Effect of Quinolizidine Alkaloid Consumption and Seed Feeding Behavior on Caterpillar Growth

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Abstract

Caterpillars who feed on a wide variety of plant hosts must adapt to the defenses of every plant they feed on. For seed-eating caterpillars this can be particularly difficult, because, as the reproductive source for the plant and thus the main determinant of evolutionary progression, the seed is one of the most heavily defended bodies on a plant. This project addressed three questions: i) what is the feeding behavior of these seed eating caterpillars, ii) how do they respond to the presence of quinolizidine alkaloids (QAs) in their seed diet, and iii) what is the fate of these compounds after they are consumed by a generalist caterpillar?

Helicoverpa zea, the corn earworm is both a seed eater and a generalist, feeding on over 30 families of plants, including both agricultural crops and native plants such as *Lupinus Texensis* (Texas bluebonnet). I observed that corn earworms exhibit highly variable feeding behavior and travel around the plant to feed from dispersed sources on the plant. In order to understand the effect of plant toxin defense on caterpillar growth, I fed corn earworms on varying concentration diets of *Lupinus texensis* seeds (containing QAs). When monitoring their growth rate and mass in response to these various levels of plant defense chemicals, no difference was found between caterpillar development across different toxin levels. Instead, it appears that caterpillars which were exposed to greater toxin levels ate greater quantities of food in order to account for the food's lower digestibility. Preliminary chemistry data suggests that the caterpillars do not house the defense chemicals in their body after consumption, instead excreting the defense chemicals in their frass (feces). These findings support the understanding that generalist caterpillars use variable food intake to promote appropriate growth and that they do not suffer from increased chemical defense in their host plants.

Keywords: *Helicoverpa zea*, corn earworm, quinolizidine alkaloids, *Lupinus texensis*, generalist, feeding behavior

Introduction

Seed Eaters and Plant Defense

Approximately half of all insects feed on plant material (Lawton & Schröder 1978); however, those which feed directly on plant reproductive bodies (pollen, flowers, ovaries and seeds) have the most significant impact on the natural selection and subsequent evolution of the plants (Schoonhoven et al. 2005; Smallegange et al. 2007). By feeding on the reproductive bodies or seeds (the next generation of plants) directly, seed-feeding herbivores have a more direct impact on plant fitness than leaf eaters (Levin 1975).

In addition to having substantial impact on their host plants, seed predators' (seed herbivores who kill the seeds they feed on) behavior and diet also affect their own growth. Seeds typically have a higher nutrient content than leaves on many plants (Smallegange et al. 2007); however, many plant species also employ greater quantities of defenses to guard against herbivory of their seeds (Wink 1992). Plant defenses can include structural defense such as thorns, spines or trichomes (small hairs), mechanical defenses such as latex (to inhibit caterpillar movement or mastication), or chemical defenses: toxic or distasteful chemical deterrents to herbivores (Fordyce & Agrawal 2001). Many plant species have been shown to accumulate higher concentrations of these toxins, called secondary metabolites, in reproductive organs than in leaves (Schoonhoven et al. 2005). Secondary metabolites are organic compounds produced by plants which are not directly involved in the normal development or reproduction of the plant, but can be used for other purposes (Rosenthal & Berenbaum 2012): in this case plant defense. This may create a trade-off for herbivorous insects feeding on reproductive organs. Seed feeding insects might have access to greater stores or greater density of essential nutrients and proteins in the seeds but they may also be more exposed to harmful chemicals via increased plant defenses.

Plants produce a wide variety of these defense toxins. These secondary metabolites act as deterrents against insect herbivores, inhibit herbivore growth, and even cause lethal toxicity

(Johnson & Bentley 1988; Wink 1992 and references within; Harborne 2007). These chemicals can vary both in composition and quantity between plant groups. Classes of these compounds include alkaloids (derived from amino acids), several subclasses of glycosides (a functional group attached to a glucose molecule), carotenoids (which affect plant coloration and sunlight exposure), and terpenoids (a diverse group of hydrocarbons which contribute to plant scent and flavor, commonly used in human medicine) among others (Balandrin 1985). Quinolizidine alkaloids (QA) are derived from the amino acid lysine, and are often called lupin alkaloids due to their abundance in plants within the genus *Lupinus* (Wink 1992). In some cases, QA-producing plants (from the *Lupinus* genus) show direct positive correlations in their plant tissues between nutrient quality and secondary metabolite levels (Rhoades & Cates 1976; Waller & Nowacki 1978; Johnson & Bentley 1988). These plants have higher concentrations of secondary metabolites in the same plant structures which have the highest nutrient and protein quantities. Herbivores eating from healthy, nutritious, QA-producing plants will be subjected to higher levels of defense chemicals. This implies that the trade-off between toxins and nutrients may be even more pertinent for caterpillars who feed on QA-producing plants as opposed to those who feed on plant species which use other defense mechanisms. Ideally, caterpillars seek out that food source which grants them access to the greatest quantity (or density) of nutrients while causing the least risk or harm to them (Stephens & Krebs 1986). However, in order to feed on the high nutrient regions of a QA-producing plant (such as the seeds), a herbivore must be able to deal with the plant's increased defenses. In fact, QA concentration in seeds has been shown to be up to ten times higher in seeds of lupine plants than in their leaves (Wink & Hartmann 1979; Wink & Witte 1984; Wink 1993). Therefore it is possible that there may be a very strong effect of QA content on seed predator growth, especially for caterpillars whose host plants are in the *Lupinus* genus.

Generalists and Caterpillar Response to Secondary Metabolites

Some caterpillars specialize on a single plant species as a host, or even a single part of that plant. There are many reasons why a caterpillar may specialize to eat only a specific plant, or closely related plant family: specialization can reduce competition with other herbivores, allow for effective camouflage or other defense against predation, or give the caterpillars access to specific nutrient profiles (Fry 1996; Futuyma & Moreno 1998). In some cases dietary specialization can also be helpful or even essential for caterpillars to develop detoxification methods against secondary metabolites because specialists generally encounter limited classes of secondary metabolites (Nishida 2002; Lampert & Bowers 2010; Ali & Agrawal 2012). Insect herbivores have typically been grouped into categories based on their degree of dietary specialization (Futuyma & Moreno 1998; Morais et al. 2011): When limited to only one or a few closely related plant taxa, often a single genus, herbivores are considered monophagous – or highly specialized. Insect herbivores that feed on many plant species within one taxonomic family are considered oligophagous. Lastly, polyphagous – highly generalized species – are those which feed on species from multiple plant families. Across all herbivorous insects, it is estimated that less than 10% feed on plants in more than three different plant families (Bernays & Graham 1988). It is estimated that only 1% of insect herbivores are fully polyphagous generalists (Forister et al. 2015).

Generalists have the potential to be exposed to a wider variety of plant defense chemicals from different host plant species (Ali & Agrawal 2012). Caterpillars use five main strategies to deal with secondary metabolite consumption when these toxins are encountered in plants (Wink 1992):

1. Avoidance: Once detecting the secondary metabolite presence, the caterpillar avoids feeding. Caterpillars are equipped with both olfactory and gustatory receptors allowing them to competently detect such chemical defenses (Kaissling 1987).

2. Detoxification: Once consumed, the secondary metabolites may be passively absorbed into the gut and detoxified through complex, typically energetically costly, enzymatic reactions (Dobler et al. 2011).
3. Excretion: Rather than having secondary chemicals absorbed into the gut, some caterpillars have developed mechanisms which allow for absorption of necessary nutrients into the gut but not plant defense chemicals (Bernays & Graham 1988; Wink & Schneider 1990). Therefore plant defenses which are not taken up from the gut may pass out directly in caterpillar frass (feces).
4. Sequestering: Some caterpillars, particularly specialists, use the chemicals to their advantage. They store consumed secondary compounds into their body in a process known as sequestration, which allows them to use these plant derived compounds for their own defense (Nishida 2002). This may allow these sequestering species to make themselves toxic to their own predators.
5. Other Specific Compound Use: A small number of species use consumed plant defense chemicals to create new compounds such as pheromones (Nickisch-Rosenegk et al. 1990; Schneider 1987). These modified chemicals are necessary to their physiology and development but are not considered sequestration as they do not aid in caterpillar defense. Caterpillars may also modify the alkaloid and then excrete it through their frass.

Caterpillars may use one or more of these strategies. Generalists may apply different strategies to their variety of host plants or for different secondary metabolite classes. In order for herbivores to use ingested chemicals to their benefit they must develop high tolerance to chemicals, feeding behavior which helps lessen the secondary metabolites' effect, or specialized chemical reactions in the gut to detoxify the chemicals (Wink 1992). All of these complicated strategies require energy and biological resources from each individual using that strategy. For herbivores exposed

to different classes of secondary metabolites (alkaloids, glycosides, terpenoids ect.) these strategies must be customized to deal with each class of defense chemcials, further taxing the development and resources of developing caterpillars (Ali & Agrawal 2012). Therefore, polyphagous herbivores typically only use Avoidance, Detoxification, or Excretion strategies as Sequestration and direct use of the compounds may have greater biological costs which make them only effective when used by specialists (Hartmann 2004; Smilanich et al. 2009; Wink 1992).

The Corn Earworm and Lupines

Helicoverpa zea, the corn earworm is both a seed eater and a polyphagous generalist; feeding on over 30 families of plants (Eger et al. 1982; Robinson 2002). They feed largely on crops of agricultural import during the fall and summer, but in winter they make generations-long migrations south from the USA-Canadian border to the northern edge of Mexico and feed on a large number of native plant species (Sandstrom et al. 2007). Corn earworm lifecycles span approximately a month and thus may spend each generation on a different host plant than prior generations (Eger et al. 1982).

Observations of corn earworm damage have been found at field sites of *Lupinus texensis* (Texas bluebonnet lupines) in Texas (Eger et al. 1982; Blanchard personal observation 2017). At these sites, corn earworms were also observed to feed in a dispersed pattern. Dispersed feeding refers to the movement of caterpillars over relatively large distances to forage, often immediately leaving areas where they have fed before the food source there was depleted (Mauricio & Bowers 1990). For the seed eating corn earworm this means penetrating the seed pod of the lupine, consuming a single seed from a pod (which typically contain five or six seeds) before moving to a different pod on a different part of the plant. For some folivorous (leaf-eating) caterpillars, dispersed feeding patterns have been shown to increase their fitness (Stamp &

Bowers 1990). Dispersed feeding can help folivourous avoid predators (Stamp & Bowers 1992), evade chemical induction – increased secondary metabolite response – by the host plant (Agrawal 2000), or introduce variability of nutrients into their diet (Stamp & Bowers 1990).

The corn earworm caterpillar, however, prefers to feed on reproductive tissues, particularly seeds, rather than leaves. Seed-feeding caterpillars act as a more direct selective force in plant reproduction than folivores as they directly eliminate seeds from the future generation. It is unknown how dispersed seed-feeding, affects the caterpillars' own success. Folivorous caterpillars experience almost no barriers to dispersed feeding, whereas the protections afforded to reproductive bodies provide deterrents for seed predators to feed dispersedly. Therefore the benefits garnered by the dispersed feeding of corn earworms on lupines must outweigh the energy and time cost to move between pods. The pods of lupines are difficult to penetrate and contain QAs but almost no nutrients (Wink 1992, Hartung personal observations 2018). It takes substantial time and energy for a corn earworm to bore through the nutrient poor, trichome-coated, and QA-containing pod. This cost to dispersed feeding implies that there may be more significant benefits to this behavior for a corn earworm than for a folivore.

The specific combination of behaviors and diet of the corn earworm make them an interesting study organism when examining the effects of QA digestion and feeding behavior. QAs have been shown to act as a feeding deterrent to some insect herbivores, whereas for other insects, QA presence can been insecticidal: leading to reduction of growth in affected population or even mortality in high dosage (Wink 1992). Observing corn earworms on bluebonnet lupines can help us gain an understanding of both their dispersed feeding behavior on seed pods and their response to QA content in food. These insights can be applied to a greater understand of QA effects on caterpillars and the feeding behaviors of generalist caterpillars. QA tolerance and

response has been studied in other lepidopteran species on related lupine species (Montllor et al. 1990; Montllor et al. 1991; Fiedler et al. 1993; Bermúdes-Torres 2009; Karban et al. 2010). These studies have generally found that generalist Lepidoptera are able to excrete QA in frass with only marginal alkaloids remaining in the caterpillar's system in response to increased QAs in their diet. However, these studies have not yet investigated the effects of QAs on seed feeders.

Research Questions

The goal of this study is to answer three main questions about *Helicoverpa zea* growth and development. Firstly, I hoped to gain insight into the dispersed feeding behavior observed at field sites. This was done through observational experiments, watching caterpillar movement and feeding behavior on lab-grown Texas bluebonnets. Next, I sought to discover how they respond physiologically to Texas bluebonnet QA levels in their diet and how these responses affect their growth. This was examined by monitoring caterpillar growth under the effect of QA in their diet, the digestibility of QA-containing diet, and chemical assays of caterpillar and frass QA content. Lastly, through QA analysis, I sought to learn the typical fate of QA after consumption by a corn earworm. This data would then be compared to the possible toxin digestion strategies outlined in Wink 1992.

Methods

Study system

Corn earworms are a common and widely spread generalist moth species, also known as the tomato fruitworm or "New World" bullworm (Eger 1982; Leite 2014). The moth caterpillars feed on a wide variety of agricultural and native plants, making them polyphagous (Robinson 2002). The moths and caterpillars are susceptible to damage and mortality due to frost in winter. They are only able to overwinter in the pupal state and cannot do so in regions of prolonged winter frost (Sandstrom et al. 2007). The moths therefore migrate south and back north throughout the year, using different plants each generation as they migrate (Robinson 2002; Sandstrom et al. 2007; Leite 2014).

The full lifecycle of a single generation of the corn earworm lasts for approximately one month. Therefore, as they migrate north, a female could potentially lay her eggs hundreds of miles from where she was born and on an entirely different plant species (Leite 2014). This lifecycle allows the corn earworm caterpillars to feed polyphagously on a wide variety of host plants from generation to generation. Corn earworms are known to feed on over 30 American crops including corn, tomato, cotton, green beans, lettuce, peppers, soybeans, and sorghum (Eger et al. 1982; Cook & Weinzierl 2004), in addition to 70 or more native plants such

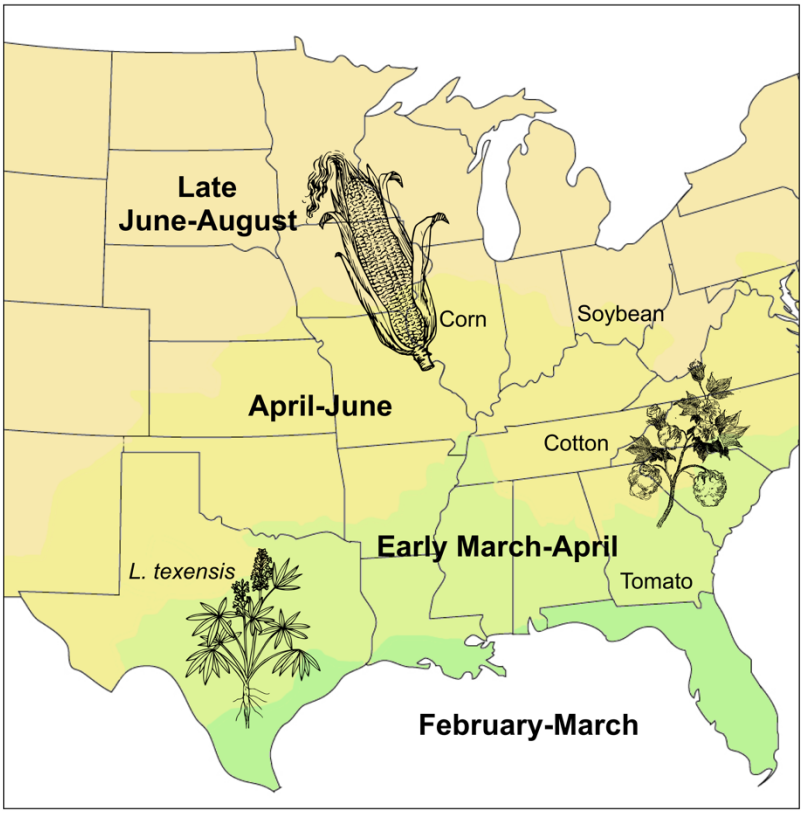


Figure 1: Annual distribution of *Helicoverpa zea*. The dates represent the first expected appearance of corn earworms through their annual migration across the midwestern United States (adapted from Sandstrom et al. 2007; and crop distributions estimated from the USDA IPAD Crop Production Maps).

as lupines, clovers, vetch, common mallow and geranium (Sudbrink & Grant 1995; Robinson 2002; Blanco et al. 2007 and references within).

Texas bluebonnet (*Lupinus texensis*), native to Texas, represents one of the first annual host plants for corn earworm migrators (Eger 1982). Bluebonnets first sprout in the fall, grow as rosettes through the winter, begin to bolt in March, bloom in May, beginning to senesce by mid- to late-May (Eger 1982; Blanchard personal observations 2017-18). My observations of plants in the lab and at Texas field sites branch at the base forming between three and twenty densely leafed branches between 15 and 40 cm long. Inflorescences (flower clusters) and subsequently groups of pods form at the tips of the branches. Corn earworm eggs are typically laid on the flowers or pods of the bluebonnet, and developing larvae feed on these structures. Texas bluebonnets contain significant amounts of all the following quinolizidine alkaloids: Lupinine, Lupanine, Multiflorine, Aphylline, Angustifoline, 13(alpha)-Tigloyl-oxylupanine, Anagyrine, Ammodendrine (Wink 1992). Wink's 1992 review describes that the total alkaloid levels in other *Lupinus* species are approximately 2 mg per gram of dry weight in the leaves and flowers, and up to 40 mg of QA per g of dry weight in seeds.

The caterpillars' capacity to take advantage of this host plant, in spite of its high QA levels, has the potential to dictate their success on crops later in the season (Eger et al. 1982). If corn earworm caterpillars grow quickly and large on early season plants, such as bluebonnet lupines, they may have more reproductive potential as they migrate further north.

Lab Population

Helicoverpa zea were acquired to build a lab population from a population cultivated by Dr. Carrie Deans at the Texas A&M Entomology department. They arrived as pupae and were allowed to breed communally for two generations before individuals were used for experimentation.

In both the adult and larval stage, individuals were kept in an environmental chamber. The incubation chamber was set to 24° C for an 15 hour lit day (beginning at 6:30 AM), and 20° C at night. Adult moths were fed on fruit punch flavor Gatorade®, commonly used in Lepidoptera studies due to its cost, manufactured consistency, and its sugar and electrolyte content (Hoang et al. 2011; Forister et al. 2013). Females oviposited on cotton squares, their food source, and nearly everywhere else in their custom-made flight cages (Figure 2). Eggs were collected from these sources daily during the breeding period.

Eggs were removed from the fight cages within a day of oviposition and were placed in communal containers containing artificial diet. Following hatching, all caterpillars were separated into individual containers each 5.5 cm by 4 cm by 3 cm. Through all instars (developmental stages), caterpillars were fed on Southland Product's *Helicoverpa zea* artificial diet until pupation. Pupae were then sexed and divided into breeding groups and placed in sanitized flight cages with fresh cotton and mesh for oviposition. Between generations, all cages and caterpillar containers were cleaned by soaking in a 1% bleach solution before rinsing in hot water. All mesh and cotton surfaces upon which eggs were oviposited were discarded after larvae hatched.

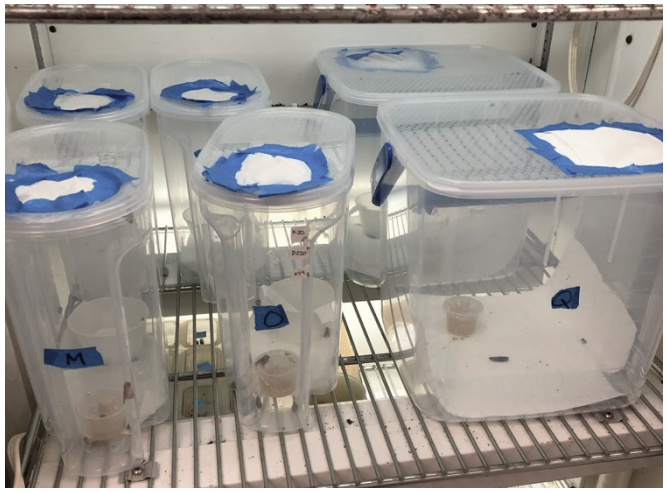


Figure 2: Corn earworm flight cages were constructed to suit the unique needs of *H. zea* moths and caterpillars. Due to the extremely small hatching size of caterpillars, and the indeterminate oviposition behavior of the females, typical mesh bug dorms were ineffective at containing early stage *H. zea* caterpillars.

Behavioral Experiment

In order to gain better understanding of corn earworm feeding behavior, an observational study was designed. The goal of this study was to understand how a corn earworm moves around a lupine as it feeds, where it feeds from, how dispersed this feeding is, and how these behavioral patterns might affect the caterpillar's growth. Due to limited plant stock, only a few iterations of the experiment were completed. Fifth instar caterpillars were starved for eight hours before the initiation of the experiment. They were then placed at the base of a fully grown and fruiting

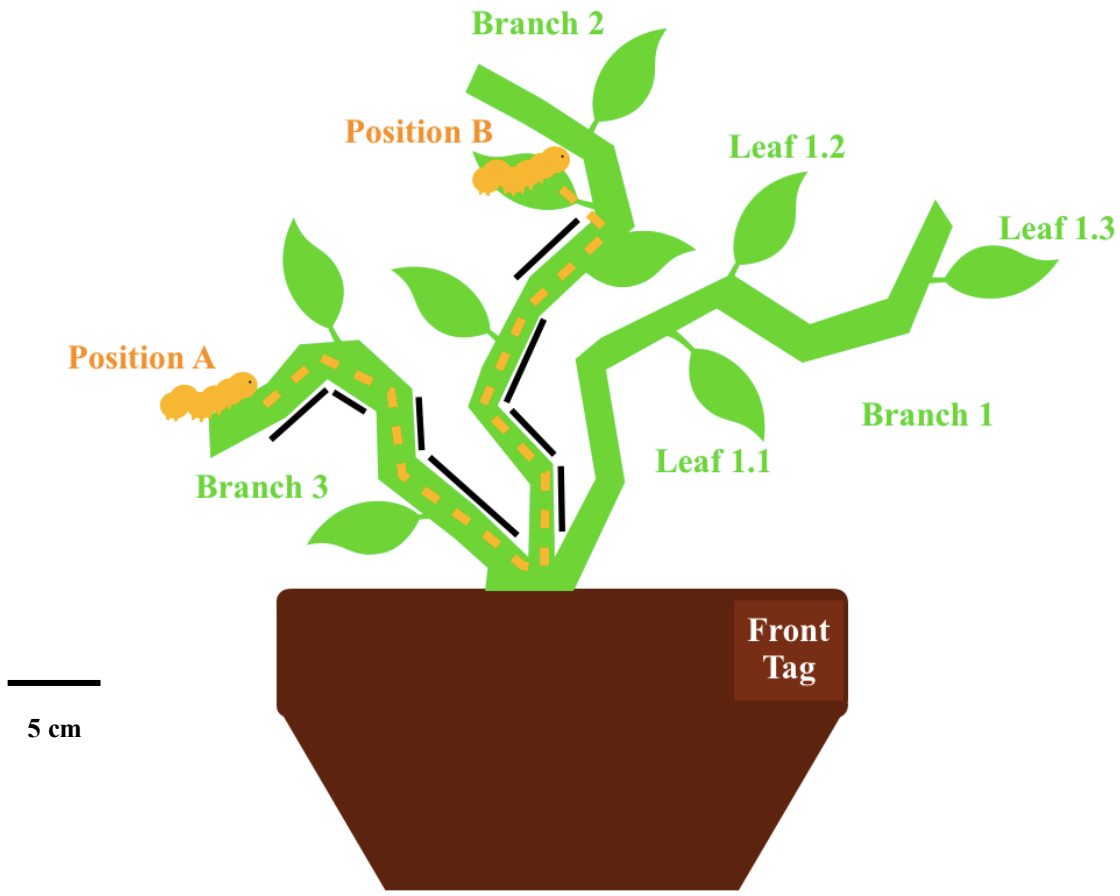


Figure 3: Simplified diagram of behavioral experiment. Each leaf, branch, sub-branch and pod were cataloged using the scheme show on Branch 1 as lupine branching structure is lateral (only one branch splits off from the main stem at one position). An example caterpillar movement is shown from position A (Tip of Branch 3) to position B (Leaf 2.3) along the given path. This distance would be measured piecewise along the branches (black lines) or leaves using a flexible measuring tape.

Lupinus texensis plant. For the next four to seven hours the caterpillars' movement distance, behavior, and location were recorded every five minutes. Movement distance was measured

along the shortest path for caterpillar could have traveled in five minutes (Figure 3). Sometimes this path was between leaves rather than along the plant stem. A caterpillar was only marked as eating if clear active mastication was visible. Caterpillars who fell off of their plant were placed back at the base of the stem. In order to catalog caterpillar location, each planet was tagged with a "front" branch and each branch was numbered sequentially from there counterclockwise around the plant. Then each offshoot (sub-branch) was recorded based on distance from the base of the branch, and each leaf and pod were documented in the same way. Data were examined holistically to determine the viability and potential value for future corn earworm behavioral studies.

Artificial diet

Basic artificial diet was purchased from Southerland Product and prepared according to their provided instructions for the *Helicoverpa zea* diet. 155ml of water was boiled before adding the dry ingredients outlined in Table 1. The diet was then cooled in square molds and cut into pieces weighing approximately 2 grams to be fed to corn ear worm caterpillars. Squares of diet were replaced every 72 hours (to prevent drying out) or when less than one sixth of the food remained (to prevent caterpillars from running out of food).

Table 1: Artificial diet recipes. Five treatments levels of different lupine seeds (and QA content) were created by changing ratios of the dry weight of ground lupine seed to the dry weight of other ingredients. The treatment names refer to this ratio. The 60% treatment contains the most lupine seed material per weight and thus the highest QA content per dry weight.

	Treatment				
	0% (Control)	**10%**	**20%**	**40%**	**60%**
Water	155 mL	155 mL	155 mL	155 mL	155 mL
Southerland Mixed Diet	27 g	24 g	21 g	15 g	9.1 g
***Lupinus tenxensis* seeds**	0 g	2.7 g	5.4 g	10.8 g	16.2 g
Agar	0 g	0.3 g	0.6 g	1.2 g	1.7 g
QA % of Dry Weight	0 %	0.64 %	1.28 %	2.56 %	3.84 %

In order to manipulate the QA content of the diets, ground *Lupinus texensis* seeds were added at different concentrations. To prevent changing the texture of the resulting artificial diet product bluebonnet seeds were ground to a fine powder using a Cyclotec Sample mill. These seeds were purchased from Eden Brothers and were organic: never treated with any chemicals or pesticides. Southerland pre-mixed *Helicoverpa zea* diet already contained appropriate agar ratios to form a solid artificial diet. Pure agar was added by dry weight to diets with lupine seeds in order to maintain appropriate consistency (firmness of the artificial diet) between different artificial diet recipes.

Growth Response to Alkaloid Content

To determine how QA levels effect corn earworm growth, groups of approximately thirty individuals were placed into treatments with artificial diets with varying QA content. Then throughout their development they were weighed to determine if their growth rates were being affected by the QA levels.

This experiment was conducted over two generations of corn earworms in order to examine data with an increased sample size. For the first generation, neonate (new born) caterpillars were placed on the control diet (0% lupine seeds) until they molted to their second instar. They were then weighed and randomly placed into one of five treatment levels: control (0%), or 60%, 40%, 20%, or 10% lupine seed by weight of dry ingredients in the artificial diet. Caterpillars were reared on the diet corresponding to their treatment until pupation and weighed 6, 9, 12, and 15 days after the reached their second instar to determine growth rate.

However, the first generation did not give us information on the effects of QA on early stage growth for the corn earworm. Bermúdes-Torres (2009) suggests that QAs have greater effect on younger caterpillars. For the second generation, eggs were collected directly from the adult flight cage, they were randomly placed into one of the five treatment levels: control (0%),

or 60%, 40%, 20%, or 10. Within 24 hours of hatching caterpillars were weighed to the nearest one-hundredth of a milligram. In each treatment, there were approximately thirty individuals which survived until pupation. Larvae were weighed on their first and third days and then every third day until they pupated (Table 2). Then final pupal weight and days from hatching to pupation were recorded.

Table 2: Weigh days for corn ear worm caterpillars in the second generation. Most entered final instar (developmental stage) and began preparing to pupate around day 15 so results beyond that day could not be compared.

X		X			X			X			X			X
Hatch	2	3	4	5	6	7	8	9	10	11	12	13	14	15

Final pupal weight, time to pupation and growth rate until last instar were compared using analysis of variance (ANOVA). Afterwards a Tukey post hoc analysis was used to identify the treatments that were statistically different. The growth rate and caterpillar mass analysis informs whether caterpillar growth is affected by QA content. Future reproductive success can be approximated using pupal weights (Gilbert 1984; Honěk 1993) so assessment of pupal weight determines whether QA content effected adult life stages or breeding of corn earworm moths.

Nutritional Indices

I used nutritional indices (Waldbauer 1968) to determine how QA content might affect quantity of food eaten or quality of food processed by corn earworms. Indices provide a mechanism to compare the ability of corn earworms' ability to digest and assimilate food and how these measures might change corn earworm feeding rate in response to QAs. To simplify, nutritional indices quantify the caterpillars' relationship to the food they feed on: the digestibility of the food, the ease with which the food can be converted to new biomass, and how much food the growing caterpillar consumes.

To calculate these indices, caterpillars were reared until molting to their forth instar (shedding the outermost layer of skin to grow quickly afterwards). In the process of molting,

caterpillars fully excrete all material in their gut (Nijhout & Williams 1974). In order to properly calculate nutritional indices, the gut must start empty so all measurement are only made with respect to food eaten since the initiation of the experiment. Once caterpillars molted into their forth instar, they were placed in fresh (5.5 cm x 4 cm x 3 cm) containers, and given food of the corresponding treatment which they had been fed on since hatching. Only individuals in the control (0%), 20% and 60% treatments were used for this experiment. In order to analyze the nutritional effect of the food according to the procedure outlined in Waldbauer 1968 (adapted by Schmidt and Resse 1985), caterpillars and their initial food were weighed. The caterpillar was then left to feed and excrete frass for 24 hours. At this point, each individual was checked to make sure it still had enough remaining food. If it had eaten more than half of the food, the caterpillar was removed to a new clean container. Its frass weight and remaining weight of food were recorded and it was given a new piece of weighed artificial diet. After the next 24 hours (totaling 48) the caterpillar, frass and remaining food were weighed and the caterpillar was placed in a fresh container without diet. The caterpillar was then left for 8 additional hours to complete its digestive cycle and excrete fully. After the 8 hours, the caterpillar and remaining frass are collected.

In order to perform the nutritional index analyses, final frass, food and caterpillars were placed in an oven for 50 °C for 48 hours. Dry weight must be used to calculate nutritional indices (Waldbauer 1968). For the caterpillars' and food initial weight which could not be dry, a wet weight to dry weight conversion factor was calculated from a separate set of fourth instar caterpillars and artificial diet samples.

Table 3: The wet to dry conversion rates found for this experiment. These were used to convert initial wet weights to usable appropriate weights. Ten caterpillars in fourth instar and three samples of each diet were used to calculate these conversion rates.

Value	Caterpillar mass	Control diet	20% artificial diet	60% artificial diet
Conversion Factor	0.183531	0.175377	0.185938	0.195138

These conversion rates were used to calculate the dry weight of the initial caterpillar and diet mass. The following equations were used to calculate nutritional indexes. All calculations use the dry weight or dry weight conversion value.

Approximate Digestibility	$AD = \frac{Ingested - Feces}{Ingested}$	(1.1)
Efficiency of Conversion of Ingested Food	$ECI = \frac{Biomass\ Gain}{Ingested}$	(1.2)
Efficiency of Conversion of Digested Food	$ECD = \frac{Biomass\ Gain}{Ingested - Feces}$	(1.3)
Relative Consumption Rate	$RCR = \frac{Ingested}{Original\ Caterpillar\ Mass * Days}$	(1.4)
Relative Growth Rate	$RGR = RCR * ECD * AD$	(1.5)

Each index was compared across treatments using an ANOVA and Tukey post-hoc analysis done in R.

Measurement of Alkaloid Content Sequestration

Generalists, are not suspected to sequester QA compounds (Fiedler 1993; Karban et al. 2010). We would therefore not expect corn earworms to sequester any of the quinolizidine alkaloids from the Texas bluebonnet seeds. To determine if or to what extent corn earworms sequester, chemically alter, or excrete alkaloid compounds, I reared 20 individuals on the highest seed treatment level, (60% lupine seed weight). All of their frass from second instar to fifth instar was collected and frozen. Then on the day they reached the last instar, caterpillars were weighed, and frozen to prepare for GC-MS analysis. Water levels, and therefore the ratio of wet to dry

mass of caterpillars vary greatly over the course of a caterpillar's development (Knerl & Bowers 2013). Thus a previous a wet-dry weight conversion rate for corn earworms was calculated by using the same method described above. Corn earworm alkaloid concentrations by dry mass can then be calculated and compared to existing data on sequestration to determine if they sequester.

To extract QAs from caterpillar sample, I followed a procedure adopted from Wink et al. 1995. Frozen larvae were further cooled in liquid nitrogen and ground with a glass pestle. Samples were then added to a 15ml test tube in addition to 2 ml of 0.5M HCl. Caterpillar frass was freeze-dried before being reconstituted in 2 ml of 0.5M HCl with approximately 2 grams of coarse sand in order to be ground. Samples where then vortexed, sonicated for ten minutes, and allowed to extract for two hours. After sonicating again, 100 ul of internal standard (0.3788 ug/ul) Sparteine and 300 ul 6M NaOH were added to the test tube. Samples were then vortexed again to distribute the base. Samples, with an additional 1 ml of NaOH to rinse, were added to 20ml EXtrelut® NT20 columns filled with Celite® diatomaceous earth (both purchased from Merck MilliporeSigma) to filter the extract. Lastly, the samples were eluted with 15 ml of dichloromethane, allowed to drain out of the EXtrelut® columns into fresh 15 ml test tubes and set to evaporate in a multi-evaporator. Dry samples were then frozen until they were aliquoted for quantification.

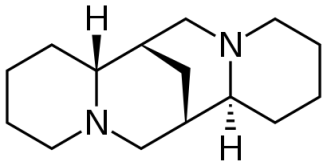


Figure 4: Sparteine alkaloid structure.

I quantified alkaloids using gas chromatography (GC). I diluted dried extracts to one third concentration (one ninth concentration for seeds and frass) with methanol and removed a 100ul aliquot. 5ul of the methanol aliquot was injected into a 6890N gas chromatograph (Agilent Technologies) with a DB-1 MS capillary column (30 m, 0.25 mm i.d., 0.10 um film thickness; Agilent J&W Scientific) coupled to a 5975 Inert mass selective detector (Agilent Technologies). Alkaloids were eluted beginning with a 3 minute hold at 120° C followed by temperature

programming from 120° to 312° C over a 10-min period, then by 3 minutes at 312° C. Alkaloids were identified by mass spectra as characterized by Wink (1995) using the Agilent Technologies NIST spectrum search. If a spectrum does not directly match one in the NIST database, the NIST spectrum search finds the most closely resembling spectrum in order to identify the compound. Alkaloids were quantified as percentage dry weight by comparison to a known percentage of sparteine added as the internal standard added to each sample.

Results

Feeding Behavior Observations

The foraging behavior of corn earworms on the bluebonnets was both surprising and erratic. Behavioral patterns varied greatly from caterpillar to caterpillar. Results from observations of twelve individual caterpillar show that a small number fed on seed pods as expected, others moved around the entire time, while some ate from other vegetative bodies on the plant (Figure 5). Out of twelve caterpillars observed, only three spent any time eating seeds or attempting to open a pod. Four spent some quantity of time eating flowers and just one chewed on several leaves before finding and eating seeds. Of the caterpillars who ate seeds, they spent on average only 14.6% of their time feeding on seeds. However, each caterpillar which ate from a seed pod ate from an average of 2.3 pods. Expanding to caterpillars who ate any plant body, typically they only ate 10.7% of the time.

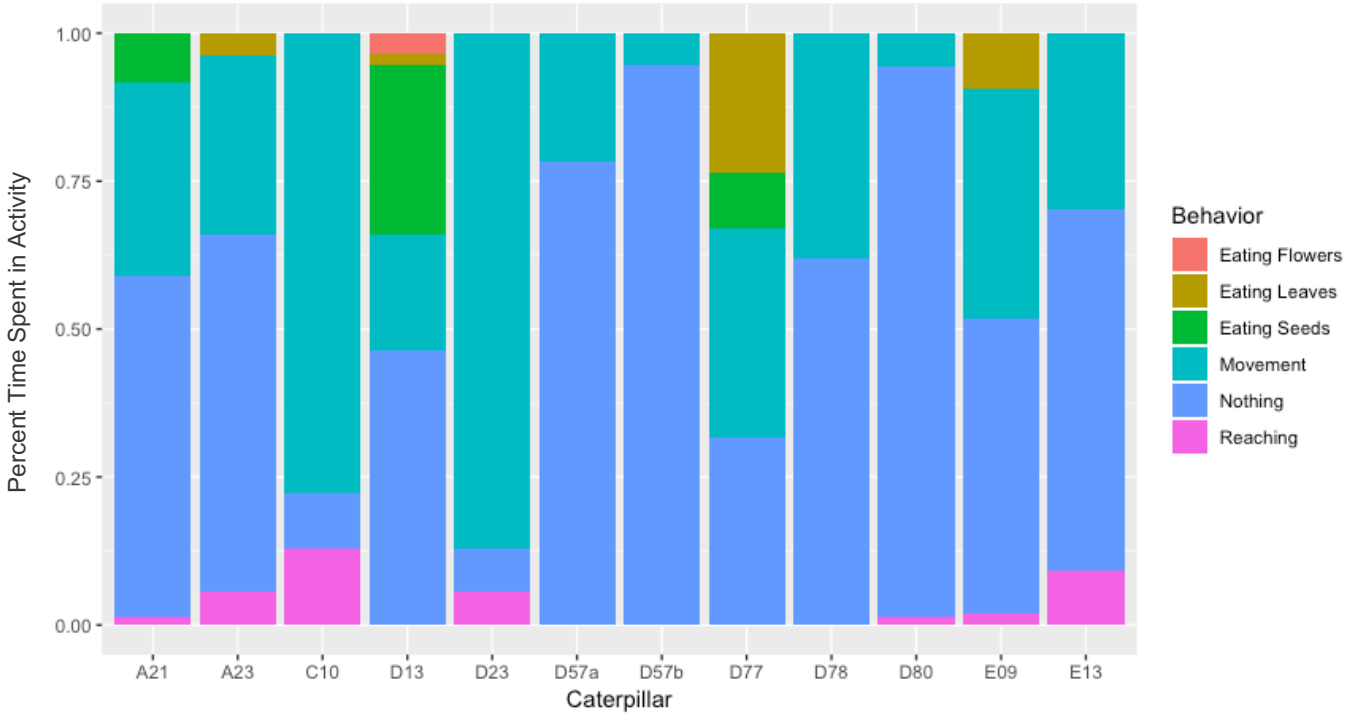


Figure 5: Behavioral results by caterpillar show percentage of caterpillar time spent doing each identifiable activity over the 4 to 7 hour observation period.

On average a caterpillar traveled 0.76 cm per minute. However, this varied widely between caterpillars and even for a single caterpillar through time (standard deviation: 0.63 cm per minute). There were certain caterpillars which almost never moved, and times, particularly during eating, when relatively "fast" caterpillars remained still for extended periods of time. In addition, I observed a behavior dubbed "reaching" in seven of the twelve caterpillars. Reaching describes the behavior of extending their full body above and beyond their current perch, only holding on with their anal prolegs (unsegmented false legs located on the last abdominal segment). The reaching caterpillar would then wave their head back and forth, possibly a behavioral display or potentially searching for a new hold or plant structure to grasp.

Caterpillar Growth in Response to Alkaloid Level

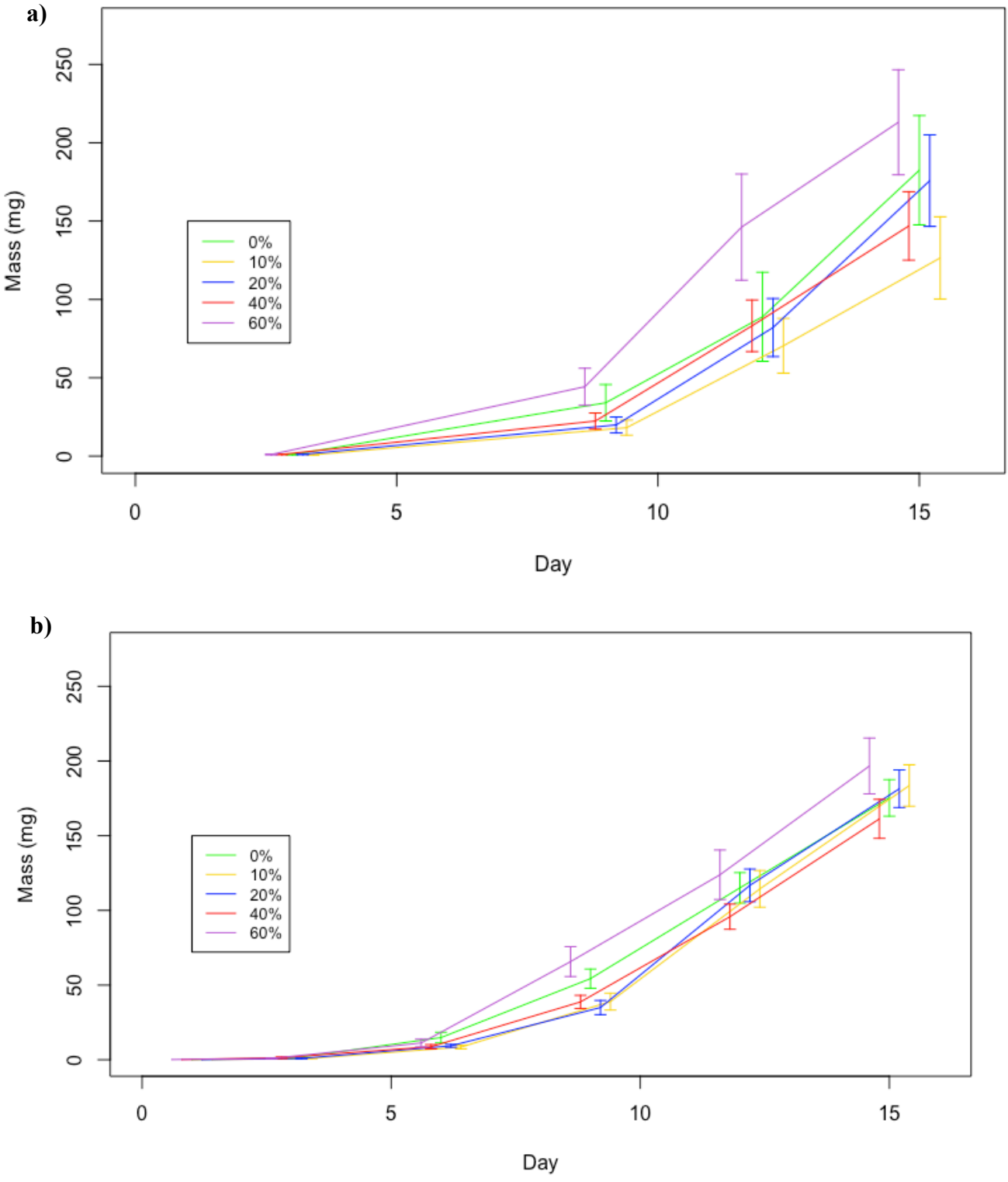
I discovered, on the whole, that the levels of seed material (and thus QA) in caterpillar diet did not affect corn earworm growth rate or mass throughout their full development time (Figure 6 and Table 4). This effect was studied in two separate generations of caterpillars and both yielded similar results. Caterpillar growth was only measured starting from second instar in Generation 1, while caterpillars where measured within 24 hours of hatching for Generation 2 to see if there was any impact on first instar development.

Table 4: ANOVA statistical values for caterpillar mass through development. Tukey Post Hoc analysis was done for those p values which were less than an alpha level of 0.05.

	Generation 1		**Generation 2**		**Tukey Post Hoc Analysis**
	F Stat	**P value**	**F Stat**	**P value**	
Hatch (Day 1)	-	-	3.6407	**0.0074**	10% was larger than 0% treatment (Gen. 2)
Day 3	0.3361	0.8528	0.7454	0.5626	
Day 6	-	-	1.6721	0.1597	
Day 9	1.8511	0.1283	4.215	**0.0030**	60% was larger than 10%, 20% & 40% (Gen. 2)
Day 12	1.5626	0.1934	0.7689	0.5472	
Day 15	1.3605	0.2562	0.7913	0.5327	

Caterpillar growth is not independent day to day, nor is it linear (Gotthard 2008). To examine caterpillar growth as a rate I divided date ranges which represent approximate linear

Figure 6: The mass of corn earworm caterpillars through time. In order to view the error bars, values are plotted slightly offset. For statistical analyses, all real values were used. a) Generation 1 data begins on day three as caterpillars typically reach second instar by their third day after hatching. b) Generation 2. Both generations depict the slightly faster growth of caterpillars in the 60% seed treatment though this difference is not statistically significant through their development. See **Table 4** for all ANOVA statistical values.



growth. I compared the semi-linear growth from hatch day until day six as well as mass growth per day during the also near linear period from day six to day fifteen. Across treatments caterpillars grew approximately 2.04 mg per day during early stages (until day six) and, on average, 178.17 mg per day in the later stages. ANOVA on both of these sets of rates yielded insignificant p values. For early development until day six, $p = 0.1619$ ($F = 1.6630$), and for late stage development after day six, $p = 0.5305$ ($F = 0.7946$). These values represent the mean slopes seen on Figure 7b.

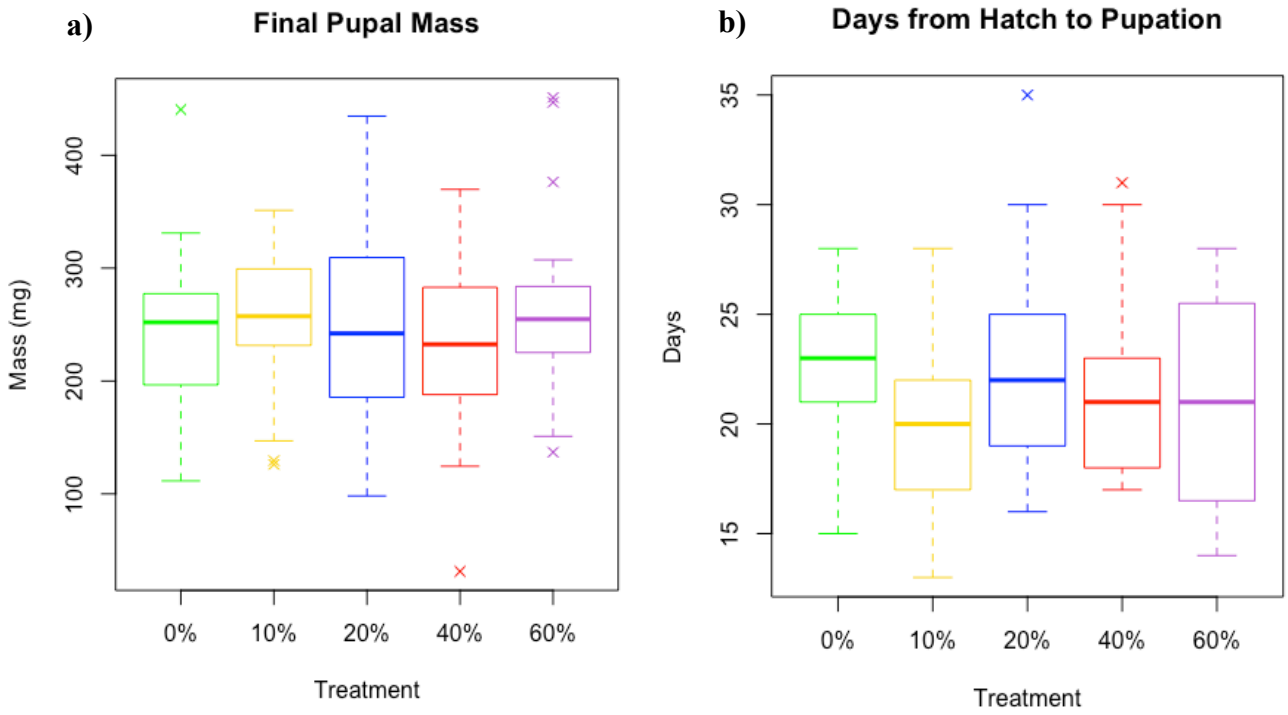


Figure 7: The final pupal mass and days from hatching to pupation from generation two. Though outliers are depicted in box and whisker blots, they were still included in ANOVA.

At the end of caterpillar growth, final pupal mass was measured but also yielded no significant difference between treatments (Figure 7a; $p = 0.5546$; $F = 0.7577$). However, time to pupation – typically around 23 days – was found to have significant differences (Figure 7b; $p =$

0.0262; F = 2.805). The Tukey Post Hoc analysis revealed that this significance was only between treatments levels 0% and 10%, meaning that this difference holds little weight biologically.

Nutritional Indices

I found significant differences in the AD, ECI and RCR indices among treatments. The Approximate Digestibility estimates the percentage of the dry diet mass that was actually consumed by the caterpillar. Many parts of the plant remain indigestible to insect herbivores and are excreted rather than digested. In the case of this study, the QA portion of the artificial diet was found to be less digestible. Using an ANOVA and a Tukey post hoc analysis, I found that while the AD of both the 20% and 0% treatments were statistically the same, the 60% treatment was less digestible (see Table 5 for F statistic and p value and Figure 8 for relationships between the means). This means that those caterpillars in the 60% treatment produced more frass per gram of diet, likely because they were excreting a larger mass of indigestible QAs.

Table 5: ANOVA statistical values for nutritional indices. Tukey Post Hoc analysis was done for those p values which were less than an alpha level of 0.05.

	F Stat	P value	Tukey Post Hoc Analysis
AD	8.3351	**0.0007**	60% was less than both other treatments
ECI	6.765	**0.0024**	60% was less than 0%
ECD	1.1687	0.3185	
RCR	3.5785	**0.0343**	0% is less than both other treatments
RGR	0.4591	0.6342	

The Efficiency of Conversion of Ingested Food describes how efficiently ingested food is digested. I found that the 60% treatment had a significantly lower conversion of ingestion to digestion than the 0% treatment. This implies that the caterpillars in the 60% treatment gained less body mass per gram of food ingested. On the other hand, ECD was not significantly different between treatments, though ECD was still lowest for the 60% treatment. The Efficiency

of Conversion of Digested Food takes into account the AD of the food to determine the ratio of digested food convergence to biomass.

The Relative Consumption Rate calculates how much food a caterpillar consumes relative to its initial body mass per day. This experiment determined that the 0% treatment caterpillars ate less food mass per day than the treatments including QAs. Given what is known about growth rate consistency across treatments, this implies that caterpillars in the 0% treatment were able to eat less food per day while still growing at the same rate as their cohorts in higher QA treatments. Relative Growth Rate describes the growth per day relative to caterpillar size.

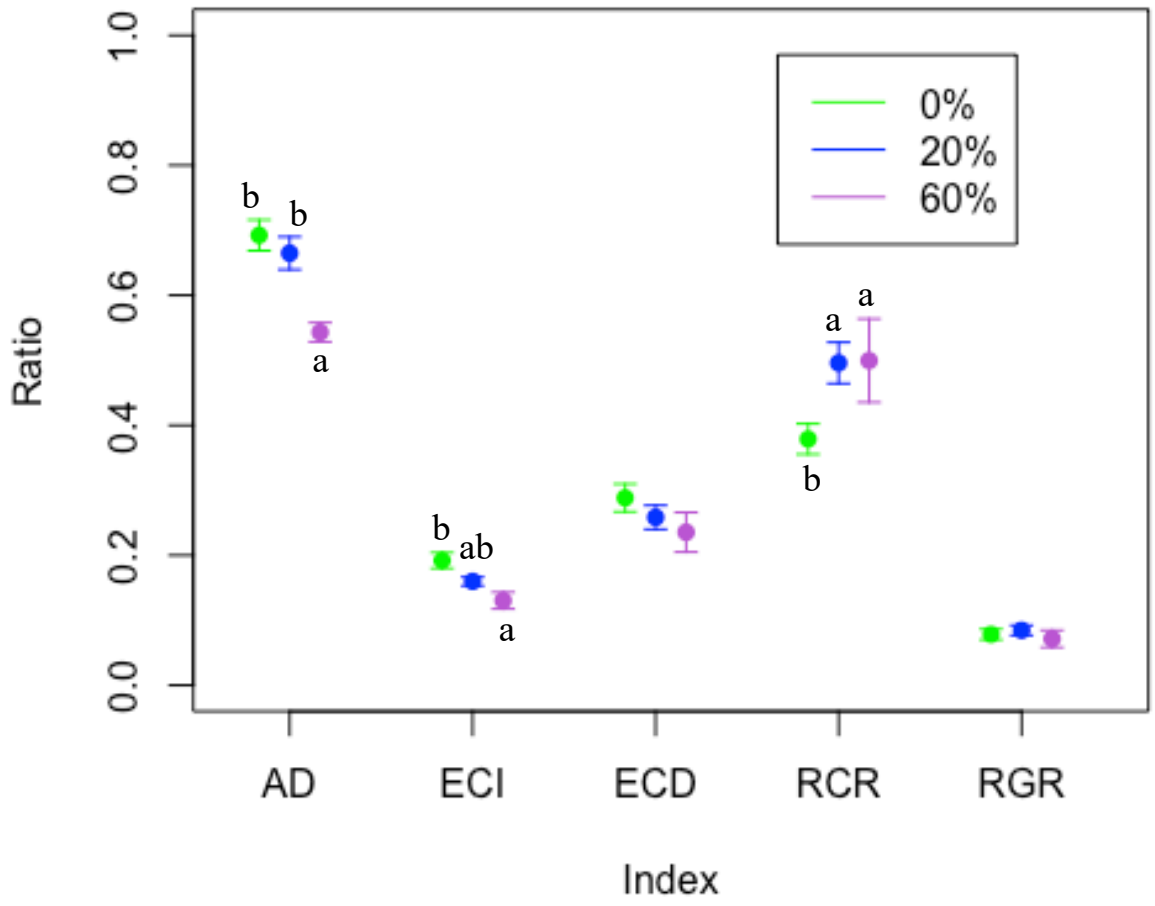


Figure 8: Means and standard errors for each of the five nutritional indices. Letters by points indicate those points are significantly different from values with different letter for that nutritional index (Table 5). Values without letters exhibit no statistical difference from the Tukey post hoc test.

The result that all treatments have statistically the same RGR is also consistent with our data from the growth rate experiment (there is no difference in growth rate between treatments).

Mass Spectrometer Gas Chromatograph

Quinolizidine alkaloids in corn earworms and frass were reported as percentage of dry weight to account for the variability in water content in caterpillars and frass. Content of QAs in caterpillars was low compared to the content of the seeds and frass (Figure 9). Content of both frass and seeds was significantly higher than that of caterpillars ($p > 0.001$; $F = 365.92$). Seeds also contained a significantly higher QA content than frass based on a Tukey post hoc analysis done on the results of the statistically significant ANOVA.

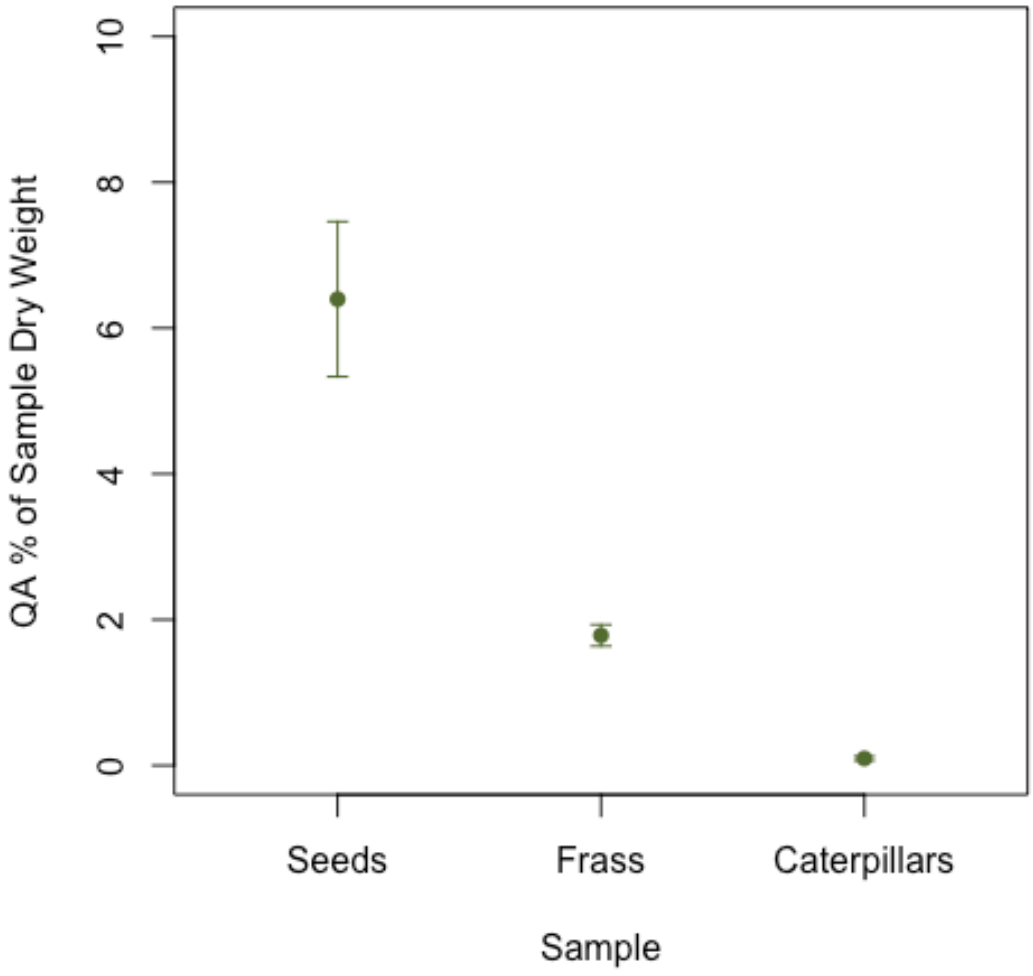


Figure 9: Means and standard errors of QA % of dry weight for each sample type**.** All three values are statistically significantly different.

Table 6: QA compounds found in samples, ordered by retention time. Different unknown QAs are indicated by Unknown A, Unknown B, etc. Bolded QAs were identified with high confidence.

Seeds	**Caterpillars**	**Frass**
Unknown A		
Unknown B		Unknown B
	17 Oxosparteine	
		Unknown C
α Isolupanine	**α Isolupanine**	**α Isolupanine**
Unknown D		Unknown D
Unknown E		
Lupanine	**Lupanine**	**Lupanine**
Unknown F		Unknown F
Unknown G-I		
Unknown J		Unknown J
Unknown K-N		
Unknown O-R		Unknown O-R
Unknown S		
Unknown T-U		Unknown T-U
Unknown V-AD		
Unknown AE		Unknown AE
Unknown AF		

A variety of Lupanine compounds were found between the three sample types. Caterpillars also contained 17-Oxosparteine, another alkaloid. The varieties of lupanine so closely resemble each other in spectra that misidentification between lupanine varieties is quite possible. Therefore we cannot draw any conclusions regarding different QA varieties and they have been qualified as Unknown in Table 6. Thirty-three total varieties of QA compounds were found in the seeds, fifteen were found in the frass, and only three were found in the caterpillars.

Discussion

This study assessed corn earworm feeding behavior and how QAs impact their performance and digestion. The main results of this study are i) corn earworms exhibit varied and erratic feeding behavior; ii) corn earworm growth and reproductive potential are not impacted by QA content in their diet; iii) in order to compensate for less digestible food, corn earworms consume greater quantities of food to maintain a consistent growth rate; and iv) corn earworms excrete plant toxins in frass. Together these results help to build a more complete understanding of how corn earworms feed and grow on lupines in nature.

The results of the behavioral experiment showed that caterpillars spent 31% of their time actively moving around the plant. 54% of the time caterpillars were still, and between all the caterpillars, they spent a mere 7% of their time eating. Those caterpillars who did feed commonly fed on more than one pod or leaf, potentially indicating a natural tendency to exhibit dispersed feeding behavior. The seed eaters fed, on average, on seeds from 2.3 pods. While dispersed seed-eaters vary widely in size and feeding range, feeding on more than on leaf or plant body without consuming the whole is generally considered to be dispersed feeding behavior (Heinrich 1979; Mauricio et al. 1990). Mauricio et al. (1990) also found that theoretically dispersed feeders traveled a distance more than ten times their body length during a seven hour day. Given the travel rate of 0.76 cm per minute found for this experiment and corn earworm late instar body length of approximately 20 mm, they have the potential to travel a distance over 150 times their body length over the course of a seven hour day. Though the results of the preliminary behavioral observations were inconclusive, both of these pieces of evidence suggest that corn earworms may be dispersed feeders. Future experiments should continue to study their feeding behavior to determine if this movement over large distances is due to avoidance of chemical induction, predators, or a response to nutrient availability.

The corn ear worm caterpillars in this experiment may have exhibited less feeding behavior that expected because they were reared on artificial diet prior to this experiment. The combination of experiencing the novel structure of wild lupine pods and presence of QAs in food could have discourage feeding behavior. Caterpillars for the behavioral experiments were all reared on the control Southland diet rather than QA-treated diets. Therefore, it is possible that olfactory or gustatory signals of toxicity from QA (Kaissling 1987) were sufficient deterrents to corn earworm feeding. Though it could have been confounded by caterpillar feeding confusion, it was unexpected to see corn earworms feed on leaves at all. Future research could be aimed at quantifying the potential for corn earworms (both captive and wild) to eat leaves. Corn earworms are generally considered to have reproductive-body feeding preference but to my knowledge there are no studies on how likely they are to eat other bodies on the plant, especially when undergoing stress or feeding confusion.

In order to examine caterpillar response to feeding on QA-food, I examined their growth in response to the addition of QA-containing *L. texensis* seed material in artificial diet. Even in instances of a six fold increases in QA content by dry weight in artificial diet, there was no statistically significant change to caterpillar growth or development. I found that between treatments there were no differences in final caterpillar size, linear growth rates or final pupal mass. This implies that there were no lasting biological effects on caterpillar performance from increased QAs in diet. On two of the dates that I measured caterpillar mass in generation 2, there were significant differences among treatment groups (Table 4). On the hatch date for generation two, the randomly assignment 10% treatment neonates were more massive that the neonates (newly hatched caterpillars) assigned to the 0% treatment. Because eggs were randomly assigned to treatments before hatching, this could not have been avoided. However, as the neonate masses from all treatments range only from 0.053 mg to 0.216 mg, and the gap between the two

treatments was closed by day three, this difference was considered biologically insignificant. Additionally, though neonates were measured within 24 hours of hatching all caterpillars were not measured at exactly the same number of hours after hatching. It is possible that variation in these early measurements can be attributed to how quickly after hatching the neonates were weighed. At day nine for generation 2, the mass of the 60% treatment group was significantly higher than the other groups (Table 4). As this gap between groups was closed by the next measurement, this was not considered to be indicative of biologically important differences in growth rates.

This conclusion was also supported by the lack of significant differences in pupal weights among treatment groups. Future reproductive success can be approximated using pupal weights (Gilbert 1984; Honěk 1993) so a lack of difference in pupal weight between treatments reinforces that the differences in mass at day nine are not meaningful. I found that the 0% treatment caterpillars took significantly longer to pupate than the 10% treatment, by 3 days. Considering the generally staggered pupal emergence and breeding time observed in lab populations (Park et al. 1998; Hartung observations 2018), this short difference in time could affect the moth's breeding capacity in the wild. However, since this difference was only apparent between the two lowest QA-content treatments (Table 4), this does not indicate that QA content in diet affected corn earworm breeding development. The culmination of the pupal results, mass measurements throughout the experiment, and estimates of growth rates all concur that corn earworm development was not significantly affected by increased QA content in their diet.

The combination of the estimates of growth rates, time to pupation, and pupal mass results support that corn earworm caterpillar development was not significantly affected by increased QA content in their diet. This is surprising given that Johnson and Bentley (1988) found that concentrations even as low as 0.02 % dry weight of QAs could have negative impacts

on the generalist caterpillar *Spodoptera eridania* (while I used up to 3.84% dry weight: Table 1). Corn earworm's ability to tolerate such high levels of QAs without reductions in performance could be attributed to their general hardiness (Robinson 2002; Sandstrom et al. 2007; Leite 2014). In the wild, corn earworms are also able to contest with human insecticides (Tabashnik et al. 2008; Welch et al. 2015).

Not only did the 60% seed meal treatment not result in reduced larval performance, but in both generations the peak caterpillar mass averages were seen in the 60% treatment throughout their growth. Though there was no biologically significant difference, due to high growth variation within treatments, the fact that this was seen in both independent generations could imply that there is some benefit to the consumption of high levels of QA diet. A possible explanation for this trend is that the diets were built with QA-containing seeds, not straight QA extracts. Bluebonnet seeds are a natural food source for corn earworms and thus contain the high nutrient values associated with seeds (Stephens & Krebs 1986). Perhaps at a certain density of lupine seeds, this additional nutrient quantity overrides the detriment of additional exposure to secondary compounds. Future experimental designs could be done with high treatment ratios of lupine seed meal to other dry ingredients to determine if this trend continues with addition of more bluebonnet material.

The nutritional indices experiment began to answer these questions by quantifying how the corn earworms digest the artificial diets and lupine material to gain new biomass. Caterpillars in the 60% seed meal group had significantly lower measures of Efficiency of Conversion of Ingested Food (ECI) and Approximate Digestibility (AD) indices (Table 5). As values of Efficiency of Conversion of Digested Food (ECD) are approximately equal across treatments, it is likely that once caterpillars have excreted the undigestible material, the treatments have the same relative success converting digested material into new biomass. Thus, the addition of QA-

containing seed material lowered the digestibility of the diet and that is driving the lowered ECI. However, the nutritional indices indicate that caterpillars in the 20% and 60% treatment groups were eating a significantly larger mass of food per day than the control group (Relative Consumption Rate in Table 5). It appears that the caterpillars in these treatment were eating more in order to compensate for lower digestibility per gram of diet. These indices together imply that the lower digestibility encouraged caterpillars to eat a greater mass of food over all. This may imply that each caterpillar was not eating the maximum quantity of food possible, but each treatment was regulating intake in order to grow at an appropriate rate (uniform across treatments). This indicates the potential for self-regulation in corn earworm caterpillars. Self-regulation has been shown to not only occur in response to toxins but also to self-select nutrient mixtures which best support their growth (Waldbauer & Friedman 1991; Lee et al. 2002; Deans et al. 2015). This sort of regulation and nutrient diversification may be observed in caterpillars as dispersed feeding. In order to perform self-regulatory feeding, caterpillars may move across a single plant or even between different species of plant (Dethier 1988). It is therefore possible that wild corn earworm caterpillars feed dispersedly on different parts of the plant in order to regulate their intake based on AD and ECI of the plant material in a similar way.

The observation of high mass in the 60% treatment could also potentially be explained by the results of the nutritional indices. These calculations made it clear that the 60% treatment caterpillars were consuming more food per day than their counterparts. Though this did not produce significant growth rate differences between treatments, this faster consumption could have contributed to faster growth. A tertiary explanation for this result is a combination of both prior explanations. Even if consuming higher quantities of food did not substantially increase corn earworm caterpillar mass because it was needed to counteract the low digestibility of the diet, it may have fostered increased nutrient intake by high lupine-level treatments. This could be

considered an alternative form of self-regulating their diet. Corn earworms have been shown to regulate their intake of carbohydrates and protein (Deans et al. 2015), and this study may indicate they also alter their behavior to regulate secondary metabolite consumption. Future experiments which could monitor the simultaneous effects of nutrient regulation and QA regulation on caterpillar behavior.

Quinolizidine Alkaloid extraction analysis revealed that the total QA concentration varied between the seeds, caterpillars and their frass (Figure 9). Seeds contained the highest quantities of QAs, and frass the second most. On average, the caterpillars' bodies contained less than 5% of the QA content of their respective frass. These data indicate that the majority of ingested QAs are excreted in corn earworm frass. The remaining QAs' presence could be explained by remaining QA-rich food in the gut rather than sequestered chemicals in other caterpillar organs. Many other species of polyphagous caterpillars are also known to eliminate QAs in frass rather than maintaining toxins in the body (Montllor et al. 1990; Wink 1992; Fiedler 1993; Karban et al. 2010). Montllor et al. (1990) posits that a QA-sequestering specialist caterpillar may contain approximately 6.8 ug of quinolizidine alkaloids per mg (0.68 % per dry weight) in late instars. The corn earworms presented an average of 0.09 % per dry weight. With this information, it can be fairly concluded that these corn earworms did not sequester QA compounds and that the fate of ingested QAs is to be excreted in the frass.

Using the NIST spectrum search, only α-isolupanine, lupanine and 17-oxosparteine could be specifically identified. In Table 6 QA compounds identified without high confidence were labeled as unknown. The seeds contained the greatest variety of QA compounds (31 unknown compounds). However, as the QAs in the seeds were significantly more concentrated than either the frass or the caterpillars, it is possible that all samples contained these compounds but were too faint to detect in caterpillar and frass samples. All unknown compounds found in the frass

were also found in the seeds except for Unknown C. If this compound is unique to the frass, that could indicate that the caterpillars may have partially broken down one of the seed compounds the into alternative QA compounds. Further extractions and access to a more specific alkaloid database will be necessary in order to fully identify these compounds and understand the possible breakdown of these alkaloids in the corn earworms' gut.

In combination with the growth response and nutritional indices, these results suggest that corn earworms are adept at processing QAs in their food and may self-regulate consumption in order to grow at an appropriate rate. These results also indicated that as a generalist, corn earworms are able to pass increased amounts of quinolizidine alkaloids without the secondary metabolites affecting their growth.

Further understanding of corn ear worm feeding behaviors is significant to the field of ecology due to the large number of native plant hosts, but it is also significant to the field of agriculture as corn earworms cause over one hundred million dollars in crop damage annually (Capinera 2001). Additionally, in Brazil, corn earworms have recently been found hybridizing with *Helicoverpa armigera* – the cotton bollworm – to produce a hybrid with even more agriculturally destructive potential (Leite 2014; Kriticos 2015; Anderson 2018). With global trends restricting agriculture into corn monoculture and the expanded range and diet of the cotton bollworm / corn ear worm hybrid, it is projected that more than 70% of United States' crops are at risk to herbivory by the Helicoverpa species (Anderson 2018). The success of corn earworms during their southern migration months, when they feed on lupines and other native plants will dictate how effective future generations are against American crops and pesticides. Learning how to manage corn earworms on native plants as well as growing our understanding of how they process toxins will contribute to agricultural management techniques for this extremely destructive species.

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References Cited

Agrawal, A.A., 2000. Specificity of induced resistance in wild radish: Causes and consequences for two specialist and two generalist caterpillars. *Oikos*, *89*(3), pp.493-500.

Ali, J.G. and Agrawal, A.A., 2012. Specialist versus generalist insect herbivores and plant defense. *Trends in Plant Science*, *17*(5), pp.293-302.

Anderson, C.J., Oakeshott, J.G., Tay, W.T., Gordon, K.H., Zwick, A. and Walsh, T.K., 2018. Hybridization and gene flow in the mega-pest lineage of moth, Helicoverpa. *Proceedings of the National Academy of Sciences*, *115*(19), pp.5034-5039.

Balandrin, M.F., Klocke, J.A., Wurtele, E.S. and Bollinger, W.H., 1985. Natural plant chemicals: Sources of industrial and medicinal materials. *Science*, *228*(4704), pp.1154-1160.

Bermúdez-Torres, K., Herrera, J.M., Brito, R.F., Wink, M. and Legal, L., 2009. Activity of quinolizidine alkaloids from three Mexican Lupinus against the lepidopteran crop pest *Spodoptera frugiperda*. *BioControl*, *54*(3), pp.459-466.

Bernays, E. and Graham, M., 1988. On the evolution of host specificity in phytophagous arthropods. *Ecology*, *69*(4), pp.886-892.

Blanco, C.A., Terán-Vargas, A.P., López, J.D., Kauffman, J.V. and Wei, X., 2007. Densities of *Heliothis virescens* and *Helicoverpa zea* (Lepidoptera: Noctuidae) in three plant hosts. *Florida Entomologist*, *90*(4), pp.742-751.

Capinera, J., 2001. *Handbook of vegetable pests*. Elsevier.

Deans, C.A., Sword, G.A. and Behmer, S.T., 2015. Revisiting macronutrient regulation in the polyphagous herbivore *Helicoverpa zea* (Lepidoptera: Noctuidae): New insights via nutritional geometry. *Journal of insect physiology*, *81*, pp.21-27.

Dethier, V.G., 1988. The feeding behavior of a polyphagous caterpillar (*Diacrisia virginica*) in its natural habitat. *Canadian Journal of Zoology*, *66*(6), pp.1280-1288.

Dobler, S., Petschenka, G. and Pankoke, H., 2011. Coping with toxic plant compounds–the insect's perspective on iridoid glycosides and cardenolides. *Phytochemistry*, *72*(13), pp.1593-1604.

Eger Jr, J.E., Sterling, W.L. and Hartstack Jr, A.W., 1982. Population dynamics of *Heliothis spp*. on *Castilleja indivisa*, an unreported host plant, and *Lupinus texensis* in Texas. *Environmental Entomology*, *11*(2), pp.327-333.

Fiedler, K., Krug, E. and Proksch, P., 1993. Complete elimination of hostplant quinolizidine alkaloids by larvae of a polyphagous lycaenid butterfly, *Callophrys rubi*. *Oecologia*, *94*(3), pp.441-445.

Fordyce, J.A. and Agrawal, A.A., 2001. The role of plant trichomes and caterpillar group size on growth and defense of the pipevine swallowtail *Battus philenor*. *Journal of Animal Ecology*, *70*(6), pp.997-1005.

Forister, M.L., Novotny, V., Panorska, A.K., Baje, L., Basset, Y., Butterill, P.T., Cizek, L., Coley, P.D., Dem, F., Diniz, I.R. and Drozd, P., 2015. The global distribution of diet breadth in insect herbivores. *Proceedings of the National Academy of Sciences*, *112*(2), pp.442-447.

Forister, M.L., Scholl, C.F., Jahner, J.P., Wilson, J.S., Fordyce, J.A., Gompert, Z., Narala, D.R., Buerkle, C.A. and Nice, C.C., 2013. Specificity, rank preference, and the colonization of a non-native host plant by the Melissa blue butterfly. *Oecologia*, *172*(1), pp.177-188.

Fry, J.D., 1996. The evolution of host specialization: are trade-offs overrated?. *The American Naturalist*, *148*, pp.S84-S107.

Futuyma, D.J. and Moreno, G., 1988. The evolution of ecological specialization. *Annual Review of Ecology and Systematics*, *19*(1), pp.207-233.

Gilbert, N., 1984. Control of fecundity in *Pieris rapae*: I. The problem. *The Journal of Animal Ecology*, pp.581-588.

Gotthard, K., 2008. Adaptive growth decisions in butterflies. *Bioscience*, *58*(3), pp.222-230.

Harborne, J.B., 2007. Role of secondary metabolites in chemical defense mechanisms in plants. In *Ciba Foundation Symposium 154-Bioactive Compounds from Plants: Bioactive Compounds from Plants: Ciba Foundation Symposium 154*. Chichester, UK: John Wiley & Sons, Ltd. pp. 126-139.

Hartmann, T., 2004. Plant-derived secondary metabolites as defensive chemicals in herbivorous insects: A case study in chemical ecology. *Planta*, *219*(1), pp.1-4.

Heinrich, B., 1979. Foraging strategies of caterpillars. *Oecologia*, *42*(3), pp.325-337.

Hoang, T.C., Pryor, R.L., Rand, G.M. and Frakes, R.A., 2011. Use of butterflies as nontarget insect test species and the acute toxicity and hazard of mosquito control insecticides. *Environmental Toxicology and Chemistry*, *30*(4), pp.997-1005.

Honěk, A., 1993. Intraspecific variation in body size and fecundity in insects: a general relationship. *Oikos*, pp.483-492.

Kaissling, R.H., 1987. Wright Lectures on Insect Olfaction Simon Fraser University Burnaby.

Knerl, A. and Bowers, M.D., 2013. Incorporation of an introduced weed into the diet of a native butterfly: consequences for preference, performance and chemical defense. *Journal of Chemical Ecology*, *39*(10), pp.1313-1321.

Johnson, N.D. and Bentley, B.L., 1988. Effects of dietary protein and lupine alkaloids on growth and survivorship of *Spodoptera eridania*. *Journal of Chemical Ecology*, *14*(5), pp.1391-1403.

Karban, R., Karban, C., Huntzinger, M., Pearse, I.A.N. and Crutsinger, G., 2010. Diet mixing enhances the performance of a generalist caterpillar, *Platyprepia virginalis*. *Ecological Entomology*, *35*(1), pp.92-99.

Kriticos, D.J., Ota, N., Hutchison, W.D., Beddow, J., Walsh, T., Tay, W.T., Borchert, D.M., Paula-Moreas, S.V., Czepak, C. and Zalucki, M.P., 2015. The potential distribution of invading *Helicoverpa armigera* in North America: is it just a matter of time?. *PLoS One*, *10*(3), p.e0119618.

Lampert, E.C. and Bowers, M.D., 2010. Host plant influences on iridoid glycoside sequestration of generalist and specialist caterpillars. *Journal of Chemical Ecology*, *36*(10), pp.1101-1104.

Lawton, J.H. and Schröder, D., 1978, August. Some observations on the structure of phytophagous insect communities: the implications for biological control. In *Proc. IV Internat. Symp. Biol. Control of Weeds. Inst. Food Agr. Sci., Univ. Fla, Gainesville* (pp. 57-73).

Leite, N.A., Alves-Pereira, A., Corrêa, A.S., Zucchi, M.I. and Omoto, C., 2014. Demographics and genetic variability of the new world bollworm (*Helicoverpa zea*) and the old world bollworm (*Helicoverpa armigera*) in Brazil. *PloS one*, *9*(11), p.e113286.

Levin, D.A., 1975. Pest pressure and recombination systems in plants. *The American Naturalist*, *109*(968), pp.437-451.

Mauricio, R. and Bowers, M.D., 1990. Do caterpillars disperse their damage?: Larval foraging behaviour of two specialist herbivores, *Euphydryas phaeton* (Nymphalidae) and *Pieris rapae* (Pieridae). *Ecological Entomology*, *15*(2), pp.153-161.

Montllor, C.B., Bernays, E.A. and Barbehenn, R.V., 1990. Importance of quinolizidine alkaloids in the relationship between larvae of *Uresiphita reversalis* (Lepidoptera: Pyralidae) and a host plant, *Genista monspessulana*. *Journal of Chemical Ecology*, *16*(6), pp.1853-1865.

Montllor, C.B., Bernays, E.A. and Cornelius, M.L., 1991. Responses of two hymenopteran predators to surface chemistry of their prey: significance for an alkaloid-sequestering caterpillar. *Journal of Chemical Ecology*, *17*(2), pp.391-399.

Morais, H.C., Sujii, E.R., Almeida-Neto, M., De-Carvalho, P.S., Hay, J.D. and Diniz, I.R., 2011. Host plant specialization and species turnover of caterpillars among hosts in the Brazilian Cerrado. *Biotropica*, *43*(4), pp.467-472.

Nickisch-Rosenegk, E., Schneider, D. and Wink, M., 1990. Time-course of pyrrolizidine alkaloid processing in the alkaloid exploiting arctiid moth, *Creatonotos transiens*. *Zeitschrift für Naturforschung: A Journal of Biosciences*, *45*(7-8), pp.881-894.

Nijhout, H.F. and Williams, C.M., 1974. Control of moulting and metamorphosis in the tobacco hornworm, *Manduca sexta* (L.): Growth of the last-instar larva and the decision to pupate. *Journal of Experimental Biology*, *61*(2), pp.481-491.).

Nishida, R., 2002. Sequestration of defensive substances from plants by Lepidoptera. *Annual Review of Entomology*, *47*(1), pp.57-92.

Park, Y.I., Shu, S., Ramaswamy, S.B. and Srinivasan, A., 1998. Mating in *Heliothis virescens*: transfer of juvenile hormone during copulation by male to female and stimulation of biosynthesis of endogenous juvenile hormone. *Archives of Insect Biochemistry and Physiology: Published in Collaboration with the Entomological Society of America*, *38*(2), pp.100-107.

Rhoades, D.F. and Cates, R.G., 1976. Toward a general theory of plant antiherbivore chemistry. In *Biochemical Interaction between Plants and Insects*. Springer, Boston, MA. pp.168-213.

Robinson, G.S., 2002. *Hostplants of the moth and butterfly caterpillars of America north of Mexico*. American Entomological Institute.

Rosenthal, G.A. and Berenbaum, M.R., 2012. *Herbivores: their interactions with secondary plant metabolites: ecological and evolutionary processes* (Vol. 2). Academic Press.

Sandstrom, M.A., Changnon, D. and Flood, B.R., 2007. Improving our understanding of *Helicoverpa zea* migration in the Midwest: Assessment of source populations. *Plant Health Progress*, *8*(1), p.63.

Schmidt, D.J. and Reese, J.C., 1986. Sources of error in nutritional index studies of insects on artificial diet. *Journal of Insect Physiology*, *32*(3), pp.193-198.

Schneider, D., 1987. The strange fate of pyrrolizidine alkaloids. In *Perspectives in chemoreception and behavior*. Springer, New York, NY. pp. 123-142

Schoonhoven, L.M., Van Loon, B., van Loon, J.J. and Dicke, M., 2005. *Insect-plant Biology*. Oxford University Press on Demand.

Smilanich, A.M., Dyer, L.A., Chambers, J.Q. and Bowers, M.D., 2009. Immunological cost of chemical defence and the evolution of herbivore diet breadth. *Ecology Letters*, *12*(7), pp.612-621.

Stamp, N.E. and Bowers, M.D., 1990. Phenology of nutritional differences between new and mature leaves and its effect on caterpillar growth. *Ecological Entomology*, *15*(4), pp.447-454.

Stamp, N.E. and Bowers, M.D., 1992. Foraging behavior of specialist and generalist caterpillars on plantain (*Plantago lanceolata*) altered by predatory stinkbugs. *Oecologia*, *92*(4), pp.596-602.

Stephens, D.W. and Krebs, J.R., 1986. *Foraging theory*. Princeton University Press.

Sudbrink Jr, D.L. and Grant, J.F., 1995. Wild host plants of *Helicoverpa zea* and *Heliothis virescens* (Lepidoptera: Noctuidae) in eastern Tennessee. *Environmental Entomology*, *24*(5), pp.1080-1085.

Tabashnik, B.E., Gassmann, A.J., Crowder, D.W. and Carrière, Y., 2008. Insect resistance to Bt crops: Evidence versus theory. *Nature Biotechnology*, *26*(2), p.199.

United States Department of Agriculture, 2016. *International Production Assessment Division: Crop Production Maps*, USDA Foreign Agricultural Service, viewed 18 March 2019, < https://ipad.fas.usda.gov/rssiws/al/global_cropprod.aspx>

Waldbauer, G.P., 1968. The consumption and utilization of food by insects. In *Advances in Insect Physiology* (Vol. 5, pp. 229-288). Academic Press.

Waldbauer, G.P. and Friedman, S., 1991. Self-selection of optimal diets by insects. *Annual Review of Entomology*, *36*(1), pp.43-63.

Waller, G.R. and Nowacki, E.K., 1978. Metabolic (catabolic) modifications of alkaloids by plants. In *Alkaloid Biology and Metabolism in Plants*. Springer, Boston, MA. pp.183-249.

Welch, K.L., Unnithan, G.C., Degain, B.A., Wei, J., Zhang, J., Li, X., Tabashnik, B.E. and Carrière, Y., 2015. Cross-resistance to toxins used in pyramided Bt crops and resistance to Bt sprays in *Helicoverpa zea*. *Journal of Invertebrate Pathology*, *132*, pp.149-156.

Wink, M., 1992. The role of quinolizidine alkaloids in plant-insect interactions. In *Insect-Plant Interactions.* CRC Press. pp. 139-174.

Wink, M., 1993. Quinolizidine alkaloids. *Methods in Plant Biochemistry*, *8*, pp.197.

Wink, M. and Hartmann, T., 1979. Cadaverine-pyruvate transamination: the principal step of enzymatic quinolizidine alkaloid biosynthesis in *Lupinus polyphyllus* cell suspension cultures. *FEBS Letters*, *101*(2), pp.343-346.

Wink, M., Meißner, C. and Witte, L., 1995. Patterns of quinolizidine alkaloids in 56 species of the genus Lupinus. *Phytochemistry*, *38*(1), pp.139-153.

Wink, M. and Schneider, D., 1990. Fate of plant-derived secondary metabolites in three moth species (*Syntomis mogadorensis*, *Syntomeida epilais*, and *Creatonotos transiens*). *Journal of Comparative Physiology B*, *160*(4), pp.389-400.

Wink, M. and Witte, L., 1984. Turnover and transport of quinolizidine alkaloids. Diurnal fluctuations of lupanine in the phloem sap, leaves and fruits of *Lupinus albus* L. *Planta*, *161*(6), pp.519-524.