Prediction Integrated Tool (GAPIT) Version 3 in R version 4.2.1 (Wang et al. 2014; Huang et al. 2019; R Core Team 2022). A Q matrix was not included as a covariate

variable to control for kinship, because all Andean lines were removed from the sample pool following the STRUCTURE analysis. Testing additional analyses with a Q matrix included will be a step taken prior to publication.

BLAST Search of White Clover Li/li and Ac/ac on Lima Bean Reference Genome

In white clover, cyanogenesis is controlled by two independently segregating Mendelian genes known as *Ac/ac* and *Li/li* (Olsen et al. 2007). *Li/li* controls the presence of the hydrolyzing enzyme linamarase. *Ac/ac* has not been as well characterized as *Li/li* but likely controls the first step in the biosynthesis of cyanogenic glucosides (Olsen et al. 2008). To identify potential homologous genes, a BLAST (Phytozome v13) search of the Lima bean reference genome Plunatus_V1_563 (https://phytozome-next.jgi.doe.gov/info/Plunatus_V1) for the white clover *Ac/ac and Li/li* genes was conducted (Altschul et al. 1997; Olsen et al. 2008; Goodstein et al. 2012; Garcia et al. 2021).

Results and Discussion

It has long been established that Lima beans were domesticated twice, once in Mesoamerica and once on the western slope of the Andes (Gutiérrez-Salgado et al. 1995). The primary gene pool of Lima bean, including domesticated lineages and the wild populations from which they were derived, can be optimally divided into five subgroups: Mesoamerican I (MI), Mesoamerican II (MII), Andean I (AI), Andean II (AII), and admixed (Chacón-Sánchez and Martinez-Castillo 2017). Given that the accessions included in this study were a diverse mixture of wild, landrace, and elite breeding lines from throughout the Americas, it was important to check for population structure prior to conducting a genome-wide association study, as the linkage disequilibrium between sites with causal

and non-causal alleles in related individuals can lead to artificial associations (Korte and Farlow 2013). As expected, a kinship matrix (Fig. 1) produced by the 'GAPIT' package in R revealed significant population structure within the 363 lines included in this study. Given that most of the available phenotypes came from the Mesoamerican gene pool, removing the Andean lines from the study was the most efficient way to remove biases introduced by this population structure.

Figure 1: Kinship matrix of 363 lines including 270 lines were drawn from the diversity panel used for the study of Lima bean domestication (Chacón-Sánchez and Martinez-Castillo 2017), 76 lines were from a diversity panel of Lima beans adapted to growth in the Central Valley of California, and an additional 20 wild lines collected in Mexico were grown for the study
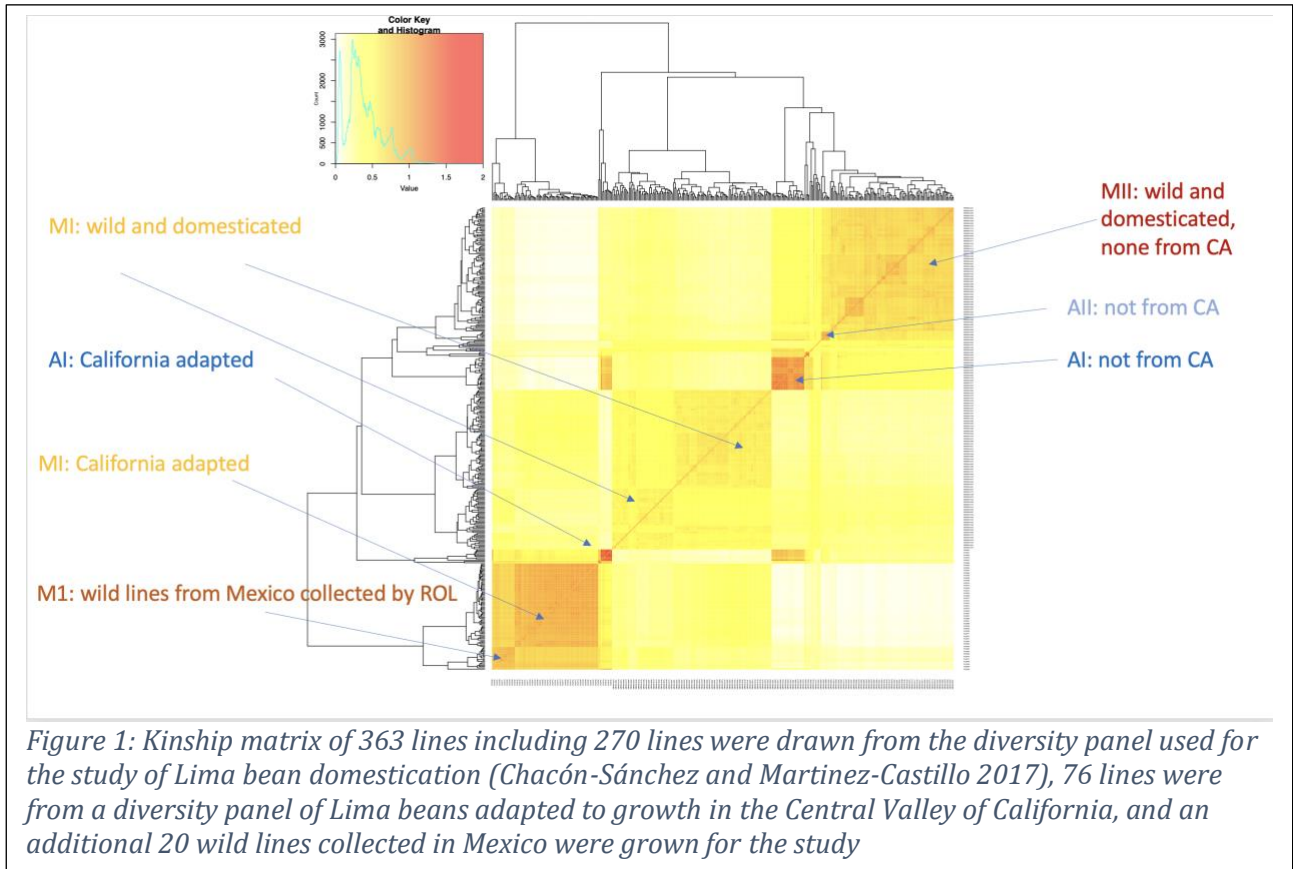
A STRUCUTRE analysis found that the optimal K was five (Fig. 2). This is the same number of populations identified by Chacón-Sánchez and Martinez-Castillo (2017). Rather than fitting neatly into the categories of MI, MII, AI, AII, and admixed, these samples were optimally divided into MII, AI, AII and two MI groups. The larger of the MI groups included a mixture of wild and domesticated lines while the smaller MI group consisted mainly of wild accessions collected in Mexico. Based on this analysis, 36 lines identified as belonging

primarily to the two Andean gene pools were removed from the study. For future

publication, a higher threshold of admixture may also be considered for removing some

additional genotypes. With the Andean lines removed the remaining population (n=327)

showed significantly less population structure (Fig. 3)

Figure 2: STRUCTURE analysis of Lima bean diversity panel (n=363)

Figure 3: Kinship matrix of only Mesoamerican accessions n=327
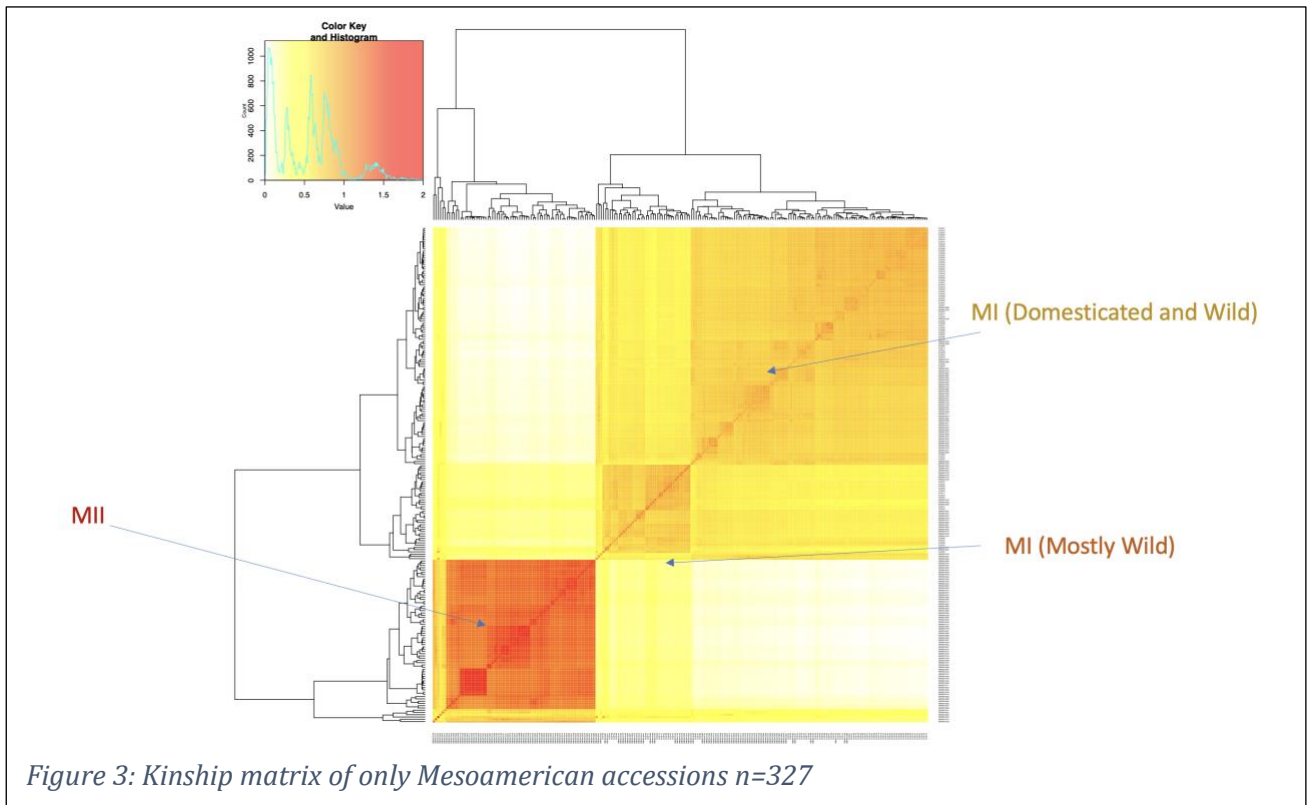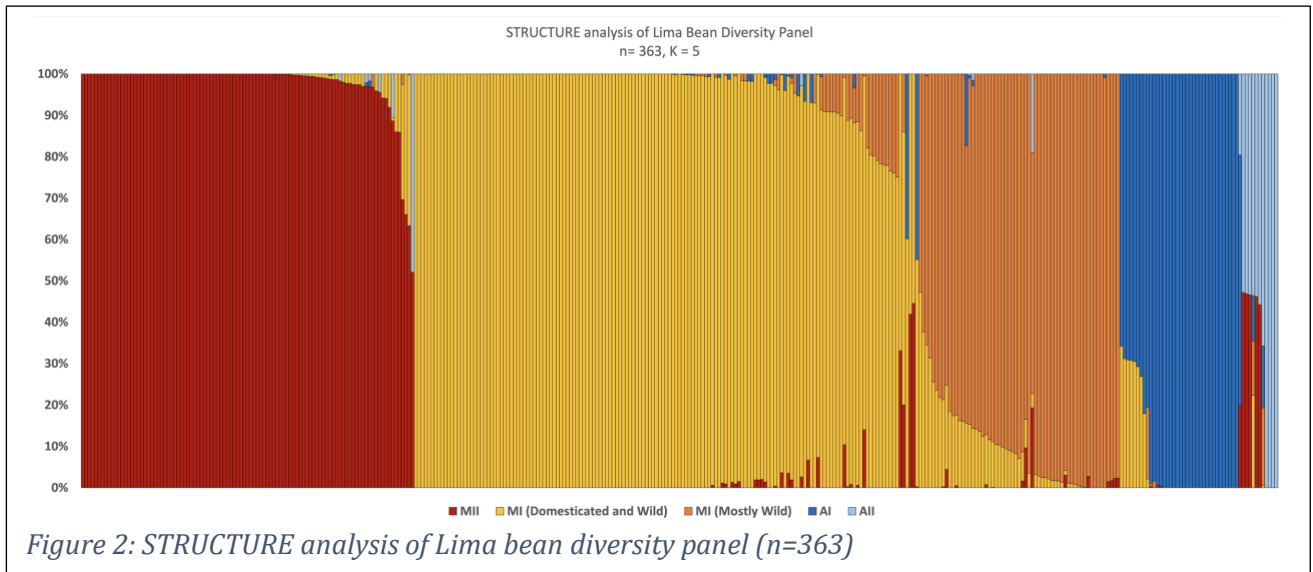
A GWAS of volatile HCN production in the first 15 minutes of tissue rupture caused by thawing, identified several significant SNPs for flower tissue and one highly significant SNP for pod tissue (Fig 4 and Table 1). The most significant SNPs for flower tissue, on Chromosomes 2 and 4 are located near matches for the BLAST search of the white clover *Li/li* sequence. The SNP identified in pods is not near the significant alignment of the BLAST search against the Lima bean reference genome of the white clover sequence or the QTL identified in the biparental population. When considering cyanogenesis as a defense trait, the immediate release of HCN following tissue disruption deters an insect herbivore and therefore serves as a resistance trait (Ballhorn et al. 2006). As such, it will be most successful against opportunistic, generalist herbivores rather than specialist herbivores which would have experineced coevolution with the crop and had more opportunity to adapt to its defenses (Gleadow and Woodrow 2002). Additional study of these findings may yield great contributions to breeding efforst for *L. hesperus* resistance.

Additional significant SNPs were found in the 15-30 minute exposure window (Fig. 5 and Table 1). In flower tissue, SNPs on chromosomes 9, 5, and 7 were closely located to significant matches from the BLAST of the white clover *Ac/ac* gene sequence on the Lima bean reference genome. In pod tissue, a signficant SNP on chromosome 6 was also closely located to a match for the *Ac/ac sequence.*

Figure 4: Manhattan plot of HCN emissions for flowers (top) and pods (bottom) 0-15 minutes after the start of thawing

Figure 5: Manhattan plot of HCN emissions for flowers (top) and pods (bottom) 0-15 minutes after the start of thawing
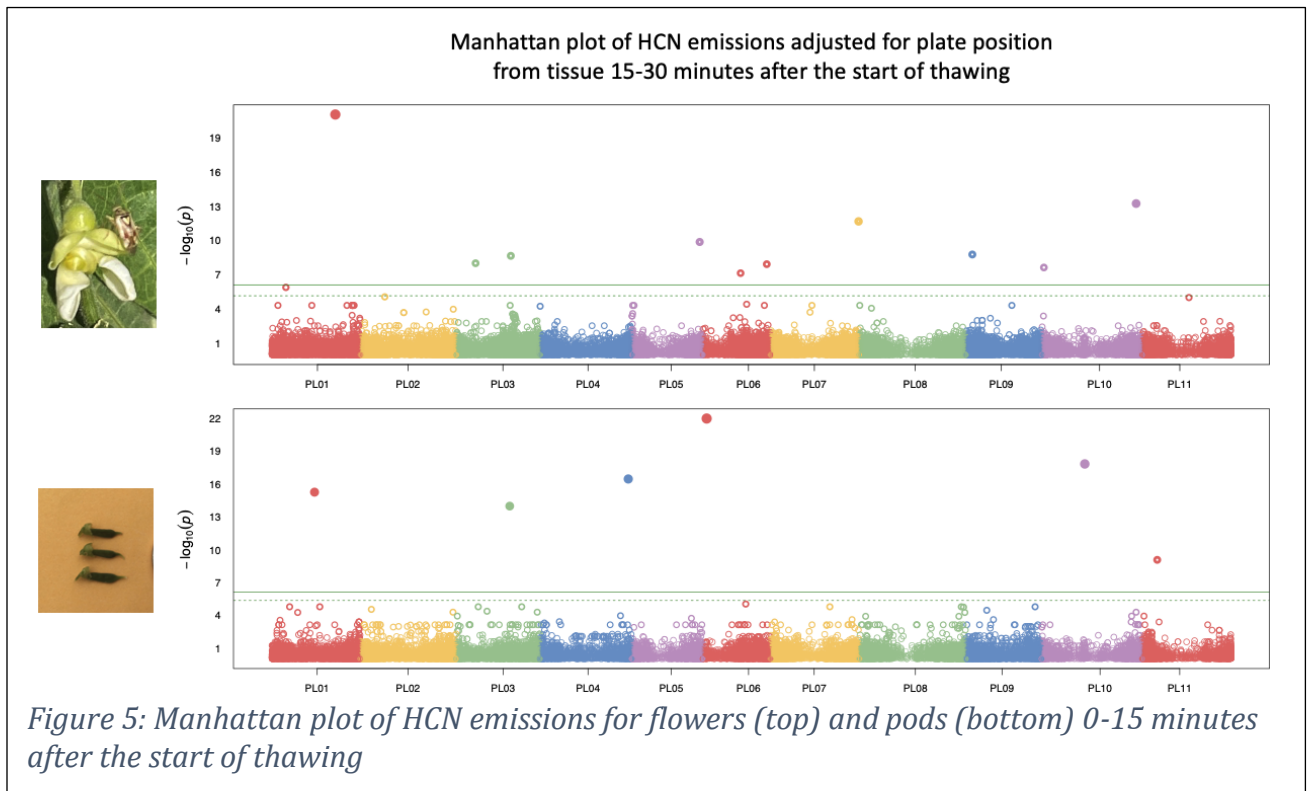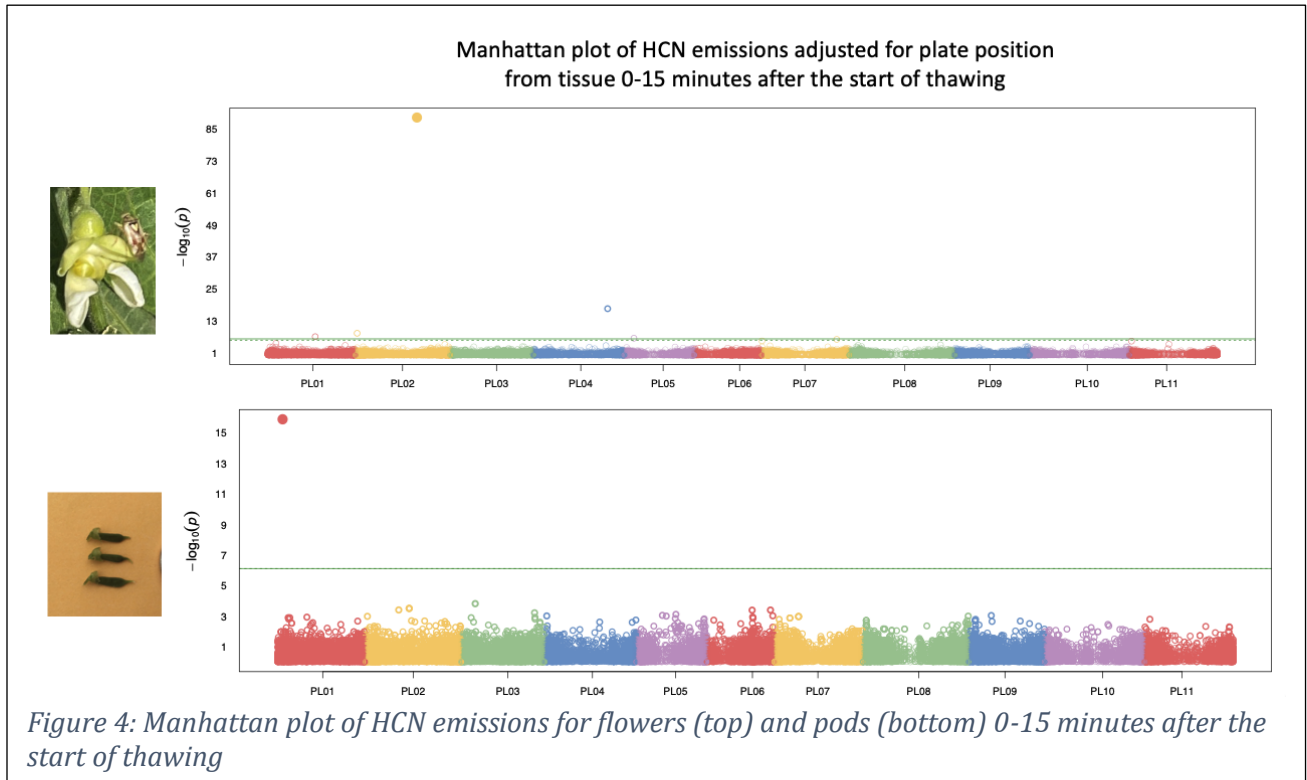
Table 1: Significant QTL identified for HCN emitted including SNP label, chromosome, position, P-value, minor allele frequency, number of observations, adjusted P-value following the Benjamini and Hochberg false discovery rate controlling procedure (Benjamini and Hochberg 1995), the allelic effect estimate, and the percent variation explained.

SNP	Chr	Pos	P.value	MAF	nobs	H&B.P.Value	Effect	PVE (%)
Significant QTL for HCN emission by flower tissue 0-15 minutes after the start of thawing								
SPL02_33506717	PL02	33506717	5.32E-90	0.006	162	3.4E-85	-0.0141	96.31
SPL04_40464404	PL04	40464404	3.48E-18	0.006	162	1.1E-13	0.0052	2.43
SPL02_1004008	PL02	1004008	6.03E-09	0.019	162	1.3E-04	0.0011	0.48
SPL01_25804374	PL01	25804374	1.13E-07	0.006	162	1.8E-03	0.0020	0.31
SPL05_5262950	PL05	5262950	4.43E-07	0.083	162	5.7E-03	-0.0004	0.08
Significant QTL for HCN emission by pod tissue 0-15 minutes after the start of thawing								
SPL01_2780894	PL01	2780894	1.34E-16	0.008	119	8.69E-12	-0.003	50
Significant QTL for HCN emission by flower tissue 15-30 minutes after the start of thawing								
SPL01_34164695	PL01	34164695	1.09E-21	0.025	162	7.1E-17	-0.0212	9.71
SPL09_3564559	PL09	3564559	1.75E-09	0.012	162	2.3E-05	0.0130	8.64
SPL10_50862236	PL10	50862236	6.33E-14	0.019	162	2.1E-09	0.0132	6.34
SPL05_36471809	PL05	36471809	1.42E-10	0.022	162	2.3E-06	0.0216	6.33
SPL03_10424455	PL03	10424455	1.01E-08	0.031	162	9.3E-05	0.0073	1.74
SPL06_34500698	PL06	34500698	1.22E-08	0.025	162	9.9E-05	0.0072	1.37
SPL03_29372256	PL03	29372256	2.29E-09	0.392	162	2.5E-05	-0.0101	0.99
SPL06_20434217	PL06	20434217	7.42E-08	0.046	162	4.8E-04	0.0089	0.96
SPL07_47371793	PL07	47371793	2.28E-12	0.145	162	4.9E-08	0.0051	0.35
SPL10_1233508	PL10	1233508	2.38E-08	0.182	162	1.7E-04	-0.0034	0.18
Significant QTL for HCN emission by pod tissue 15-30 minutes after the start of thawing								
SPL06_2184041	PL06	2184041	1.15E-22	0.008	119	7.4E-18	-0.0206	32.43
SPL01_22985159	PL01	22985159	6.03E-16	0.004	119	9.8E-12	0.0323	23.32
SPL04_47531226	PL04	47531226	3.78E-17	0.008	119	8.2E-13	-0.0227	18.39
SPL10_23229417	PL10	23229417	1.60E-18	0.008	119	5.2E-14	0.0311	9.82
SPL03_28779663	PL03	28779663	1.13E-14	0.303	119	1.5E-10	-0.0083	3.80
SPL11_8225056	PL11	8225056	9.07E-10	0.017	119	9.8E-06	-0.0085	1.75
Significant QTL for HCN emission by flower tissue 30-60 minutes after the start of thawing								
SPL06_35243954	PL06	35243954	2.04E-07	0.268519	162	0.013	-0.0134	50
No Significant QTL for HCN emission by pod tissue 30-60 minutes after the start of thawing								
Significant QTL for HCN emission by flower tissue 60-90 minutes after the start of thawing								
SPL02_50012693	PL02	50012693	2.05E-09	0.037037	162	0.000	-0.0263	77.99
SPL08_1493876	PL08	1493876	5.29E-07	0.41358	162	0.017	-0.0090	7.34
No Significant QTL for HCN emission by pod tissue 60-90 minutes after the start of thawing								

Table 3: Results of BLAST search of white clover genes on the Lima bean reference genome

Ac/ac				
Chromosome	Score (Bits)	E-value	Position	Length
Pl06	590	1.00E-166	Pl06:24333127..24335090	1.96 Kb
Pl10	527	1.00E-147	Pl10:42823180..42826109	2.93 Kb
Pl09	127	4.00E-27	Pl09:36844721..36844944	224 b
Pl07	59	2.00E-06	Pl07:42769004..42769171	168 b
Pl11	53.6	7.00E-05	Pl11:33860033..33860164	132 b
Pl01	48.2	3.00E-03	Pl01:39235421..39235513	93 b
Li/li				
Chromosome	Score (Bits)	E-value	Position	Length
Pl11	309	5.00E-82	Pl11:4728127..4732222	4.1Kb
Pl01	196	9.00E-48	Pl01:10080482..10083186	2.71 Kb
Pl04	150	3.00E-34	Pl04:37697601..37699769	2.17 Kb
Pl05	136	7.00E-30	Pl05:35407270..35410168	2.9 Kb
Pl03	86	1.00E-14	Pl03:6608414..6608693	280 b
Pl07	60.8	5.00E-07	Pl07:31786574..31786676	103 b
Pl02	60.8	5.00E-07	Pl02:33417198..33418215	1.02 Kb
Pl08	50	8.00E-04	Pl08:9447102..9447195	94 b
Pl06	46.4	1.00E-02	Pl06:14388483..14388576	94 b

Table 2: QTL for HCN identified in a biparental RIL population

Tissue	QTL Peak	Flanking Markers of QTL Peak	LOD	%PVE	Source
Floral Bud	Pl05.57.4	Pl05_35520814 - Pl05_35804750	68.33	93.26	Zullo
Floral Bud	Pl10.27	Pl10_46286504 - Pl10_46521900	9.98	2.79	Zullo
Floral Bud	Pl08.24.6	Pl08_2715645 - Pl08_2791797	5.98	1.53	Zullo
Immature Pod	Pl05.56.8	Pl05_35359560 - Pl05_35364729	53.84	88.69	Zullo
Immature Pod	Pl10.29	Pl10_49100171 - Pl10_50371482	8.3	3.72	Zullo
Immature Pod	Pl01.42	Pl01_6146858 - Pl01_10207081	8.04	3.58	Zullo
Immature Pod	Pl11.17	Pl11_1100133 - Pl11_1568555	5.91	2.5	Zullo
Young Leaves	-	Pl0537094525 - Pl05_37249873	14.71	44.25	Dohle

Prior QTL analysis of HCN in floral buds, immature pods, and leaves of a RIL population identified significant loci for volatile HCN on chromosome 5 (Table 3) (Dohle 2017; Zullo 2021). This QTL is very close in position to one found by the GWAS analysis of HCN in flowers defrosting for 15-30 minutes, PL05_36471809. There is also a significant alignment with the white clover sequence for the *Li/li* gene in a nearby region of chromosome 5 (Table 2). It is interesting to note that there is evidence of β-glucosidase activity being induced by the presence of insect herbivores (Ballhorn et al. 2009). The greenhouse from which the samples in this study were collected had a stable infestation of thrips but was free of the larger herbivores typically found in field settings. It is therefore possible that if this study were repeated with field-collected samples, this locus would have a stronger effect.

Conclusion

Cyanogenesis is a complex trait in Lima bean with multiple SNPs closely associated with the expression of cyanogenesis. Highly significant SNPs found in flowers during the first 15 minutes after tissue disruption are close matches for the white clover *Li/li* gene sequence. This could contribute to the effectiveness of cyanogenesis as a resistance trait that deters insect herbivores (Gleadow and Woodrow 2002). Additional SNPS on chromosomes 9, 5, 7, and 6 found in the 15-30 minute exposure window may be associated with the biosynthesis of cyanogenic glucosides as they are close to matches of the white clover *Ac/ac* gene sequence. Finally, a QTL on chromosome 5 was in close proximity to previously identified QTL for cyanogeneis in flowers, pods, and leaves as well as the white clover sequence for *Li/li.* Further analysis and research is needed to clarify the function and

expression of genes located near the significant SNPs identified by this study and solidfy understanding of the genetic architecture of cyanogenesis in Lima beans.

Several additional steps will be take to advance this research prior to publicaiton. First, the STRUCTURE and GWAS analyses will be reexamined to consider higher thresholds of admixture. Next, confidence intervals and markers flanking the significant SNPS will be analyzed to increase certainty about the relationship between these finidings and the BLAST search maches as well as previously identified QTL from the RIL population. A study of genome annotations and the expression atlas will also be undertaken to identify clues about the function of genes near these significant SNPs. The results from wild and domesticated accessions will also be compared to determine how the matches for *Li/li* and *Ac/ac* genes may have been affected by domestication. Lastly accessions with extreme phenotypes will be identified and their associated genotypes used for breeding, futher mapping, and validation studies.

References

Adeparusi, E. O. (2001). Effect of processing on the nutrients and anti-nutrients of Lima bean (*Phaseolus lunatus* L.) flour. *Food/Nahrung*, *45*(2), 94-96. doi: 10.1002/1521-3803(20010401)45:2<94::AID-FOOD94>3.0.CO2-E

Alonso-Amelot, M. E., & Oliveros-Bastidas, A. (2005). Kinetics of the natural evolution of hydrogen cyanide in plants in neotropical *Pteridium arachnoideum* and its ecological significance. *Journal of Chemical Ecology*, *31*(2), 315-331. doi: 10.1007/s10886-005-1343-z

Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J. H., Zhang, Z., Miller, W., & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research*, 25(17), 3389–3402. doi: 10.1093/nar/25.17.3389

Alvarado-Rodriquez, B., Leigh, T. F., & Foster, K. W. (1986). Oviposition site preference of *Lygus hesperus* (Hemiptera: Miridae) on common bean in relation to bean age and

genotype. *Journal of Economic Entomology*, 79(4), 1069–1072. doi: 10.1093/jee/79.4.1069

Angelani, C. R., Carabias, P., Cruz, K. M., Delfino, J. M., de Sautu, M., Espelt, M. V., Ferreira-Gomes, M. S., Gómez, G. E., Mangialavori, I. C., Manzi, M., Pignataro, M. F., Saffioti, N. A., Salvatierra Fréchou, D. M., Santos, J., & Schwarzbaum, P. J. (2018). A metabolic control analysis approach to introduce the study of systems in biochemistry: the glycolytic pathway in the red blood cell. *Biochemistry and Molecular Biology Education*, *46*(5), 502-515. doi: 10.1002/bmb.21139

Ariani, A., Berny Mier y Teran, J. C., & Gepts, P. (2016). Genome-wide identification of SNPs and copy number variation in common bean (*Phaseolus vulgaris* L.) using genotyping-by-sequencing. *Molecular breeding*, *36*(7), 1-11. doi: 10.1007/s11032-016-0512-9

Ballhorn, D. J., Heil, M., & Lieberei, R. (2006). Phenotypic plasticity of cyanogenesis in Lima bean *Phaseolus lunatus*-Activity and activation of β-glucosidase. *Journal of Chemical Ecology*, 32(2), 261–275. doi: 10.1007/s10886-005-9001-z

Ballhorn, D. J., Kautz, S., Heil, M., & Hegeman, A. D. (2009). Cyanogenesis of wild Lima bean (*Phaseolus lunatus* L.) is an efficient direct defence in Nature. *PloS One*, 4(5), e5450–e5450. doi: 10.1371/journal.pone.0005450

Benjamini, Y. & Hochberg, Y. Controlling the false discovery rate: a practical and powerful approach to mutliple testing. *J.R. Statis. Soc. B.* 57, 289–300 (1995).

Bolarinwa, I. F., Oke, M. O., Olaniyan, S. A. & Ajala, A. S., 2016. A review of cyanogenic glycosides in edible plants. *Toxicology–New Aspects to This Scientific Conundrum*. IntechOpen doi: 10.5772/64886

Chacón-Sánchez, M. I. & Martínez-Castillo, J. (2017). Testing domestication scenarios of Lima bean (*Phaseolus lunatus* L.) in Mesoamerica: insights from genome-wide genetic markers. *Frontiers in Plant Science*, 8, 1551–1551. doi: 10.3389/fpls.2017.01551

Cruz, D. F., Hoz, J. F. D. L., Perea, C. S., Quintero, J. C., & Duitama, J. (2014). NGSEP (Next Generation Sequencing Eclipse Plugin). CGIAR. https://hdl.handle.net/10568/68332

Cuny, M. A. C., La Forgia, D., Desurmont, G. A., Glauser, G., & Benrey, B. (2019). Role of cyanogenic glycosides in the seeds of wild Lima bean, *Phaseolus lunatus*: defense, plant nutrition or both? *Planta*, 250(4), 1281–1292. doi: 10.1007/s00425-019-03221-3

Dohle, S. (2017). *Development of resources for Lima bean (Phaseolus lunatus) breeding and genetics research*. University of California, Davis.

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular ecology*, *14*(8), 2611-2620. doi: 10.1111/j.1365-294X.2005.02553.x

Feigl, F., & Anger, V. (1966). Replacement of benzidine by copper ethylacetoacetate and tetra base as spot-test reagent for hydrogen cyanide and cyanogen. *Analyst*, *91*(1081), 282-284. doi: 10.1039/an9669100282

Francis, R. M. (2017). pophelper: an R package and web app to analyze and visualize population structure. *Molecular ecology resources*, *17*(1), 27-32. doi: 10.1111/1755-0998.12509

Frehner, S. M., & Conn, E. E. (1990). Pattern of the cyanide-potential in developing fruits: Implications for plants accumulating cyanogenic monoglucosides (*Phaseolus lunatus*) or cyanogenic diglucosides in their seeds (*Linum Usitatissimum*, *Prunus amygdalus*). *Plant Physiology* (Bethesda), 94(1), 28–34. doi: 10.1104/pp.94.1.28

Garcia T., Duitama, J., Smolenski Zullo, S., Gil, J., Ariani, A., Dohle, S., Palkovic, A., Skeen, P., Bermudez-Santana, C., Debouck, D. G., Martinez-Castillo, J., Gepts, P., & Chacón-Sánchez, M. I. (2021) Comprehensive genomic resources related to domestication and crop improvement traits in Lima bean. Nature Comm 12:702 doi: 10.1038/s41467-021-20921-1

Gleadow, R. M. & Woodrow, I. E. (2002). Constraints on effectiveness of cyanogenic glycosides in herbivore defense. *Journal of Chemical Ecology*, 28(7), 1301–1314. doi: 10.1023/A:1016298100201

Goodstein, D. M., Shu, S., Howson, R., Neupane, R., Hayes, R. D., Fazo, J., Mitros, T., Dirks, W., Hellsten, U., Putnam, N., & Rokhsar, D. S. (2012). Phytozome: a comparative platform for green plant genomics. *Nucleic Acids Research*, 40(D1), D1178–D1186. doi: 10.1093/nar/gkr944

Gutiérrez-Salgado, A., Gepts, P., & Debouck, D. G. (1995). Evidence for two gene pools of the Lima bean, *Phaseolus lunatus* L., in the Americas. *Genetic Resources and Crop Evolution*, *42*(1), 15-28. doi: 10.1007/BF02310680

Hartmann, T. (2007). From waste products to ecochemicals: fifty years research of plant secondary metabolism. *Phytochemistry*, *68*(22-24), 2831-2846. doi: 10.1016/j.phytochem.2007.09.017

Huang, M., Liu, X., Zhou, Y., Summers, R. M. & Zhang, Z. BLINK: A package for the next level of genome-wide association studies with both individuals and markers in the millions. Gigascience giy154 (2019). doi:10.1093/gigascience/giy154

Hughes, M. A. (1991). The cyanogenic polymorphism in *Trifolium repens* L. (white clover). *Heredity*, 66(1), 105–115. doi: 10.1038/hdy.1991.13

Jones, D. A., 1998. Why are so many food plants cyanogenic? *Phytochemistry*, *47*(2), pp.155-162. doi: 10.1016/S0031-9422(97)00425-1

Korte, A., & Farlow, A. (2013). The advantages and limitations of trait analysis with GWAS: a review. *Plant methods*, *9*(1), 1-9. doi: 10.1186/1746-4811-9-29

Lenth, R. (2022). emmeans: Estimated marginal means, aka least-squares means. R package version 1.8.2, https://CRAN.R-project.org/package=emmeans

Lieberei, R. (1988). Relationship of cyanogenic capacity (HCN-c) of the rubber tree *Hevea brasiliensis* to susceptibility to *Microcyclus ulei*, the agent causing South American leaf blight. *Journal of Phytopathology*, 122(1), 54–67. doi: 10.1111/j.1439-0434.1988.tb00990.x

Loyd, R. C. & Gray, E. (1970). Amount and distribution of hydrocyanic acid potential during the life cycle of plants of three sorghum cultivars. *Agronomy Journal*, 62(3), 394–397. doi: 10.2134/agronj1970.00021962006200030025x

Meyer, R. S. & Purugganan, M. D., 2013. Evolution of crop species: genetics of domestication and diversification. *Nature Reviews Genetics*, *14*(12), 840-852. doi: 10.1007/s10722-010-9630-0

Money, D., Gardner, K., Migicovsky, Z., Schwaninger, H., Zhong, G. Y., & Myles, S. (2015). LinkImpute: fast and accurate genotype imputation for nonmodel organisms. *G3: Genes, Genomes, Genetics*, *5*(11), 2383-2390. doi: 10.1534/g3.115.021667

National Research Council. (2002). *Tropical Legumes: Resources for the future*. The Minerva Group, Inc.

Olsen, K. M., Hsu, S.-C., & Small, L. L. (2008). Evidence on the molecular basis of the Ac/ac adaptive cyanogenesis polymorphism in White Clover (*Trifolium repens* L.). *Genetics* (Austin), 179(1), 517–526. doi: 10.1534/genetics.107.080366

Ospina, M. A., Pizarro, M., Tran, T., Ricci, J., Belalcazar, J., Luna, J. L., Londoño, S. S., Ceballos, H., Dufour, D., & Becerra Lopez-Lavalle, L. A. (2021). Cyanogenic, carotenoids and protein composition in leaves and roots across seven diverse population found in the world cassava germplasm collection at CIAT, Colombia. *International Journal of Food Science & Technology*, 56(3), 1343-1353. doi: 10.1111/ijfs.14888

Pichersky, E., & Lewinsohn, E. (2011). Convergent evolution in plant specialized metabolism. *Annual Review of Plant Biology*, *62*(1), 549-566. doi: 10.1146/annurev-arplant-042110-103814

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, *155*(2), 945-959. doi: 10.1093/genetics/155.2.945

R Core Team (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Ragir, S. (2000), Diet and food preparation: Rethinking early hominid behavior. *Evolutionary Anthropology*, 9: 153-155. doi: 10.1002/1520-6505(2000)9:4<153::AID-EVAN4>3.0.CO;2-D

Sánchez-Pérez, R., Pavan, S., Mazzeo, R., Moldovan, C., Aiese Cigliano, R., Del Cueto, J., Ricciardi, F., Lotti, C., Ricciardi, L., Dicenta, F., López-Marqués, R. L., & Møller, B. L. (2019). Mutation of a bHLH transcription factor allowed almond domestication. *Science (American Association for the Advancement of Science)*, 364(6445), 1095–1098. doi: 10.1126/science.aav8197

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature methods*, *9*(7), 671-675. doi: 10.1038/nmeth.2089

Shlichta, J. G., Cuny, M. A., Hernandez-Cumplido, J., Traine, J. & Benrey, B. (2018). Contrasting consequences of plant domestication for the chemical defenses of leaves and seeds in lima bean plants. *Basic and Applied Ecology*, *31*, 10-20. doi: 10.1007/s10886-014-0434-0

Smartt, J. (1988). Morphological, physiological and biochemical changes in *Phaseolus* beans under domestication. In *Genetic resources of Phaseolus beans* (pp. 143-161). Springer, Dordrecht. doi: 10.1007/s13580-015-0127-9

Tello, D., Gonzalez-Garcia, L. N., Gomez, J., Zuluaga-Monares, J. C., Garcia, R., Angel, R., Mahecha, D., Duarte, E., Leon, M. D. R., Reyes, F., Escobar-Velásquez, C., Linares-Vásquez, M., Cardozo, N., & Duitama, J. (2022). NGSEP 4: Efficient and accurate identification of orthogroups and whole genome alignment. *bioRxiv.* doi: 0.1101/2022.01.27.478091

Vanderborght, T. (1979). Analysis of cyanide content in Lima bean [toxic factor in edible legumes, varietal variations]. In *Annales de Gembloux*.

Wang, Q., Tian, F., Pan, Y., Buckler, E. S. E. S. & Zhang, Z. A SUPER powerful method for genome wide association study. *PLoS One* 9, e107684 (2014). doi: 10.1371/journal.pone.0107684

Wilson, W. M. & Dufour, D. L. (2002). Why "bitter" cassava? Productivity of "bitter" and "sweet" cassava in a Tukanoan Indian settlement in the northwest Amazon. *Economic Botany*, 56(1), 49–57. doi: 10.1663/0013-0001(2002)056[0049:WBCPOB]2.0.CO;2

Wrangham, R. & Conklin-Brittain, N. (2003). Cooking as a biological trait. *Comparative Biochemistry and Physiology, Part A,* 136(1), 35–46. doi: 10.1016/S1095-6433(03)00020-5

Zullo, S. S. Developing a molecular linkage map for and understanding the biochemical mechanisms and underlying genetic architecture of biotic stress resistance in Lima bean *(Phaseolus lunatus L.)*. Diss. University of California, Davis, 2021.

Chapter 3: Characterization and variation of defense against *Lygus hesperus* in *Phaseolus lunatus*

Abstract

Understanding the defense traits plants have evolved to defend themselves from insect herbivores may help with efforts to improve crop varieties and reduce yield losses. In the specific case of Lima bean (*Phaseolus lunatus*) production in California, more research is needed to characterize the plant's protective adaptations against the native polyphagous insect, the Western Tarnished Plant Bug (*Lygus hesperus*). This chapter presents four related investigations. The first is a study of yield in a diversity panel of Lima beans with and without insecticide protection. This data shows that there is extensive variation in the susceptibility of Lima bean to damage by *L. hesperus.* This is further supported by the second study of field samples of *L. hesperus* from nine Lima bean varieties. Finally, two studies explore cyanogenesis as a Lima bean defense trait. Cyanogenesis is an effective defense against insect herbivores in many crop plants but has been selected against during domestication due to the risk of intoxication. There is strong evidence that cyanogenic capacity is markedly differentiated among different plant organs, ontogenetic stages, and in the presence of some insects. Data on plants in a greenhouse study showed that cyanogenesis in immature pods of Lima bean is increased by the presence of the generalist herbivore *L. hesperus*. There was also a negative correlation between *L. hesperus* populations development and the cyanogenic capacity of young pods. This indicates that cyanogenesis may be induced and contributes to the defense of Lima bean plants against *L. hesperus*.

Introduction

Amplifying defense traits that protect plants from insect herbivores through plant breeding has the potential to increase yields while reducing pesticide use and associated concerns for human and environmental health (Arthurs and Dara 2019, Isman 2020). This is a particularly important strategy for organic systems in which conventional pesticides cannot be used. Lima beans are an important grain legume globally and the most economically important dry bean grown in California where their primary insect pest is the Western Tarnished Plant Bug (*Lygus hesperus*) (Long et al. 2014).

Lima beans are a model experimental organism for studying anti-herbivore defense traits (Arimura et al. 2000, 2002; Heil 2004a, 2004b; Kost and Heil 2005, 2006; Heil et al. 2008; Moreira et al. 2015). Within this body of literature, many studies have focused on the trait of cyanogenesis (Ballhorn et al. 2006, 2008, 2009, 2014; Shlichta et al. 2014, 2018; Cuny et al. 2019; Lai et al. 2020). Several experiments have been conducted in recent years to identify specific mechanisms that contribute to the tolerance or resistance traits that protect some Lima bean accessions from damage by *L. hesperus* (Dohle 2017; Zullo 2021). One mechanism that has been considered is the production of various polygalacturonase inhibiting proteins (PGIP) in the cell walls of Lima bean that bind to *L. hesperus* salivary enzymes and mitigate attempted digestion of the cell wall (Dashner 2016; Zullo 2021). This trait was found to be strongly influenced by environmental variables such as pest pressure and insecticide treatments but the study design did not permit differentiation of these results as the primary goal was QTL mapping (Zullo 2021). Cyanogenesis is a trait of particular interest since it is known to be an effective anti-herbivore defense trait in wild Lima beans that has been selected against during domestication (Vanderborght 1979;

59

Baudoin et al. 1991; Ballhorn et al. 2009; Shlichta et al., 2018). Several QTL have been

identified for cyanogenesis in flowers, immature pods, and leaves (Dohle 2017; Zullo

2021). However, these studies have not yet determined if cyanogenesis is an effective trait

in the defense of Lima beans against *L. hesperus* specifically.

    L. hesperus predominantly feed on the flowers and immature pod tissue of Lima

bean and if cyanide is an effective deterrent or toxin for *L. hesperus* then increased

expression of cyanogenesis in these tissue types could be amplified through breeding

without risk to the human consumers of mature seeds which are known to have low

cyanogenic capacity (Long et al. 2014; Shlichta et al. 2014). The final part of this study aims

to determine how cyanogenesis affects *L. hesperus* survival and development as well as test

if cyanogenic capacity can be induced by the presence of *L. hesperus*.

Cyanogenesis in Lima Beans and Related Species

    Many economically important crops with high protein content and great importance

for indigenous food systems are members of the legume family (Cullis and Kunert 2017;

Jimenez-Lopez et al. 2020). Several of these legume crops are cyanogenic (Honig et al.

1983; Okolie and Ugochukwu 1989). It appears that this trait has evolved independently

several times in the legume family. Within the legume genus *Phaseolus*, there are five

domesticated crop species but only one, Lima bean, is cyanogenic (Baudoin et al. 1991). In

addition to Lima bean, five other cross-compatible, *Phaseolus* species within the

Polystachios group of section Paniculati are also cyanogenic (Baudoin et al. 1991; Lai et al.

2020). This and other evidence indicate that despite being a widespread trait, cyanogenesis

evolved independently multiple times through the recruitment of similar genes (Takos et

al. 2011; Lai et al. 2020).

In an extensive screening of wild, weedy, and cultivated forms of Lima bean, all were found to be cyanogenic. However, there is variability within and between populations (Baudoin et al. 1991). Domesticated forms typically have much less cyanogenic potential (HCNp), the amount of stored cyanogenic glucosides, and cyanogenic capacity (HCNc), the amount of cyanide released when damage occurs (Loyd and Gray 1970; Vanderborght 1979; Lieberei 1988; Adeparusi 2001). Cyanogenic potential is determined by the biosynthesis and accumulation of cyanogenic glucosides (Loyd and Gray 1970). Cyanogenic capacity is primarily determined by genetic factors but there is also a significant influence of plant age and other environmental factors (Lieberei 1988).

Cyanogenesis is typically considered a constitutive trait with strong genetic control by two Mendelian genes (Olson et al. 2008; Ballhorn et al. 2009). However, there is great variation in the trait within populations and even within an individual plant (Till 1987). Previous studies have found cyanogenic potential and capacity to vary based on the age and tissue type being measured (Martin et al. 1938; Gleadow and Woodrow 2000; Ballhorn et al. 2005; Shlichta et al. 2014). For example, in Lima bean, young leaves have higher cyanogenic potential than mature leaves (Ballhorn et al. 2005). Wild Lima bean seeds by contrast have very high cyanogenic potential but low cyanogenic capacity, likely due to the low moisture content inhibiting β-glucosidase activity (Shlichta et al. 2014). Additionally, there is evidence that cyanogenic capacity may be locally induced by the presence of insect herbivores even if cyanogenic potential is constitutive (Ballhorn et al. 2006). Temperature, humidity, seasonal dynamics, water-stress, and nutrient availability may also affect cyanogenesis (Jones 1966; Dement and Mooney 1974; Cooper-Driver and Swain 1976; Kooyers et al 2014).

Cyanogenesis is very nitrogen intensive with a one-to-one ratio of nitrogen and carbon in each molecule of hydrogen cyanide (Kakes 1990). The availability of nitrogen can be a limiting factor for plant growth (Bloom 1977). It has also been shown that cyanogenic glucosides can be catabolized for protein synthesis (Blumenthal-Goldschmidt et al. 1963; Floss et al. 1965; Castric et al. 1972). Therefore, it has been hypothesized that cyanogenic glucosides evolved first as an intermediate nitrogen storage compound and only later evolved into a defense compound (Forslund et al. 2007; Møller 2010; Cuny et al. 2019). In the case of Lima beans, evidence supports the hypothesis that cyanogenic glucosides primarily serve as an anti-herbivore defense more so than a nitrogen storage mechanism (Cuny et al. 2019).

Lima bean plants with high cyanogenic glucoside content in leaves had lower above ground biomass than low cyanogenic glucoside content plants when no herbivores are present, but this difference was less in the presence of herbivores (Ballhorn et al. 2014). This could indicate that there is a high cost to producing cyanogenic glucosides. Alternatively, these plants may be investing in a strong defense of their vegetative tissue so that a smaller above ground biomass can produce higher yield. Additionally, seeds of Lima bean with high cyanogenic glucoside content had lower germination rates but produce seedlings that had high cyanogenic glucoside content and supported lower growth rates of the generalist herbivore *Spodoptera littoralis* (Cuny et al. 2019). In addition to having tradeoffs with growth and vigor, plants with high cyanogenic glucosides have lower investment in other defense mechanisms (Ballhorn et al. 2008). In Lima bean, a negative correlation was found between cyanogenic glucosides and volatile organic compound emissions (Ballhorn et al. 2008). This evidence indicates that in Lima bean, cyanogenic

glucosides serve primarily as an antiherbivore defense compound rather than a nitrogen storage mechanism.

Cyanogenesis as a Defense Against Insect Herbivores

Cyanogenesis is an anti-herbivore defense trait found in many plant families (Poulton 1990; Møller and Seigler 1999). It is especially common in crop plants. While an estimated 11% of all plant species are cyanogenic, the trait is present in approximately 21% of the major world food crops (Jones 1998). Given that humans have long known of several effective methods of detoxifying cyanogenic foods, including leaching, cooking, and fermenting, it is possible that crops with this trait were specifically selected by early farmers for their superior defense against insect herbivores (Jones 1998; Ragir 2000; Wilson and Dufour 2002; Wrangham and Conklin 2003).

Despite its value as an anti-herbivore defense trait, cyanogenesis has been selected against during the process of domestication. With the notable exception of sorghum, most crops have lower levels of pre-cyanogenic compounds than their wild relatives (Ballhorn et al. 2009; Shlichta et al. 2018; Sánchez-Pérez et al. 2019; Ospina et al. 2021; Cowan et al 2021). This may be because, though satisfactory, our methods of detoxifying foods do not fully eliminate cyanide. Specifically, in the example of Lima bean, the enzyme linamarase rapidly hydrolyzes cyanogenic glucosides during cooking but becomes denatured at 141 °C (Liener 1962). If cyanogenic glucosides remain unhydrolyzed when that cooking temperature is reached, their cyanide will be released within the consumers digestive track (Poulton 1983). This can be tolerated at low levels, but chronic cyanide intoxication can cause severe symptoms including degenerative neuropathy, paralysis, blindness, and pre-mature death (Osuntokun 1969, 1994; Van Heijst et al. 1994; *Román 2014).* Given the

severe consequence of chronic or acute cyanide intoxication as well as the bitter taste, it is understandable that cyanogenesis was selected against in crop plants (Ladizinsky 1999).

The toxicity of HCN comes from asphyxiation when it binds to cytochrome oxidase, a key enzyme in the mitochondrial respiratory pathway (Antonini et al. 1971; Cooper and Brown 2008). This chemistry makes it toxic to both animal and plant cells. It is therefore necessary for plants to store pre-cyanogenic compounds, typically cyanogenic glycosides, separately from enzymes that cleave the compound and form HCN (Kakes 1990; Vetter 2000). The result of this arrangement is a possible difference in the HCNp and the HCNc of a plant. The cyanogenic potential is measured as the amount of pre-cyanogenic compounds contained in the plant tissue (Loyd and Gray 1970). The cyanogenic capacity is measured as the amount of cyanide released by a given quantity of plant tissue over a unit of time (Lieberei 1988). The enzymatic activity, which can be variable across species and genotypes, will determine how closely measurements of cyanogenic potential and cyanogenic capacity correspond (Ballhorn et al. 2005).

The interaction between cyanogenic plants and insect herbivores is complex and maybe be affected by several biotic and abiotic factors (Bernays et al. 1977; Gleadow and Woodrow 2002). As with other chemical defense mechanisms, specialist insect herbivores may have adaptations that protect them from cyanide produced by their host plant including metabolizing it for use of the nitrogen in protein synthesis (Witthohn and Naumann 1984; Engler et al. 2000) or sequestering it for their own defense (Schappert and Shore 2000; Pinheiro de Castro et al. et al. 2020). Generalist herbivores may avoid cyanide intoxication by balancing their diet with cyanogenic and acyanogenic foods (Gleadow and Woodrow 2002; Ballhorn et al. 2005).

As a defense mechanism, cyanogenesis can operate in two ways depending on the cyanogenic capacity of a given plant tissue: deterrence or intoxication (Ballhorn et al. 2006). Cyanogenesis is not a universally effective deterrent and for some insects cyanide may even act as a phagostimulant (Brattsten et al. 1983). Cyanogenesis seems most effective as a deterrent when the cyanogenic capacity is high, release is rapid, and the insect herbivore is an opportunistic generalist rather than a well-adapted specialist (Gleadow and Woodrow 2002). Below a certain threshold, dependent on the herbivore, cyanogenesis is ineffective as a deterrent (Ballhorn et al. 2005). Intoxication by cyanide consumption typically occurs when herbivores consume large amounts of plant material with lower cyanogenic capacity (Ballhorn et al. 2006). In this sort of situation, cyanide may be released within the digestive track, causing lethal damage or inhibiting growth of the insect (Alonso-Amelot and Oliveros Bastidas 2005; Ballhorn et al. 2005). Plants with higher cyanogenic capacity may be rejected before an insect can consume a sufficient dose.

There is variation in the susceptibility of Lima bean to damage by *L. hesperus* but this has only been catalogued within a small number of commercial cultivars. The first step of this study is to catalogue this variation within a more diverse panel of accessions. From this research, the University of California Davis Dry Bean Breeding program will be able to select better parents and introduce more diversity into the Lima Bean breeding pipeline. In addition to characterizing the variation in *L. hesperus* tolerance or resistance, understanding more about the possible mechanisms that contribute to this phenotype will help target selection in breeding. To do this a field study in which multiple varieties were vacuum sampled will be analyzed to demonstrate the choice of *L. hesperus* in the field when multiple varieties are present. It is unknown if cyanogenesis is an effective defense against

L. hesperus in Lima bean. In combination, these studies illustrate the extent of variation in *L. hesperus* resistance or tolerance phenotypes in Lima bean as well as determine if cyanogenesis is induced by *L. hesperus* presence and if there is a negative correlated between cyanide and *L. hesperus* population growth.

Materials and Methods

Diversity Panel Yield Trial of Insecticide-Treated and Non-Insecticide-Treated Plots

To study the phenotypic range of tolerance or resistance to *Lygus spp.* 77 diverse varieties of Lima bean from 18 countries were planted during the 2019 and 2021 field seasons at the Plant Sciences Field Facility (PSFF) of the University of California Davis on June 9, 2019, and on May 14, 2021, at 38°32'03.3"N 121°46'44.9"W and 38°32'16.9"N 121°47'19.2"W, respectively. The experiment was divided into two neighboring blocks separated by a buffer of four rows. One of the blocks received insecticide treatments on August 20, 2019, and July 2, 2021. The other block was a control not treated with insecticides. In 2019, UC 92 plots were vacuumed to in each block to verify that *L. hesperus* levels were lower in the insecticide treated plots. In 2021, the vacuum equipment was unavailable, so water traps were used to verify the difference in *L. hesperus* pressure between blocks.

Both fields were drip irrigated and conventionally managed. Each variety was planted in a single row in eight plots of 4.5 meters (15 feet). The plots were randomized within eight sub-blocks. Field notes on days to flowering, growth habit, seed color, and flower color were taken. Plots were cut and threshed in the field with the same machinery each year. Yield and 100-seed-weight measurements were conducted after harvest. Four of

the lines were excluded from the analysis due to poor germination or photoperiod sensitivity.

Vacuum sampling of a diverse selection of California-adapted Lima bean cultivars

In a small trial at the University of California Davis Student Organic Farm, nine Lima bean varieties were planted on June 1, 2017, at 38°32'32.5"N 121°46'01.3"W (Table 4). The field was flood irrigated and organically managed. Every variety was planted in two plots, one of which was randomly assigned a location within each of two blocks. Each plot was 20 feet long and six 30-inch rows wide. Starting at the time of flowering, July 21, 2017, the middle two rows of every plot were vacuumed each week between 11am and 1pm. Samples for each plot were bagged and then frozen. Insects were then transferred to vials of ethanol and adult *L. hesperus* in the sample were counted and sexed. Adult *L. hesperus* are highly mobile and can readily fly between small plots. Nymph counts would therefore have been a better measurement to take. However, nymphs were not counted because many were crushed by the force of the vacuum or were two small to be accurately identified with the available expertise. The middle two rows of each plot were harvested measured for total yield and 100 seed weight. The variety UC Lee was removed from the study due to poor germination rates.

Table 4. Varieties of Lima bean planted at the UC Davis Student Organic Farm for the vacuum sampling study in 2017				
Variety	**Growth Habit**	**Seed Size**	**Seed Color**	*L. hesperus* **Susceptibility**
UC Haskell	Vine	Small	White	Low
UC Cariblanco N	Vine	Small	White	High
UC Beija Flor	Bush	Small	White	Low
Henderson Bush	Bush	Small	White	High
UC 92	Bush	Large	White	High
UC Lee	Vine	Large	White	High
Dixie Speckled	Bush	Small	Red, pink	Low
Jackson Wonder	Bush	Small	Brown, purple	Low
Calico Cat (G26451)	Vine	Small	Black, brown, white	Low

Table 5. List of plant materials used in the Cyanogenic Response to L. hesperus study and their known traits				
Variety	**Growth Habit**	**Seed Size**	**Seed Color**	*L. hesperus* **Susceptibility**
UC Haskell	Vine	Small	White	Low
UC Beija Flor	Bush	Small	White	Low
Henderson Bush	Bush	Small	White	High
UC 92	Bush	Large	White	High
UC Lee	Vine	Large	White	High

Cyanogenic Response to L. hesperus

Five cultivars (Table 5) of California-adapted Lima bean were selected for this study with the aim of representing the diversity of seed size, growth habit, cyanogenic capacity, and tolerance of *L. hesperus* (Long et al. 2014; Dohle 2017; Zullo 2021; Gibson, Palkovic, and Gepts unpublished results). All the varieties had white seed coats as this is the market standard for dry Lima beans produced in California. Prior research found that there is not a correlation between seed coat color and cyanogenesis (Baudoin et al. 1991). Originally, one wild accession was included for comparison but due to photoperiod sensitivity and delayed phenology, it proved infeasible to collect samples from these plants in synchrony with the others.

Figure 7. Layout of experimental greenhouse.

In four greenhouse plantings, plants of each variety were individually germinated from seed in azalea pots with approximately two liters of UC Agronomy potting soil mix. Each pot was placed in its own cage (BugDorm-2120 Insect Rearing Tent, MegaView Science Co., Ltd., Taichung, Taiwan) with drip line for water and fertilizer (Fig. 7). Each cage was randomly assigned a position in the greenhouse. Three flowers and three young pods

of each plant were collected one, two, and three weeks after flowering. Flowers were selected with white petals, indicating that the day of sampling was their first to open. Immature pods were approximately 2cm long (Fig. 8). Additionally, succulent mature seed tissue was collected four weeks after flowering were sampled. Mature seed samples consisted of a slice of the bean from the opposite side of the hilum from the micropyle of approximately 200mg that would fit into a 96-well plate collection tube (Fig. 8). All samples were frozen at -80°C and later analyzed for cyanogenic capacity using the Feigl-Anger paper assay (Feigl and Anger 1966). While some have critiqued this method for being only semiquantitative, it was selected based on the available resources and practicality for analyzing large numbers of samples (Ballhorn et al. 2005).

Figure 8. (Left) Flowers were sampled on the first day they were opened. This was determined by their color which was white on the first day and yellow thereafter. (Top right) Pods were sampled based on approximate size rather than a specific developmental age. (Bottom right) Mature seeds were harvested, shelled from their pods, and then sliced on the opposite side of the hilum from the micropyle to create a piece of approximately 200 mg that would fit into a 96-well plate collection tube.

Half of the plants were randomly assigned to a treatment group which had adult *L. hesperus* added to their bug dorm one week after flowering. In the pilot study, 25 adult *L. hesperus* were added. The resulting level of herbivory was high and, as a result, the susceptible varieties, UC 92 and UC Lee, had insufficient flowers survive for sampling or pod development. In the subsequent rounds of the experiment a total of only 14 adult *L. hesperus* were added, seven one-week-old adult males and seven one-week-old adult females. This level of herbivory preserved sufficient flowers for sampling and pod development on susceptible plants. The one-week-old adult insects were added after the week one flower and pod samples were collected so that those samples had no interaction with the *L. hesperus*. All cages had a low level of thrips infestation; however, the greenhouses were not treated with insecticide during the study.

All *L. hesperus* introduced to cages in the experiment were one-week post-emergence adults reared in a colony founded by individuals collected from Lima bean and alfalfa fields in the fall of 2019 and 2020 (Fig. 9). The colony was maintained at 20°C and 12 h of photoperiod. Adults were held in 30.5cm cube collapsible cage (BioQuip Products, Rancho Dominguez, CA) with a bedding of shredded white printer paper, a water-soaked organic cotton round (Swisspers®, Parkdale Inc., Gastonia, NC), hulled sunflower seeds, and fresh organic green beans supplied three times a week. The egg-laid beans were moved to rearing tubs (1-Gallon Ice Cream Tub with Lid, Polyfarm, Amazon.com) where they emerged in approximately 7-10 days. Nymphs were supplied with a bedding of shredded white printer paper and green beans three times per week and moved to adult cages at the time of emergence.

Figure 9. Colony of L. hesperus maintained in the laboratory for this experiment

The pilot study, September 2019-December 2020 was conducted in a small greenhouse at the Orchard Park Greenhouse Facility at UC Davis (38°32'36.0"N 121°45'49.3") and included only two plants of each variety. The second round conducted in December 2019-March 2020 was conducted in the same greenhouse. In round two, six plants of each variety were planted and divided equally between the two treatment groups. The third round, March 2020-May 2020, was conducted in a neighboring greenhouse that had been enclosed with black plastic drapes to exclude all-natural light. Artificial lights lit the greenhouse in a 12-hour photoperiod. This was done in an attempt to include a photoperiod sensitive wild Lima bean accession in the study but even with this treatment, it was not possible to synchronize its flowering with that of the commercial cultivars. In round three, six plants of each variety were planted and divided equally between the two treatment groups. In the fourth round of the study, January 2021-April 2021, the

experiment was planted in a neighboring greenhouse with natural light. In round four, eight plants of each variety were planted and divided equally between the two treatment groups.

In each round of the experiment, an equal number of plants for each variety and treatment group were planted. However, due to poor germination, not every plant survived to participate in the study. Across the three plantings there were a total of 16 replicates of each variety divided between two treatment groups – so eight plants per treatment. In all rounds of the studies, cages were randomly assigned positions within the greenhouse using the Microsoft Excel random number generator function.

All the flower and pod samples were frozen at -80°C for several months before they were processed with a colorimetric assay with Feigl-Anger paper (Feigl and Anger, 1966) (Fig. 4). Defrosting samples were exposed to Feigl-Anger paper for 0-15 minutes, 15-30 minutes, 30-60 minutes, and 60-90 minutes after being removed from the freezer. The results of the assay were scanned and analyzed using the readplate2 plugin on ImageJ 1.52q (Schneider et al. 2012; Angelani et al. 2018). This semiquantitative method provided a measurement of the intensity of blue produced by the interaction of volatilized HCN and the chemical treatment of the Feigl-Anger paper. Since the volatilized HCN had to be synthesized from enzymatic activity in thawing sample tissues, a standard of KCN in NaOH solution, used in other studies, was not considered sufficiently comparable to use in estimating the quantities of cyanide released from tissues during each exposure window.
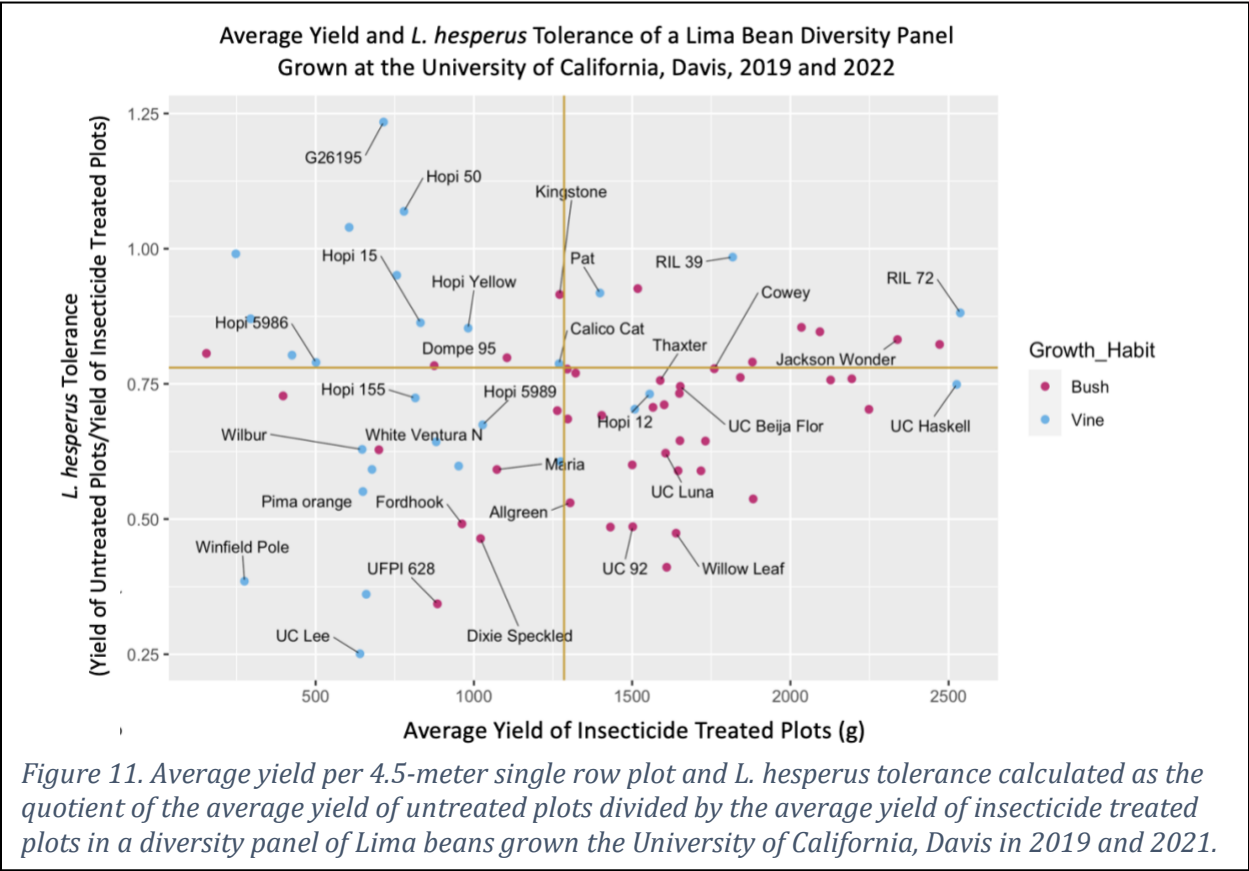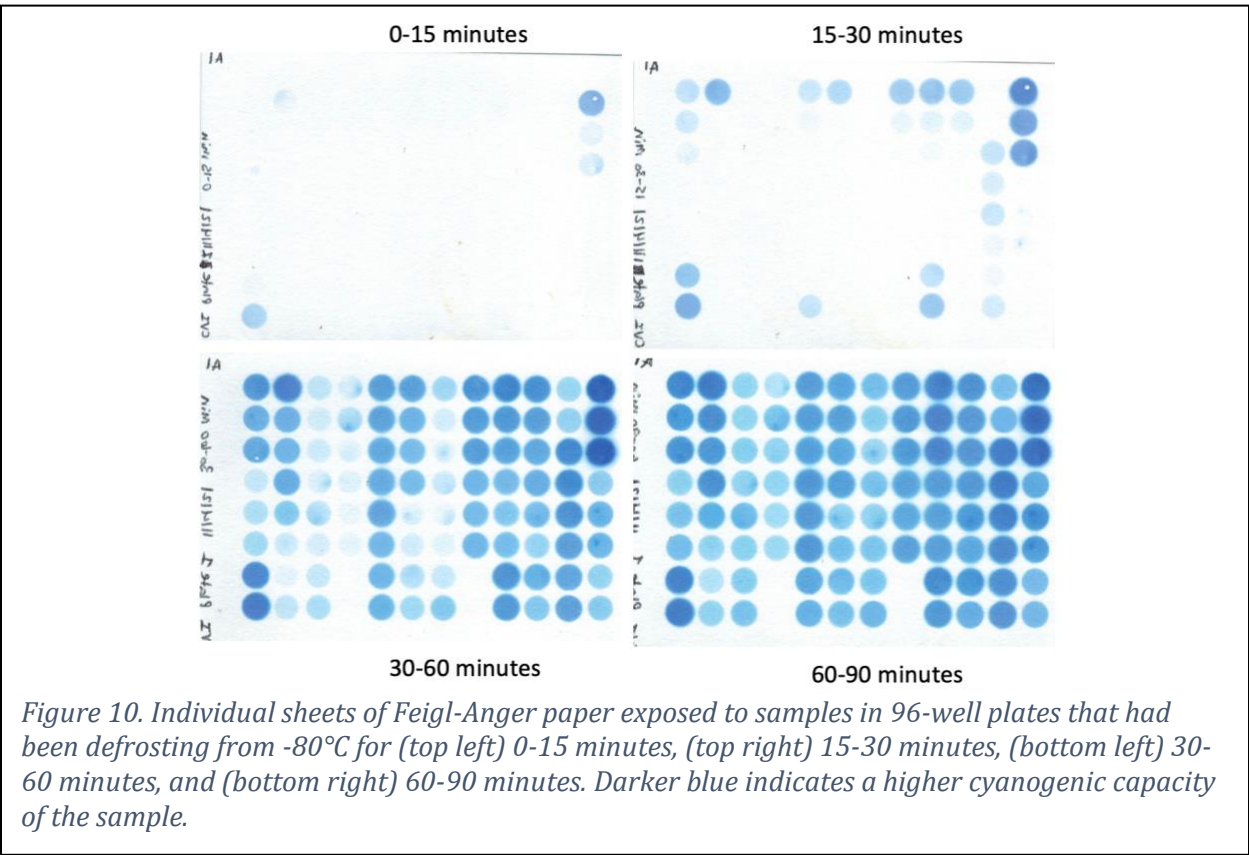
L. hesperus Survival and Reproduction

To understand how well *L. hesperus* survive and reproduce on varieties of Lima bean, populations founded by adult *L. hesperus* added to cages in the cyanogenic response

experiment were collected and analyzed after three weeks. The development of *L. hesperus* is temperature dependent but takes approximately 4 weeks at 20°C, with this being the approximate temperature of the greenhouses in which this experiment was conducted (Butler and Wardecker 1971). Three weeks is therefore not enough time for eggs to be laid and the resulting offspring to develop into adults and so the adults collected were survivors of the initial introduction rather than newly developed adults. The number of surviving adults and nymphs were counted for each cage in the treatment group. In rounds three and four, the nymphal instars were identified to indicate the speed of development for *L. hesperus* on the various varieties of Lima bean.

Analysis

All statistical analysis was conducted in R version 4.2.1 (R Core Team 2022). For the final analysis, only the 60–90-minute exposure window was used due to concerns that plate position may have affected the results of earlier windows since samples on the outside of the 96-well plate may have defrosted more quickly than samples in the interior of the 96-well plate (Fig. 10). This design flaw was not apparent in the time trial since a smaller number of samples were used and so the thermal mass of the plate was lower. It has been noted as an important lesson in experimental uniformity. Prior to publication, a statistical model including plate position may be tested to analyze results from earlier exposure windows.

Figure 10. Individual sheets of Feigl-Anger paper exposed to samples in 96-well plates that had been defrosting from -80°C for (top left) 0-15 minutes, (top right) 15-30 minutes, (bottom left) 30-60 minutes, and (bottom right) 60-90 minutes. Darker blue indicates a higher cyanogenic capacity of the sample.

Figure 11. Average yield per 4.5-meter single row plot and L. hesperus tolerance calculated as the quotient of the average yield of untreated plots divided by the average yield of insecticide treated plots in a diversity panel of Lima beans grown the University of California, Davis in 2019 and 2021.

Results and Discussion

Diversity Panel Yield Trial of Insecticide-Treated and Non-Insecticide-Treated Plots

Plots of UC 92 were vacuumed in 2019 and monitored with water traps in 2021 to check the difference in *L. hesperus* densities. In 2019, plots in the sprayed block had on average 0.5 *L. hesperus* while plots in the unsprayed block had on average 1.5 *L. hesperus.* In 2021, water traps collected every 3 days for 2 weeks after the insecticide treatment indicated that in UC 92 plots there were an average of 0.125 *L. hesperus* in the sprayed section and an average of 0.375 *L. hesperus* in the unsprayed section. These data indicate that there was a difference in *L. hesperus* densities between the two treatment blocks.

High tolerance of damage by *L. hesperus* was defined by dividing the yield of untreated plots by the yield of insecticide treated plots (Fig. 11). If the quotient was high, the variety was highly defended against *L. hesperus.* The most desirable varieties for breeding are those with high tolerance to *L. hesperus* and high yield. It should be noted that the term "tolerance" is used here as a place holder for the true mechanism of resilience. It is possible that some varieties may be resistant rather than tolerant. A resistant variety can avoid damage while a tolerant variety can recover from damage.

Sixteen commercial cultivars from the United States were included in this study (Table 3). On average, these elite lines yielded 65% as much in conditions unprotected by insecticide as compared to conditions with protection of insecticide. Pat (92%) and Kingstone (92%) had the highest *L. hesperus* tolerance while UC Lee (25%), UC 92 (49%), and Fordhook (49%) had the lowest. UC Haskell performed the best when both yield (2525.5 g/plot) and *L. hesperus* tolerance (75%) are accounted for.
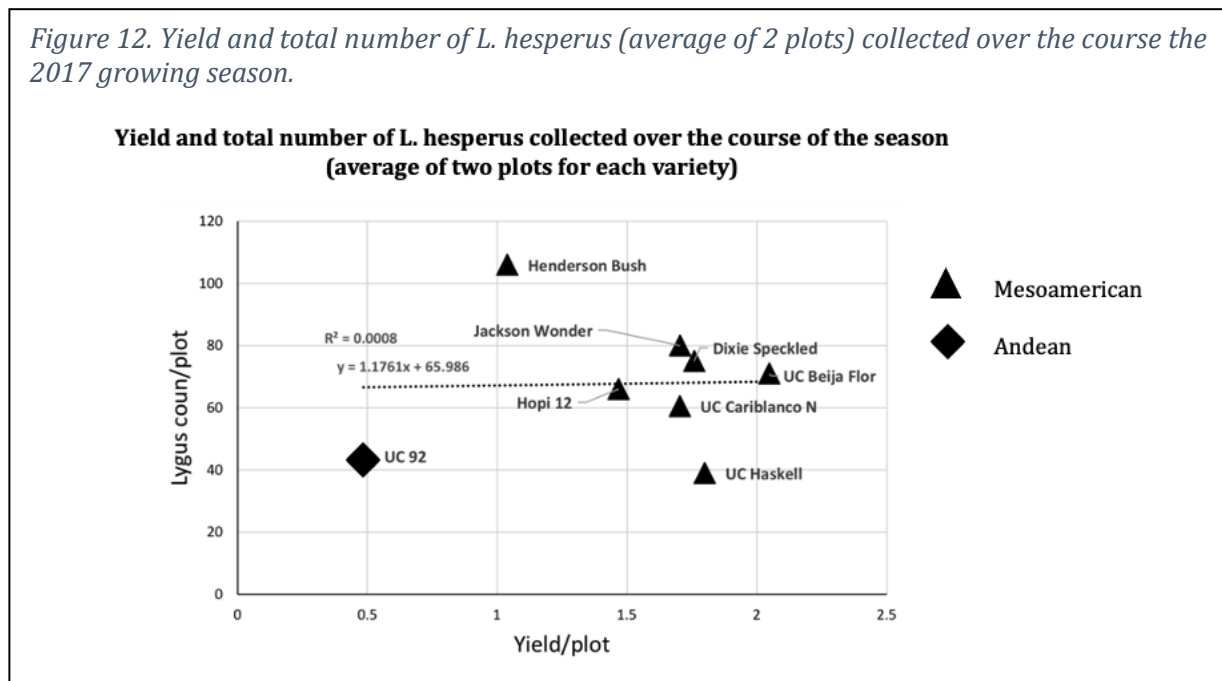
Table 6. Results from 2019 and 2021 Field Study of Commercial Varieties				
Variety	Growth Habit	Yield with Insecticides (g/15ft plot)	Yield w/out Insecticides (g/15ft plot)	% Change in Yield (No Insecticides/ Insecticides)
UC Lee	Vine	640.0	160.6	25%
UC 92	Bush	1501.8	729.7	49%
Fordhook	Bush	962.2	472.6	49%
Allgreen	Bush	1304.0	691.2	53%
Maria	Bush	1072.4	634.5	59%
UC Cariblanco N	Vine	951.7	569.2	60%
UC Luna	Bush	1605.5	998.4	62%
White Ventura N	Bush	699.6	439.4	63%
Wilbur	Vine	646.8	406.8	63%
Henderson Bush	Bush	1296.4	888.1	69%
UC Beija Flor	Bush	1651.9	1231.3	75%
UC Haskell	Vine	2525.5	1891.4	75%
Thaxter	Bush	1588.6	1201.5	76%
Dompe 95	Bush	874.5	685.4	78%
Kingstone	Bush	1270.8	1163.1	92%
Pat	Vine	1398.3	1283.6	92%

Among the 56 heirloom and international cultivars, the average yield in unprotected conditions compared to protected conditions was 73%. Noir De Kisenyi - G26196 (123%), a baby, black-and-white-seeded, bush type from Rwanda, and Hopi 50 (106%), a baby, white-seeded, vine type from the United States, were the least susceptible to *L. hesperus* but did not compete well on overall yield. The American heirloom variety, Jackson Wonder, had the best combination of high *L. hesperus* tolerance (90%) and an average yield of 2338.4 g/plot when treated with insecticide and 1945.4 g/plot when unprotected by insecticide. Many of the international and heirloom varieties included in this study have already been incorporated into the UC Davis Lima Bean Breeding Program. These results will offer additional guidance in parent selection and the choice of varieties used in future research on insect defense mechanisms.

Vacuum sampling of a diverse selection of California-adapted Lima bean cultivars

When comparing total number of *L. hesperus* per plot and yield per plot, yield did not appear to be affected by the density of *L. hesperus*, as shown by the nearly horizontal trend line (Fig. 12). In this group of varieties, UC 92 appeared to be an outlier with low yields but also a low number of *L. hesperus*. UC 92 is the only variety in this study from the Andean gene pool of Lima bean. While the yields of Andean and Mesoamerican varieties are comparable, Andean lines tend to have much larger seeds. As a result, the loss of a single seed due to *L. hesperus* herbivory would constitute a loss of a higher percentage of yield than when a single small seed is lost (Chacón-Sánchez and Martinez-Castillo, 2017).

Figure 12. Yield and total number of L. hesperus (average of 2 plots) collected over the course the 2017 growing season.

Yield and total number of L. hesperus collected over the course of the season (average of two plots for each variety)

$R^2 = 0.0008$

$y = 1.1761x + 65.986$

It should also be noted that data from this experimental design may over represent resistance or tolerance traits. On a typical California farm, a single variety will be planted across several if not tens of acres. While *L. hesperus* adults are highly mobile, they have less choice in an actual farm setting than in this experimental field. Nymphs were not considered in this study because they were crushed by the strong pressure of the vacuum

and difficult to identify with the available expertise. Nymphs are much less mobile than adults and therefore would have less ability to choose between varieties.

It is interesting to note the strong difference in the average number of *L. hesperus* per plot between the two highest yielding varieties, UC Haskell and UC Beija Flor. The large difference in average *L. hesperus* might be attributable to sampling bias caused by the denser canopy of the indeterminate UC Haskell compared to the determinate UC Beija Flor. It could also be that these two varieties have different mechanisms of resistance or tolerance to *L. hesperus.* UC Haskell and UC Beija Flor share as a parent UC Cariblanco N, but their resistance probably comes from their other respective parents. UC Beija Flor is an F10 progeny from a cross of UC Cariblanco N and CIAT accession G25165 (USDA 2010a; Genesys-PGR: https://www.genesys-pgr.org/10.18730/PH1KM). This accession was included in the diversity panel yield comparison under insecticide treated and untreated conditions described above. It has an average sprayed yield of 1650.8 g per plot and an average unsprayed yield of 1106.4 g per plot. This puts its average yield with insecticide protection above average but its estimated tolerance of *L. hesperus* below average. UC Haskell is an F10 progeny from a cross of UC Cariblanco N and an accession introduced from CIAT accession "P&T 4255" (USDA 2010b). This accession has been less well studied at it is unknown what degree or mechanism of resilience it may offer to insect herbivory.

These results open several avenues of research to pursue in the future. From further analysis of these and other data it may be possible to distinguish resistance and tolerance traits. Another area of future exploration should be the differences between the defense phenotypes of Lima beans in the Andean and Mesoamerican gene pools. Unlike the Mesoamerican gene pool, the range of Andean Lima beans falls completely outside the

native range of the genus *L. hesperus* and therefore may have fewer defense adaptations to it and closely related species (Kelton 1975; Gutiérrez Salgado et al. 1995). In figure 12, the only Andean variety, UC 92, supports very low *L. hesperus* densities. However, as shown in Figure 11, it is very sensitive to *L. hesperus* with nearly a 50% drop in yield in the unsprayed plots compared to the sprayed plots. This combination of data may indicate that the variety has some resistance traits but very little tolerance to *L. hesperus* herbivory. Future studies should include additional Andean lines for a more robust comparison of defense phenotypes between the two gene pools.

Cyanogenic response to L. hesperus

There was a very significant effect of variety on levels of cyanide in flowers, but the effect was not significant in pods (Tables 7A and 7B). Large-seeded Lima beans, UC 92 and UC Lee had significantly less cyanide in their flowers than UC Haskell. Henderson Bush and UC Beija Flor had intermediate levels of cyanide in flowers that were not significantly different from UC Haskell, UC 92, or UC Lee (Table 8). Cyanide was not detectable in the mature seeds.

The presence of *L. hesperus* had no effect on the level of cyanide in flowers but it did affect the level of cyanide in pods (Tables 7A and 7B). Pods collected from plants with *L. hesperus* had higher levels of cyanide (Table 9). This may be due to increased enzymatic activity increasing the cyanogenic capacity rather than potential as this was found in Lima bean leaves (Ballhorn et al. 2005). There was no significant difference in cyanide levels when considering the interactions between treatment and variety. The duration of the *L. hesperus* presence, as measured by "Time" in the study, did not affect the level of cyanide in

flowers or pods. Nor was there a significant difference in cyanide levels when considering the interaction of treatment and time, or time and variety.

Table 7A. Type III Analysis of Variance Table with Kenward-Roger's Method – Flowers							
	Sum sq	Mean sq	NumDF	DenDF	F value	Pr(>F)	Signifi-cance
Time	0.00200	0.00200	1	564.8	3.20	0.0743	
Treatment	0.00005	0.00005	1	65.92	0.08	0.7786	
Variety	0.01393	0.00348	4	55.27	5.57	0.0008	***
Treatment*Variety	0.00332	0.00083	4	65.88	1.33	0.2694	
Time*Treatment	0.00005	0.00005	1	569.83	0.07	0.7857	
Time*Variety	0.00410	0.00103	4	568.79	1.64	0.1621	

Table 7B. Type III Analysis of Variance Table with Kenward-Roger's Method - Pods							
	Sum sq	Mean sq	NumDF	DenDF	F value	Pr(>F)	Signifi-cance
Time	0.00070	0.00070	1	307.98	0.56	0.4559	
Treatment	0.00952	0.00952	1	32.79	7.56	0.0097	**
Variety	0.00146	0.00036	4	29.41	0.29	0.8827	
Treatment*Variety	0.00421	0.00105	4	29.89	0.83	0.5139	
Time*Treatment	0.00175	0.00175	1	302.81	1.39	0.2393	
Time*Variety	0.00316	0.00079	4	299.70	0.63	0.6430	

Table 8. Compact Letter Display of All Pairwise Comparisons - Flowers						
Variety	pmmean	SE	df	lower.CL	upper.CL	Group
UC 92	0.038	0.011	1.28	0.038	0.038	1
UC Lee	0.039	0.011	1.26	0.039	0.039	1
Henderson Bush	0.047	0.012	1.34	0.046	0.047	12
UC Beija Flor	0.054	0.011	1.28	0.054	0.054	12
UC Haskell	0.062	0.011	1.30	0.062	0.063	2

Table 9. Compact Letter Display of All Pairwise Comparisons - Pods						
Treatment	pmmean	SE	df	lower. CL	upper.CL	Group
No *L. hesperus*	0.052	0.003	24.40	0.052	0.052	1
L. hesperus	0.063	0.003	43.80	0.063	0.063	2

L. hesperus *Survival and Reproduction*

From the greenhouse experiment of cyanogenic capacity with and without the presence of *L. hesperus,* insects from each of the cages (Fig. 7) were counted four weeks after flowering and three weeks after the *L. hesperus* had been introduced. For thorough counts, all plants were completely deconstructed with each leaf carefully checked for nymphs and the surface of the potting soil carefully searched. The count per cage of each variety was averaged to estimate survival and reproduction (Table 10). It is interesting to note that the two varieties known to be resistant or tolerant of *L. hesperus*, UC Haskell and UC Beija Flor, have very different numbers of surviving adults and new nymphs. It is possible that cyanogenesis may be responsible for this difference. In quantified studies of greenhouse grown samples without *L. hesperus*, UC Beija Flor was estimated to have a cyanogenic capacity of 153.4 nM/30 minutes of volatile HCN released from floral bud samples and 224.1 nM/30 minutes of volatile HCN released from immature pod samples (Zullo 2021). In the same study, UC Haskell only had a cyanogenic capacity of 126.86 nM/30 minutes of volatile HCN released from floral bud samples and 63.4 nM nM/30 minutes of volatile HCN released from immature pod samples. Given that survival of adult and nymph *L. hesperus* is more strongly correlated with cyanide in immature pods than in flowers (Figs. 8 and 9) this difference in cyanogenic capacity could be contributing to the higher number of insects surviving on UC Haskell as compared to UC Beija Flor. It should be noted however that this was a no-choice experiment and in the field collected vacuum samples, in which there were many varieties to choose from, more *L. hesperus* were captured on UC Beija Flor than UC Haskell. This could be due to sampling bias due to the

denser canopy of the indeterminate UC Haskell or it could indicate that a mechanism other than cyanogenesis is contributing to *L. hesperus* preference and survival.

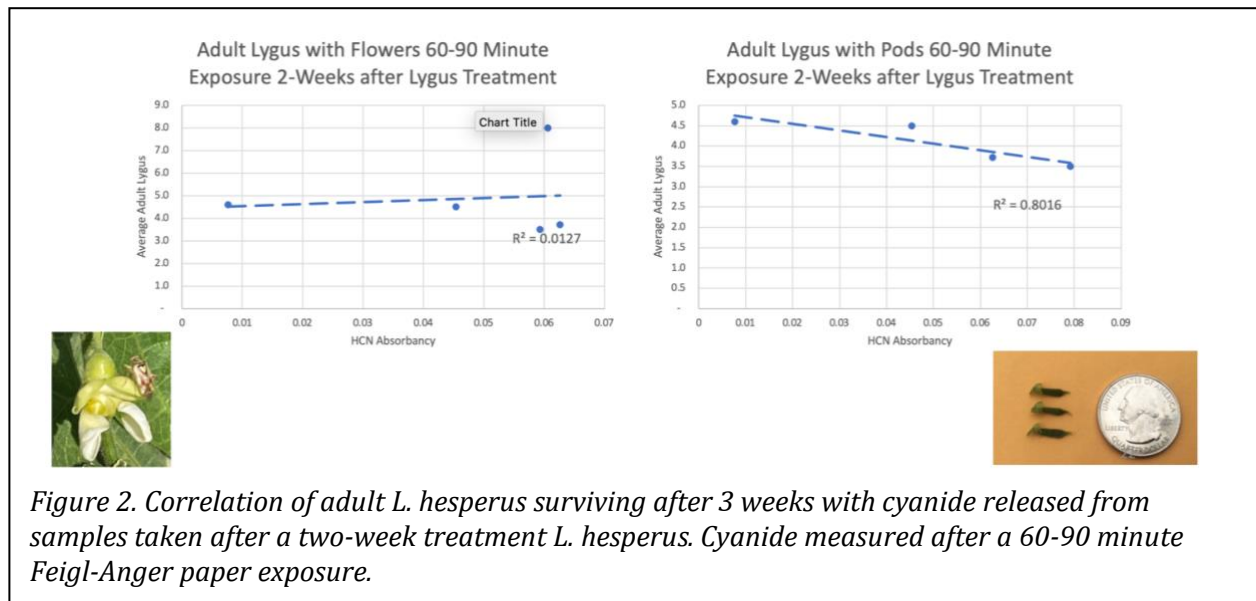| Table 10. Average numbers of L. hesperus captured in cages with plants of each variety 3 weeks after 14 1-week post-emergence adults were introduced			
Variety	Average Adults	Average Nymphs	Average Total
Henderson Bush	4.6	101	105.6
UC 92	3.7	40.3	44
UC Beija Flor	4.5	46.7	51.2
UC Haskell	8	112.7	120.7
UC Lee	3.5	48.3	51.8

Figure 2. Correlation of adult L. hesperus surviving after 3 weeks with cyanide released from samples taken after a two-week treatment L. hesperus. Cyanide measured after a 60-90 minute Feigl-Anger paper exposure.
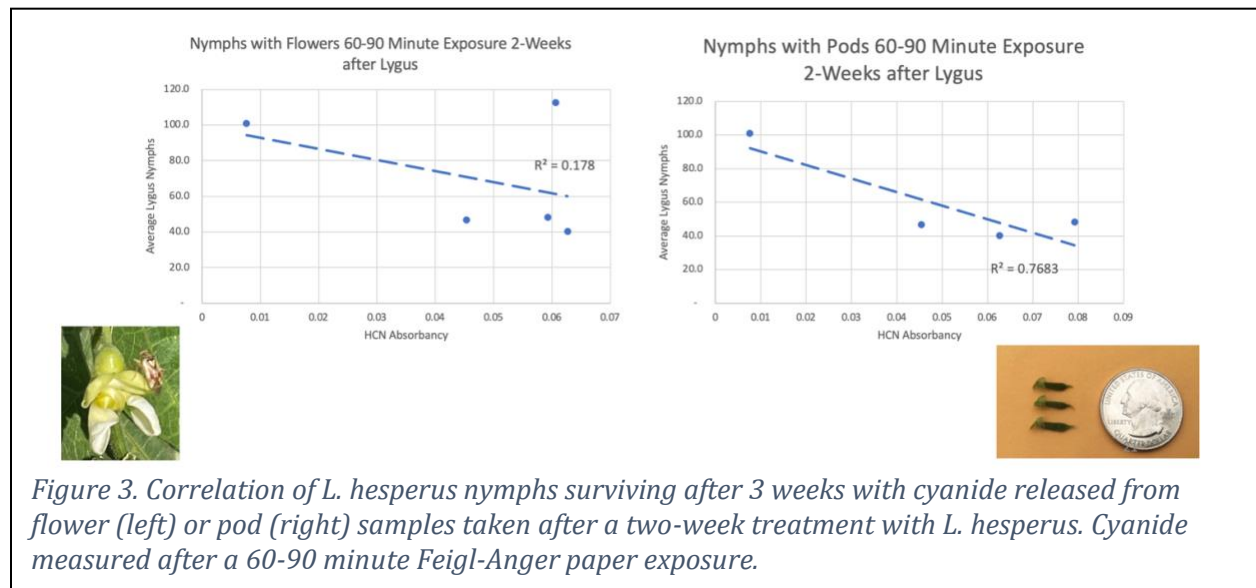

Figure 3. Correlation of L. hesperus nymphs surviving after 3 weeks with cyanide released from flower (left) or pod (right) samples taken after a two-week treatment with L. hesperus. Cyanide measured after a 60-90 minute Feigl-Anger paper exposure.

This study has several limitations. One is that no wild or weedy types were included for comparison with the domesticated forms. While growing these accessions can be challenging in confined greenhouse space, including them in a similar analysis could shed light on how the trait of cyanogenesis has been affected by domestication. Another limitation of the study was the use of discrete semiquantitative measurements using Fiegl-Anger paper. While this method of measuring cyanogenic capacity is well established, safe, and high throughput, it does not provide a true quantitative measurement, or the continuous sampling provided by the enclosed detection systems used for studies of volatilized cyanide (Ballhorn et al. 2005).

Conclusion

This study found that the survival and reproduction of *L. hesperus* was negatively correlated with the cyanogenic capacity of their host plant. This indicates that selecting plants with higher cyanogenic capacity in their flowers and young pods may be an effective way to control *L. hesperus.* The evidence does not support the hypothesis that cyanogenic capacity was induced by the presence of *L. hesperus.* For consumer safety, future research should determine if there is a relationship between the cyanogenic glucoside content of flowers, immature pods, and the mature seeds they grow into as a correlation has been found between the cyanogenic content of mature seeds and the cotyledons from the seedlings those seeds grow into (Shlichta et al. 2014)

References

Adeparusi, E. O. (2001). Effect of processing on the nutrients and anti-nutrients of lima bean (*Phaseolus lunatus* L.) flour. *Food/Nahrung*, *45*(2), 94-96. doi: 10.1002/1521-3803(20010401)45:2<94::AID-FOOD94>3.0.CO;2-E

Alonso-Amelot, M. E., & Oliveros-Bastidas, A. (2005). Kinetics of the natural evolution of hydrogen cyanide in plants in neotropical *Pteridium arachnoideum* and its ecological significance. *Journal of Chemical Ecology*, *31*(2), 315-331. doi: 10.1007/s10886-005-1343-z

Angelani, C. R., Carabias, P., Cruz, K. M., Delfino, J. M., de Sautu, M., Espelt, M. V., Ferreira-Gomes, M. S., Gómez, G. E., Mangialavori, I. C., Manzi, M., Pignataro, M. F., Saffioti, N. A., Salvatierra Fréchou, D. M., Santos, J., & Schwarzbaum, P. J. (2018). A metabolic control analysis approach to introduce the study of systems in biochemistry: the glycolytic pathway in the red blood cell. *Biochemistry and Molecular Biology Education*, *46*(5), 502-515. doi: 10.1002/bmb.21139

Antonini, E., Brunori, M., Rotilio, G. C., Greenwood, C., & Malmström, B. G. (1971). The interaction of cyanide with cytochrome oxidase. *European Journal of Biochemistry*, *23*(2), 396-400. doi: 10.1111/j.1432-1033.1971.tb01633.x

Arimura, G. I., Ozawa, R., Shimoda, T., Nishioka, T., Boland, W., & Takabayashi, J. (2000) Herbivore-induced volatiles elicit defense genes in lima bean leaves. Nature 406: 512–515. doi: 10.1038/35020072

Arimura, G. I., Ozawa, R., Nishioka, T., Boland, W., Koch, T., Kühnemann, F., & Takabayashi, J. (2002). Herbivore-induced volatiles induce the emission of ethylene in neighboring lima bean plants. *The Plant Journal* 29(1), 87–98. doi: 10.1046/j.1365-313x.2002.01198.x

Arthurs, S., & Dara, S. K. 2019**.** Microbial biopesticides for invertebrate pests and their markets in the United States. *Journal of Invertebrate Pathology*: 13-21. doi: 10.1016/j.jip.2018.01.008

Ballhorn, D. J., Godschalx, A. L., Smart, S. M., Kautz, S., & Schädler, M. (2014). Chemical defense lowers plant competitiveness. *Oecologia*, 176(3), 811–824. doi: 10.1007/s00442-014-3036-1

Ballhorn, D. J., Heil, M., & Lieberei, R. (2006). Phenotypic plasticity of cyanogenesis in Lima bean *Phaseolus lunatus*-Activity and activation of β-glucosidase*. Journal of Chemical Ecology*, 32(2), 261–275. doi: 10.1007/s10886-005-9001-z

Ballhorn, D. J., Kautz, S., Lion, U., & Heil, M. (2008). Trade-offs between direct and indirect defences of lima bean (*Phaseolus lunatus*). *The Journal of Ecology*, 96(5), 971–980. doi: 10.1111/j.1365-2745.2008.01404.x

Ballhorn, D. J., Kautz, S., Heil, M., & Hegeman, A. D. (2009). Cyanogenesis of wild lima bean (*Phaseolus lunatus L.)* is an efficient direct defence in nature. *PloS One*, 4(5), e5450–e5450. doi: 10.1371/journal.pone.0005450

Baudoin, J. P., Barthelemy, J. P., & Ndungo, V. (1991). Variability of cyanide contents in the primary and secondary genepools of the Lima bean, *Phaseolus lunatus* L. *Bulletin des Ressources Genetiques Vegetales (CIRP/FAO); Noticiario de Recursos Geneticos Vegetales (CIRF/FAO); Plant Genetic Resources Bulletin (IBPGR/FAO)*.

Bernays, E. A., Chapman, R. F., Leather, E. M., McCaffery, A. R., & Modder, W. W. D. (1977). The relationship of *Zonocerus variegatus* (L.) (Acridoidea: Pyrgomorphidae) with cassava (*Manihot esculenta*). *Bulletin of Entomological Research*, 67(3), 391–404. doi: 10.1017/S0007485300011202

Bloom, A. J. (1997). Nitrogen as a limiting factor: crop acquisition of Ammonium and Nitrate. In: Jackson, L. (ed.) *Ecology in agriculture*, Academic Press, San Diego, pp. 145-172.

Blumenthal-Goldschmidt, S., Butler, G. W., & Conn, E. E. (1963). Incorporation of hydrocyanic acid labelled with carbon-14 into asparagine in seedlings. *Nature*, *197*(4868), 718-719. doi: 10.1038/197718a0

Brattsten, L. B., Samuelian, J. H., Long, K. Y., Kincaid, S. A., & Evans, C. K. (1983). Cyanide as a feeding stimulant for the southern army worm, *Spodoptera eridania*. *Ecological Entomology*, *8*(2), 125-132. doi: 10.1111/j.1365-2311.1983.tb00490.x

Castric, P. A., Farnden, K. J., & Conn, E. E. (1972). Cyanide metabolism in higher plants: V. The formation of asparagine from β-cyanoalanine. *Archives of Biochemistry and Biophysics*, *152*(1), 62-69. doi: 10.1016/0003-9861(72)90193-2

Cooper, C. E., & Brown, G. C. (2008). The inhibition of mitochondrial cytochrome oxidase by the gases carbon monoxide, nitric oxide, hydrogen cyanide and hydrogen sulfide: chemical mechanism and physiological significance. *Journal of Bioenergetics and Biomembranes*, *40*(5), 533-539. doi: 10.1007/s10863-008-9166-6

Cooper-Driver, G. A., & Swain, T. (1976) Cyanogenic polymorphism in bracken in relation to herbivore predation. *Nature* 260.5552: 604-604.

Cowan, M. F., Blomstedt, C. K., Møller, B. L., Henry, R. J., & Gleadow, R. M. (2021). Variation in production of cyanogenic glucosides during early plant development: A comparison of wild and domesticated sorghum. *Phytochemistry*, *184*, 112645. doi: 10.1016/j.phytochem.2020.112645

Cullis, C., & Kunert, K. J. (2017). Unlocking the potential of orphan legumes. *Journal of Experimental Botany*, *68*(8), 1895-1903. doi: 10.1093/jxb/erw437

Cuny, M. A. C., La Forgia, D., Desurmont, G. A., Glauser, G., & Benrey, B. (2019). Role of cyanogenic glycosides in the seeds of wild Lima bean, *Phaseolus lunatus*: defense, plant nutrition or both? *Planta*, 250(4), 1281–1292. doi: 10.1007/s00425-019-03221-3

Dashner, Z. (2016). *Examination of Lygus bug resistance in Lima bean: Polygalacturonase inhibiting proteins as candidate traits*. University of California, Davis.

Dement, W. A., & Mooney, H. A. (1974). Seasonal variation in the production of tannins and cyanogenic glucosides in the chaparral shrub, *Heteromeles arbutifolia*. *Oecologia*, 15(1), 65–76. doi: 10.1007/BF00345228

Dohle, S. (2017). *Development of resources for Lima bean (*Phaseolus lunatus*) breeding and genetics research*. PhD thesis, University of California, Davis.

Engler, H. S., Spencer, K. C., & Gilbert, L. E. (2000). Insect metabolism preventing cyanide release from leaves. *Nature* (London), 406(6792), 144–145. doi: 10.1038/35018159

Floss, H. G., Hadwiger, L., & Conn, E. E. (1965). Enzymatic formation of β-cyanoalanine from cyanide. *Nature*, *208*(5016), 1207-1208. doi: 10.1038/2081207a0

Feigl, F., & Anger, V. (1966). Replacement of benzidine by copper ethylacetoacetate and tetra base as spot-test reagent for hydrogen cyanide and cyanogen. *Analyst*, *91*(1081), 282-284. doi: 10.1039/an9669100282

Forslund, K., & Jonsson, L. (1997). Cyanogenic glycosides and their metabolic enzymes in barley, in relation to nitrogen levels. *Physiologia Plantarum*, 101(2), 367–372. doi: 10.1111/j.1399-3054.1997.tb01010.x

Gleadow, R. M. & Woodrow, I. E. (2000). Temporal and spatial variation in cyanogenic glycosides in *Eucalyptus cladocalyx*. *Tree Physiology*, 20(9), 591–598. doi: 10.1093/treephys/20.9.591

Gleadow, R. M. & Woodrow, I. E. (2002). Constraints on effectiveness of cyanogenic glycosides in herbivore defense. *Journal of Chemical Ecology*, 28(7), 1301–1314. doi: 10.1023/A:1016298100201

Gutiérrez Salgado, A., Gepts, P., & Debouck, D. (1995). Evidence for two gene pools of the Lima bean, *Phaseolus lunatus* L., in the Americas. *Genetic Resources and Crop Evolution*, 42(1), 15–28. https://doi.org/10.1007/BF02310680

Heil, M. (2004a). Direct defense or ecological costs: responses of herbivorous beetles to volatiles released by wild Lima bean (*Phaseolus lunatus*). *Journal of Chemical Ecology*, 30(6), 1289–1295. doi:10.1023/B:JOEC.0000030299.59863.69

Heil, M. (2004b). Induction of two indirect defences benefits Lima bean (*Phaseolus lunatus*, Fabaceae) in nature. *The Journal of Ecology*, 92(3), 527–536. doi: 10.1111/j.0022-0477.2004.00890.x

Heil, M., Lion, U., & Boland, W. (2008). Defense-inducing volatiles: in search of the active motif. *Journal of Chemical Ecology*, 34(5), 601–604. doi: 10.1007/s10886-008-9464-9

Honig, D. H., Hockridge, M. E., Gould, R. M., & Rackis, J. J. (1983). Determination of cyanide in soybeans and soybean products. *Journal of Agricultural and Food Chemistry*, *31*(2), 272-275. doi: 10.1021/jf00116a021

Horiuchi, Arimura, G., Ozawa, R., Shimoda, T., Takabayashi, J., & Nishioka, T. (2003). A comparison of the responses of *Tetranychus urticae* (Acari: Tetranychidae) and *Phytoseiulus persimilis* (Acari: Phytoseiidae) to volatiles emitted from Lima bean leaves with different levels of damage made by *T. urticae* or *Spodoptera exigua* (Lepidoptera: Noctuidae). *Applied Entomology and Zoology*, 38(1), 109–116. doi: 10.1303/aez.2003.109

Isman, M. (2020). Botanical insecticides in the twenty-first century - fulfilling their promise? *Annual Review of Entomology*. 65: 233-249. doi: 10.1146/annurev-ento-011019-025010

Jimenez-Lopez, J. C., Singh, K. B., Clemente, A., Nelson, M. N., Ochatt, S., & Smith, P. M. (2020). Legumes for global food security. *Frontiers in Plant Science*, *11*, 926. doi: 10.3389/fpls.2020.00926

Jones, D. A. (1966). On the polymorphism of cyanogenesis in *Lotus corniculatus*. I. Selection by animals. *Canadian Journal of Genetics and Cytology*, *8*(3), 556-567. doi: 10.1139/g66-067

Jones, D. A., 1998. Why are so many food plants cyanogenic? *Phytochemistry*, *47*(2), pp.155-162. doi: 10.1016/S0031-9422(97)00425-1

Kakes, P. (1990). Properties and functions of the cyanogenic system in higher plants. *Euphytica*, 48(1), 25–43. doi: 10.1007/BF00028958

Kelton, L. (1975). The Lygus bugs (Genus *Lygus* Hahn) of North American (Heteroptera: Miridae). Memoirs of the Entomological Society of Canada, 107(S95), 5–101. doi: 10.4039/entm10795fv

Kooyers, N. J., Gage, L. R., Al-Lozi, A., & Olsen, K. M. (2014). Aridity shapes cyanogenesis cline evolution in white clover (*Trifolium repens* L.). *Molecular Ecology*, *23*(5), 1053-1070. doi: 10.1111/mec.12666

Kost, C. & Heil, M. (2005). Increased availability of extrafloral nectar reduces herbivory in Lima bean plants (*Phaseolus lunatus*, Fabaceae). *Basic and Applied Ecology*, 6(3), 237–248. doi: 10.1016/j.baae.2004.11.002

Kost, C. & Heil, M. (2006). Herbivore-induced plant volatiles induce an indirect defence in neighbouring plants. *The Journal of Ecology*, 94(3), 619–628. doi: 10.1111/j.1365-2745.2006.01120.x

Ladizinsky, G. (1999). On the origin of almond. *Genetic Resources and Crop Evolution*, 46(2), 143–147. doi: 10.1023/A:1008690409554

Lai, D., Maimann, A. B., Macea, E., Ocampo, C. H., Cardona, G., Pičmanová, M., Darbani, B., Olsen, C. E., Debouck, D., Raatz, B., Møller, B. L., & Rook, F. (2020). Biosynthesis of cyanogenic glucosides in *Phaseolus lunatus* and the evolution of oxime-based defenses. *Plant Direct*, 4(8), e00244–n/a. doi: 10.1002/pld3.244

Lieberei. (1988). Relationship of cyanogenic capacity (HCN-c) of the rubber tree *Hevea brasiliensis* to susceptibility to *Microcyclus ulei*, the agent causing South American leaf blight. *Journal of Phytopathology*, 122(1), 54–67. doi: 10.1111/j.1439-0434.1988.tb00990.x

Liener, I. E. (1962). Toxic factors in edible legumes and their elimination. *The American Journal of Clinical Nutrition*, *11*(4), 281-298.

Long, R., Temple, S., Meyer, R., Schwankl, L., Godfrey, L., Canevari, M., & Roberts, P. (2014). Lima bean production in California. ANR Publication 8505. https://escholarship.org/uc/item/4cb5376v

Loyd, R. C. & Gray, E. (1970). Amount and distribution of hydrocyanic acid potential during the life cycle of plants of three sorghum cultivars. *Agronomy Journal*, 62(3), 394–397. doi: 10.2134/agronj1970.00021962006200030025x

Martin, J. H., Couch, J. P., & Briese, R. R. (1938). Hydrocyanic acid content of different parts of the sorghum plant. *Agronomy Journal*, 30(9), 725–734. doi: 10.2134/agronj1938.00021962003000090003x

Møller, B. L., and D. S. Seigler. (1999) Biosynthesis of cyanogenic glycosides, cyanolipids and related compounds." *Plant Amino Acids Biochemistry and Biotechnology*: 563-609.

Møller, B. L. (2010). Functional diversifications of cyanogenic glucosides. *Current opinion in plant biology*, *13*(3), 337-346. doi: 10.1016/j.pbi.2010.01.009

Moreira, X., Abdala-Roberts, L., Hernández-Cumplido, J., Cuny, M. A. C., Glauser, G., & Benrey, B. (2015). Specificity of induced defenses, growth, and reproduction in lima bean (*Phaseolus lunatus*) in response to multispecies herbivory. *American Journal of Botany*, 102(8), 1300–1308. doi: 10.3732/ajb.1500255

Okolie, N. P., & Ugochukwu, E. N. (1989). Cyanide contents of some Nigerian legumes and the effect of simple processing. *Food Chemistry*, *32*(3), 209-216. doi: 10.1016/0308-8146(89)90049-6

Olsen, K. M., Hsu, S.-C., & Small, L. L. (2008). Evidence on the molecular basis of the *Ac/ac* adaptive cyanogenesis polymorphism in white clover (*Trifolium repens* L.). *Genetics*, 179(1), 517–526. doi: 10.1534/genetics.107.080366

Ospina, M. A., Pizarro, M., Tran, T., Ricci, J., Belalcazar, J., Luna, J. L., Londoño, L. F., Salazar, S., Ceballos, H., Dufour, D., Becerra Lopez-Lavalle, L. A. (2021). Cyanogenic, carotenoids and protein composition in leaves and roots across seven diverse population found in the world cassava germplasm collection at CIAT, Colombia. *International Journal of Food Science & Technology*, 56(3), 1343-1353. doi: 10.1111/ijfs.14888

Osuntokun, B. O. (1969). *Chronic cyanide intoxication and a degenerative neuropathy in Nigeria*. PhD thesis, University of Ibadan, Nigeria

Osuntokun, B. O. (1994). Chronic cyanide intoxication of dietary origin and a degenerative neuropathy in Nigerians. *Acta Horticulturae*, 375, 311–321. https://doi.org/10.17660/ActaHortic.1994.375.31

Pinheiro de Castro, E. C., Demirtas, R., Orteu, A., Olsen, C. E., Motawie, M. S., Zikan Cardoso, M., Zagrobelny, M., & Bak, S. (2020). The dynamics of cyanide defences in the life cycle of an aposematic butterfly: Biosynthesis versus sequestration. *Insect Biochemistry and Molecular Biology*, 116, 103259–103259. doi: 10.1016/j.ibmb.2019.103259

Poulton, J. E. (1983). Cyanogenic compounds in plants. *Handbook of Natural Toxins*, *1*, 117.

Poulton, J. E. (1990). Cyanogenesis in plants. *Plant Physiology*, 94(2), 401–405. doi: 10.1104/pp.94.2.401

R Core Team (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Ragir, S. (2000), Diet and food preparation: Rethinking early hominid behavior. *Evol. Anthropol.*, 9: 153-155. doi: 10.1002/1520-6505(2000)9:4<153::AID-EVAN4>3.0.CO;2-D

Román, G. C. (2014). Tropical myelopathies. *Handbook of Clinical Neurology*, *121*, 1521–1548. doi: 10.1016/B978-0-7020-4088-7.00102-4

Sánchez-Pérez, R., Pavan, S., Mazzeo, R., Moldovan, C., Aiese Cigliano, R., Del Cueto, J., Ricciardi, F., Lotti, C., Ricciardi, L., Dicenta, F., López-Marqués, R. L., & Møller, B. L. (2019). Mutation of a bHLH transcription factor allowed almond domestication. *Science*, 364(6445), 1095–1098. doi: 10.1126/science.aav8197

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature methods*, *9*(7), 671-675. doi: 10.1038/nmeth.2089

Shlichta, J. G., Cuny, M. A. C., Hernandez-Cumplido, J., Traine, J., & Benrey, B. (2018). Contrasting consequences of plant domestication for the chemical defenses of leaves and seeds in lima bean plants. *Basic and Applied Ecology,* 31, 10–20. doi: 10.1016/j.baae.2018.05.012

Shlichta, J. G., Glauser, G., & Benrey, B. (2014). Variation in cyanogenic glycosides across populations of wild Lima beans (*Phaseolus lunatus*) has no apparent effect on bruchid beetle performance. *Journal of Chemical Ecology*, 40(5), 468–475. doi: 10.1007/s10886-014-0434-0

Takos, A. M., Knudsen, C., Lai, D., Kannangara, R., Mikkelsen, L., Motawia, M. S., Olsen, C. E., Sato, S., Tabata, S., Jørgensen, K., Møller, B. L., & Rook, F. (2011). Genomic clustering of cyanogenic glucoside biosynthetic genes aids their identification in Lotus japonicus and suggests the repeated evolution of this chemical defence pathway. *The Plant Journal: for Cell and Molecular Biology*, 68(2), 273–286. doi: 10.1111/j.1365-313X.2011.04685.x

Till, I. (1987). Variability of expression of cyanogenesis in white clover (*Trifolium repens* L.). *Heredity*, *59*(2), 265-271. doi: 10.1038/hdy.1987.122

United States Department of Agriculture (USDA 2010b) Agricultural Marketing Service Science and Technology – Plant Variety Protection Office. PVPO Number 201000306 "UC Beija-Flor." Filed May 17, 2010. Accessed November 13, 2022. https://apps.ams.usda.gov/CMS//AdobeImages/201000306.pdf

United States Department of Agriculture (USDA 2010b) Agricultural Marketing Service Science and Technology – Plant Variety Protection Office. PVPO Number 201000307 "UC Haskell." Filed May 18, 2010. Accessed November 13, 2022. https://apps.ams.usda.gov/CMS//AdobeImages/201000307.pdf

Vanderborght, T. (1979) Le dosage de l'acide cyanhydrique chez *Phaseolus lunatus* L. Annales de Gembloux 85:29-41

Van Heijst, A. N. P., Maes, R. A. A., Mtanda, A. T., Chuwa, L. M. M., Rwiza, H. T., & Moshi, N. H. (1994). Chronic cyanide poisoning in relation to blindness and tropical neuropathy. *Journal of Toxicology. Clinical Toxicology*, 32(5), 549–556. doi: 10.3109/15563659409011059

Vetter, J. (2000). Plant cyanogenic glycosides. *Toxicon* (Oxford), 38(1), 11–36. doi: 10.1016/S0041-0101(99)00128-2

Wilson, W. M. & Dufour, D. L. (2002). Why "bitter" cassava? productivity of "bitter" and "sweet" cassava in a Tukanoan Indian settlement in the Northwest Amazon. *Economic Botany*, 56(1), 49–57. doi: 10.1663/0013-0001(2002)056[0049:WBCPOB]2.0.CO;2

Witthohn, K. & Naumann, C. M. (1984). Qualitative and quantitative studies on the compounds of the larval defensive secretion of *Zygaena trifolii* (Esper, 1783) (Insecta, Lepidoptera, Zygaenidae). *Comparative Biochemistry and Physiology. C, Comparative Pharmacology and Toxicology*, 79(1), 103–106. doi: 10.1016/0742-8413(84)90170-1

Wrangham, R. & Conklin-Brittain, N. (2003). Cooking as a biological trait. *Comparative Biochemistry and Physiology, Part A,* 136(1), 35–46. doi: 10.1016/S1095-6433(03)00020-5

Zullo, S. S. Developing a molecular linkage map for and understanding the biochemical mechanisms and underlying genetic architecture of biotic stress resistance in Lima bean *(Phaseolus lunatus L.)*. Ph.D. Diss. University of California, Davis, 2021.

Conclusion

Plants and insects have a long coevolutionary history that was complicated by the domestication of crop plants and rise of agriculture approximately 12,000 years ago (Vialatte et al. 2011; Midamegbe et al. 2011; Bourguet et al. 2014). While mobile insects have evolved new ways to utilize plants for food and shelter, stationary plants are evolving physical and chemical ways of defending themselves (Ehrlich and Raven 1964). The domestication of crop plants has given insects a significant advantage by narrowing genetic diversity and imposing selection pressures that prioritize agronomic traits over defense (Johnson 2008; Tooker and Frank 2012; Dar et al. 2021). This can be seen in the reduction of physical defense mechanism that are unpalatable as well as in chemical defense mechanism that are toxic (Mitchell et al. 2016). However, several mechanisms of anti-herbivore defense have survived in crop plants and even been selected under the pressures of cultivation and domestication. The goal of my doctoral research was to study the evolution of anti-herbivore defense mechanisms during domestication and specifically the trait of cyanogenesis as a defense mechanism in Lima beans under pressure from the insect pest, *Lygus hesperus*, commonly known as the Western Tarnished Plant Bug. This was accomplished through a comprehensive literature review of how domestication has affected the genetics of anti-herbivore defense traits in crop plants and three research chapters which presented results from a genome-wide association study of cyanogenesis and lima beans, and a collection of studies characterizing defense phenotypes against *L. hesperus* in Lima beans.

Reviewing the literature on the genetic effect of domestication on anti-herbivore defense mechanisms in crop plants yielded several insights in chapter 1. First, reductions in

genetic diversity of gene pools under domestication has led to a general trend of reduction in anti-herbivore defense mechanisms, especially those which may be unpalatable or toxic to human consumers (Evans 1993; Benrey et al. 1998; Wittkop et al. 2009; Mitchell et al. 2016; Whitehead et al. 2017). Polyploidization, a recurrent phenomenon in plants that is especially common in crop plants, has also affected anti-herbivore defense compounds (Renny-Byfield and Wendel 2014). While the evidence is mixed, some research indicates that novel biochemistry in polyploids may serve as a source of natural variation from which crop plants can evolve or retain defense mechanism (Renny-Byfield and Wendel 2014). Selection under domestication has also resulted in differential expression of anti-herbivore defense mechanisms in harvested organs and reproductive tissues of crop plants (Zhoray et al. 2004). Genes that stop the expression of defense mechanisms in these tissues while allowing expression elsewhere in the plant provide compromises between the selection pressure of domestication and selection pressures from insect herbivores (Sánchez-Pérez et al. 2007; McCall and Fordyce 2010; Shang et al. 2014; Whitehead et al. 2017).

While many of the examples presented in the review are drawn from other crops, Lima beans are a valuable model organism for the study of anti-herbivore defense traits in crop plants. The availability of diverse accessions from two domestication groups and from wild populations makes possible facilitates comparison and association studies (Gutiérrez-Salgado et al. 1995; Andueza-Noh et al. 2013). Investigations in this system are also aided by advanced genetic resources including a reference genome, recombinant inbred line population, and large collection of sequenced accessions (Chacón-Sánchez and Martinez-Castillo 2017; Dohle 2017; Zullo 2021; Garcia et al. 2021). The experiments presented in

the remaining chapters build on this existing body of research and raise new questions for future studies, especially those that consider the evolutionary dynamics of multi-trait defense syndromes under domestication.

In chapter 2, a genome-wide association study of cyanogenesis in a Mesoamerican diversity panel of Lima beans was conducted to elucidate the genetic architecture of cyanogenesis in Lima bean and to ascertain the effect of domestication on this trait. Several significant SNPs were identified for the phenotype of volatilized cyanide collected 0-15 minutes and 15-30 minutes after tissue disruption. When the known sequences of genes for cyanogenesis in white clover were BLAST searched against the Lima bean reference genome, there were many close matches in location to the significant SNPs identified by the GWAS. Some of the SNPs for the 0-15 minute collection were close to matches for the *Li/li* gene that controls synthesis of the β-glucosidase which cleaves cyanide from a cyanogenic glucoside. By contrast, some of the SNPs for the 15–30 minute collection were close to matches for the *Ac/ac* gene which controls synthesis of the cyanogenic glucoside. A significant SNP on chromosome 5 was closely located to QTL identified for cyanogenesis in flowers, pods, and leaves of a biparental recombinant inbred line population of Lima beans (Dohle 2017; Zullo 2021). Further analysis will be conducted prior to publication to check the confidence intervals and flanking markers to increase certainty about a relationship between the significant SNPs, QTL, and BLAST matches. Additional study of the genome annotations and expression atlas for clues about the function of genes near these regions will also be undertaken. To examine the effect of domestication on cyanogenesis genes, the GWAS results of wild and domesticated accessions will be compared. Finally, accessions

with extreme phenotypes and their associated genotypes will be identified for use in breeding and further mapping and validation.

In chapter 3, four related experiments were presented to characterize the phenotypes of defense against *L. hesperus* herbivory in Lima beans. First, to explore the variation in the resistance or tolerance to *L. hesperus* herbivory, a diversity panel of California-adapted Lima beans was grown in a yield trial under treatment with and without insecticides. This revealed that there is variation in the trait or suit of traits that contribute to *L. hesperus* tolerance or resistance. Second, a study of the cyanogenic response to *L. hesperus* was conducted in which flowers, young pods, and mature succulent seeds were sampled for volatile cyanide at 1, 2 and 3 weeks after the start of flowering from plants with and without exposure to *L. hesperus* adults. This study demonstrated that there is a significant effect of variety on level of cyanide in flowers and a significant effect of *L. hesperus* exposure for cyanide levels in pods. This indicates that cyanogenesis may be induced by *L. hesperus* in pods. This same study design was used to examine the effect of cyanogenesis on Lygus survival and reproduction. Insects that had been introduced to the cages 1 week after flowering were found after 4 weeks to have rates of survival and reproduction that were correlated with the cyanogenic capacity of each variety. These four studies combine to indicate that cyanogenesis may be part of the defensive mechanism in Lima beans under pressure from *L. hesperus.* Future research should be done to study how cyanide affects nymphal development and what the lethal dose of cyanide is for *L. hesperus* at each life stage.

This work was built on a body of prior research on anti-herbivore defense traits in crop plants generally and Lima beans specifically. By increasing understanding of the

mechanisms and multi trait dynamic that contribute to plant defense against insects, targeted breeding and management practices may be undertaken to reduce yield loss from insect pests and improve global food security. Future research is needed to continue this endeavor. In the case of Lima beans and *L. hesperus* future work should focus on clearly identifying the operative mechanism or mechanisms of resistance or tolerance. With the existing data, more analysis can be conducted to identify target genes for breeding differential expression of cyanogenesis in reproductive tissues for increased protection. Additionally, further study of nymphal development of *L. hesperus* on cyanogenic Lima beans may indicate additional strategies for reducing herbivory. Ongoing research in these areas will be of great value to Lima bean producers in California and will likely also contribute to understanding of how to defend other crops from similar generalist herbivores.

References

Andueza-Noh, R. H., Serrano-Serrano, M. L., Chacón Sánchez, M. I., Sánchez del Pino, L., Camacho-Pérez, J., Coello-Coello, J., Mijangos Cortes, J., Debouck, D. G., & Martínez-Castillo, J. (2013). Multiple domestications of the Mesoamerican gene pool of Lima bean (*Phaseolus lunatus* L.): evidence from chloroplast DNA sequences. *Genetic Resources and Crop Evolution*, *60*(3), 1069-1086. doi: 10.1007/s10722-012-9904-9

Benrey, B., Callejas, A., Rios, L., Oyama, K. and Denno, R.F., 1998. The effects of domestication of *Brassica* and *Phaseolus* on the interaction between phytophagous insects and parasitoids. *Biological Control*, *11*(2), pp.130-140. doi:10.1006/bcon.1997.0590

Bourguet, D., Ponsard, S., Streiff, R., Meusnier, S., Audiot, P., et al. (2014). Becoming a species by becoming a pest' or how two maize pests of the genus *Ostrinia* possibly evolved through parallel ecological speciation events. *Molecular Ecology* **23**: 325–342. doi: 10.1111/mec.12608.

Chacón-Sánchez, M. I. & Martínez-Castillo, J. (2017). Testing Domestication Scenarios of Lima Bean (*Phaseolus lunatus* L.) in Mesoamerica: Insights from Genome-Wide Genetic Markers. Frontiers in Plant Science, 8, 1551–1551. doi: 10.3389/fpls.2017.01551

Dar, M. S., Dholakia, B. B., Kulkarni, A. P., Oak, P. S., Shanmugam, D., Gupta, V. S., & Giri, A. P. (2021). Influence of domestication on specialized metabolic pathways in fruit crops. *Planta*, 253(2), 61–. doi10.1007/s00425-020-03554-4

Dohle, S. (2017). *Development of resources for Lima bean (Phaseolus lunatus) breeding and genetics research*. University of California, Davis.

Ehrlich, P. R., & Raven, P. H. (1964). Butterflies and plants: a study in coevolution. *Evolution*, 586-608. doi: 10.1111/j.1558-5646.1964.tb01674.x

Garcia T., Duitama, J., Smolenski Zullo, S., Gil, J., Ariani, A., Dohle, S., Palkovic, A., Skeen, P., Bermudez-Santana, C., Debouck, D. G., Martinez-Castillo, J., Gepts, P., Chacón-Sánchez, M. I. (2021) Comprehensive genomic resources related to domestication and crop improvement traits in Lima bean. Nature Comm 12:702 doi: 10.1038/s41467-021-20921-1

Gutiérrez-Salgado, A., Gepts, P., & Debouck, D. G. (1995). Evidence for two gene pools of the Lima bean, *Phaseolus lunatus* L., in the Americas. *Genetic Resources and Crop Evolution*, *42*(1), 15-28. doi: 10.1007/BF02310680

Johnson, M. T. J. (2008). Bottom-up effects of plant genotype on aphids, ants, and predators. *Ecology*, *89*(1), 145-154. doi: 10.1890/07-0395.1

McCall, A. C., & Fordyce, J. A. (2010). Can optimal defence theory be used to predict the distribution of plant chemical defences. *The Journal of Ecology*, 98(5), 985–992. doi: 10.1111/j.1365-2745.2010.01693.x

Midamegbe, A., Vitalis, R., Malausa, T., Delava, É., Cros-Arteil, S., et al. (2011). Scanning the European corn borer (*Ostrinia spp.*) genome for adaptive divergence between host-affiliated sibling species. *Molecular Ecology* **20**: 1414–1430. doi: 10.1111/j.1365-294X.2011.05035.x.

Mitchell, C., Brennan, R. M., Graham, J., & Karley, A. J. (2016). Plant defense against herbivorous pests: exploiting resistance and tolerance traits for sustainable crop protection. *Frontiers in Plant Science*, *7*, 1132. doi: 10.3389/fpls.2016.01132

Renny-Byfield, S., & Wendel, J. F. (2014). Doubling down on genomes: polyploidy and crop plants. *American Journal of Botany*, *101*(10), 1711-1725. doi: 10.3732/ajb.1400119

Sánchez-Pérez, R., Ortega, E., Duval, H., Martínez-Gómez,, P., & Dicenta, F. (2007). Inheritance and relationships of important agronomic traits in almond. *Euphytica* **155**, 381–391. doi: 10.1007/s10681-006-9339-5

Shang, Y., Ma, Y., Zhou, Y., Zhang, H., Duan, L., Chen, H., Zeng, J., Zhou, Q., Wang, S., Gu, W., Liu, M., Ren, J., Gu, X., Zhang, S., Wang, Y., Yasukawa, K., Bouwmeester, H. J., Qi, Z., Zhang, Z., Lucas, W. J., & Huang, S. (2014). Biosynthesis, regulation, and domestication of bitterness in cucumber. *Science*, *346*(6213), 1084-1088. doi: 10.1126/science.1259215

Tooker, J. F., & Frank, S. D. (2012). Genotypically diverse cultivar mixtures for insect pest management and increased crop yields. *Journal of Applied Ecology*, *49*(5), 974-985. doi: 10.1111/j.1365-2664.2012.02173.x

Vialatte, A., Dedryver, C. A., Simon, J. C., Galman, M., & Plantegenest, M. (2005). Limited genetic exchanges between populations of an insect pest living on uncultivated and related cultivated host plants. *Proceedings of the Royal Society B-Biological Sciences p*272: 1075–1082. doi: 10.1098/rspb.2004.3033

Whitehead, S. R., Turcotte, M. M., & Poveda, K. (2017). Domestication impacts on plant–herbivore interactions: a meta-analysis. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *372*(1712), 20160034. doi: 10.1098/rstb.2016.0034

Wittkop, B., Snowdon, R. J., & Friedt, W. (2009). Status and perspectives of breeding for enhanced yield and quality of oilseed crops for Europe. *Euphytica*, *170*(1), 131-140. doi: 10.1007/s10681-009-9940-5

Zohary, D. (2004). Unconscious selection and the evolution of domesticated plants. *Economic botany*, *58*(1), 5-10. doi: 10.1663/0013-0001(2004)058[0005:USATEO]2.0.CO;2

Zullo, S. S. Developing a Molecular Linkage Map for and Understanding the Biochemical Mechanisms and Underlying Genetic Architecture of Biotic Stress Resistance in Lima Bean *(Phaseolus lunatus L.)*. Diss. University of California, Davis, 2021.