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ECOLOGICAL AND EVOLUTIONARY INTERACTIONS AMONG PLANT
RESISTANCE, HERBIVORES, AND PREDATORS

by

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Dissertation submitted in partial fulfillment of
the requirements for the degree of Doctor
of Philosophy in the Department of
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ABSTRACT

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Abstract

To understand how plant defensive traits will evolve, we need to consider the biotic context for plant-herbivore interactions. I investigated how predators affect selection on defensive traits in plants. First, I established the timing of resistance in three soybean genotypes. Next, I examined the combined effects of resistance and predators on plant fitness. I reared Mexican bean beetles (MBBs) with or without spined soldier bugs (SSBs) on soybeans with constitutive resistance (CR) or no resistance (NR). SSBs fed more on MBBs that fed on NR than on CR plants, and this translated into an increased fitness benefit from predators for NR plants over CR plants. Selection for some types of resistance in plants should thus be stronger with lower predation rates. Similarly, I reared MBBs with or without SSBs on soybeans with early induced resistance (EI), late induced resistance (LI), or CR. SSBs fed more on MBBs reared on LI plants than on beetles raised on CR plants, but no more on beetles reared on EI plants than on beetles reared on CR plants. LI plants were the only of the three soybean varieties to receive a fitness benefit from predators, which could help explain the evolution of this type of plant defense. The results of both experiments also suggest that predator introductions may be more beneficial to LI or NR crop plants than EI or CR crops. Finally, I present a model that determines the optimum amount of induced resistance (IR) and CR for a plant growing with and without neighbors. Unlike earlier models, our plants have a probability of being attacked that is modified by short- and long-term feedback of plant defenses to herbivores. Higher costs of defense favor IR

over CR, while increasing herbivore attack rates or increasing the overall effectiveness of defense results in more CR. Plants with neighbors might be selected to evolve higher or lower levels of CR than if they were growing alone. Adding neighbors also selects for more mixed induced/constitutive strategies for all parameters. Having defended neighbors could thus be part of the reason why plants have evolved such mixed defense strategies.

Contents

Abstract.....	iv
List of Tables	ix
List of Figures.....	x
Acknowledgements.....	xiii
1. Introduction.....	1
1.1 Overview of the problem	1
1.2 Past work on how selection for plant defensive traits is affected by the biotic and abiotic environment	2
1.3 The effect of predators on plant defenses against herbivores.....	5
1.4 Potential influence of specific characteristics of plants, herbivores, and predators on the beneficial effect of predators on plant fitness	6
1.5 Summary of my research and the following chapters.....	11
2. Timing of induced resistance in three soybean genotypes.....	13
2.1 Introduction.....	13
2.2 Methods	14
2.2.1 Study system	14
2.2.2 Experimental design.....	15
2.3 Results.....	18
2.4 Discussion.....	18
3. Negative interactions between chemical defense and predators affect fitness in soybeans.....	23
3.1 Introduction.....	23

3.2 Materials and methods	25
3.2.1 Experimental organisms.....	25
3.2.2 Experimental design.....	26
3.2.2.1 Experiment 1	26
3.2.2.2 Experiment 2	27
3.2.3 Analysis.....	29
3.3 Results.....	29
3.3.1 Experiment 1	29
3.3.2 Experiment 2	31
3.4 Discussion.....	34
4. Timing of resistance changes interactions between plant chemical defenses and predators in soybeans.....	40
4.1 Introduction.....	40
4.2 Materials and methods	42
4.2.1 Experimental organisms.....	42
4.2.2 Experimental design.....	44
4.2.2.1 Experiment 1	44
4.2.2.2 Experiment 2	46
4.2.3 Analysis.....	46
4.3 Results.....	47
4.3.1 Experiment 1	47
4.3.2 Experiment 2	49
4.4 Discussion.....	54

5. The effects of neighbors on the strength and timing of optimum levels of defense in plants.....	59
5.1 Introduction.....	59
5.2 Methods	62
5.2.1 Overview.....	62
5.2.2 Model formulation	63
5.2.3 Adding immigration.....	66
5.2.4 Model analysis	68
5.3 Results.....	69
5.3.1 Determining the CSS	69
5.3.2 Effect of cost of defense.....	71
5.3.3 Effect of relative effectiveness of induced defense	75
5.3.4 Effect of overall effectiveness of defense	75
5.3.5 Effects of changing attack rate.....	78
5.3.6 Effects of adding immigration	78
5.4 Discussion.....	81
References.....	86
Biography.....	94

List of Tables

Table 1: Default parameter values for all runs unless otherwise noted. The investment in constitutive defense (d) was always varied between 0 and 1 with the amount of induced defense equal to $(1-d)$ since the total defense always summed to 1. $B = \frac{\beta h}{m}$ and $D = \frac{\delta}{r}$ were parameters created when we non-dimensionalized the model as described above.. 71

List of Figures

- Figure 1: Timing of induced resistance in 4 soybean genotypes as measured by Preference Index (PI; mean \pm s.e.). A PI > 1 indicates that Mexican bean beetles preferred leaf discs from undamaged plants to discs from previously damaged plants. .. 19
- Figure 2: Theoretical explanation of the additive effects of induced (I) and constitutive (C) resistance in the H, M, and HM genotypes. In the H and M genotypes, Mexican bean beetles are repelled by the combined induced and constitutive resistance when the induced resistance is at its peak, so the beetles prefer previously undamaged plants. In the HM genotype, beetles are always repelled by the constitutive component of resistance and cannot detect the additional resistance conferred by the induced component. 21
- Figure 3: Seed counts per plant (mean \pm s.e.) for non-resistant (NR) and resistant (R) soybeans grown in the absence and presence of Mexican bean beetle (MBB) herbivores in Experiment 1. 30
- Figure 4: Percent leaf area damaged (mean \pm s.e.) by MBBs in each treatment in Experiment 2. A damage index was visually estimated for each plant in each replicate. The midpoint of the damage index range was used as the percent damaged value for each plant, and the mean of the values for all plants in a replicate was used as the value for that replicate. 32
- Figure 5: Seed counts per plant (mean \pm s.e.) for non-resistant (NR) and resistant (R) soybeans (both with MBBs) either with (Pred) or without (No Pred) predator additions in Experiment 2. 33
- Figure 6: Average proportion (\pm s.e.) of spined soldier bugs feeding on Mexican bean beetles caged on non-resistant (NR) vs. resistant (R) plants during 3 observation periods in Experiment 2. 35
- Figure 7: (a) Percent leaf area damaged (mean \pm s.e.) by MBBs in each treatment in Experiment 1. A damage index was visually estimated for each plant in each replicate. The midpoint of the damage index range was used as the percent damaged value for each plant, and the mean of the values for all plants in a replicate was used as the value for that replicate. (b) Seed counts per plant (mean \pm s.e.) for late induced (LI) and constitutively resistant (CR) soybeans (both with MBBs) either with (Pred) or without (No Pred) predator additions in Experiment 1. 48
- Figure 8: Average proportion (\pm s.e.) of spined soldier bugs feeding on Mexican bean beetles caged on late induced (LI) vs. constitutively resistant (CR) soybeans during 3 observation periods in Experiment 1. 50

Figure 9: (a) Percent leaf area damaged (mean +/- s.e.) by MBBs in each treatment in Experiment 2. A damage index was visually estimated for each plant in each replicate. The median of the damage index range was used as the percent damaged value for each plant, and the mean of the values for all plants in a replicate was used as the value for that replicate. (b) Seed counts per plant (mean +/- s.e.) for late induced (LI) and constitutively resistant (CR) soybeans (both with MBBs) either with (Pred) or without (No Pred) predator additions in Experiment 1..... 51

Figure 10: Average proportion (+/- s.e.) of spined soldier bugs feeding on Mexican bean beetles caged on early induced (EI) vs. constitutively resistant (CR) soybeans during 3 observation periods in Experiment 2. 53

Figure 11: Graphical representation of the relationship between the fraction of plants attacked in a given year (f_A') and the average final herbivore biomass per plant from the previous year. The biomass of herbivores from the previous year is a function of the equilibrium herbivore biomass per attacked plant (\bar{H}) and the fraction of plants attacked (f_A) that year. f_A' is a saturating function of $f_A \bar{H}$ with a half-saturation constant of z 67

Figure 12: Invasion analysis using default parameter values. The lines shown are the contours of the invader's relative fitness for varying levels of investment in constitutive defense (d) for the resident and invader plants. In regions marked with + the invader's fitness is higher than the resident's fitness, while in regions marked with - the invader's fitness is lower than the resident's fitness. The value of d for the resident that corresponds with the crossing of the two equal fitness lines (labeled with a "1") indicates an Evolutionarily Singular Strategy with convergence stability (also known as a CSS). 70

Figure 13: Effects of varying the cost of defense (c) on the optimal level of constitutive defense for both the invader-resident system (crosses) and plants grown alone (open circles). The parameter values were chosen to reflect reasonable values that did not cause the fraction of plants attacked to be negative. A value of 1 for d denotes all constitutive defense, a value of 0 for d denotes all induced defense, and intermediate values denote a mix of the two strategies. 72

Figure 14: The equilibrium sizes of (a) attacked plants, (b) unattacked plants (b), and (c) the fraction of plants attacked at the CSS level of defense for the invader-resident system shown in Fig. 13..... 74

Figure 15: Effects of varying four of the non-dimensionalized model parameters on the optimal level of constitutive defense for both the invader-resident system (crosses) and plants grown alone (open circles). All parameter values were chosen to reflect reasonable values that did not cause the fraction of plants attacked to be negative. 76

Figure 16: Effects of varying the cost of defense (c) on the optimal level of constitutive defense for both the invader-resident system (crosses) and plants grown alone (open circles) at a lower (a) and higher (b) value of z than that used in Fig. 3. The parameter values were chosen to reflect reasonable values that did not cause the fraction of plants attacked to go negative. Note that no values of c less than 0.15 were used in (b) to prevent the fraction of plants attacked from going below zero. 79

Figure 17: Effects of varying the herbivore immigration rate (I) on the optimal level of constitutive defense for both the invader-resident system (crosses) and plants grown alone (open circles). 80

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1. Introduction

1.1 Overview of the problem

Plants possess many defensive traits that can enhance their fitness in the presence of herbivores. These plants traits can be physical attributes such as thorns on branches or trichomes on leaves. Plants tissues can also contain a variety of chemical compounds such as phenolics or alkaloids. Some of these chemicals are always present in plant tissues, while others are only produced after the plant is damaged. All of these traits have the potential to increase plant fitness through the reduction of herbivore damage, but can potentially be costly to the plant producing them (Agrawal et al. 1999; Mauricio 1998). However, plants and their herbivores do not exist in isolation. Instead, they are influenced by other environmental factors, including interactions with other organisms such as other plants, other herbivores, plant and herbivore pathogens, and predators of the herbivores. The presence of these other species can alter the pattern of natural selection acting on defensive traits. Other plants may influence the relative fitness costs of producing defensive traits for a focal plant. The strength or even the direction of selection on plant defensive traits by one herbivore may be modified by the presence of another herbivore. Chemical defenses can have increased fitness benefits to plants in the presence of the herbivores' pathogens by increasing the susceptibility of herbivores to those pathogens. Conversely, the fitness benefits to plants can be decreased if herbivore pathogens are negatively influenced by plant chemical defenses. Likewise, plant defensive traits can either increase or decrease the effectiveness of predators and their indirect benefits on

plant fitness. Similarly, abiotic factors can alter selection on defensive traits. I discuss specific examples of all of these influences below.

Thus, to fully understand how defensive traits will evolve, we need to consider the biotic and abiotic context in which plant-herbivore interactions take place. In the following sections, I review previous studies on how abiotic factors and the biotic community context affect selection on plant defense traits. I then discuss features of plants, herbivores, and predators that may influence whether defense enhances or detracts from the predator benefit. Finally, I outline my research on part of the biotic context for plant-herbivore interactions.

1.2 Past work on how selection for plant defensive traits is affected by the biotic and abiotic environment

Abiotic conditions and the biotic community can both affect the pattern of natural selection on plant defensive traits in the presence of an herbivore. Abiotic factors can affect the ability of plants to produce defensive compounds as well as the costs associated with such production. For example, plants might produce more alkaloids simply because they grow in soils with higher nitrogen concentrations (Rausher 1992). Plants growing in soil with less nutrient availability might also be more strongly favored to produce defensive compounds, as they may be less able to afford to lose to herbivores what nutrients they have acquired (Bryant et al. 1993; Herms and Mattson 1992). Humidity, temperature, and light, as well numerous other abiotic factors can also affect the ability of plants to produce defensive compounds (Gouinguene and Turlings 2002).

Likewise, the presence of other plants of the same or different species can alter the pattern of natural selection imposed by one herbivore species on plant defensive traits. Other plants may influence the relative fitness costs of producing defensive traits. Plants may have to “choose” between allocating limited resources to grow fast enough to compete vs. investing these resources in secondary metabolites to maintain defense against herbivores (Siemens et al. 2002; Uriarte et al. 2002; Weis and Hochberg 2000). Plants may benefit indirectly from the defense of their neighbors due to “herd immunity” (Anderson and May 1990; Frank 1998; Poitrineau et al. 2004). If a plant’s neighbors are producing large amounts of expensive herbivore defenses, that plant may be selected to produce less costly defense itself and instead parasitize its neighbors’ defenses by benefiting from the lower herbivore attack rate in its neighborhood.

The presence of multiple herbivores can also alter the pattern of natural selection acting on plant defensive traits. The strength or direction of selection on plant defensive traits by one herbivore may be modified by the presence of another herbivore. Pilson (1996) found that selection for resistance to flea beetles (*Phyllotreta cruciferae*) in wild mustard plants (*Brassica rapa*) was lessened with increasing numbers of diamondback moths (*Plutella xylostella*). Similar diffuse coevolution patterns (where the presence of multiple herbivores influenced resistance to one of those herbivores) were found by Juenger and Bergelson (1998) looking at herbivores of scarlet gilia (*Ipomopsis aggregata*), by Stinchcombe and Rausher (2001) investigating deer and generalist insect herbivores on morning glories (*Ipomoea hederacea*), and in various other systems (Leimu and Koricheva 2006). Conversely, Hougeneitzman and Rausher (1994) found that one

insect herbivore did not affect the evolution of resistance to a different insect herbivore in morning glories, suggesting that such diffuse coevolution patterns are not always present with multiple herbivores.

The presence of an herbivore's pathogens can also alter the pattern of natural selection acting on plant defensive traits. Chemical defenses can have increased fitness benefits to plants in the presence of herbivore pathogens by increasing the susceptibility of herbivores to those pathogens (Price et al. 1980). For example, Stubblebine and Langenheim (1977) found that leaf resins from the legume *Hymenaea courbaril* made beet armyworm (*Spodoptera exigua*) much more susceptible to viral infection. Similarly, Hountondji et al. (2006) determined chemicals induced by cassava green mites on cassava made the mites more susceptible to the pathogenic fungus *Neozygites tanajoae*. Conversely, the fitness benefits to plants of chemical defenses can be decreased if herbivore pathogens are negatively influenced by plant chemical defenses. Hoover et al. (1998) found decreased noctuid moth larval mortality caused by baculoviruses with increasing phenolic concentrations in their host plants. Raymond et al. (2002) found similar effects of plant phenolics on nucleopolyhedroviruses in winter moths (*Operopherta brumata*). Experiments have also shown increasing tannin concentration in oaks decreased gypsy moth (*Lymantria dispar*) infection rates by a baculovirus (Hunter and Schultz 1993; Keating et al. 1988). However, field studies that accounted for the phenology of natural gypsy moth populations suggested tannins have no effect on baculovirus infection rates (D'Amico et al. 1998; Dwyer et al. 2005).

1.3 The effect of predators on plant defenses against herbivores

Predators can affect selection on herbivore resistance in plants. Plants can benefit indirectly from the activities of predators through reduced herbivore damage. This reduction can result from changes in the number, feeding habits, location of feeding, etc. of the herbivore. However, plant resistance factors will often affect the magnitude of indirect benefits plants receive from predators. There is evidence that defensive traits may either enhance or detract from the indirect fitness benefits of predators. One such positive (enhancement) effect might occur if herbivores on resistant plants are more susceptible to predators. An example of this is the work of Starks et al. (1972) who noted that parasitoids had higher attack rates and were more effective in reducing aphid density on resistant than non-resistant plants. Kalule and Wright (2002a) found a similar effect in their parasitoid-aphid system. Another type of positive effect of plant resistance on predators would be a chemical released by resistant plants serving as attractors for predators (Price et al. 1980). Thaler (1999) noted parasitoids locating herbivore prey using chemicals released by tomatoes after being damaged by those herbivores. Ninkovic et al. (2001) found ladybird beetles (*Coccinella septempunctata*) were attracted by defensive chemicals released by barley being fed upon by aphids. Alborn et al. (1997) and Havill and Raffa (2000) found similar effects in other systems.

One type of negative effect of plant resistance factors on predators would be lower attack rates of predators on herbivores due to lowered herbivore palatability caused by plant defensive chemicals (Brower et al. 1967; Dyer 1995; Eisner 1970; Price et al. 1980; Sipura 2002). Another example of a negative effect of plant resistance factors on

predator effectiveness would be lower fecundity, survivorship, and/or body size for predators feeding on herbivores eating resistant plants (Price et al. 1980; Turlings and Benrey 1998). Kauffman and Flanders (1985) found that the higher the resistance exhibited by soybean cultivars to herbivorous Mexican bean beetles, the greater the negative impact was on eulophid parasitoid wasp survivorship and body size. Dover et al. (1987) found a similar effect in the same three-species system, and Campbell and Duffey (1979), Reitz and Trumble (1996), Fuentes-Contreras et al.(1998), Havill and Raffa (2000), and Hufbauer (2001) found comparable effects in other plant-herbivore-parasitoid systems. Agrawal et al. (2002) found that plants producing more cucurbitacins were less attractive to predatory mites and reduced the fecundity of mites that did attack herbivores on resistant plants. Finally, physical plant defenses such as trichomes and other mechanical defenses can interfere with the searching behavior and prey capture of predators such as parasitoids (Bottrell et al. 1998), predaceous bugs (Bjorkman and Ahrne 2005), and spiders (Denno et al. 2002; Riihimaki et al. 2006).

1.4 Potential influence of specific characteristics of plants, herbivores, and predators on the beneficial effect of predators on plant fitness

The interaction between plant defenses against herbivores and predators of those herbivores may change as specific characteristics of the organisms involved in the interaction vary. One example of the potential importance of such characteristics of the organisms that may be common across many systems is shown by the “nasty host hypothesis” of Gauld et al. (1992). They noted that tropical woody plants have more

secondary compounds than their temperate counterparts. Thus, hosts might be less available to tropical than to temperate parasitoids because tropical host tissues are more toxic than are the tissues of temperate hosts. Certain parasitoid groups are relatively more abundant than others in the tropics, and Gauld et al. hypothesized this might be due to their ability to deal with “nasty” hosts. Parasitoids that develop inside the living host are potentially exposed continuously to any plant chemicals the host is eating. Such parasitoids are among the least common in the tropics. Conversely, parasitoids that develop on the outside of the host and paralyze their host at the time of attack are more common in the tropics. These parasitoids would not be continuously exposed to chemical plant defenses, and many of the plant chemicals already present in the host would degrade by the time the parasitoid began feeding on the host (though this assumption that externally feeding parasitoids receive less chemical exposure has not yet been experimentally tested). Thus, the characteristic type of attack by the parasitoid may determine the interaction with plant chemical resistance factors. This idea is supported by the many examples of negative effects of resistant plants on the development and survivorship of parasitoids that develop inside hosts (Campbell and Duffy 1979; Dover et al. 1987; Fuentes-Contreras et al. 1998; Havill and Raffa 2000; Hufbauer 2001; Kauffman and Flanders 1985; Reitz and Trumble 1996) and lack of similar examples from external parasitoids.

There are numerous plant characteristics that may influence the interactions between plant resistance factors and predators. One such plant characteristic is whether the type of chemical resistance in the plant is primarily antibiotic or anti-feeding.

Antibiotic resistance negatively affects the metabolism of herbivores and slows their development, while anti-feeding resistance causes the herbivores to consume less plant material. If the resistance is antibiotic, it might increase predator effectiveness by causing herbivores to stay in a susceptible stage for a longer period of time, cause the predators to consume more prey than they otherwise would, or make the herbivores less able to escape predation. On the other hand, antibiotic resistance might instead make the predators less effective if the resistance factors cause the herbivores to be distasteful to the predators or render the herbivores less mobile and harder for the predators to find. Similarly, anti-feeding resistance could make the predators more effective by causing the herbivores to feed on a more exposed area of the plant, or less effective by causing the herbivores to feed on a less exposed area of the plant.

Another potentially important characteristic of plant resistance is the timing of plant resistance, and whether the resistance is constitutive or inducible. In constitutive resistance to herbivore damage, the defenses are always expressed in the plant, while induced resistance is a response to herbivore damage that reduces herbivore survival, reproductive output, or preference for a plant (Karban and Baldwin 1997). This could present a clear difference as to how much the herbivore has been affected by plant resistance before the attack of the predator, especially with a predator that attacks soon after the herbivore begins feeding. The potential effects of timing of defense are discussed in more detail in Chapter 3.

As with plant characteristics, various herbivore characteristics could have an influence on the outcome of the interaction between plant resistance factors and

predators. One such characteristic is the location on the plant where the herbivore typically feeds. That location may determine how much the herbivore is exposed to plant resistance factors, especially if the plant tends to localize where it produces those factors (e.g. only in new leaves or primarily in the stem). The life-cycle stage(s) of the herbivores when they feed on the plant is another potentially important characteristic. If only adults are feeding on the plants, they may not be as exposed to resistance factors as herbivores that have both larvae and adults that feed on the plants. Additionally, the timing of the herbivore's feeding relative to the timing of induction of plant resistance is of potential influence.

The degree of specialization in feeding by the herbivore is another characteristic that could influence the outcome of the interaction between plant resistance factors and predators. If the herbivore is a specialist feeding only on the focal plant (or only on closely related plants), such as the monarch caterpillar studies by Brower et al. (1967), the herbivore may be better adapted to detoxify or sequester herbivore-resistant chemicals produced by the plant than would be a generalist herbivore. The specialist herbivore may be better at avoiding predation than the generalist (either by sequestering these chemicals and therefore being unpalatable or by detoxifying the compounds and thus being healthier and better able to avoid predators). In this case a plant fed on by a generalist herbivore might receive a greater fitness benefit from a predator than a plant fed on by a specialist herbivore. This idea is supported by Dyer (1995), who found that specialist caterpillars were more likely than generalists to be rejected by ant predators, and that this difference appeared to be based on the ability of specialist caterpillars to

utilize plant defensive compounds for their own defense. Conversely, if the specialist herbivore quickly rids itself of the plant resistance chemicals while the generalist cannot get rid of them as quickly, the generalist may be the more unpalatable of the two, and the plant fed on by a specialist herbivore might receive the greater fitness benefit from predators.

Finally, various predator characteristics could have an influence on the outcome of the interaction between plant resistance factors and predators. As discussed above, the timing of the predator attack relative to the timing of plant resistance induction and the timing of the herbivore feeding may influence the interaction. Additionally, the general feeding strategy (i.e. internal parasitoid or external predator) and degree of specialization of the predator might influence the interaction between plant resistance factors and predators. A generalist predator that eats only part of an herbivore at one specific point in time might be less likely to be strongly exposed to any plant resistance factors than parasitoids that develop inside a host (as discussed above). This idea is supported by Osier et al. (1996), who found no negative effect of plant resistance chemicals in tomato leaves on a generalist predator even though those defensive chemicals did negatively affect the herbivore (*Manduca sexta*). Furthermore, as with herbivores, specialist predators might be better adapted at dealing with any plant resistance chemicals they do come into contact with than generalists. This effect was observed by Rank and Smiley (1994), who found that generalist predators were repelled by chrysomelid beetle secretions that contain salicin from their host plants, but a specialist fly predator was not

deterred by the same secretions. Harvey et al. (2005) found a similar negative effect of plant chemicals on a generalist parasitoid but lack of effect on a specialist parasitoid.

Although I have discussed a number of plant, herbivore, and predator life history characteristics that could have an influence on the outcome of the interaction between plant resistance factors and predators, there are many more that may be of importance that I did not mention. The above characteristics are some that might reasonably be manipulated in an experimental setting, are widespread, and could clearly have a large influence on the outcome of the interaction. Due to time constraints it was impossible for me to investigate all of the characteristics mentioned above. Therefore, I chose to concentrate on the type and timing of plant resistance, as that had already been shown to be important in the dynamics of my plant (soybeans) and herbivore (Mexican bean beetles) of choice (Underwood 1999; Underwood and Rausher 2000).

1.5 Summary of my research and the following chapters

I investigated how predators affect selection on defensive traits in plants, as well as how this interaction between herbivore defense and predators changed with different timing and strengths of plant defenses. In Ch. 1 I discuss a laboratory experiment that determined the timing of resistance in the plants I chose for my experimental study system. In the second chapter, I describe experiments that established that a constitutive resistance in soybeans to herbivorous Mexican bean beetles reduced the fitness benefit plants received from predaceous spined soldier bugs. My study was the first to measure

the direct fitness consequence for plants of a negative interaction between resistance and predators.

In the third chapter, I discuss two additional field experiments that established that this negative interaction between predators and resistance for constitutive resistance was not consistent across different resistance types. Plants with late induced resistance received a fitness benefit from predators, while plants with early induced resistance and constitutive resistance did not. This result illustrated for the first time that resistance types with different timing can vary in their effects on the fitness benefit plants receive from predators.

Finally, in the last chapter I discuss a model that determined that the optimal type of defense in plants changed in the presence of conspecific neighbors relative to the optimum defense in the absence of neighbors. Plants with neighbors could be selected to evolve higher or lower levels of constitutive defense than if they were growing alone. Adding neighbors also tended to select for more mixed induced/constitutive strategies over some range for all parameters, leading to the novel suggestion that neighbors could be part of the reason why some plants have evolved such mixed defense strategies.

2. Timing of induced resistance in three soybean genotypes

2.1 Introduction

Plant traits that negatively affect herbivores can be classified as constitutive if they are always expressed in the plant or induced if they are produced only in response to herbivore damage. In turn, the timing of induced resistance responses varies greatly between and within plant species, ranging from minutes (Green and Ryan 1972; McCloud et al. 1995) to years (Haukioja 1980) after the damage occurs. Understanding the time course of induced resistance is necessary to understand the potential ecological interactions of that resistance with herbivores and higher trophic levels. The timing of induced resistance has already been shown to be important in the dynamics of plants and herbivores (Underwood 1999; Underwood and Rausher 2000). Likewise, plant resistance has been shown to interact either positively (i.e. James and Price 2004) or negatively (i.e. Harvey et al. 2005) with predators. The timing of induced resistance is fundamental to understanding this interaction of plant resistance with predators since the time at which induced resistance peaks could determine how much the herbivore has been affected by plant resistance before the predator attacks.

Despite the multitude of studies that have investigated the effects of induced resistance, the genetic basis for induced resistance has rarely been examined (Karban and Baldwin 1997), nor has the question of whether the same genes can contribute to both induced and constitutive resistance. As part of a larger study investigating the interaction between plant defenses of different types and predators, I examined the course of the

induced response to damage by Mexican bean beetles in four genotypes of soybeans. Three of these genotypes were known to be chemically resistant to Mexican bean beetles from field experiments (Narvel et al. 2001). For each of these genotypes one to two quantitative trait loci (QTL) that formed part of the basis for this resistance had been identified. However, the type of resistance in the three resistant genotypes was unknown. Therefore, I quantified the timing of induced responses to damage in all of the soybean genotypes using a bioassay. The results of this bioassay allowed me to examine how genetic factors, alone and in combination, can determine the type and timing of resistance.

2.2 Methods

2.2.1 Study system

Soybeans (*Glycine max*) have well-characterized, closely related genotypes that differ in both the strength and type of resistance. Chemical resistance factors in soybeans include phenolics (such as isoflavonoids, pterocarpan, and coumestans), peroxidases, proteinase inhibitors, phytoalexins, and many others (Kogan and Fischer 1991). Some of these resistance factors are constitutive defenses (such as isoflavonoids) while others are induced defenses (such as phytoalexins). I focused on four closely related soybean genotypes (obtained from Dr. R. Boerma, Univ. of Georgia) that differed in chemical resistance characters but were morphologically indistinguishable (Boerma and Walker 2005; Narvel et al. 2001; Walker et al. 2004). All four genotypes I used were created by crossing the highly insect resistant soybean cultivar PI 229358 with the higher yielding

Cobb cultivar. The presence or absence of two resistance QTLs from PI 229358, designated H and M, was identified through analysis of cultivars resulting from this cross (Narvel et al. 2001; Rector et al. 1999). Soybean genotypes with either the H or M resistance QTLs are known to provide functional resistance to Mexican bean beetles in the field. The first genotype I used possessed both the H and M resistance alleles; I refer to it as the HM genotype. The second genotype I used possessed only the H resistance allele; I refer to it as the H genotype. The third genotype I used possessed only the M resistance allele; I refer to it as the M genotype. The final genotype I used in my experiments possessed neither the H nor M resistance alleles; I refer to it as the L (for lacking) genotype.

Mexican bean beetles (*Epilachna varivestis*) are specialists on legumes and are widespread economic pests of soybeans. Both adults and larvae feed by scraping leaf tissues, so it is easy to quantify damage as percent leaf area scraped. Beetles used in this experiment were obtained from a laboratory colony reared on pinto beans (*Phaseolus vulgaris*).

2.2.2 Experimental design

I designed my experiment to damage the soybeans in one discrete event then measure the induced response by bioassay with Mexican bean beetles at four one-week intervals following damage. I followed a similar protocol to Underwood (1998), as that study investigated the timing of induced effects in the same system but with different soybean genotypes. Thus, I chose to use a bioassay to determine the induced response of

soybeans to Mexican bean beetle damage. I also chose my sample intervals based on the results of Underwood's study, which suggested that induced responses in soybeans to Mexican bean beetle damage were primarily present in the first twenty days following damage.

I grew 40 soybean plants of each of the 4 genotypes in a greenhouse in Durham, NC from April-June 2002. Plants were grown in 2.8 L pots filled with Fafard 3B potting soil. When plants had expanded their third true leaf, I chose half of the plants from each genotype to receive damage. I bagged enough third instar and larger Mexican bean beetle larvae on plants in the damaged treatment to consume approximately 60% of the total leaf area within 72 hours. I added or removed beetle larvae at 24 and 48 hours to achieve this damage level. Control plants were similarly bagged but no beetle larvae were added.

I determined the feeding preference of Mexican bean beetle adults for undamaged leaves from control versus previously damaged plants at four sampling dates (3, 10, 17, and 24 days) after the damage period ended. This bioassay provided a measure of the level of induced resistance at each of the sampling dates. At each date, I randomly chose five pairs of previously damaged and control plants from each genotype. Using a 2-cm-diameter cork borer, I cut leaf discs from the most recently expanded undamaged leaf for each plant. This method has been shown not to cause induced resistance in the leaf discs (Kogan and Fischer 1991; Underwood 1998). I discarded plants after sampling, so that no plant was used more than once. I placed two leaf discs from each member of each pair of control and damaged plants on opposite sides of a 9-cm-diameter Petri dish lined

in damp filter paper. I placed one female Mexican bean beetle, previously starved for 24 hours in each dish. The beetles were left in the dish to feed until they had consumed at least 25% of the leaf area in the dish or for 48 hours, whichever came first. I scanned all leaf discs on a flatbed scanner, and then measured the leaf area of each disc consumed by the beetles using Adobe Photoshop (Adobe 2002) with a custom surface area measurement plug-in (Reindeer Games 1999).

I used the area of consumed leaf tissue for the control (c) and damaged (d) discs for each pair to calculate a preference index [$PI=2(c/(c+d))$] analogous to Underwood (1998) and Kogan (1972). A PI of 1 thus indicated no preference for either damaged or control plants, while a PI of 2 indicated a complete preference for control over previously damaged plants. The mean PI from all samples of a given genotype for a given time since damage was therefore used as the measure of induced resistance for that time and genotype.

For statistical analyses I used the ratios of leaf area damaged ($c/(c+d)$) rather than the PIs, and arcsine square-root transformed these ratios to normalize their distributions. I used an ANOVA with main effects of genotype and sample time and an interaction term of genotype with sample time to determine if the four genotypes differed in the timing of their induced response. I also performed a two-tailed t-test to determine if the PI for each genotype at each sample time was significantly different from 1 (no preference). I used S-Plus 2000 software (MathSoft 1999) for both analyses.

2.3 Results

The level of induced resistance did vary among genotypes ($F_{3,64} = 3.213$, $p = .029$). The main effect of time since damage was not significant ($F_{3,64} = 1.383$, $p = .256$). However, the interaction term between genotype and time since damage indicated that the timing of induced resistance varied among genotypes ($F_{9,64} = 3.860$, $p < .001$; Fig. 1). The H genotype exhibited a relatively rapid induced response, peaking in resistance at 10 days after initial damage followed by a decline to no preference for undamaged plants by day 17. The M genotype showed a more delayed induced reaction, peaking in resistance at 24 days after initial damage (the latest time at which preference was measured). A series of two-tailed t-tests confirmed that those two peaks ($p < .05$ for both) were the only genotype by time combinations significantly different from 1. Neither HM nor L showed any induced resistance. None of my genotypes showed significant induced susceptibility, defined as an increased vulnerability to herbivore damage following initial herbivore attack.

2.4 Discussion

The time course of induced resistance I observed in my four genotypes was very different from the time course of induced resistance observed in similar experiments, but with different soybean genotypes, performed by Underwood (1998), who also used feeding preferences of Mexican bean beetles to quantify induced resistance. Underwood found a peak of induced resistance at three days after damage for all genotypes tested. I found peaks in induced resistance at ten days for the H genotype and 24 days for the M

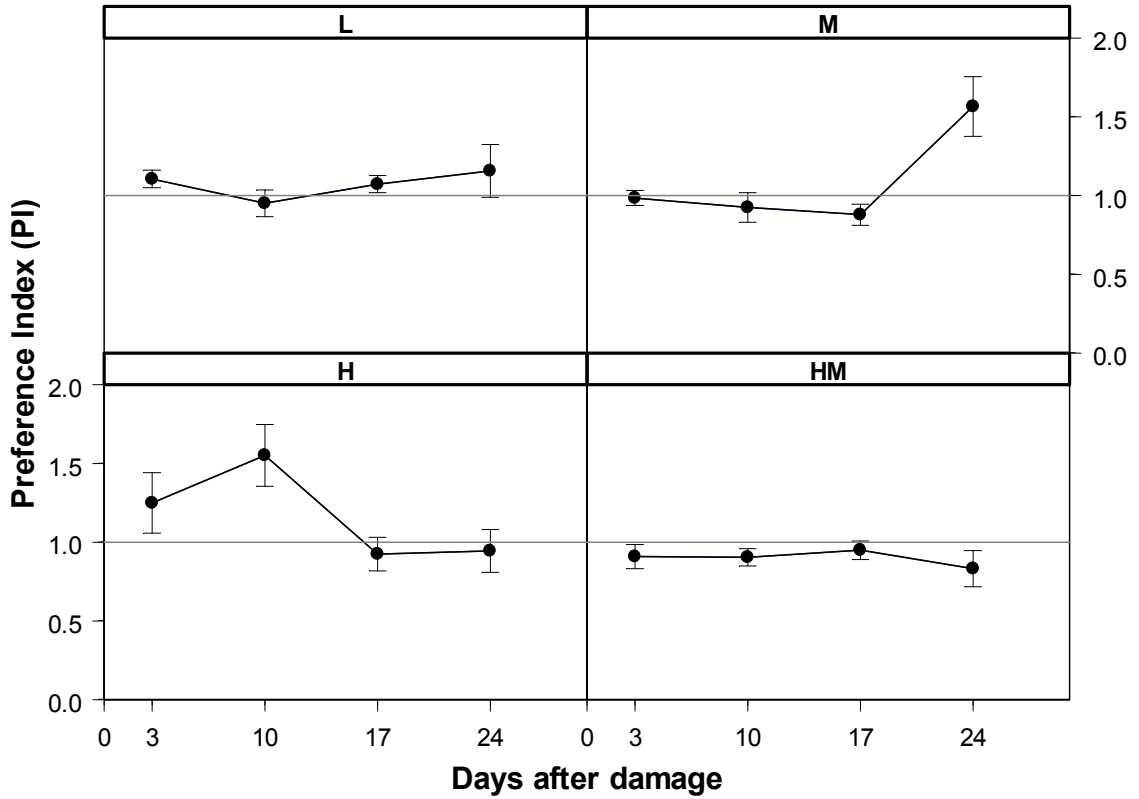


Figure 1: Timing of induced resistance in 4 soybean genotypes as measured by Preference Index (PI; mean +/- s.e.). A PI > 1 indicates that Mexican bean beetles preferred leaf discs from undamaged plants to discs from previously damaged plants.

genotype, and no induced resistance in the other two genotypes. This is likely due to differences in the underlying genetic basis of resistance for these genotypes. Underwood used common varieties of soybeans, which all have a similar genetic background (T.Carter, personal communication, March 2001) and are not considered to be insect resistant. The genotypes of soybeans I used had different genetic bases for resistance, which lead to very different strengths and timing of induced resistance.

The lack of induced resistance in the HM genotype, whereas both the H and M genotypes showed induced resistance, has several potential explanations. The HM genotype is known to have strong resistance to Mexican bean beetles in the field (Narvel et al. 2001). Since I found no induced component to that resistance, HM must be constitutively resistant. When both the H and M QTLs are present, an epistasis effect may prevent the expression of induced resistance. Both the H and M QTLs also likely have a constitutive component to the resistance they confer. When combined, the constitutive components of this resistance may effectively overwhelm the induced components (Fig. 2). The effect of resistance factors in combination is often very different than the simple sum of their individual effects (Karban and Baldwin 1997), so the H and M QTLs acting constitutively in combination is not surprising.

The system of genotypes I have described with differing strengths and time courses of induced resistance should be ideal for experiments testing the effects on herbivores and higher trophic levels of resistance with different timing. I have illustrated a series of four related soybean genotypes that have alternately either no resistance (L), early induced resistance (H), late induced resistance (M), and constitutive resistance but

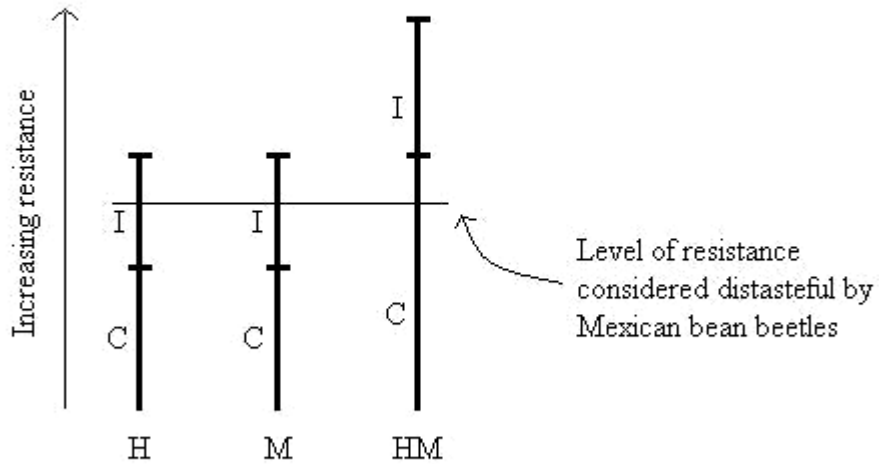


Figure 2: Theoretical explanation of the additive effects of induced (I) and constitutive (C) resistance in the H, M, and HM genotypes. In the H and M genotypes, Mexican bean beetles are repelled by the combined induced and constitutive resistance when the induced resistance is at its peak, so the beetles prefer previously undamaged plants. In the HM genotype, beetles are always repelled by the constitutive component of resistance and cannot detect the additional resistance conferred by the induced component.

no induced resistance (HM). Since all four genotypes are closely related, they should be similar in most non-resistance characters.

3. Negative interactions between chemical defense and predators affect fitness in soybeans

3.1 Introduction

Plants possess many defensive traits, such as thorns, trichomes, tough leaves, and a broad arsenal of secondary chemical compounds. Many studies have demonstrated that these traits negatively affect herbivores, and so frequently enhance the fitness of plants that possess them. Yet, herbivores are also attacked by their own predators. By reducing herbivore density or feeding rate, predators of herbivores can provide indirect benefits to plants. However, plant defensive traits will often affect the magnitude of indirect benefits plants receive from predators.

Numerous studies have found that plant defensive traits may enhance the indirect benefits plants receive from predators (Alborn et al. 1997; Gomez and Zamora 1994; Havill and Raffa 2000; Kalule and Wright 2002b; Mantyla et al. 2004; Ninkovic et al. 2001; Osier et al. 1996; Price et al. 1980; Rank and Smiley 1994; Romeis et al. 2005; Starks et al. 1972; Thaler 1999; Turlings and Benrey 1998). Various other studies have found that plant defensive traits may detract from those benefits (Agrawal et al. 2002; Bottrell et al. 1998; Brower et al. 1967; Campbell and Duffy 1979; Denno et al. 2002; Dover et al. 1987; Dyer 1995; Eisner 1970; Fuentes-Contreras et al. 1998; Gassmann and Hare 2005; Harvey et al. 2005; Havill and Raffa 2000; Hufbauer 2001; Kauffman and Flanders 1985; Ode et al. 2004; Orr and Boethel 1986; Price et al. 1980; Rank and Smiley

1994; Reitz and Trumble 1996; Romeis et al. 2005; Sipura 2002; Turlings and Benrey 1998). However, all of these studies have measured indirect benefits in the currencies of herbivore damage or predator abundance or vitality, rather than measuring components of plant fitness. Damage does not necessarily translate in a linear fashion into a reduction in plant fitness. Many plants are able to tolerate or compensate for a substantial amount of damage without showing a concomitant reduction in fitness (Mauricio et al. 1997; Rosenthal and Kotanen 1994; Strauss and Agrawal 1999; Trumble et al. 1993; van der Meijden et al. 1988). Likewise, any effects on predator abundance or vigor may not translate into effects on plant fitness, even when these effects on predators lead to reduced herbivore damage. Similarly, lower predator density does not necessarily cause a linear reduction in the number of herbivores consumed. Only if defensive traits alter the indirect effect of predators on plant fitness will the abundance of predators influence selection on those defensive traits. This selective pressure could be positive or negative (i.e. selecting for more or less defense). Therefore, it is important to quantify actual fitness effects mediated through predators of herbivores.

Here, I report the effect of predatory spined soldier bugs (*Podisus maculiventris*) on the fitness of two different soybean (*Glycine max*) genotypes that differ in their level of herbivore resistance when fed upon by Mexican bean beetles (*Epilachna varivestis*). Previous studies of the effects of plant defenses on predators in similar soybean systems (tritrophic systems involving soybeans and at least one of the two insects I used) have shown positive (Price et al. 1980), negative (Kauffman and Flanders 1985; Orr and

Boethel 1986), or no effects (Orr and Boethel 1986) of defenses on predators. However, these studies concentrated on effects of plant defenses on the predators themselves, not on plant fitness effects. As soybeans are annuals, I used total number of seeds produced by a plant to quantify plant fitness. This metric avoids the problems of tolerance and compensation described above and is more relevant than simply measuring damage from an applied perspective since farmers are most interested in yields (total seed counts for all plants in a field).

3.2 Materials and methods

3.2.1 Experimental organisms

Soybeans (*Glycine max*) have well-characterized, closely related genotypes that differ in both the strength and type of resistance. Chemical resistance factors in soybeans include phenolics (such as isoflavonoids, pterocarpan, and coumestans), peroxidases, proteinase inhibitors, phytoalexins, and many others (Kogan and Fischer 1991). Some of these resistance factors are constitutive defenses (such as isoflavonoids) that are likely to always be present in plant tissues. Others are induced defenses (such as phytoalexins) that are only produced after the plant is damaged, and have high rates of turnover (Kogan and Fischer 1991). I focused on two closely related soybean genotypes (obtained from Dr. R. Boerma, Univ. of Georgia) that differed in chemical resistance characters but were morphologically indistinguishable (Boerma and Walker 2005; Narvel et al. 2001; Walker et al. 2004). The first genotype possessed the H and M resistance QTLs (quantitative trait

loci), which confer a strong constitutive defense against Mexican bean beetles; I refer to it as the resistant (R) genotype. In previous choice test experiments (Bartlett 2006, Chapter 1), Mexican bean beetles did not show any preference for previously undamaged R soybeans over previously damaged R soybeans up to 24 days after damage, demonstrating that the combined resistance conferred by the H and M resistance QTLs is constitutive. The second genotype lacked the H and M QTLs, and I refer to it as the non-resistant (NR) genotype. For information on the origin of these genotypes, see Narvell et al. (2001).

Mexican bean beetles (*Epilachna varivestis*) are specialists on legumes and are widespread economic pests of soybeans. These beetles feed by scraping leaf tissues, so it is easy to quantify damage as percent leaf area scraped. Beetles used in this experiment were obtained from a laboratory colony reared on pinto beans (*Phaseolus vulgaris*).

The spined soldier bug (*Podisus maculiventris*) is a common generalist predator that attacks a variety of soft-bodied insects. These bugs regularly attack third and larger larval instars and pupae of Mexican bean beetles and are considered a beneficial insect regularly found on soybeans (Deitz et al. 1976). Soldier bugs used in this experiment were obtained from the commercial supplier Arizona Biological Control, Inc.

3.2.2 Experimental design

3.2.2.1 Experiment 1

I designed my first experiment to measure the effect of Mexican bean beetles on seed set for the NR and R soybean genotypes. The experiment was carried out at the

Duke University Zoology Department field station in Durham County, North Carolina from May to October 2002. It was a 2x2 factorial experiment with the following four treatment combinations:

1. NR soybeans grown alone
2. R soybeans grown alone
3. NR soybeans exposed to Mexican bean beetles
4. R soybeans exposed to Mexican bean beetles

Each treatment combination had a total of seven replicates. Each replicate included 4 soybean plants in 3.8 L pots enclosed in a 2.25 m² by 2 m tall cage (2 plants per pot, 2 pots per cage). The cages consisted of a wooden frame covered with black fiberglass porch screening. Replicates were equally divided among 2 spatial blocks and treatments were randomly assigned within each block.

When the plants had expanded their 4th true leaf, I added 5 1st instar Mexican bean beetles to each plant in the appropriate cages. Mexican bean beetle densities were chosen to reflect typical densities and damage levels seen in the field. I left the Mexican bean beetle larvae in the cages to feed until pupation, and then removed all pupae. At the end of the experiment, I counted the seeds produced by each plant. I used the average number of seeds produced by the 4 plants in each cage as the measure of fitness.

3.2.2.2 Experiment 2

Having demonstrated the effect herbivores have on plants and seed set in Experiment 1 (see Results), I designed Experiment 2 to determine differences in the

interaction between predatory spined soldier bugs and chemical plant defenses in the NR and R genotypes. The experiment was carried from May-October 2003 with methods identical to Experiment 1 except as noted. It was a 2x2 factorial experiment with the following four treatment combinations:

1. NR soybeans exposed to Mexican bean beetles only
2. R soybeans exposed to Mexican bean beetles only
3. NR soybeans exposed to both Mexican bean beetles and spined soldier bugs
4. R soybeans exposed to both Mexican bean beetles and spined soldier bugs

Each treatment combination had a total of twenty replicates. Replicates were equally divided among 4 spatial blocks and treatments were randomly assigned within each block.

I added 3 spined soldier bugs to the appropriate cages when the Mexican bean beetles had reached their third instar. Spined soldier bug densities were chosen to reflect typical densities seen in the field. The spined soldier bugs were left in the cages for 72 hours and then removed. While the spined soldier bugs were in the cages, I counted once per day the number of bugs in each cage that were actively feeding on Mexican bean beetles. Twice each week, I visually estimated a “damage index” for each replicate based on the percent of leaf area scraped by Mexican bean beetle larvae (1=0-5% damaged, 2=5-10%, 3=10-20%, 4=20-40%, 5=40-60%, 6=60-80%, 7=over 80%).

3.2.3 Analysis

I performed a 3-way G-test (log-likelihood ratio test) with 2 damage classes (<30% and >30%) to determine the effects of plant defense and predators on percent leaf area damaged. To indicate the interactive function of plant defenses and predators on plant fitness, I performed an ANOVA including main effects of block, genotype, and predators, and a genotype x predator interaction. I employed two-sample tests for equality of proportions (prop.test, MathSoft 1999) with continuity correction (to account for small sample sizes) to test for differences in the proportion of spined soldier bugs observed feeding on Mexican bean beetles when the beetles were feeding on different soybean genotypes. To investigate the direct effect of resistance on plant fitness, I used Welch Modified (to account for unequal variances between samples) Two-Sample t-Tests. I used S-Plus 2000 software (MathSoft 1999) for all statistical analyses.

3.3 Results

3.3.1 Experiment 1

A Welch Modified Two-Sample t-Test confirms the two soybean genotypes (NR and R) did not significantly differ ($t = 0.639$, $df = 10.598$, $p = 0.536$) in the number of seeds they produced in the absence of herbivores in the field (Fig. 3). Both genotypes showed a reduction in seed set with the addition of Mexican bean beetles, but the reduction was larger in NR plants than in R plants. I first performed an ANOVA including main effects of block, genotype, and herbivores, and a genotype x herbivore

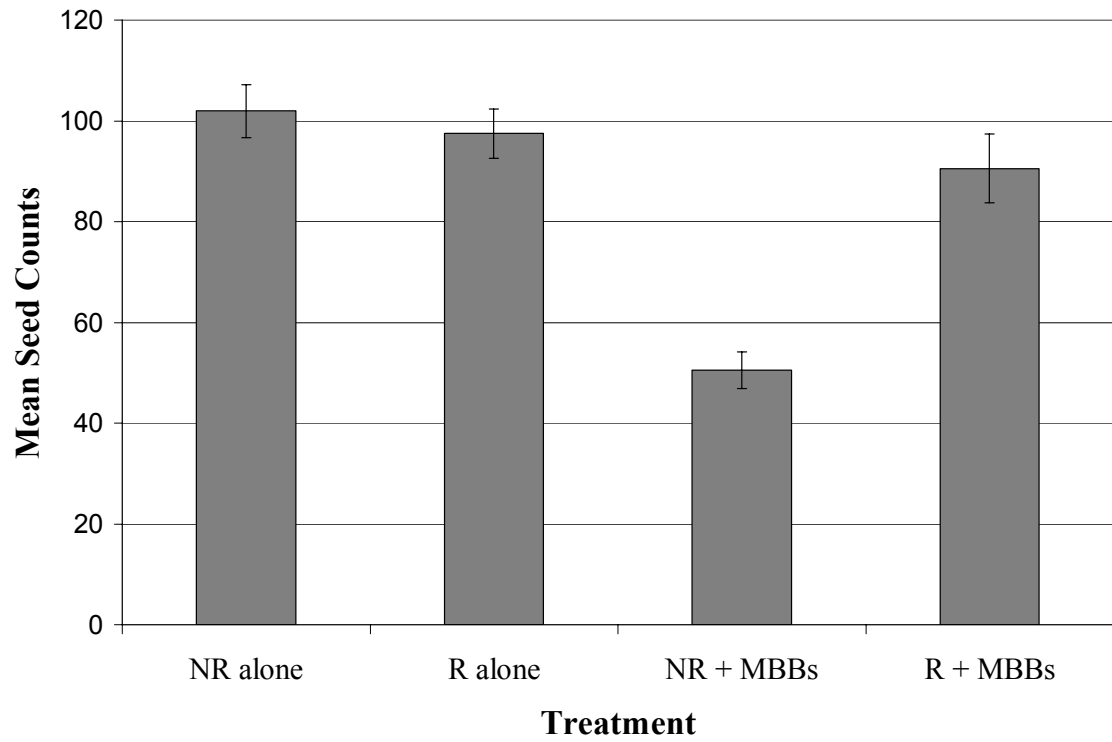


Figure 3: Seed counts per plant (mean +/- s.e.) for non-resistant (NR) and resistant (R) soybeans grown in the absence and presence of Mexican bean beetle (MBB) herbivores in Experiment 1.

interaction. Since the block effect was not significant ($F_{1,23}=0.0396$, $p=0.844$), I omitted it from the model. In the simplified model, genotype ($F_{1,24}=7.177$, $p=.013$), herbivores ($F_{1,24}=20.190$, $p<.001$), and the interaction between genotype and herbivores ($F_{1,24}=13.569$, $p=.001$) all significantly influenced seed count. The significance of this interaction term demonstrated the reduction in seed set for NR plants over R plants with herbivores present was significant.

3.3.2 Experiment 2

Resistant plants received significantly less damage from herbivores than did non-resistant ones ($G=41.100$, $d.f.=1$, $p<.001$), and predators reduced overall herbivore damage ($G=10.767$, $d.f.=1$, $p<.001$), especially for the non-resistant plants (Fig. 4). The interactive effect of predators and resistance on damage was also significant ($G=65.926$, $d.f.=3$, $p<.001$). These reductions in damage translated directly into increased fitness (seed counts). Thus, resistant plants had higher seed counts than non-resistant ones, and predators had a positive effect on plant fitness, especially for the non-resistant plants (Fig. 5). In my ANOVA testing for the interactive effect of plant defense and predators on plant fitness, the block effect was not significant ($F_{3,73}=0.553$, $p=0.648$), thus I omitted it from the model. In the simplified model both genotype ($F_{1,76}=228.298$, $p<.001$) and predators ($F_{1,76}=30.325$, $p<.001$) significantly influenced seed count. Additionally, the interaction between genotype and predators ($F_{1,76}=6.69$, $p=.001$) significantly influenced

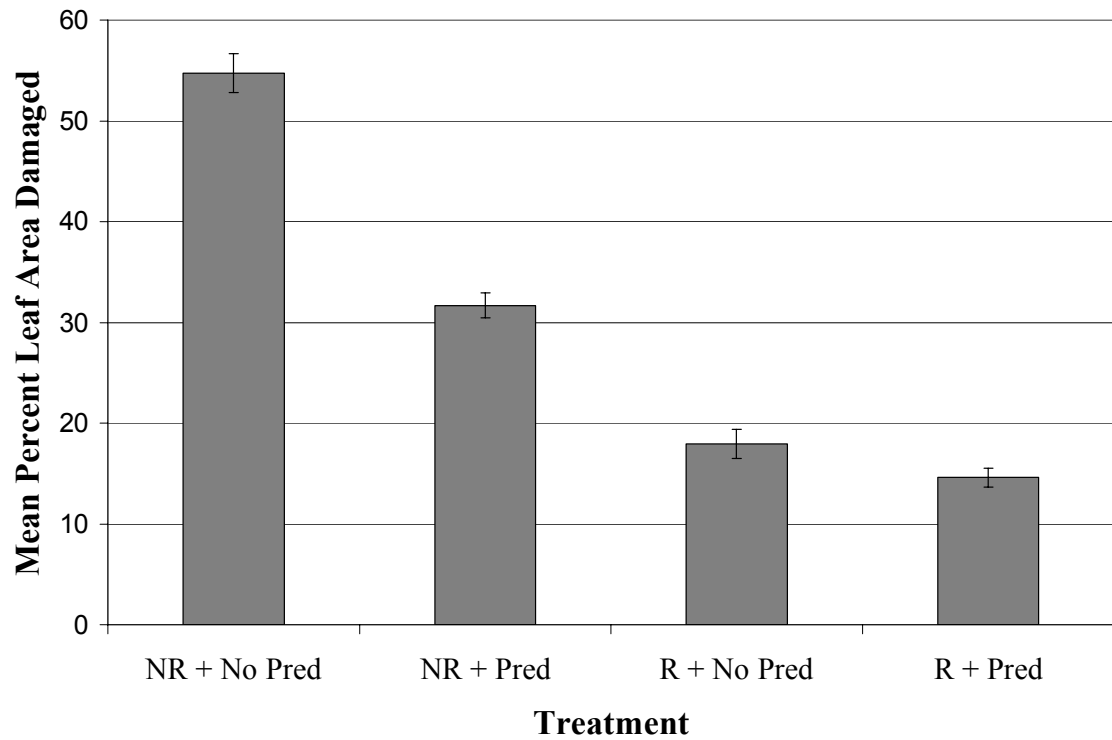


Figure 4: Percent leaf area damaged (mean +/- s.e.) by MBBs in each treatment in Experiment 2. A damage index was visually estimated for each plant in each replicate. The midpoint of the damage index range was used as the percent damaged value for each plant, and the mean of the values for all plants in a replicate was used as the value for that replicate.

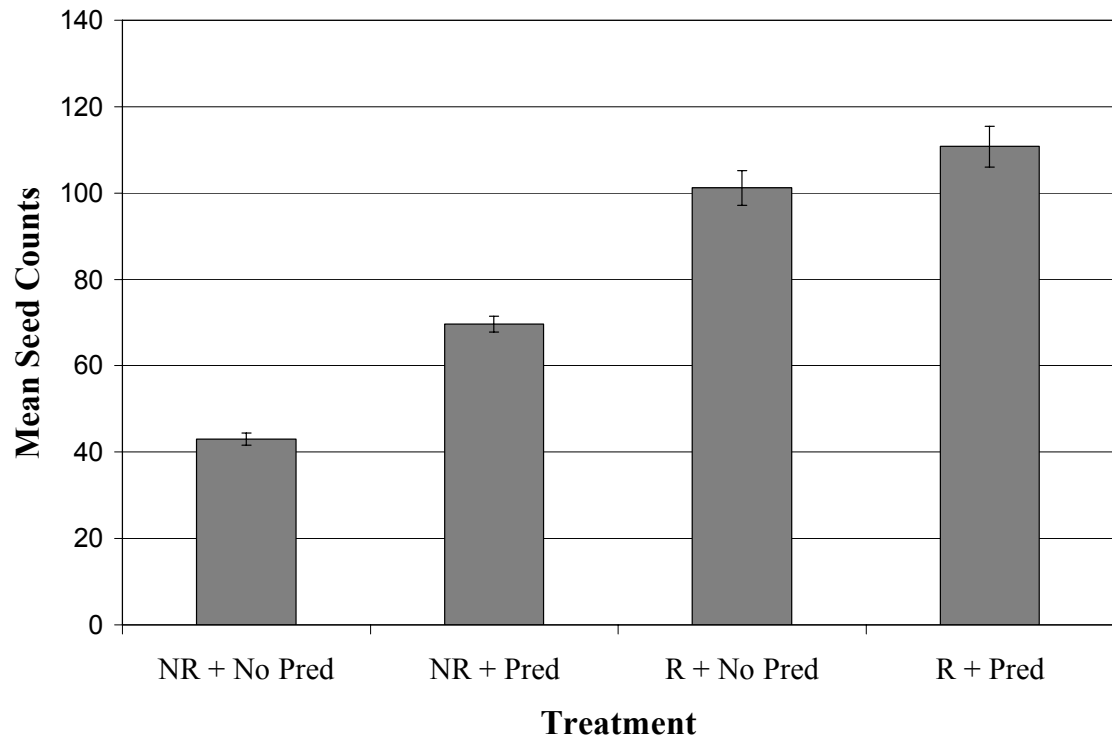


Figure 5: Seed counts per plant (mean +/- s.e.) for non-resistant (NR) and resistant (R) soybeans (both with MBBs) either with (Pred) or without (No Pred) predator additions in Experiment 2.

seed count, demonstrating the negative interaction between predators and plant resistance was significant. A Welch Modified Two-Sample t-Test verified that R plants had significantly higher seed set than NR plants in the absence of predators ($t = 13.765$, $df = 23.957$, $p < .001$), showing a direct positive effect of resistance on plant fitness.

A greater proportion of spined soldier bugs was observed feeding on Mexican bean beetles when the beetles were feeding on non-resistant plants compared to when beetles were feeding on resistant plants (Fig. 6). This result was consistent over the full duration of the predator addition. A two-sample test for equality of proportions suggested that this increased rate of predation on herbivores eating NR plants over herbivores eating R plants was significant over all three observation periods ($p = .01$, $p < .01$, and $p = .06$ for days one, two, and three respectively). This confirmed a negative interaction between predators and resistance.

3.4 Discussion

My experiments demonstrate that predatory spined soldier bugs had higher feeding rates on Mexican bean beetles that were feeding on non-resistant plants than on beetles that were feeding on resistant plants, and illustrate a negative interaction between the effects of plant defenses and predators on plant fitness. Unlike all previous studies I show that the effect of defense on predators does translate to a difference in the fitness benefit resistant and non-resistant plants receive from predators. Fig. 5 illustrates that when plant resistance is high, predators such as spined soldier bugs may be less effective

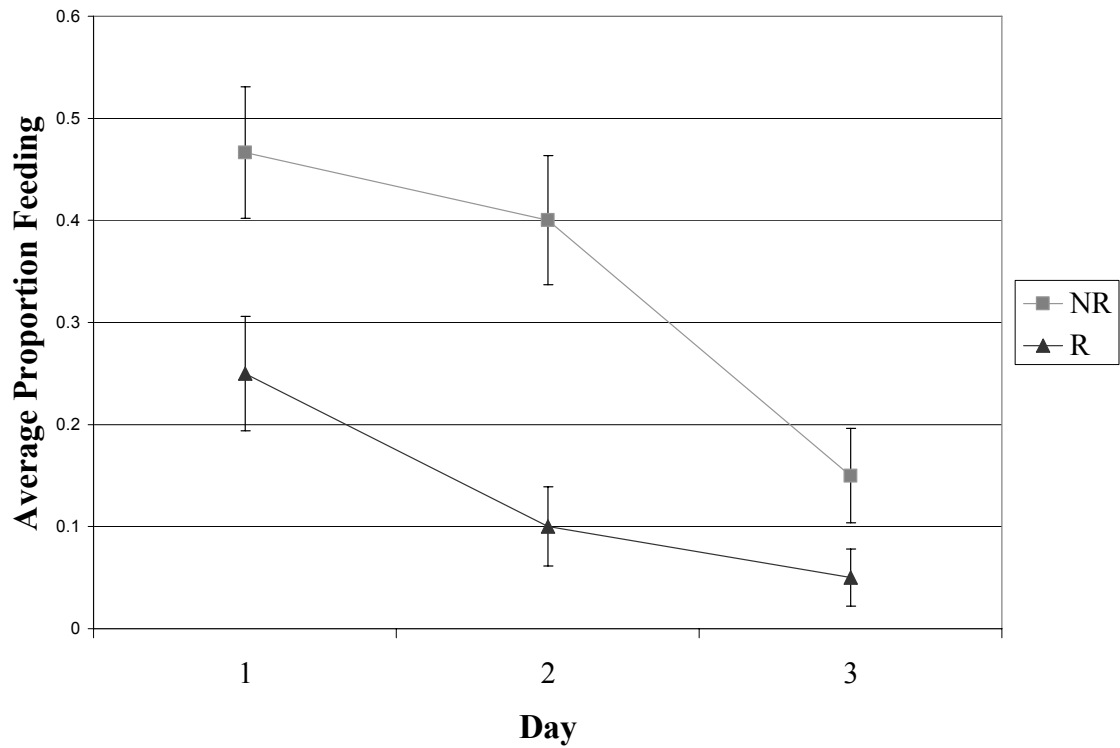


Figure 6: Average proportion (+/- s.e.) of spined soldier bugs feeding on Mexican bean beetles caged on non-resistant (NR) vs. resistant (R) plants during 3 observation periods in Experiment 2.

in increasing plant fitness through predation on herbivores such as Mexican bean beetles. This difference suggests that there would be stronger selection for the production of at least some types of chemical resistance in plants in the absence of predators than in their presence. Fig. 6 implies the difference in fitness effects between the 2 genotypes was due to higher attack rates of spined soldier bugs on Mexican bean beetles on non-resistant plants than on resistant plants.

There are several potential explanations for the higher attack rates of spined soldier bugs shown in Fig. 6. One is lowered beetle palatability caused by plant defensive chemicals. Reduced palatability could be due to plant chemicals being sequestered by the herbivore for its own defense, changes in hemolymph composition due to unavoidable uptake of compounds from the midgut, or chemicals that are simply in transit through the gut. Reduction in attack rates by predators due to plant chemicals in herbivores has been noted in a variety of systems, with one classical example being the work of Brower et al. (1967) on monarch butterflies (*Danaus plexipus*) feeding on milkweed and milkweed relatives. They found that the attack rates of jays on monarchs were negatively correlated with cardenolide content in the plants on which the monarchs were feeding. Similar examples can be found in Eisner (1970), Dyer (1995), and Sipura (2002), who found that ant predators preferred herbivores that had eaten less chemically defended plants. This explanation seems very plausible in my system given the feeding mechanism of spined soldier bugs. The bugs feed by sucking hemolymph from live prey, thus it seems very likely that they would be exposed to chemicals eaten by their prey that

do not immediately break down. Since the soldier bugs were lab reared and had not encountered Mexican bean beetles that had fed on soybeans prior to the experiment, it is unlikely that the soldier bugs had developed any preference for bean beetles raised on either NR or R soybeans prior to the start of the experiment. Soldier bugs in the field could potentially encounter bean beetles feeding on multiple soybean varieties, and thus might learn to avoid bean beetles on chemically defended varieties. This could lead to an even stronger difference in the feeding rates of soldier bugs than I saw in my caged experiments.

Changes in the behavior of the Mexican bean beetles themselves in response to plant defenses could also explain the results seen in Fig. 6. For example, chemical defenses could have caused the beetles to feed on a different part of the plant or to move less than they would otherwise. This might have lead to the beetles on the resistant plants being less easy for the soldier bugs to find. However, I did not observe different movement behavior of the beetles on the resistant plants over the course of the experiment (R. Bartlett, personal observation).

It is important to note that this study looked only at a constitutive chemical resistance to herbivores. Many plants, including soybeans, also exhibit induced resistance that is expressed only after herbivore damage occurs (Karban and Baldwin 1997). With an inducible chemical defense, the herbivore might not have induced any plant resistance factors at the time the predator attacks, which may mean that the predator won't encounter the plant resistance factor. The timing of defense induction is thus a

potentially important factor in determining the interaction between plant resistance and predators, as it has already been shown to be important in the dynamics of soybean and its herbivores (Underwood 1999; Underwood and Rausher 2000). A plant that induces defense early after the initial herbivore damage would likely cause the herbivore to be exposed to that resistance before the predator attacks. However, a plant with late defense induction relative to the start of herbivore feeding may not expose the herbivore to the resistance factors prior the predator attack.

In my study there was a negative interaction between plant defenses and predators. If predator attacks are rare, this would not be of great detriment to the plants because the potential fitness benefit from predators would be small. However, if predation rates are higher, selection would be stronger for the plant to maximize the benefit from predators. Thus, the plant would be selected to produce either less defense or defense that did not interfere with the predator as much. Likewise, predators that often attack herbivores on defended plants would be selected towards methods of dealing with those defenses.

The outcome of this experiment also sheds some light on the likely success of predators for biological control of herbivores in soybeans and other economically important plants. The increased fitness benefit from predators for NR over R plants suggests that predator introductions would be much more effective on plants without a strong constitutive chemical resistance to herbivores. The overall effectiveness of resistance in increasing seed set relative to the overall success of predators also suggests

that breeding soybeans for insect resistance may be much more beneficial than predator introductions. There did not appear to be a significant cost of resistance to plant fitness in my caged experiments. Fig. 3 shows that there was no significant difference in the seed set of NR and R plants in the absence of herbivores. However, this cost may be more evident in plants grown in more stressful field conditions.

4. Timing of resistance changes interactions between plant chemical defenses and predators in soybeans

4.1 Introduction

Many plants possess defensive traits such as secondary chemical compounds that negatively affect herbivores, and thus enhance the fitness of plants that possess them. Plants can also benefit indirectly from the activities of predators through reduced herbivory. However, defensive traits will often affect the magnitude of indirect benefits plants receive from predators. Numerous studies have found that plant defensive traits may enhance the indirect benefits plants receive from predators (Alborn et al. 1997; Gomez and Zamora 1994; Havill and Raffa 2000; Kalule and Wright 2002b; Mantyla et al. 2004; Ninkovic et al. 2001; Osier et al. 1996; Price et al. 1980; Rank and Smiley 1994; Romeis et al. 2005; Starks et al. 1972; Thaler 1999; Turlings and Benrey 1998). Various other studies have found that plant defensive traits may detract from those benefits (Agrawal et al. 2002; Bottrell et al. 1998; Brower et al. 1967; Campbell and Duffy 1979; Denno et al. 2002; Dover et al. 1987; Dyer 1995; Eisner 1970; Fuentes-Contreras et al. 1998; Gassmann and Hare 2005; Harvey et al. 2005; Havill and Raffa 2000; Hufbauer 2001; Kauffman and Flanders 1985; Ode et al. 2004; Orr and Boethel 1986; Price et al. 1980; Rank and Smiley 1994; Reitz and Trumble 1996; Romeis et al. 2005; Sipura 2002; Turlings and Benrey 1998). Much of this variation in observed

outcomes may be due to variation in characteristics of the specific organisms involved in the interaction.

One such characteristic is the type of herbivore resistance exhibited by the plant. Plants may exhibit chemical resistance to herbivores that is either constitutive or inducible, and these different resistance types have often been shown to contrast in their short- and long-term effects on herbivores in the absence of predators (Karban and Baldwin 1997; Underwood and Rausher 2002). In constitutive resistance to herbivore damage, the defenses are always expressed in the plant, while induced resistance is a response to herbivore damage that reduces herbivore survival, reproductive output, or preference for a plant (Karban and Baldwin 1997). Constitutive and induced resistances are likely to differ in the degree to which the herbivore will have been exposed to the defense compounds before the predator attacks. With constitutive defense, even if the predator attacks soon after the herbivore begins feeding, the herbivore will likely have been exposed to the resistance factors. With an inducible chemical defense, the herbivore might not have induced any plant resistance factors at the time the predator attacks, which may mean that the predator won't encounter the plant resistance factor.

The timing of defense induction is thus a potentially important factor in determining the interaction between plant resistance and predators, and it has already been shown to be important in the dynamics of plants and herbivores (Underwood 1999; Underwood and Rausher 2000). A plant that induces defense early after herbivore feeding begins may cause the herbivore to be exposed to that resistance before the

predator attacks. However, a plant that induces relatively late after herbivore feeding begins may mean that the herbivore has not experienced the resistance factors when the predator attacks.

I have previously demonstrated a negative interaction between the effects of plant defenses and predators on plant fitness in a system of soybeans (*Glycine max*) with strong constitutive defense, herbivorous Mexican bean beetles (*Epilachna varivestis*), and predaceous spined soldier bugs (*Podisus maculiventris*) (Bartlett in review). Here, I report the effect of predatory spined soldier bugs on the fitness of two additional soybean genotypes that exhibit induced herbivore defense that peaks at different times after feeding by Mexican bean beetles begins. Previous studies of the effects of plant defenses on predators in similar soybean systems (tritrophic systems involving soybeans and at least one of the two insects I used) have shown positive (Price et al. 1980), negative (Kauffman and Flanders 1985; Orr and Boethel 1986), or no effects (Orr and Boethel 1986) of defenses on predators. Some of this unexplained variation may be due to differences in the timing of defense in the soybeans used, as this was not quantified in any of those studies.

4.2 Materials and methods

4.2.1 Experimental organisms

Soybeans (*Glycine max*) have well-characterized, closely related genotypes that differ in both the strength and type of resistance. Chemical resistance factors in soybeans

include phenolics (such as isoflavonoids, pterocarpan, and coumestans), peroxidases, proteinase inhibitors, phytoalexins, and many others (Kogan and Fischer 1991). Some of these resistance factors are constitutive defenses (such as isoflavonoids) while others are induced defenses (such as phytoalexins). I focused on three closely related soybean genotypes (obtained from Dr. R. Boerma, Univ. of Georgia) that differed in chemical resistance characters but were morphologically indistinguishable (Boerma and Walker 2005; Narvel et al. 2001; Walker et al. 2004). The first genotype possessed the H and M resistance QTLs (quantitative trait loci), which confer a strong constitutive defense against Mexican bean beetles; I refer to it as the constitutively resistant (CR) genotype. In a previous choice test experiment (Bartlett 2006, Chapter 1), Mexican bean beetles did not show any preference for previously undamaged CR soybeans over previously damaged CR soybeans up to 24 days after damage, demonstrating that the combined resistance conferred by the H and M resistance QTLs is constitutive. A genotype possessing only the H resistance QTL peaked in resistance by 10 days after damage; I refer to it as the early induced (EI) genotype. A genotype possessing only the M resistance QTL peaked in resistance 24 days after damage; I refer to it as the late induced (LI) genotype. Soybean genotypes with either the H or M resistance QTLs are known to be resistant to Mexican bean beetles when compared to non-resistant soybean genotypes, although the exact chemical basis for this resistance is unknown. For information on the origin of these genotypes, refer to Narvel et al. (2001).

Mexican bean beetles (*Epilachna varivestis*) are specialists on legumes and are widespread economic pests of soybeans. These beetles feed by scraping leaf tissues, so it is easy to quantify damage as percent leaf area scraped. Beetles used in this experiment were obtained from a laboratory colony reared on pinto beans (*Phaseolus vulgaris*).

The spined soldier bug (*Podisus maculiventris*) is a common generalist predator that attacks a variety of soft-bodied insects. These bugs regularly attack third and larger larval instars and pupae of Mexican bean beetles and are considered a beneficial insect regularly found on soybeans (Deitz et al. 1976). Soldier bugs used in this experiment were obtained from the commercial supplier Arizona Biological Control, Inc.

4.2.2 Experimental design

4.2.2.1 Experiment 1

I designed Experiment 1 to determine if the CR and LI genotypes differ in the interaction between predatory spined soldier bugs and chemical plant defenses. The experiment was carried out at the Duke University Zoology Department field station in Durham County, North Carolina from May to October 2004. It was a 2x2 factorial experiment with the following four treatment combinations:

1. CR soybeans exposed to Mexican bean beetles only
2. LI soybeans exposed to Mexican bean beetles only
3. CR soybeans exposed to both Mexican bean beetles and spined soldier bugs
4. LI soybeans exposed to both Mexican bean beetles and spined soldier bugs

Each treatment combination had a total of twenty replicates. Each replicate included 4 soybean plants in 3.8 L pots enclosed in a 2.25 m² by 2 m tall cage (2 plants per pot, 2 pots per cage). The cages consisted of a wooden frame covered with black fiberglass porch screening. Replicates were equally divided among 4 spatial blocks and treatments were randomly assigned within each block.

When the plants had expanded their 4th true leaf, I added 5 1st instar Mexican bean beetles to each plant in the appropriate cages. I added 3 spined soldier bugs to the appropriate cages when the Mexican bean beetles had reached their third instar. Mexican bean beetle and spined soldier bug densities were chosen to reflect typical densities and damage levels seen in the field. The spined soldier bugs were left in the cages for 72 hours and then removed. While the spined soldier bugs were in the cages, I counted once per day the number of bugs in each cage that were actively feeding on Mexican bean beetles. Twice each week, I visually estimated a “damage index” for each replicate based on the percent of leaf area scraped by Mexican bean beetle larvae (1=0-5% damaged, 2=5-10%, 3=10-20%, 4=20-40%, 5=40-60%, 6=60-80%, 7=over 80%). I left the Mexican bean beetle larvae in the cages to feed until pupation, and then removed all pupae. At the end of the experiment, I counted the seeds produced by each plant. I used the average number of seeds produced by the 4 plants in each cage as the measure of fitness.

4.2.2.2 Experiment 2

I designed Experiment 2 to determine differences in the interaction between predatory spined soldier bugs and chemical plant defenses in the CR and EI genotypes. I used identical experimental procedures as in Experiment 1 except that I carried out the experiment from May to October 2005 with the following four treatment combinations:

1. CR soybeans exposed to Mexican bean beetles only
2. EI soybeans exposed to Mexican bean beetles only
3. CR soybeans exposed to both Mexican bean beetles and spined soldier bugs
4. EI soybeans exposed to both Mexican bean beetles and spined soldier bugs

4.2.3 Analysis

I performed a 3-way G-test (log-likelihood ratio test) with 2 damage classes (<20% and >20%) to determine the effects of plant defense and predators on percent leaf area damaged. To indicate the interactive function of plant defenses and predators on plant fitness, I performed an ANOVA including main effects of block, genotype, and predators, and a genotype x predator interaction. I employed two-sample tests for equality of proportions (prop.test, MathSoft 1999) with continuity correction (to account for small sample sizes) to test for differences in the proportion of spined soldier bugs observed feeding on Mexican bean beetles when the beetles were feeding on different soybean genotypes. I used S-Plus 2000 software (MathSoft 1999) for all statistical analyses.

4.3 Results

4.3.1 Experiment 1

CR plants received significantly less damage from herbivores than did LI plants ($G=9.748$, $d.f.=1$, $p=.002$), and predators reduced overall herbivore damage ($G=21.743$, $d.f.=1$, $p<.001$), especially for the LI plants (Fig. 7a). The interactive effect of predators and resistance on damage was also significant ($G=36.106$, $d.f.=3$, $p<.001$). These reductions in damage translated directly into increased fitness (seed counts). Thus, CR plants had higher seed counts than LI plants, and predators had a positive effect on plant fitness, especially for LI plants (Fig. 7b). In my ANOVA testing for the interactive effect of plant defenses and predators on plant fitness, the block effect was not significant ($F_{3,73}=2.120$, $p=0.104$), thus I omitted it from the model. In the simplified model, both genotype ($F_{1,76}=59.893$, $p<.001$) and predator treatment ($F_{1,76}=12.040$, $p<.001$) significantly influenced seed count. Additionally, the interaction between genotype and predators ($F_{1,76}=4.282$, $p=.042$) significantly influenced seed count, demonstrating that spined soldier bugs were less effective in increasing plant fitness for soybeans with constitutive resistance to herbivores than those with late induced resistance.

A greater proportion of spined soldier bugs was observed feeding on Mexican bean beetles when the beetles were feeding on LI compared to CR plants (Fig. 8). This result was consistent over the full duration of the predator addition. A two-sample test for equality of proportions suggested that this increased rate of predation on herbivores

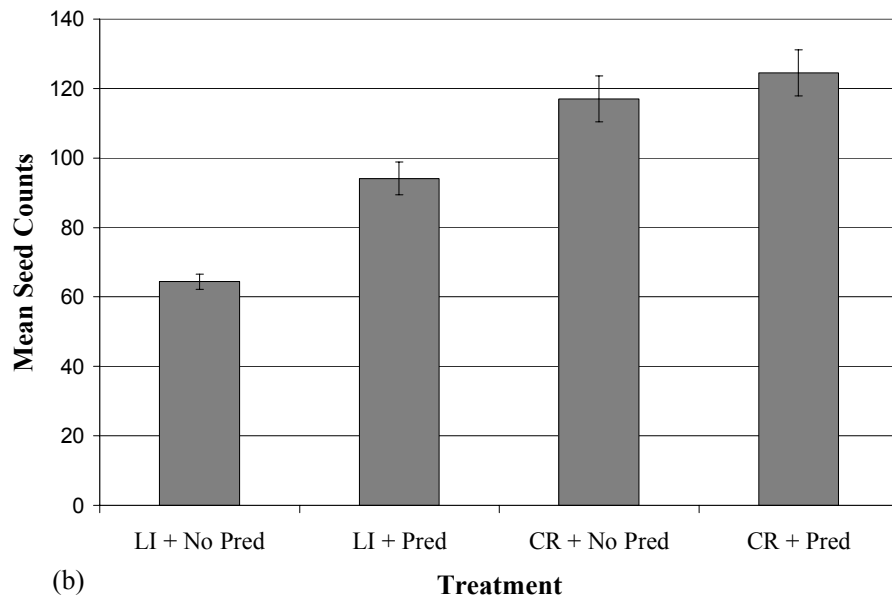
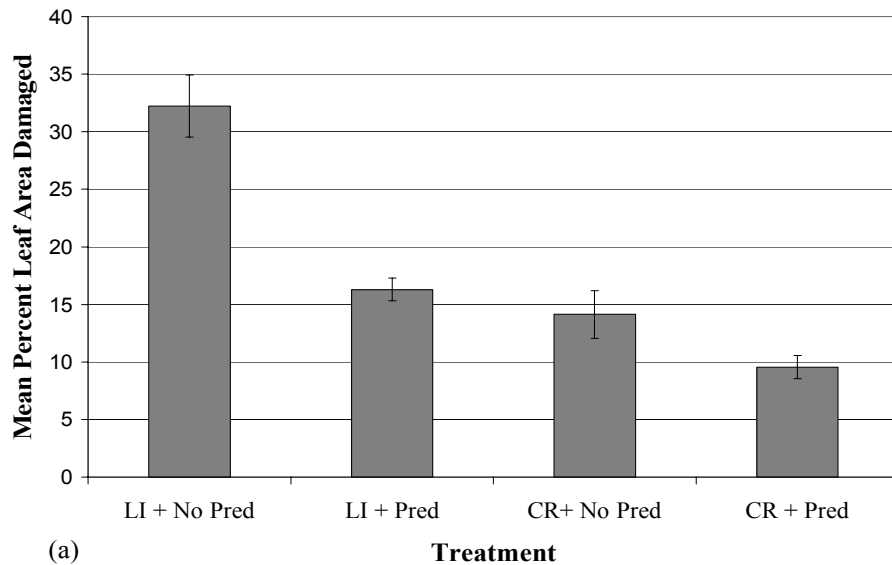


Figure 7: (a) Percent leaf area damaged (mean +/- s.e.) by MBBs in each treatment in Experiment 1. A damage index was visually estimated for each plant in each replicate. The midpoint of the damage index range was used as the percent damaged value for each plant, and the mean of the values for all plants in a replicate was used as the value for that replicate. (b) Seed counts per plant (mean +/- s.e.) for late induced (LI) and constitutively resistant (CR) soybeans (both with MBBs) either with (Pred) or without (No Pred) predator additions in Experiment 1.

eating LI plants over herbivores eating CR plants was significant over the first two observation periods ($p=.001$, $p=.022$ for days one and two). The difference was not significant ($p=.10$) for the third observation period due to the overall lower number of predators still feeding. The increased rate of predation on herbivores eating LI plants over the rate of predation on herbivores eating CR plants again demonstrated that spined soldier bugs were less effective on soybeans with CR resistance than on soybeans with LI resistance.

4.3.2 Experiment 2

CR plants received less damage from herbivores than did EI plants ($G=29.430$, $d.f.=1$, $p<.001$). However, predators did not significantly reduce overall herbivore damage for the CR and EI plants ($G=0.465$, $d.f.=1$, $p=.495$) (Fig. 9a). The interactive effect of predators and resistance on damage was significant ($G=31.592$, $d.f.=3$, $p<.001$). The reductions in damage due to plant genotype translated directly into increased fitness (seed counts). Thus, CR plants had higher seed counts than EI plants, but predators had little effect on plant fitness (Fig. 9b). In an ANOVA investigating the interactive effect of plant defenses and predators on plant fitness, the block effect was not significant ($F_{3,73}=2.105$, $p=0.107$), therefore I omitted it from the model. In the simplified model, genotype ($F_{1,76}=63.760$, $p<.001$) significantly influenced seed count. However, predators ($F_{1,76}=1.864$, $p=.176$) had no significant effect on seed count. Likewise, the interaction between genotype and predators was not significant ($F_{1,76}=0.021$, $p=.884$),

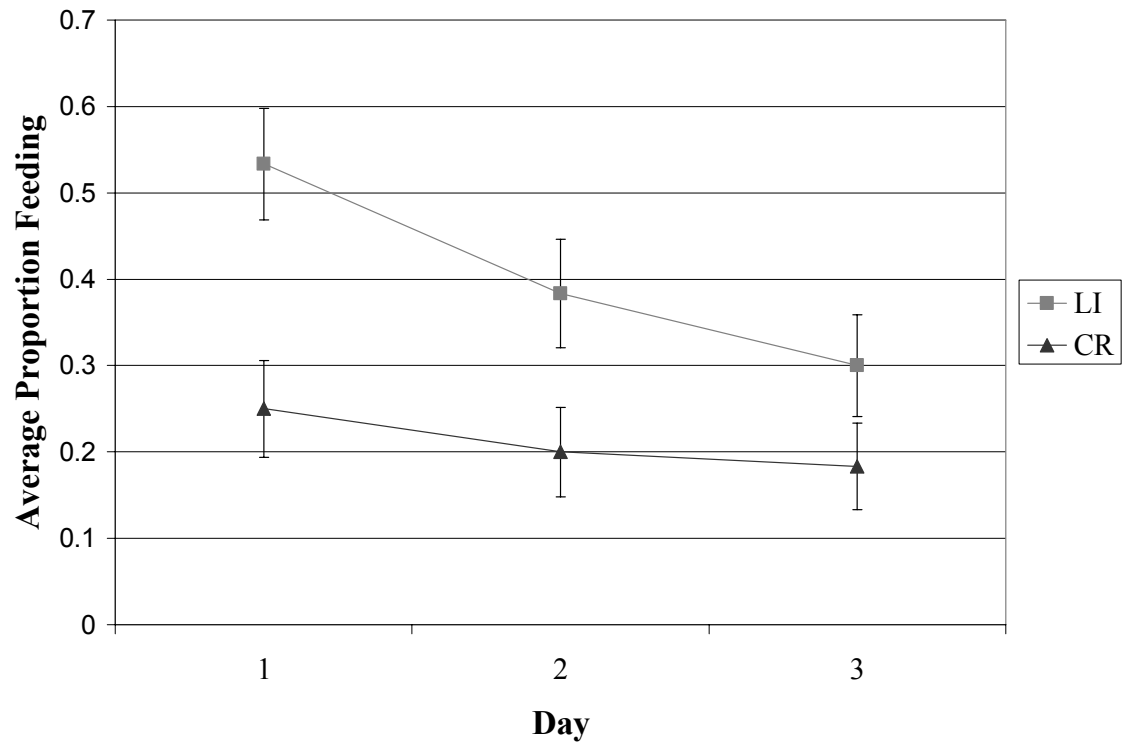


Figure 8: Average proportion (+/- s.e.) of spined soldier bugs feeding on Mexican bean beetles caged on late induced (LI) vs. constitutively resistant (CR) soybeans during 3 observation periods in Experiment 1.

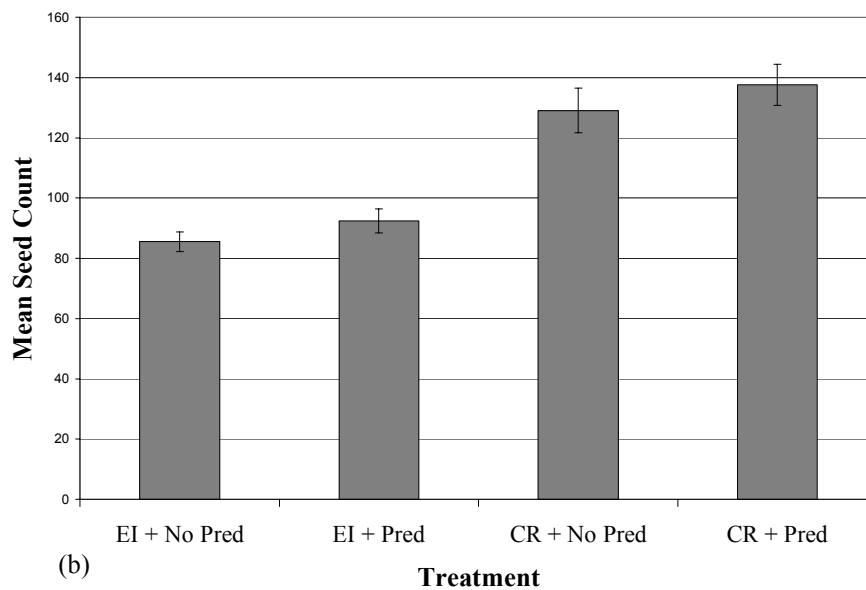
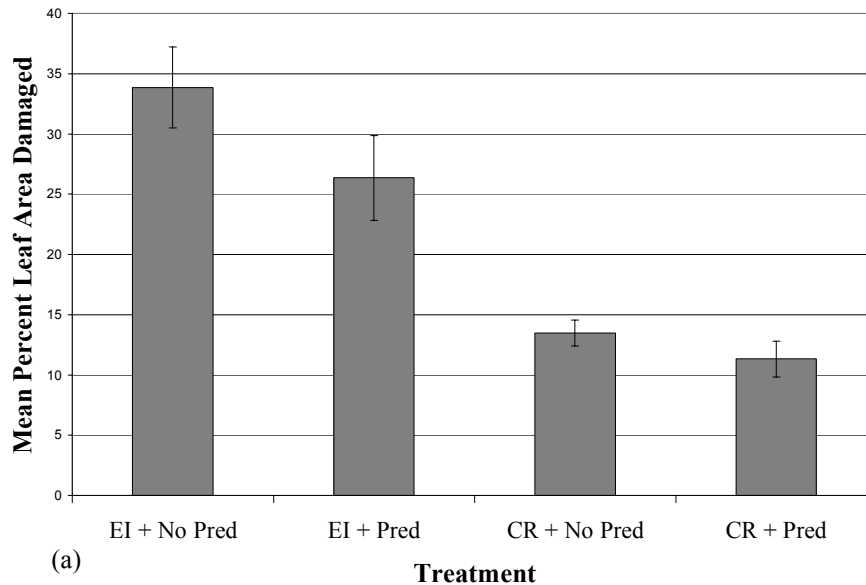


Figure 9: (a) Percent leaf area damaged (mean \pm s.e.) by MBBs in each treatment in Experiment 2. A damage index was visually estimated for each plant in each replicate. The median of the damage index range was used as the percent damaged value for each plant, and the mean of the values for all plants in a replicate was used as the value for that replicate. (b) Seed counts per plant (mean \pm s.e.) for late induced (LI) and constitutively resistant (CR) soybeans (both with MBBs) either with (Pred) or without (No Pred) predator additions in Experiment 2.

demonstrating that spined soldier bugs were ineffective in increasing plant fitness for both soybeans with constitutive resistance and soybeans with early induced resistance. Although the soldier bugs did have an effect on damage for the early induced genotype, this effect was not strong enough to translate into a fitness difference.

Similar proportions of spined soldier bugs were observed feeding on Mexican bean beetles when the beetles were feeding on EI plants compared to when beetles were feeding on CR plants (Fig. 10). This result was consistent over the full duration of the predator addition. A two-sample test for equality of proportions verified that the rates of predation on herbivores eating EI plants and herbivores eating CR plants were not significantly different for any of the three observation periods ($p=.218$, $p=.207$, and $p=.405$ for days one, two, and three respectively). The lack of difference in predation rates on herbivores eating EI plants and herbivores eating CR plants again demonstrated that spined soldier bugs were not significantly more effective on soybeans with EI resistance than on soybeans with CR resistance.

4.4 Discussion

My experiments show that predatory spined soldier bugs had higher feeding rates on Mexican bean beetles that were feeding on plants with late induced resistance than on beetles that were feeding on constitutively resistant plants. However, there was no significant difference between the soldier bug feeding rates on beetles that fed on plants with early induced resistance or constitutive resistance. Thus, early induced resistance

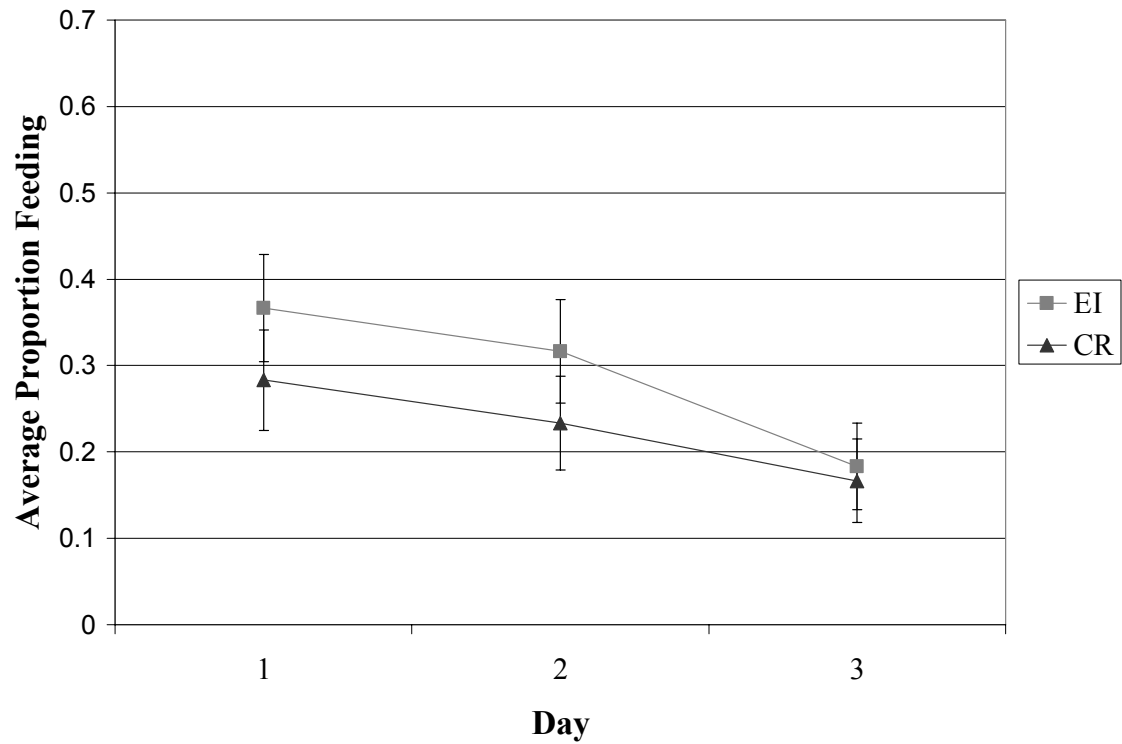


Figure 10: Average proportion (+/- s.e.) of spined soldier bugs feeding on Mexican bean beetles caged on early induced (EI) vs. constitutively resistant (CR) soybeans during 3 observation periods in Experiment 2.

negatively affected predators in a similar manner to constitutive resistance, while late induced resistance had less effect on predators, similar to plants with no insect resistance (Bartlett in review). These effects on predators also translated into fitness effects for the plants. Thus, LI plants received a fitness benefit from predator additions (Fig. 7b) similar to that previously seen for non-resistant plants (Bartlett in review), while EI plants did not receive a fitness benefit from predator additions (Fig. 9b) similar to CR plants. that spined soldier bugs were not significantly more effective on soybeans with EI resistance than on soybeans with CR resistance.

4.4 Discussion

My experiments show that predatory spined soldier bugs had higher feeding rates on Mexican bean beetles that were feeding on plants with late induced resistance than on beetles that were feeding on constitutively resistant plants. However, there was no significant difference between the soldier bug feeding rates on beetles that fed on plants with early induced resistance or constitutive resistance. Thus, early induced resistance negatively affected predators in a similar manner to constitutive resistance, while late induced resistance had less effect on predators, similar to plants with no insect resistance (Bartlett in review). These effects on predators also translated into fitness effects for the plants. Thus, LI plants received a fitness benefit from predator additions (Fig. 7b) similar to that previously seen for non-resistant plants (Bartlett in review), while EI plants did not receive a fitness benefit from predator additions (Fig. 9b) similar to CR plants.

There are several mechanisms that might explain this negative interaction between spined soldier bugs and resistance that was observed in the CR and EI soybeans but absent in the LI soybeans. One is lowered beetle palatability caused by plant defensive chemicals, as discussed in Bartlett (in review). Reduced predator attack rates due to plant chemicals present within the herbivores have been described in a diversity of systems (i.e. Brower et al. 1967; Dyer 1995; Eisner 1970; Sipura 2002). For my constitutively resistant plants or plants that induced defense early after herbivore attack, it is likely that the herbivore would have been exposed to the chemical resistance factors prior to the predator attack. Thus, the predators may have been repelled by plant resistance factors present in the herbivore. For my plants that induced resistance late, the herbivores were not exposed to peak resistance levels prior to the three days of predator attacks shown in Fig. 8. Therefore, it is possible that these herbivores had relatively lower levels of plant resistance factors present in their bodies, which lead to relatively higher predator attack rates.

Modified behavior by Mexican bean beetles in response to the differing types of plant defenses could also explain the negative interaction between predators and plant resistance that was present in the CR and EI genotypes but absent in the LI genotype. For example, CR and EI resistance could have caused the beetles to feed on a different part of the plant or to move less (and thus encounter the predators less) than they did on LI plants. However, the beetles appeared to display similar feeding and movement behavior

on all of the plant genotypes over the course of the study (R. Bartlett, personal observation).

The spined soldier bugs I used were lab reared and had not encountered Mexican bean beetles that had fed on soybeans prior to the experiment. Thus, it is unlikely that the soldier bugs had developed any preference for bean beetles raised on either EI, LI, or CR soybeans prior to the start of the experiment. Soldier bugs in the field could possibly encounter bean beetles feeding on multiple soybean varieties, and therefore may learn to avoid bean beetles that have consumed chemically defended varieties. This could lead to an even stronger difference in the feeding rates of soldier bugs than I observed in my caged experiments.

My results present several implications for the evolution of chemical defenses in plants and the responses of predators to those defenses. In my study plants with late induced resistance received greater fitness benefits from predators than plants with either early induced resistance or constitutive resistance. If this effect translates to other systems, plants with potentially high predation rates on their herbivores over a discrete period of time could be favored to produce chemical resistance after the predator attack window. This selection towards less interference of plant defenses with predators would increase as the potential fitness benefit from predators increased relative to the potential benefits from earlier defense. If the cost of producing induced resistance is low (relative to producing constitutive resistance) and the potential benefits from predation high, plants would be favored even more to produce induced resistance that does not interfere with

predators. This interaction with predators is a potential explanation for the evolution of induced resistance in some plants. Likewise, predators that are negatively affected by plant resistance factors would be favored to time their attacks to avoid peaks in plant resistance that they encounter often. Predators would also undergo selection towards better detection of plant defenses, as well as methods of dealing with those defenses. This could lead to the type of positive interaction between predators and inducible plant defense described by Thaler (1999), in which parasitoids were attracted by an induced plant defense and were more likely to attack herbivores on plants with induced defense than herbivores on plants without induced defense. The scenarios of selection I described above are unlikely to occur in soybeans, as they are not free to evolve in the wild, but could occur in other plants that produce herbivore defenses.

My study also gives an indication of the likelihood of success for predator introductions for biocontrol of pest insects on crop plants of different resistance types. The increased fitness benefits of predators for LI plants over EI and CR plants suggests that crops with late induced resistance would receive more benefit from predator introductions than would crops with constitutive resistance or early induced resistance. The overall effectiveness of constitutive resistance in both experiments also suggests that breeding crop plants for a strong constitutive resistance may be more beneficial than predator introductions. However, there may be a fitness cost to such resistance for plants grown under field conditions.

While this study investigated different types of plant resistance, there is a range of other organism characteristics common across many systems that could also affect the magnitude and direction of the interaction between predators and plant defenses. For example, the degree of specialization in feeding of the predator could affect this interaction. In my study I used a generalist predator that feeds on a wide variety of insects. More specialized predators may be better adapted to deal with plant chemicals, and there is some evidence of this in other systems (Dyer 1995). Likewise, more specialized herbivores may be better at using plant chemicals for their own defense than less specialized herbivores. Prior knowledge of how all of these characteristics influence the interaction between predators and resistance would give a useful picture of how effective predators may be when introduced into a system with plants that are resistant to herbivores, as well as the likely selective pressures on all of the organisms involved.

5. The effects of neighbors on the strength and timing of optimum levels of defense in plants

5.1 Introduction

Plants possess a wide variety of defenses against herbivores. These defenses can vary broadly in the strength of their effects on herbivores, as well as the timing of those effects. Defenses against herbivore damage can be constitutive, always expressed in the plant, or they can be induced, produced only in response to herbivore damage (Karban and Baldwin 1997). Plants may also pay a cost for the production of herbivore defense of either type in terms of decreased fitness.

For any given plant, there should be an optimum timing and strength of herbivore defense based on the cost of that defense and the timing and intensity of herbivore attacks. However, plants generally do not grow in isolation from other plants. Anderson and May (1985; 1990) formalized the concept of “herd immunity” with their description of disease epidemics in humans. Disease epidemics die out quickly and cannot spread when the majority of individuals in a group are immunized or have acquired immunity during past disease outbreaks. Thus, even individuals that are not immune benefit from the immunity of other members of the group. If there is a cost to immunity, an individual may be favored to forgo immunity to optimize its own cost/benefit ratio. Frank (1998) pointed out that the same herd immunity argument also applies to defense induction in other organisms.

Therefore, plants may be favored to produce different amounts and types of herbivore defense when they are growing alone than when they are growing surrounded by conspecific neighbors also producing herbivore defense. If a plant's neighbors are producing large amounts of expensive defense to reduce herbivore attack, that plant may be favored to produce less defense itself and benefit from the lower herbivore attack rate in its neighborhood, thus parasitizing its neighbors' defense investment. In order to reduce the costs of defense, plants may simply produce a smaller amount of defense, or they may produce defense with lower costs, such as inducible defense. Inducible defense may cost less than constitutive defense as plants may pay little or no cost when not under attack (Agrawal and Karban 1999). Inducible defense may also be less effective than constitutive defense, since there is a lag between herbivore attack and the onset of effectual defense. However, if most of the plants in an area adopt a low-defense strategy, an individual plant may outperform its neighbors by producing more defense or more effective defense than they do.

Herd immunity scenarios can be modeled with an invader-resident (also called mutant-resident) system where the resident has a set defense strategy, the invader uses a different strategy, and the invader replaces the resident if the invader has higher fitness. This theoretical approach was used by Poitrineau et al. (2004) to investigate the evolution of the amount of defense and inducibility of defense in a plant-herbivore system. Assuming a fixed probability of herbivore attack, they found that the level of defense generally increased and inducibility decreased as the frequency of herbivore attacks

increased. Induced and constitutive defenses have also been shown to differ in both short- and long-term effects on herbivore population dynamics (Underwood and Rausher 2002). Over the short-term, defense can affect the number or size of herbivores on a single plant. Over the long-term, defense can affect the size of the herbivore population, and thus the likelihood of attack for a plant's progeny (or that same plant in subsequent years). In turn, these effects of defense on herbivores might change the optimal type of defense.

Previously no theoretical model has combined herd immunity effects with the feedback to and from herbivores on the evolution of plant herbivore defense. Both of these effects could simultaneously influence the optimum type and level of defense in a plant. We develop a model in which plants can produce either induced or constitutive herbivore defense and have a probability of being attacked by herbivores that is modified by short- and long-term feedback of plant defenses to and from herbivores. We determine the optimum levels of induced and constitutive defenses for a plant that is growing alone, and then use an invader-resident framework to determine how that optimum changes when a plant has conspecific neighbors. Further, we consider the effects of various factors such as the cost of defense and the relative effectiveness of induced and constitutive defenses on the evolution of optimal herbivore defense for plants both with and without neighbors.

5.2 *Methods*

5.2.1 Overview

Our model evaluates the growth of plants and herbivores in a well-mixed system with no explicit space¹. A single plant has a probability of being attacked in a season that is dependent on the total biomass of herbivores (from all plants) in the system from the previous season. Plants can produce constitutive and induced defense to reduce damage from herbivores, but their total investment in defense is limited. Both types of defense negatively affect herbivore growth and so also act as herbivore resistance (Karban and Baldwin 1997). Herbivore biomass on a single attacked plant grows as a function of the consumption rate of the herbivores on that plant, and total herbivore biomass is proportional to the herbivore biomass on a single attacked plant times the fraction of plants that are attacked. This leads to an optimum level of constitutive and induced defense that is dependent on the herbivore attack rate, which is set by herbivore biomass. We find the optimum level of each defense type (constitutive and induced) for plants growing alone, and then investigate the effects of herd immunity using an invader-resident system in which the optimum is the Convergently Stable Strategy (CSS). The CSS, also known as an Evolutionary Singular Strategy with convergence stability, is the strategy that cannot be successfully invaded by any other strategy (Waxman and Gavrillets 2005). Herbivores receive feedback from plant defense type over a single

¹ Although space in our model is not explicit, we nevertheless assume the plants in the neighborhood affect the local herbivore density experienced by a focal plant.

season since plant defense levels affect herbivore biomass, which in turn affects the amount of herbivore attack on a plant. One year's defense also affects the next year's herbivore numbers, which in turn affect optimal defense.

5.2.2 Model formulation

We begin by considering a simple model in which herbivore attack is initiated at one discrete time in the growing season. An example would be a scenario in which adults oviposit at the start of the season, and then emerging larvae commence feeding. Plants grow at a rate that depends on whether they are attacked or unattacked. Herbivore biomass grows at a rate determined by the changing biomass of attacked plants only. We model the within-season growth of plants and herbivores as follows:

$$\begin{aligned}
 \frac{dP_A}{dt} &= (r - m(1+c)P_A)P_A - \frac{hHP_A}{1 + \rho(\varepsilon(1-d) + d)} \\
 \frac{dP_U}{dt} &= (r - m(1+cd)P_U)P_U \\
 \frac{dH}{dt} &= \frac{\beta hP_A H}{1 + \rho(\varepsilon(1-d) + d)} - \delta H
 \end{aligned} \tag{1}$$

$P_A(t)$, $P_U(t)$ are the biomass of attacked and unattacked plants at time t (the dependence of the state variables on time is suppressed in Eq. (1)). Both attacked and unattacked plants grow at an initial, instantaneous, per-unit-biomass rate r , pay background maintenance costs at a rate m per unit biomass, experience a cost of herbivore defense (measured as a fractional increase in maintenance costs) equal to c , and invest d in constitutive defense. We assume that plants have a total amount of defense equal to 1, which is an arbitrary index, because we use other parameters to scale it to cost and effectiveness. Thus, the

amount of induced defense found in attacked plants is $(1-d)$. Unattacked plants do not have induced defense, so their cost, measured as an increase in maintenance, is only cd (compared to c for attacked plants since they pay the costs of both induced and constitutive defense and $c(d+1-d)=c$). The overall effectiveness of defense is ρ . As ρ increases the reduction in herbivory due to plant defense increases. The relative effectiveness of induced defense (compared to constitutive defense) is ε . Induced defense is considered to be less effective than constitutive defense since there is a lag time between initial herbivore damage and maximum levels of defense. Therefore, ε is always less than 1. The overall reduction in herbivory due to plant defenses is thus a function of d , ρ , and ε . Herbivore biomass, $H(t)$, grows as a function of attacked plant biomass only and measures the total biomass of herbivores on a single plant rather than herbivore number. Herbivore biomass is more relevant to herbivory rates than number of herbivores, since large herbivores can eat more plant than small herbivores. Herbivores feed at a rate h per unit of herbivore biomass. Herbivores convert ingested plant material into new herbivore biomass with an efficiency β . Herbivore biomass dies or is metabolized at rate δ .

To reduce the number of parameters, we define new state variables and parameters: $\tilde{P}_A = \frac{m}{r} P_A$, $\tilde{P}_U = \frac{m}{r} P_U$, $\tilde{H} = \frac{h}{r} H$, $\tilde{t} = rt$, $B = \frac{\beta h}{m}$, and $D = \frac{\delta}{r}$.

Substituting these into Eq. (1), simplifying the result, and dropping the tildes produces the non-dimensionalized model:

$$\begin{aligned}
\frac{dP_A}{dt} &= (1 - (1+c)P_A)P_A - \frac{HP_A}{1 + \rho(\varepsilon(1-d) + d)} \\
\frac{dP_U}{dt} &= (1 - (1+cd)P_U)P_U \\
\frac{dH}{dt} &= \frac{BP_AH}{1 + \rho(\varepsilon(1-d) + d)} - DH
\end{aligned} \tag{2}$$

which has six parameters (c , ρ , ε , d , B , and D). We assume the season is long enough for plant and herbivore biomass to come to equilibrium. We then solve for the equilibrium sizes of attacked and unattacked plants and the equilibrium herbivore biomass per attacked plant at the end of the season, which are given by:

$$\begin{aligned}
\bar{P}_A &= \frac{D(1 + \rho(\varepsilon(1-d) + d))}{B} \\
\bar{P}_U &= \frac{1}{1+cd} \\
\bar{H} &= [1 + \rho(\varepsilon(1-d) + d)] \left[1 - \frac{D}{B} (1 + \rho(\varepsilon(1-d) + d))(1+c) \right]
\end{aligned} \tag{3}$$

We link the within-season dynamics to between-season dynamics as follows. We assume that the fraction of plants that will be attacked next year (f_A') is a saturating function (Fig. 11) of the average biomass of herbivores per plant (including both attacked and unattacked plants) this year (which is $f_A\bar{H}$). Specifically, we use the Michaelis-Menton function

$$f_A' = \frac{f_A\bar{H}}{z + f_A\bar{H}} \tag{4}$$

in which z is the half-saturation constant. We solve this at equilibrium for $f_A = f_A' = \overline{f_A}$ to generate the equilibrium fraction of plants attacked (which is also the probability of one plant being attacked):

$$\overline{f_A} = \frac{\overline{H} - z}{\overline{H}} \quad (5)$$

The parameter z thus relates herbivore biomass to herbivore attack rate. If $\overline{H} < z$, the herbivore is driven to extinction. We assume herbivores can evolve counter-defenses to avoid extinction so we only explore parameter values that yield \overline{H} greater than z . As \overline{H} increases relative to z , the attack rate increases. We assume all plant “sites” are occupied each year regardless of the herbivore attack rate the year before. Our plants are either annuals with annual seed production to fill all sites or perennials with no between-year induced resistance and no effect of last year’s damage on the rate of growth next year. However, we imagine that the fraction of sites taken by different defense types could change over time if the fitnesses of those types differ.

The average fitness (w) of a single plant, taking into account its probability of being attacked, is:

$$w = \overline{f_A} \overline{P_A} + (1 - \overline{f_A}) \overline{P_u} \quad (6)$$

5.2.3 Adding immigration

Thus far we have assumed that the plant and herbivore represent a closed system.

However, we can also allow for outside herbivore biomass coming into the system by

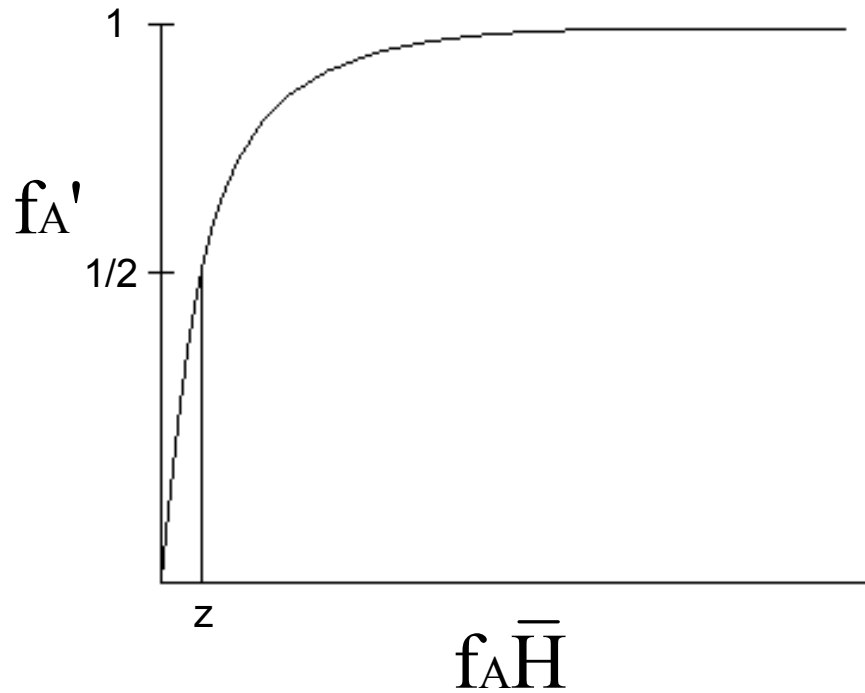


Figure 11: Graphical representation of the relationship between the fraction of plants attacked in a given year (f_A') and the average final herbivore biomass per plant from the previous year. The biomass of herbivores from the previous year is a function of the equilibrium herbivore biomass per attacked plant (\bar{H}) and the fraction of plants attacked (f_A) that year. f_A' is a saturating function of $f_A \bar{H}$ with a half-saturation constant of z .

adding an immigration term. The immigrants might be herbivores that feed on the same plant species but are coming from a different population, or might be generalist herbivores that come from other plant species in the same location (and have their population densities on those other plants set by factors other than defense in the focal plant). This immigration rate, I , increases the herbivore biomass in the system and thus increases the fraction of plants being attacked by herbivores. This modifies the fraction of plants attacked in a given year (Eq. 4) as follows:

$$f_A' = \frac{f_A \bar{H} + I}{z + f_A \bar{H} + I} \quad (7)$$

Again solving at equilibrium for $f_A = f_A' = \bar{f}_A$, the new equilibrium fraction of plants attacked is:

$$\bar{f}_A = -\left(\frac{z + I - \bar{H}}{2\bar{H}}\right) + \sqrt{\frac{(z + I - \bar{H})^2}{4\bar{H}^2} + \frac{I}{\bar{H}}} \quad (8)$$

The calculation for fitness (Eq. 6) remains unchanged.

5.2.4 Model analysis

With this framework, it is relatively simple to determine what the “best” strategy is for a plant growing alone with regard to the fraction of all defense investment allocated to constitutive defense (d) for any given set of parameters. The best strategy is simply the level of d that maximizes fitness (w).

We can also use this model to examine herd immunity effects using invader-resident systems, and thus address the issue of whether the optimum defense is the same

when a plant is growing alone vs. when it has neighbors with their own defenses. Specifically we allow a resident population of plants to set the herbivore population (H) given their investment in constitutive defense (d). A low-frequency mutant may have a different d but would experience the level of H set by the residents. The optimum strategy in this system is the CSS, which gives the plant the highest fitness relative to its neighbors and cannot be invaded by other strategies. This may or may not be the same optimum d as if the plant were growing alone with no neighbors. For all numerical analyses of this model we used MATLAB Version 7 (The MathWorks 2004).

5.3 Results

5.3.1 Determining the CSS

Fig. 12 shows a fitness contour for a default set of parameter values (Table 1) in an invader-resident system. The resident sets the herbivore population size, but herbivores attack both the resident and invader. Contours show the invader's relative fitness expressed as invader fitness divided by resident fitness, given d of the invader and of the resident. The invader succeeds if its relative fitness is greater than 1. The fitness of the invader is always equal to the fitness of the resident when both use the same strategy (the 1:1 line). For some parameter values, including the default parameters, there is a second line where different strategies lead to the same fitness. The location x where this line crosses the 1:1 line is the CSS. This point exhibits convergence stability

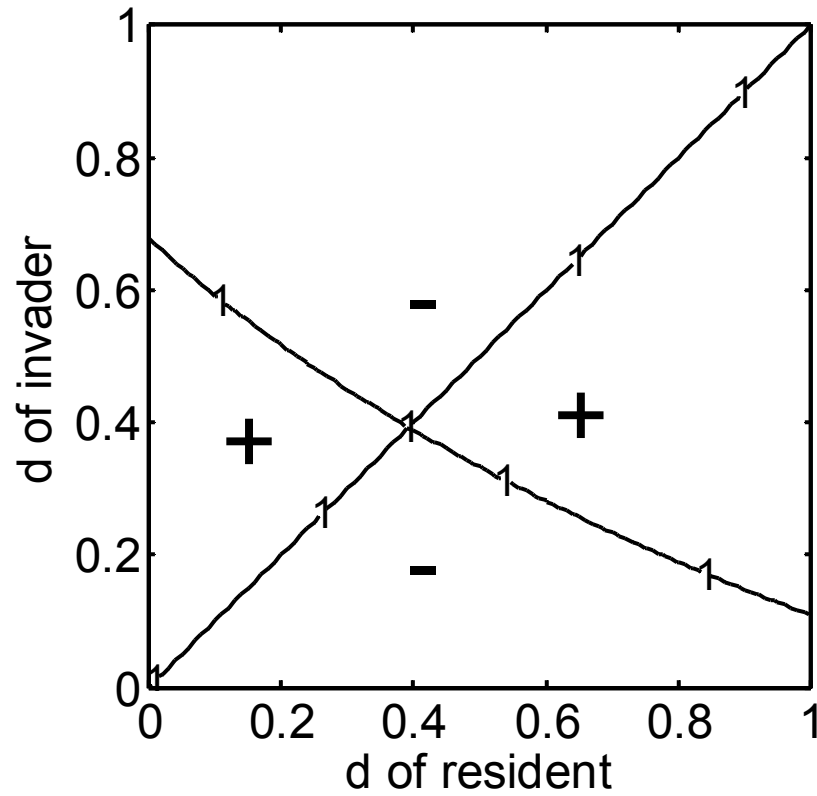


Figure 12: Invasion analysis using default parameter values. The lines shown are the contours of the invader's relative fitness for varying levels of investment in constitutive defense (d) for the resident and invader plants. In regions marked with + the invader's fitness is higher than the resident's fitness, while in regions marked with - the invader's fitness is lower than the resident's fitness. The value of d for the resident that corresponds with the crossing of the two equal fitness lines (labeled with a "1") indicates an Evolutionarily Singular Strategy with convergence stability (also known as a CSS).

Table 1: Default parameter values for all runs unless otherwise noted. The investment in constitutive defense (d) was always varied between 0 and 1 with the amount of induced defense equal to $(1-d)$ since the total defense always summed to 1.

$B = \frac{\beta h}{m}$ and $D = \frac{\delta}{r}$ were parameters created when we non-dimensionalized the model as described above.

Parameter	Default Value
c (cost of defense)	.3
d (amount of investment in constitutive defense)	always varied from 0 to 1
ε (relative effectiveness of induced defense)	.5
ρ (overall effectiveness of defense)	2
B (combination of herbivore conversion rate, herbivory rate, and plant maintenance rate)	.3
D (combination of herbivore death rate and plant growth rate)	.4
z (attack rate constant)	.5
I (immigration rate of herbivores from outside the system)	0

since the resident's phenotype can only be successfully invaded by mutants with a phenotype closer to x than the phenotype of the resident (Waxman and Gavrilets 2005).

5.3.2 Effect of cost of defense

For any given set of parameter values, we can determine the optimum defense strategy (value of d) for a plant that is growing alone and the optimum defense strategy (CSS) for a plant growing with neighbors. Fig. 13 shows the result of this comparison over a range of values for the parameter c (cost of defense), keeping all other parameters at their default values (Table 1). For plants growing alone, low costs of defense lead to completely constitutive defense (the more costly but also more effective type of defense). Higher costs of defense lead to completely inducible defense (the less costly but also less

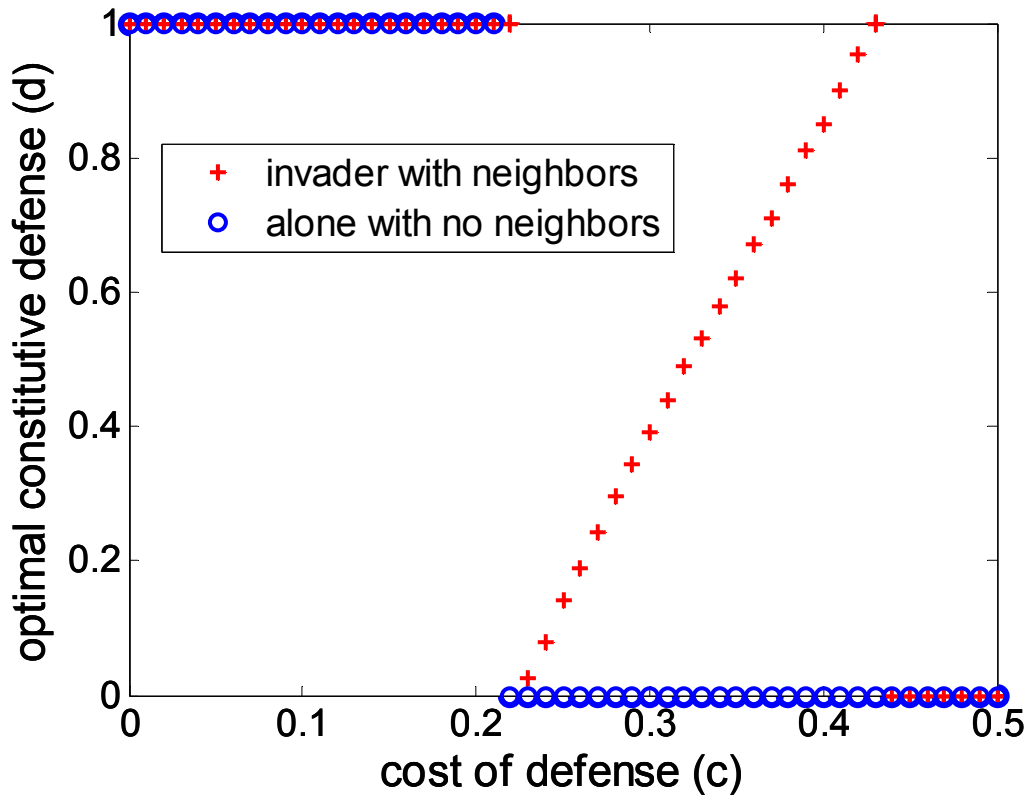


Figure 13: Effects of varying the cost of defense (c) on the optimal level of constitutive defense for both the invader-resident system (crosses) and plants grown alone (open circles). The parameter values were chosen to reflect reasonable values that did not cause the fraction of plants attacked to be negative. A value of 1 for d denotes all constitutive defense, a value of 0 for d denotes all induced defense, and intermediate values denote a mix of the two strategies.

effective type of defense in isolated plants). We see similar results at either end of the invader-resident system. However, for mid-range values of c in the invader-resident system, the best (CSS) strategy is a mix of constitutive and induced defense. As the cost of defense (c) increases, this leads to an increasing optimum level of constitutive defense over this range of c . The increase in the optimum d over this range may at first seem counter-intuitive. This result is due to the fact that increasing the cost of defense decreases the equilibrium size of unattacked plants (Eq. 3b), but does not change the equilibrium size of attacked plants (Eq. 3a). Thus, the size of attacked plants relative to the size of unattacked plants with any $d > 0$ increases with increasing c . At the same time, low levels of constitutive defense lead to high levels of H , which in turn selects for $d > 0$. The difference in relative sizes of attacked and unattacked plants increases the relative importance of the size of attacked plants, especially with high herbivore attack rates, and selects for more constitutive defense with increasing c . This effect is best described by Fig. 14, which demonstrates how the size of attacked plants is increasing and the size of unattacked plants is decreasing while the fraction of plants attacked remains relatively high at the optimum strategy over the range of interest for values of c . Essentially, if herbivore attack is common, plants are selected to increase their size when they are attacked by defending more against herbivores, even if their potential size if they are not attacked decreases.

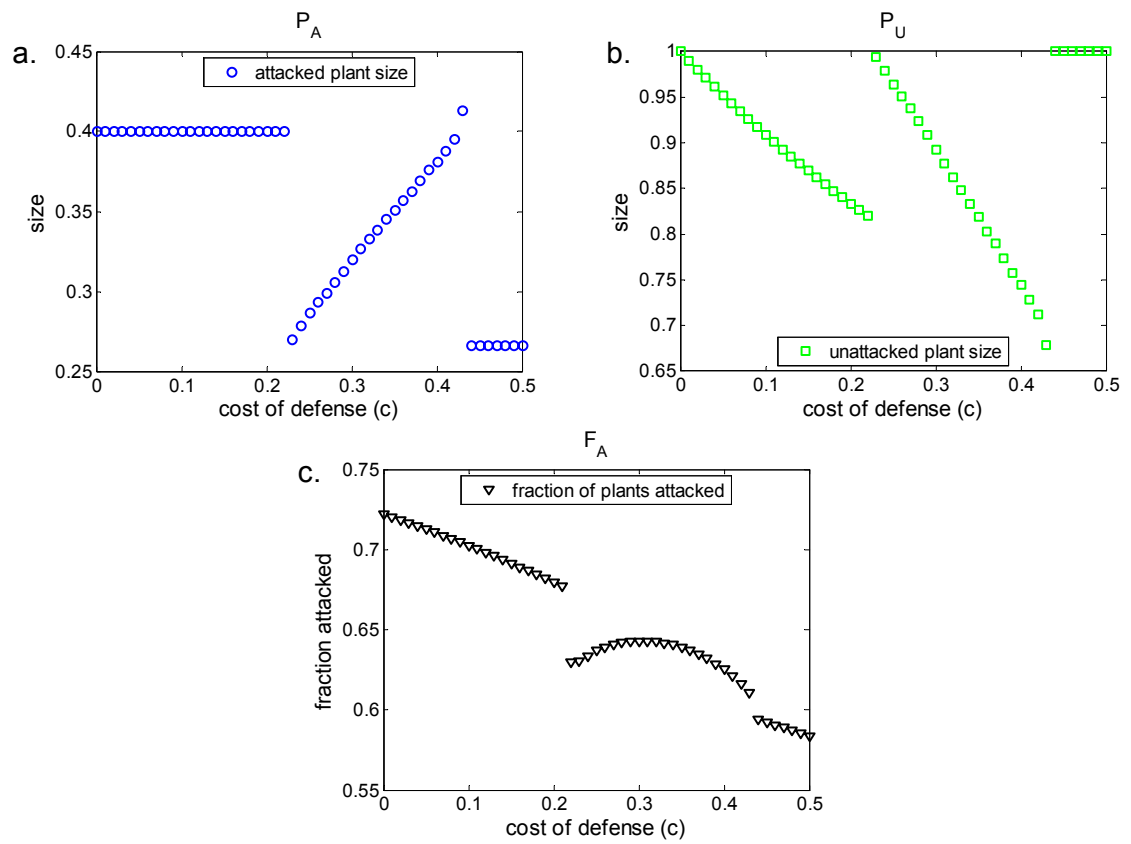


Figure 14: The equilibrium sizes of (a) attacked plants, (b) unattacked plants (b), and (c) the fraction of plants attacked at the CSS level of defense for the invader-resident system shown in Fig. 13.

5.3.3 Effect of relative effectiveness of induced defense

Fig. 15a shows the effect of varying ε , the effectiveness of induced defense relative to the effectiveness of constitutive defense, maintaining all other parameters at their default values (Table 1). For a plant growing alone, regardless of the value of ε with all other parameters at default levels, it is not worth paying the higher cost of constitutive defense, so these plants always exhibit induced defense (constitutive defense was possible with low levels of ε with c lower than default). However, in an invader-resident system, plants that exhibit some level of constitutive defense out-compete their less resistant neighbors due to the high biomass of herbivores present in the system. The level of constitutive defense required for an advantage (and thus the CSS) increases as the relative effectiveness of induced defense increases. Yet, as the relative effectiveness of induced defense nears the same level of effectiveness as constitutive defense, this advantage is no longer possible and all plants are selected to have induced defense.

5.3.4 Effect of overall effectiveness of defense

Fig. 15b shows the effect of varying ρ , the overall effectiveness of both types of defense, keeping all other parameters at their default values (Table 1). For plants growing alone, the less effective but less costly induced defense is favored when overall effectiveness is low and thus the absolute difference in effectiveness between induced and constitutive defense is smaller (Eq. 2). As overall effectiveness of defense increases, the difference in effectiveness between induced and constitutive defense also increases.

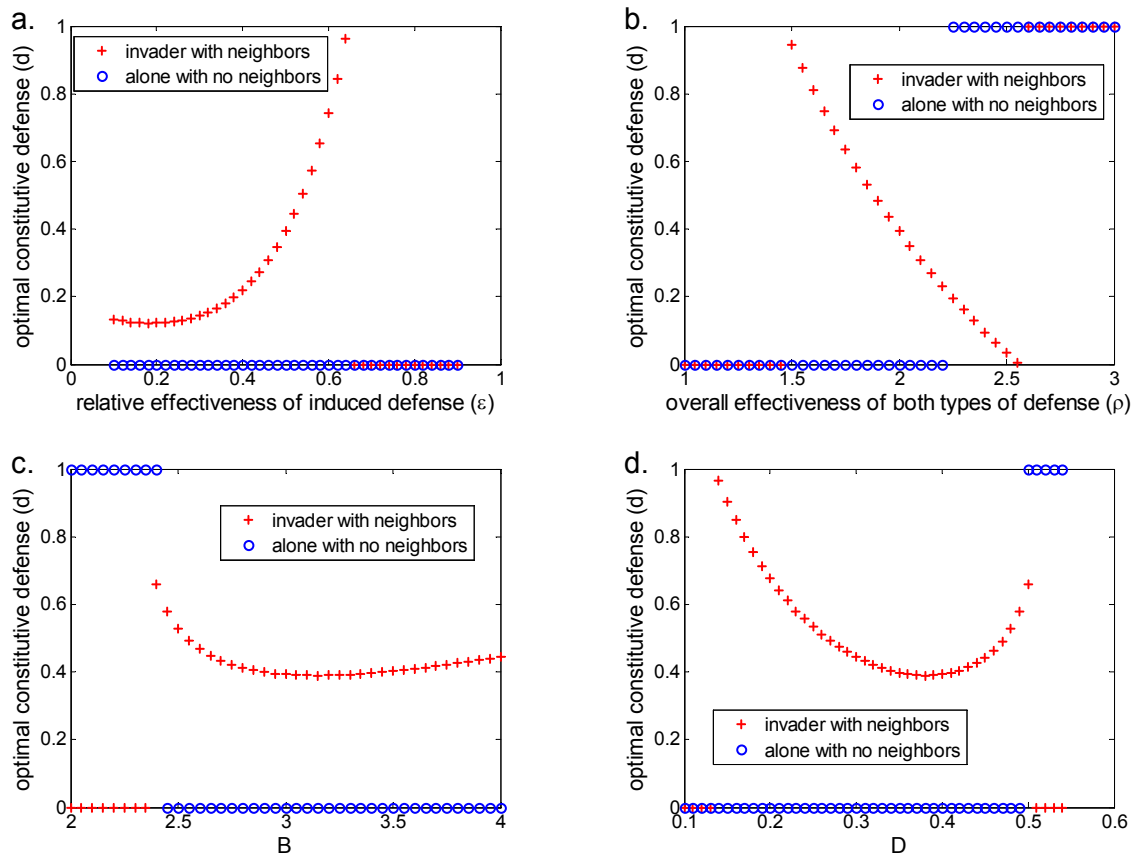


Figure 15: Effects of varying four of the non-dimensionalized model parameters on the optimal level of constitutive defense for both the invader-resident system (crosses) and plants grown alone (open circles). All parameter values were chosen to reflect reasonable values that did not cause the fraction of plants attacked to be negative.

When this difference is great enough (and constitutive defense is much more effective than induced), the best strategy for plants growing alone switches to constitutive defense. A similar effect is present at either end of the range of values for overall effectiveness for the invader-resident system. Over the middle range of values for overall effectiveness, the CSS for the invader-resident system is a mix of induced and constitutive defenses. The mixed defense strategy starts at mostly constitutive and moves towards more induced defense. When the CSS is for more constitutive defense than the best strategy for plants growing alone, the invader increases the amount of constitutive defense since it is able to out-compete its less resistant neighbors due to the high biomass of herbivores present in the system. As the overall effectiveness of defense goes up, this herbivore biomass goes down so the amount of constitutive defense that gives a competitive advantage decreases. When the CSS moves to less constitutive defense than the best growing-alone strategy, the invader is effectively able to parasitize the defense of the resident and drives the system towards less constitutive defense. Thus, there is selection for more “cheating” by producing less costly defense than the defense of neighbors as the effectiveness of defense goes up. However, when the overall effectiveness of defense is high enough, constitutive defense is so effective relative to induced it is always the best strategy.

The effects of varying the other model parameters from Eq. 2 (B and D) led to complex nonlinear effects (Fig. 15c,d) and are not described in detail.

5.3.5 Effects of changing attack rate

We also investigated the effects of varying the other parameters given different values of z , the half saturation constant for herbivore attack rate. Low values of z lead to high attack rates, and vice-versa (see Eq. 5). Fig. 16 shows the result of varying c (cost of defense) with a higher and a lower value for z than that used in Fig. 13, keeping all other parameters at their default values (Table 1). We saw similar results for all of the parameters shown in Fig. 15 using these values of z . Increasing the herbivore attack rate selects for constitutive defense and lowering the herbivore attack rate selects for induced defense regardless of the other parameter values. This is similar to the results of other models (Adler and Karban 1994; Clark and Harvell 1992; Poittrineau et al. 2004; Tollrian and Harvell 1999; van Baalen 1998) that suggest induced defense is futile when attack is almost certain but much more useful when attack is less certain.

5.3.6 Effects of adding immigration

Increasing immigration in the model (see Eqs. 7 and 8) tends to select for purely constitutive defense for plants growing alone, since increasing immigration increases the herbivore attack rate on plants (Fig. 17). In the invader-resident system, the CSS moves from more constitutive defense than the best growing-alone strategy at lower immigration levels to parasitizing the neighbors' defenses at higher immigration levels. As herbivore pressure increases, the tendency would be for plants to produce more constitutive defense, but higher levels of constitutive defense are more vulnerable to "cheaters". However, when the immigration level (and thus the attack rate) is high enough, the best

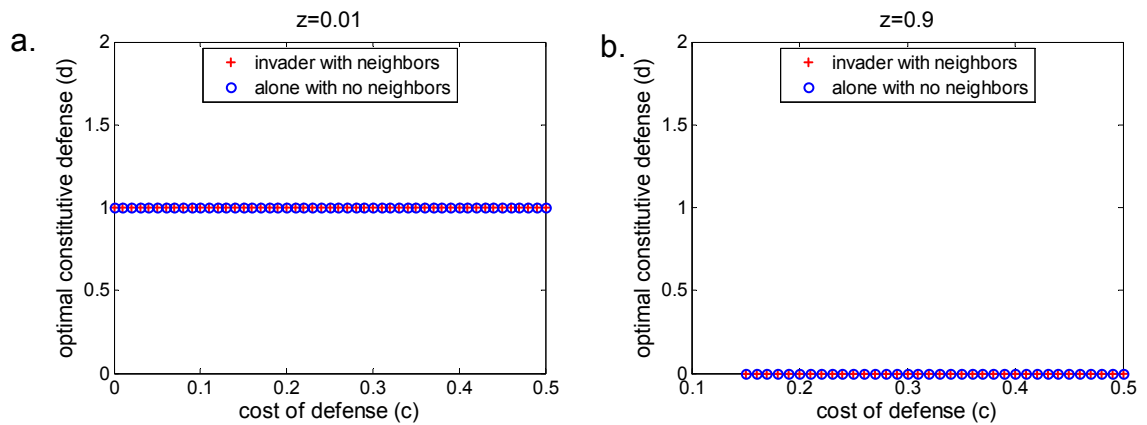


Figure 16: Effects of varying the cost of defense (c) on the optimal level of constitutive defense for both the invader-resident system (crosses) and plants grown alone (open circles) at a lower (a) and higher (b) value of z than that used in Fig. 3. The parameter values were chosen to reflect reasonable values that did not cause the fraction of plants attacked to go negative. Note that no values of c less than 0.15 were used in (b) to prevent the fraction of plants attacked from going below zero.

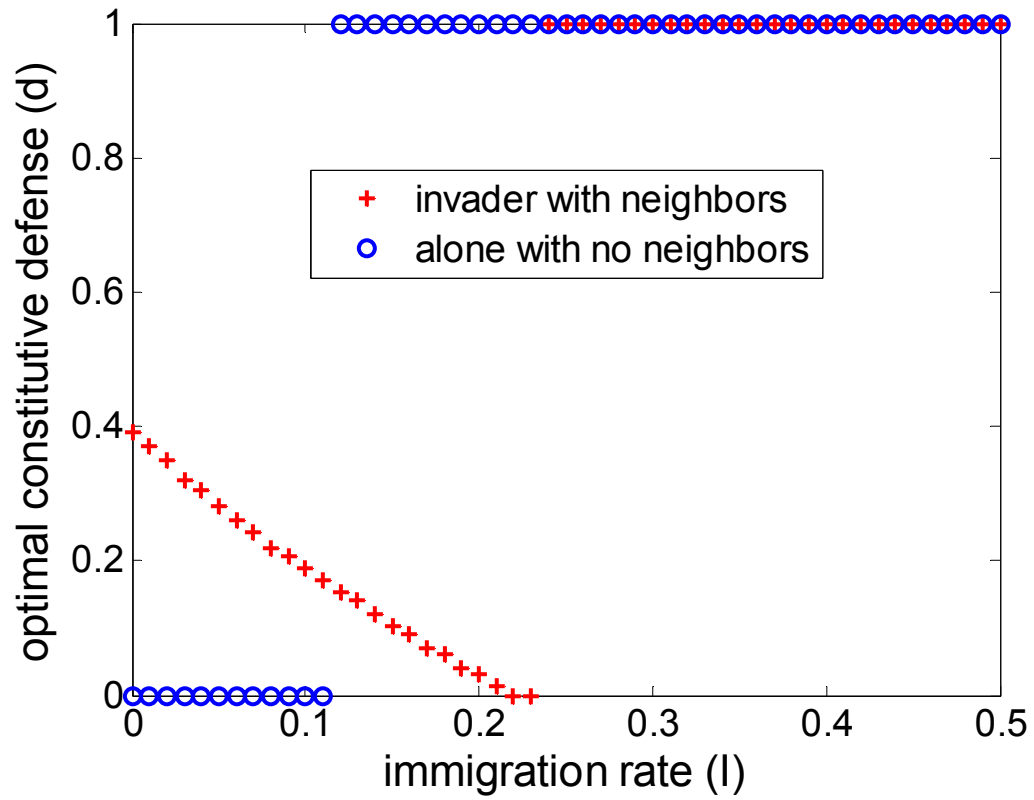


Figure 17: Effects of varying the herbivore immigration rate (I) on the optimal level of constitutive defense for both the invader-resident system (crosses) and plants grown alone (open circles).

strategy is always to use constitutive defense regardless of whether a plant is growing alone or has neighbors.

5.4 Discussion

All induced defense, all constitutive defense, or some mix of the two may be favored for plants under different conditions in our model. The results of our analysis suggest that having conspecific neighbors should influence the evolution of the type of defense produced by plants. For plants growing alone, the optimum type of defense was generally all induced defense or all constitutive defense over large ranges for all parameters. Considering the influence of neighboring conspecific plants often selected for defense strategies that differed from the best strategy in isolation over broad parameter ranges (Figs. 13 & 15). Plants with neighbors could be selected to evolve higher levels of constitutive defense (as a result of an “arms race” to be more defended than one’s neighbor when herbivore attack is common) or lower levels of constitutive defense (to exploit herd immunity) than would be optimal for plants growing alone. Thus, the density of conspecific neighbors is a potentially important factor in determining the optimal type of defense in a plant.

Adding neighbors into a system with feedback from herbivores also tended to select for more mixed induced/constitutive strategies over some range for all parameters relative to the best growing-alone strategy. Many plants do employ a mix of induced and constitutive defense strategies (Karban and Baldwin 1997). Most of these plants grow in the vicinity of conspecific neighbors (as do most plants in general). Therefore, it is

possible that having defended neighbors could be part of the reason why plants have evolved such mixed defense strategies.

The fitness of our plants was proportional to their final size, not their growth rate, as size was more readily quantified in our model. However, the growth rate of plants could also be used as a measure of fitness, and may be more important than size in determining how much a plant should be defended against herbivores (Coley et al. 1985). Although size is usually proportional to growth rate, this is not always true. For example, herbivores may respond to a higher growth rate by consuming more plant, leading to a smaller final size than with a lower growth rate.

Although we modeled our system as a plant-herbivore system that could vary in defense type (inducible or constitutive), this model may also be applicable to a variety of other systems with inducible and constitutive defenses. For example, some sessile marine invertebrates can produce spines and thicker or differently-shaped shells as induced responses to predation (Harvell 1990; Harvell 1992; Lively 1986). These organisms have other defenses against predation that are always present (constitutive). Therefore, they exhibit a mixed induced/constitutive strategy for defense against predation. All of these marine organisms are surrounded by conspecific neighbors in the ocean, so the same herd immunity arguments discussed earlier for plants and herbivores would apply to these invertebrates and their predators. Our model could therefore help to explain why some of these colonial invertebrates employ these mixed defense strategies.

One limitation of our model was that we did not explicitly investigate the total amount of defense that plants should produce, but instead concentrated on the optimal allocation of a fixed amount of defense between two types of defense (constitutive vs. induced). It was clear in some cases that this resulted in plants producing induced defense since it was less costly, when such plants likely would be selected to produce fewer or no defenses had that been possible (i.e. the plants growing alone in Fig. 15a). This may have overestimated in some cases the amount of induced defense that a plant should produce. The overestimation of induced defenses should have only occurred where the cost of defense was high relative to the benefits of defense, as this is the scenario likely to lead to no defenses. As most of the parameter space we explored was in regions where the benefits of defense were not greatly outweighed by the costs, we suspect that the overall result that we found of more mixed strategies for plants with neighbors would have still occurred had we included the option for plants to produce smaller amounts of defense. However, the parameter ranges for mixed strategies may have been smaller than we observed in our analysis.

Our results are similar in many respects to those of Poitrineau et al. (2004), who also modeled the evolution of inducible defenses in a similar invader-resident system. They modeled inducibility and level of defense as separate quantities, unlike our model of the optimal allocation to the two types of defense. In their model increasing costs of defense tended to decrease defense, similar to our reduction in constitutive defense at high costs. However, increasing costs of defense also decreased

inducibility in their model, whereas inducible defenses were favored in our model since they were the less costly defense type. They also found that increasing the attack rate increased defense and decreased inducibility, similar to our result. However, for cost of defense and attack rate, regardless of the degree of herd immunity, they found sharp transitions from low to high defense and inducibility. This result was significantly different from the regions of gradual shift in strategies we saw in our systems with neighbors for all parameters (Figs. 13 and 15). The difference was likely due to the feedback we included to and from defenses on herbivores that constantly modified the optimal level of defense and attack rates, whereas their model considered attack rates to be a fixed quantity and did not explicitly model the attackers.

Our model also sheds some light on the question raised in various other studies of how plants should optimize the tradeoff between competitive ability and defense against herbivores (Donaldson et al. 2006; Uriarte et al. 2002; Weis and Hochberg 2000). These studies have suggested that increasing levels of herbivore resistance should either increase (Weis and Hochberg 2000), decrease (Donaldson et al. 2006; Weis and Hochberg 2000), or have no effect on (Uriarte et al. 2002) competitive ability, as defined by the ability of plants to gather resources. Although we do not directly address tradeoffs between defense and competitive ability, our analysis suggests that selection for defense types in plants surrounded by neighbors can be highly sensitive to small parameter changes. Thus, selective pressures for levels of resistance even in the absence of tradeoffs with competitive ability could be highly variable and would likely become even

more variable with the addition of additional pressures from selection on competitive ability.

The feedback between herbivores and plant defenses is one of the key differences between our model and earlier models of similar systems, and has helped lead to some predictions not made by previous models. One such prediction is that plants with neighbors should very commonly adopt different defense strategies than plants growing alone. Another prediction is that a mix of constitutive and induced defense strategies should evolve more often in plant-herbivore systems in which the plant is growing surrounded by conspecific neighbors than systems in which the plant is growing alone. Both of these predictions could help explain the diverse pattern of plant defenses that have been observed in natural systems (Karban and Baldwin 1997) and might provide a basis for experimental tests of the relationship between plant density and defense strategies.

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