

**RESPONSE OF INSECT PEST AND BENEFICIAL SPECIES TO THE TIMING
AND SEVERITY OF DICAMBA INJURY IN SOYBEAN**

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The undersigned, appointed by the dean of the Graduate School, have examined the
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**RESPONSE OF INSECT PEST AND BENEFICIAL SPECIES TO THE TIMING
AND SEVERITY OF DICAMBA INJURY IN SOYBEAN**

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TABLE OF CONTENTS

Acknowledgements	ii
List of Tables	v
List of Figures	vi
Abstract	vii
CHAPTER I: Literature Review	1
Influence of Dicamba Injury on Soybean Insects	1
Justification	1
Situation with Off-Target Movement (OTM) of Dicamba in US Agriculture	2
Potential Methods of OTM of Dicamba or Other Herbicides	6
Japanese Beetle	8
Summary and Objectives	9
Literature Cited	11
CHAPTER II: Response of Insect Pest and Beneficial Species to the Timing and Severity of Dicamba Injury in Soybean	15
Abstract	15
Introduction	17
Materials and Methods	19
Site Descriptions	19
Treatment Information	20
Data Collection	21
Statistical Analysis	21
Results and Discussion	22
Response of All Insects Combined	22
Response of Bean Leaf Beetle (<i>Cerotoma trifurcata</i>)	23
Response of Soybean Thrips (<i>Neohydatothrips variabilis</i>)	24
Response of Green Cloverworm (<i>Hypena scabra</i>)	24
Response of Japanese Beetle (<i>Popillia japonica</i>)	25
Response of Family <i>Cicadellidae</i>	26
Response of Family <i>Pentatomidae</i>	26
Species Diversity Response	28
Yield Response	28
Discussion	29
Literature Cited	31

LIST OF TABLES

Table		Page
CHAPTER II		
2.1	Site description, planting information, and herbicide injury application dates ...	35
2.2	Soybean yield response to V3 and R1 herbicide injury applications.....	44

LIST OF FIGURES

Figure		Page
CHAPTER II:		
1	Mean number of insect species collected following V3 and R1 herbicide injury applications	36
2	Mean number of bean leaf beetle collected following V3 and R1 herbicide injury applications	37
3	Mean number of soybean thrips collected following V3 and R1 herbicide injury applications	38
4	Mean number of green cloverworm collected following V3 and R1 herbicide injury applications	39
5	Mean number of Japanese beetle collected following V3 and R1 herbicide injury applications	40
6	Mean number of insects in family <i>Cicadellidae</i> collected following V3 and R1 herbicide injury applications	41
7	Mean number of insects in family <i>Pentatomidae</i> collected following V3 and R1 herbicide injury applications	42
8	Shannon Diversity Index values following V3 and R1 herbicide injury applications	43

Abstract

Off-target movement (OTM) of dicamba (3,6-dichloro-2-methoxybenzoic acid), to sensitive crops has been one of the most significant issues to affect non-dicamba-resistant (non-DR) soybean (*Glycine max* (L.) Merr.) production in recent years. Although the yield response of non-DR soybean to OTM of dicamba has been extensively studied, few studies have been conducted to understand the effects that dicamba injury has on insect infestations in non-DR soybean. A field experiment was conducted at four locations in Missouri in 2018 and three locations in 2019 to determine if dicamba injury to non-DR soybean has any effect on the prevalence of insect species throughout the growing season. At each location, dicamba was applied to non-DR soybean at rates corresponding to 1/10th, 1/100th, 1/1,000th, and 1/10,000th of the labeled used rate (560 g ae ha⁻¹) at either the V3 or R1 stage of growth. In this study, dicamba doses associated with vapor drift resulted in increased visitation to soybean from bean leaf beetle (*Cerotoma trifurcata*). However, the most common response was reduced insect visitation after V3 or R1 applications of dicamba at 1/10th or 1/100th the labeled rate compared to the non-treated control. This response is likely related to the reduction in soybean growth and biomass observed following treatment with the higher rates of dicamba, which in turn influenced the habitat preferences exhibited by the species observed in this study. As the use of dicamba increases in U.S. soybean production, it is important to understand the effects OTM of dicamba has on insect species in agricultural production environments.

CHAPTER I

INFLUENCE OF DICAMBA INJURY ON SOYBEAN INSECTS

Justification

The increase in the number of herbicide-resistant and multiple herbicide-resistant weed species in U.S. corn, cotton, and soybean has created a need for new approaches in weed management. One of the newest options for the control of dicotyledenous weed species are soybean (*Glycine max* (L.) Merr.) and cotton (*Gossypium hirsutum* L.) cultivars that have been genetically engineered to withstand applications of dicamba (3,6-dichloro-2-methoxybenzoic acid). Historically, dicamba applications had been restricted from use in soybean and cotton due to the sensitive nature of these crop species to this herbicide (Wax et al. 1969, Behrens and Lueschen 1979, Al-Khatib and Peterson 1999, Egan et al. 2014a, Solomon and Bradley 2014). The recent deregulation of dicamba for use in dicamba-resistant (DR) cultivars quickly became an attractive weed control option for many producers with herbicide-resistant weed populations as dicamba and other synthetic auxin herbicides have been shown to select for resistance slower than most other commonly used herbicide sites of action (Heap 1997, Sterling and Hal 1997, Heap 2014).

Following the release of dicamba resistant crops into the marketplace, reports of off-target movement of dicamba began to surface in the United States. Dicamba applications made to soybean with DR genetics in close proximity to susceptible soybean varieties results in increased risk for injury due to extreme sensitivity of non-DR soybean, even at sublethal rates (Wax et al. 1969, Behrens and Lueschen 1979, Solomon and Bradley 2014). Although not known to be directly toxic to most insect species, dicamba

may have an indirect effect on insect species commonly present in a given crop or environment (Bohnenblust et al. 2013, Egan et al. 2014b, Bohnenblust et al. 2016, Freydier and Lundgren 2016). Bohnenblust et al. (2016) found that sublethal doses of dicamba in alfalfa resulted in reduced visitation by pollinator species. Herbicide injury is a well-known issue for agricultural crops, however, it is not fully understood how plant injury caused by off-target movement of herbicides affects insect species that inhabit these environments. Therefore, the objectives of this research are to determine species diversity response to varying levels of dicamba injury in soybean at multiple growth stages.

Given the extremely low dose of dicamba required to exhibit injury symptoms in susceptible soybean, it is plausible that soybean injury from dicamba could occur through insect transfer. Pollinator species have been shown to carry pesticide residues from treated field areas back to beehives, suggesting the potential for insects to serve as a vector for off-target movement of dicamba. The second objective of this research is to determine if Japanese beetle (*Popillia japonica* Newman) have the potential to serve as a method for off-target movement of dicamba.

Situation with Off-target Movement (OTM) of Dicamba in U.S. Agriculture

As herbicide-resistant weeds continue to dominate U.S corn, cotton, and soybean production, farmers are left with fewer chemical weed control options (Norsworthy et al. 2012). Dicamba-resistant soybean varieties were first commercially available for planting in the United States during the 2016 growing season in an effort to provide an additional chemical weed control option against herbicide-resistant weeds. Prior uses of dicamba had largely been limited to the control of emerged broadleaf weed species in

cereal crops, pastures and noncropland areas (Burnside and Lavy 1966, Hahn et al. 1969, Gold et al. 1988, Egan and Mortensen 2012). The introduction of DR crops allows POST applications of dicamba to be made in soybean and cotton through the plants ability to metabolize the herbicide dicamba to a herbicidally-inactive product (Behrens et al. 2007). Although DR soybean were deregulated for planting for the 2016 growing season, the Environmental Protection Agency (EPA) did not approve any dicamba formulations for POST applications until 2017. During the 2016 growing season, it is suspected that illegal applications of dicamba may have been made to DR crops prior to herbicide approval, and this resulted in off-target movement to sensitive crops and other plant species. During this time, the Missouri Department of Agriculture received 130 dicamba injury complaints throughout the state (Bradley 2017aa).

Although dicamba was discovered in 1942 by Zimmerman and Hitchcock, it was not used in a commercial setting until the 1960's (Ross and Lembi 2009). Early formulations utilized the dimethylamine (DMA) salt of dicamba and were sold under the trade name Banvel®. Several years after the introduction of the DMA formulation, additional formulations were introduced onto the market such as the diglycolamine (DGA) salt. More recently, the DGA plus VaporGrip technology (Xtendimax with VaporGrip ®; FeXapan plus VaporGrip ®) and the N,N-bis-(aminopropyl) methylamine (BAPMA) salt (Engenia ®) were introduced and are now the only dicamba formulations labeled for use on DR soybean and cotton varieties.

During the inaugural growing season for the application of these formulations to DR soybean and cotton, there were over 2,700 official dicamba-related injury investigations conducted by state Departments of Agriculture in the United States. It is

estimated that those cases of off-target movement impacted 3.6 million acres of non-DR soybean in 2017 (Bradley 2017bb). Bradley (2018) also reported that as of July 15, 2018, over 600 official dicamba-injury complaints had been filed with state Departments of Agriculture by those states who chose to participate in the survey, and that these complaints are believed to have injured an estimated 1.1 million acres of non-DR soybean alone.

Soybean are extremely sensitive to dicamba even if only exposed to driftable fractions (Wax et al. 1969, Behrens and Lueschen 1979, Solomon and Bradley 2014). Following reports of dicamba injury to non-DR soybean fields, farmers began to question if they would see yield reductions in those areas where injury occurred. A meta-analysis conducted by Egan et al. (2014a) indicated that exposure of non-DR soybean to 0.56 g dicamba ha⁻¹ at the flowering stage resulted in an average yield loss of 1%. However, the same exposure dose resulted in little to no yield reduction when the exposure occurred during vegetative growth stages. Yield reductions of 8.7 and 3.7% were observed following application of 5.6 g ha⁻¹ dicamba at flowering and vegetative stages, respectively (Egan et al. 2014a). A more recent meta-analysis found that soybean yield could be reduced by 5% in non-DR soybean following exposure to dicamba at 1.9 g ha⁻¹ during vegetative growth stages (V1 to V3) or 0.89 g ha⁻¹ during the flowering stage (R1 to R2), respectively (Kniss 2018). Although injury from driftable fractions of dicamba has shown the potential for smaller yield reductions, exposure to a higher rate of 56.1 g ha⁻¹ resulted in yield losses of 48% or more, regardless of growth stage (Egan et al. 2014a).

In addition to yield reduction, dicamba exposure also has the potential to reduce overall crop height, density, seed oil percentage, and can delay soybean maturity (Wax et al. 1969, Solomon and Bradley 2014). While the adoption of new herbicide-resistant crops can often provide benefits to producers such as reduced herbicide use and new weed management options, the effects of these new technologies on insect species diversity isn't always a primary consideration (Fernandez-Cornejo and Caswell 2006, Baumgart-Getz et al. 2012, Fernandez-Cornejo et al. 2014a). A limited number of studies have been conducted to determine how insect populations are affected by herbicide treatment and/or herbicide injury. Previous research revealed that insect response to glyphosate treatment in glyphosate-resistant soybean varieties varied between individual species as some species were more abundant, while others were less abundant following glyphosate applications (Jackson and Pitre 2004a, b). Similarly, Buckelew et al. (2000) found that soybean treated with glyphosate seemed to be preferable for Potato leafhoppers (*Empoasca fabae*) as a result of weed control achieved through herbicide treatment. However, the response of insect species that exist within non-DR soybean that has been injured through off-target movement of dicamba is not fully understood.

Driftable fractions and even labeled use rates of dicamba are not known to be directly toxic to insects (Bohnenblust et al. 2013, Freydier and Lundgren 2016). However, dicamba injury in susceptible plant species has been known to alter plant and insect biodiversity levels (Bohnenblust et al. 2013, Egan et al. 2014b, Bohnenblust et al. 2016). For example, Bohnenblust et al. (2013) found that painted lady butterfly (*Vanessa cardui*) caterpillars and pupae accumulated less mass when feeding in thistle plants treated with dicamba versus thistle plants with no herbicide treatment. Egan et al.

(2014b) also determined that populations of soybean aphid (*Aphis glycines*) and potato leafhopper decreased in soybean and alfalfa injured by sublethal rates of dicamba while Bohnenblust et al. (2016) found that decreases in flowering in alfalfa (*Medicago sativa*) caused by dicamba injury led to a reduction in pollinator visitation. This previous research implies that the recent increases in the number of dicamba injury complaints could have an effect on the insect species present in field crops like soybean.

Potential Methods of OTM of Dicamba or Other Herbicides

Herbicides like dicamba have a variety of routes through which they may move away from their intended target and cause injury to adjacent susceptible plant species. Physical pesticide drift is defined as the movement of pesticide dust or droplets through the air at or soon after the time of application to any site other than the area intended (EPA 2018). Droplet size, nozzle type, spray pressure, and application height are factors known to influence the risk of physical drift when making herbicide applications (Dexter 1993, Nuyttens et al. 2007). However, dicamba acid has also been known to volatilize following application, causing injury to soybean up to four days after application (Behrens and Lueschen 1979, Weidenhamer et al. 1989). Volatilization, defined as the physical change of a liquid or a solid compound into a gaseous state, is influenced by environmental factors such as formulation, temperature, relative humidity, and rainfall with respect to dicamba (Behrens and Lueschen 1979, Ross and Lembi 2009). Since many environmental factors are outside of the control of the individual applicator, vapor drift of dicamba poses a serious threat for off-target movement to susceptible areas. Off-target injury from dicamba can also result from contaminated spray tanks, make-up water

or nurse trucks, transfer hoses, measuring containers, or chemical storage containers (Boerboom 2004).

Many studies have been conducted to examine how insects interact with a variety of pesticides that are commonly used in field settings. Although insecticides may appear to be a more direct threat to insect species, increased herbicide use paired with declining insecticide use leaves herbicides among the most heavily scrutinized pesticides today. In addition, reduction in host plants that occur as weed species and are controlled by herbicides in row crops is believed to affect insect population levels in a variety of cropping systems (Buckelew et al. 2000, Jackson and Pitre 2004b, Sharma et al. 2018). In 1960, insecticides and herbicides accounted for 58 and 18% of the total pounds of pesticides applied in the United States, respectively. By 2008, however, insecticides and herbicides accounted for 6 and 76% of pounds of pesticides applied in the United States, respectively (Fernandez-Cornejo et al. 2014b). For example, the western honey bee (*Apis mellifera* L.) provides pollination to an estimated 77% of commodities grown around the world (Delaplane et al. 2000). The reliance of honey bee species for pollination of field crops has gained more attention in recent years due to declining honey bee populations both domestically and abroad (Gallai et al. 2009, Pettis and Delaplane 2010). Johnson et al. (2010) also found that wax, pollen, bees, and associated beehives all contained detectable levels of herbicides, insecticides, and fungicides. Among the herbicides found in beehives were atrazine (6-chloro-*N*-ethyl-*N*-(1-methylethyl)-1,3,5-triazine-2,4-diamine) and *S*-metolachlor (2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-[(1*S*)-2-methoxy-1-methylethyl]acetamide) which were among the four most heavily used herbicides in 2008 (Kearney et al. 1964, Prueger and Pfeiffer 1994, Johnson et al. 2010, Fernandez-Cornejo

et al. 2014b). As these herbicides are not prone to vapor drift, it is reasonable to suspect that they may have been introduced to beehives from pollinating bees carrying herbicide residues from treated field crop areas.

Japanese beetle

Given the sensitivity of soybean to dicamba and the potential for herbicides to be transported via insect movement, it is reasonable to suspect that off-target movement of dicamba to susceptible, non-DR soybean could be a result of movement of insect pests from soybean fields treated with dicamba. Although pollinator species such as honey bees are present in soybean during the time of field applications, pest species such as Japanese beetle (*Popillia japonica*) are commonly present in soybean when applications of dicamba are commonly made as well. Japanese beetle are known to feed on soybean during the middle of the growing season, prior to silk development in corn (Turnipseed and Kogan 1976).

Although Johnson et al. (2010) found pesticide residues in pollen collected from honey bees, many of the pesticides detected were likely physically transported to the beehives via honey bees themselves. It is also interesting to note the anatomical similarities between an adult honey bee and an adult Japanese beetle. The honey bee has hardened body plates and is covered with hairs (Gould and Gould 1988, Winston 1991). Similarly, the Japanese beetle contains a hardened exoskeleton and has body segments with hairs extruding from the abdomen and ventral surfaces (Fleming 1972).

Japanese beetle originated in Japan but was first discovered in the United States in the summer of 1916 in southern New Jersey (Fleming 1972). Soon after its establishment in the United States, Japanese beetle became a troublesome pest due to nondiscriminatory

feeding preferences among plant species (Fleming 1972, Potter and Held 2002). As the species continued to spread throughout the country, agricultural crops such as corn and soybean soon became targets of Japanese beetle feeding. Adults are known to cause particularly devastating levels of defoliation in soybean (Turnipseed and Kogan 1976, Potter and Held 2002). Continuous and sporadic mating behavior has been shown to prolong the presence of adult beetles throughout the growing season as new generations of adults seem to occur continuously (Fleming 1972, Potter and Held 2002). Japanese beetle are known to be a restless insect with constant movement from one location to another (Fleming 1972). Fleming (1972) also noted that beetle flight increases with temperature up to 35° C, as well as noted increases in flight during periods of relative humidity below 60%.

Extended presence of Japanese beetle throughout soybean development, especially during vegetative stages where field applications of dicamba are being made, create a unique situation for potential of off-target movement of dicamba to occur. This research will investigate the possibility of transport of dicamba through the movement of Japanese beetle from soybean fields treated with dicamba to non-DR soybean.

Summary and Objectives

The continual increase in the number of herbicide-resistant weeds has led to the introduction of new weed control systems that allow for POST applications of dicamba to be made in soybean for the first time in cotton and soybean. These new systems have resulted in a recent surge in cases of off-target movement of dicamba to susceptible species and brought about many unanswered questions for soybean producers in the United States. One question seeks to understand how insect species respond to dicamba-

injured plant species. The objective of this portion of the research is to determine how soybean insect pest and beneficial species respond to the timing and severity of dicamba injury in soybean. To quantify this response, research will determine species diversity response to varying levels of dicamba injury in soybean at multiple soybean growth stages.

Multiple methods of off-target movement of dicamba have been identified including physical drift, tank contamination, volatility, and others. Studies have also shown that some insects demonstrate the ability to act as a vector for movement of pesticides from treated field areas. It is plausible that a common soybean insect pest, such as Japanese beetle, could serve as a vector for off-target movement of dicamba due to its feeding and flight behavior in soybean. Given the small amount dicamba required to result in soybean injury, it is reasonable to conclude that Japanese beetle could transport injurious levels of dicamba. The objective of this portion of the research is to determine if Japanese beetle have the potential to serve as a method for off-target movement of dicamba.

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Chapter II

RESPONSE OF INSECT PEST AND BENEFICIAL SPECIES TO THE TIMING AND SEVERITY OF DICAMBA INJURY IN SOYBEAN

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ABSTRACT

Off-target movement (OTM) of dicamba (3,6-dichloro-2-methoxybenzoic acid), to sensitive crops has been one of the most significant issues to affect non-dicamba-resistant (non-DR) soybean (*Glycine max* (L.) Merr.) production in recent years. Although the yield response of non-DR soybean to OTM of dicamba has been extensively studied, few studies have been conducted to understand the effects that dicamba injury has on insect infestations in non-DR soybean. A field experiment was conducted at four locations in Missouri in 2018 and three locations in 2019 to determine if dicamba injury to non-DR soybean has any effect on the prevalence of insect species throughout the growing season. At each location, dicamba was applied to non-DR soybean at rates corresponding to 1/10th, 1/100th, 1/1,000th, and 1/10,000th of the labeled used rate (560 g ae ha⁻¹) at either the V3 or R1 stage of growth. In this study, dicamba doses associated with vapor drift resulted in increased visitation to soybean from bean leaf beetle (*Cerotoma trifurcata*). However, the most common response was reduced insect visitation after V3 or R1 applications of dicamba at 1/10th or 1/100th the labeled rate compared to the non-treated control. This response is likely related to the reduction in soybean growth and biomass observed following treatment with the higher rates of dicamba, which in turn influenced the habitat preferences exhibited by the species observed in this study. As the use of

dicamba increases in U.S. soybean production, it is important to understand the effects OTM of dicamba has on insect species in agricultural production environments.

INTRODUCTION

The predominance of herbicide-resistant weeds in U.S. agriculture has limited the number of effective management options for these species in row crop production systems (Norsworthy et al. 2012). In an effort to address this problem, Monsanto genetically engineered soybean (*Glycine max* (L.) Merr.) and cotton (*Gossypium hirsutum* L.) cultivars to withstand applications of dicamba (3,6-dichloro-2-methoxybenzoic acid), thus providing a new mode of action that had not previously been available for use in these crops. Historically, dicamba had been limited to use in cereal crops, pastures, and non-cropland areas (Burnside and Lavy 1966, Hahn et al. 1969, Gold et al. 1988, Egan and Mortensen 2012) and was not labeled for post-emergence (POST) use in soybean or cotton due to the high sensitivity of these crops to this herbicide (Wax et al. 1969, Behrens and Lueschen 1979, Al-Khatib and Peterson 1999, Egan et al. 2014a, Solomon and Bradley 2014). Soybean are especially sensitive to even low doses of dicamba, and can exhibit injury symptoms even if exposed to 1/1000th or 1/10,000th of the labeled use rate of this herbicide (Wax et al. 1969, Behrens and Lueschen 1979, Solomon and Bradley 2014). In addition to the possibility of yield loss, susceptible soybean injured by dicamba also have the potential to exhibit reductions in height, seed oil content, and seed germination, and may also experience delays in maturity, depending on the dose and stage of growth that soybean are in when the dicamba exposure occurs (Wax et al. 1969, Auch and Arnold 1978, Solomon and Bradley 2014, Jones et al. 2019).

Dicamba-resistant (DR) soybean varieties were made commercially available for planting in the United States in 2016. Although DR soybean were de-regulated for

planting in 2016, the Environmental Protection Agency (EPA) did not approve any formulations of dicamba for post-emergence (POST) applications until the 2017 growing season. Unfortunately, in this inaugural year for application of approved dicamba formulations in DR crops, there were many reports of dicamba moving off-target and injuring nearby sensitive plant species in high soybean production areas of the United States. Collectively, state departments of agriculture reported 2,708 dicamba-related investigations and university extension weed scientists estimated 1.5 million hectares of non-dicamba tolerant soybean were injured by dicamba in 2017 (Bradley 2017a; Benbrook 2017). The following year, Bradley (2018) reported that over 600 official dicamba complaints had been filed with State Departments of Agriculture but also that many states refused to provide numbers related to their dicamba-related investigations at that time. In 2019, Scott (2019) reported that there were 1,526 official dicamba complaints to the Departments of Agriculture in the top ten soybean-producing states in the U.S.

Studies have been conducted that illustrate commercial formulations of dicamba are not directly toxic to species such as *Coleomegilla maculate* (DeGeer), *Hippodamia convergens* (Guérin-Méneville), and *Apis mellifera* (L.) (Morton et al. 1972, Michaud and Vargas 2010, Bohnenblust et al. 2013, Egan et al. 2014b, Freydier and Lundgren 2016). Although Freydier and Lundgren (2016) found that *Coleomegilla maculate* (DeGeer) longevity was reduced by dicamba, commercial formulations of dicamba did not show the same result, suggesting that the additional ingredients used to create commercial formulations of dicamba are not directly toxic to many insect species. However, dicamba injury to susceptible plant species has been shown to have the

potential to alter plant and insect biodiversity levels (Bohnenblust et al. 2013, Egan et al. 2014b, Bohnenblust et al. 2016). Bohnenblust et al. (2013) found that painted lady butterfly (*Vanessa cardui*) caterpillars and pupae accumulated less mass when feeding in thistle plants treated with dicamba than thistle plants that received no herbicide treatment. In the same study, Bohnenblust et al. (2013) suggested that if a large portion of field-edge plant species were exposed to sublethal doses of dicamba, insect species may be required to travel further to find quality food sources. It is reasonable to believe that as these species begin to travel further from their field habitat for food sources, the number of species remaining within a field area may be altered from their original levels. Egan et al. (2014b) also determined that populations of soybean aphid (*Aphis glycines*) and potato leafhopper decreased in soybean and alfalfa injured by sublethal rates of dicamba while Bohnenblust et al. (2016) found that reductions in flowering in alfalfa (*Medicago sativa*) as a result of dicamba injury reduced overall pollinator visitation. Results from these studies indicate that sublethal doses of dicamba to susceptible plant species will likely lead to altered insect biodiversity levels within field crops as these species are required to travel further in search of sufficient food and pollen sources. The objectives of this research were to determine how soybean insect pest and beneficial species respond to the timing and severity of dicamba injury in soybean.

MATERIALS AND METHODS

Site Descriptions

Replicated field experiments were conducted in Buchanan, Boone, Knox and Moniteau counties in Missouri in 2018 and repeated in Warren, Jackson and Scott counties in 2019. A non-DR soybean variety was planted at each trial site between April

30 and May 9 in 2018 and between May 13 and June 11 in 2019 at a rate of 345,000 seeds ha⁻¹. Site-specific soil properties and planting dates can be found in Table 2.1.

Individual plots were 6 x 6 m and arranged in a randomized complete block design. Treatments were replicated six times at all sites except for the trial in Moniteau county where size constraints limited the experiment to four replications. Herbicide treatments were applied with a CO₂-pressurized backpack delivering 140 L ha⁻¹ at 152 kPa with a constant speed of 1.34 m/s. Treatments containing dicamba were made using TTI 11002 nozzles and all other herbicides were applied through XR11002 flat fan nozzles (Tee Jet Spraying Systems Co. P.O. Box 7900, Wheaton, IL 60189). All plots were surrounded by a 1.5 m non-treated buffer in order to avoid any cross-plot contamination of dicamba.

Treatment Information

In each experiment, the diglycolamine salt of dicamba (Xtendimax® with VaporGrip Technology, Bayer CropScience, St. Louis, MO) was applied at sublethal doses of 56, 5.6, 0.56, and 0.056 g ae ha⁻¹ at either the V3 or R1 stage of soybean growth. These application rates correspond to 1/10th, 1/100th, 1/1,000th, and 1/10,000th of the current labeled rate, respectively. Lactofen (Cobra®, Valent USA LLC, Walnut Creek, CA) was also applied at 175 g ae ha⁻¹ at the V3 or R1 stage of growth as a comparison treatment. Lactofen is a common diphenyl ether herbicide used to control broadleaf weed species in soybean and often results in soybean leaves with necrotic spots and/or crinkled leaf edges (Wichert and Talbert 1993, Bradley 2009). The appearance of leaf injury as a result of lactofen injury is much different from that caused by off-target movement of dicamba.

Data Collection

Insect census data were collected via weekly sweep net samples (38-cm Heavy Duty Sweep Net; BioQuip Products, Rancho Dominguez, CA). Ten sweeps were conducted in a different 3-m row segment of each plot each week. Time of day has been known to influence the number of insect species caught in sweep net samples (Dumas et al. 1962, 1964, Studebaker et al. 1991). Sweep net sampling was conducted at each site beginning no earlier than 10:00 a.m. and no later than 2:00 p.m. during times when there was no dew present to maximize the number of species collected. Once collected, insects were stored in unused, plastic bags and frozen for subsequent identification. Sweep net sampling began the day of the first V3 herbicide application prior to any herbicide applications and continued weekly at each trial site until the R5 stage of soybean growth which occurred in August at all trial sites in both years. Sweep net sampling occurred between May 31 and August 6 in 2018 and between June 18 and August 28 in 2019. After collection, species were visually identified down to the taxonomic family level. Soybean yield was determined by harvesting the innermost two soybean rows within each half of each plot with a small plot combine (Kincaid®, Haven, KS) and moisture was adjusted to 13%.

Statistical Analysis

All data were subject to analysis in SAS, version 9.4 (SAS ® Institute Inc., Cary, NC) using the PROC GLIMMIX procedure. Herbicide treatment and application stage were considered fixed effects while year and trial site were considered to be random effects. Year and trial site were considered random effects so that conclusions about herbicide treatments or application stages can be made over a wide range of

environments and locations (Carmer et al. 1989, Blouin et al. 2011). All insect species were tested for normality and the transformation that provided the best fit was selected for each respective group. Total insects collected were analyzed to capture any trends that may exist from all insects present throughout the experiment. Only the four most abundant species and two most abundant families were selected for individual analysis. In the case of Japanese beetle (*Popillia japonica*), only data from 2018 is included due to a lack of species presence in the 2019 trial sites. Species diversity was quantified using Shannon's Diversity Index and all means were separated using Fisher's protected LSD at $P \leq 0.05$.

RESULTS AND DISCUSSION

Response of all insects combined

In general, the number of insects present during the experiment increased from the time of V3 applications through 5 weeks after application (WAA) (Figure 1). The rate of dicamba also influenced the likelihood of insect visitation; 1/10th the labeled rate of dicamba reduced visitation from insect species each week compared to the total number of insects collected in the non-treated control areas during that same week ($P < 0.0001$). Similarly, the 1/100th rate of dicamba reduced insect visitation compared to the non-treated control for each week except 1 WAA. Insect visitation was not different between the non-treated control and soybean treated with the 1/1000th and 1/10,000th rate of dicamba or lactofen at any time interval following application. Following the R1 herbicide injury applications, reductions in insect visitation were observed through 4 WAA with dicamba at 1/10th the labeled rate, and through 3 WAA with dicamba at 1/100th the labeled rate (Figure 1). There were very few differences in insect visitation

between the non-treated control and soybean treated with any of the remaining dicamba treatments or lactofen at any other time interval following treatment. Bohnenblust et al. (2016) also found that pollinator visitation to alfalfa was reduced following applications of sublethal rates of dicamba. The results from this experiment indicate that sublethal doses of dicamba greater than 5.6 g ae ha⁻¹ can lead to reductions in the total number of insects present in soybean. Based on previous work that revealed that susceptible soybean injured by dicamba will exhibit reductions in height as well as reduced biomass, leaf area, and canopy closure (Wax et al. 1969, Auch and Arnold 1978, Solomon and Bradley 2014, Perry 2018), it is likely that the reductions in insect visitation observed in this research could be attributed to the lack of adequate food source and/or habitat for the species present. This reduction in insect visitation may also indicate a desire for these species to seek out neighboring vegetation or field areas to fulfill food and habitat requirements.

Response of bean leaf beetle (*Cerotoma trifurcata*)

Very few differences in bean leaf beetle populations were observed between herbicide treatments and the non-treated control following either the V3 or R1 herbicide injury applications (Figure 2). However, five weeks after the R1 application there was an increase in bean leaf beetle populations in response to dicamba at 1/1,000th and 1/10,000th the labeled rate. This response can likely be explained by results from a study conducted by Buckelew et al. (2000) who determined that bean leaf beetle preferred soybean with greater plant height. Given that sublethal doses of dicamba are known to reduce plant height and canopy width, it is reasonable to expect that bean leaf beetle would prefer soybean injured by less injurious rates of dicamba that do not cause significant height or

biomass reductions (Wax et al. 1969, Auch and Arnold 1978, Kelley et al. 2005, Solomon and Bradley 2014, Perry 2018). However, this does not explain why bean leaf beetle seemed to prefer these treatment areas over the non-treated control.

Response of soybean thrips (*Neohydatothrips variabilis*)

Only one herbicide injury treatment resulted in a reduction in soybean thrips compared to the non-treated control within the same collection week following the V3 applications (Figure 3). However, all dicamba treatments reduced visitation of soybean thrips from two through five weeks after R1 applications. Lactofen injury also resulted in reductions in soybean thrips from two through four weeks after the R1 application timing. By two weeks after the R1 application, all soybean exhibited various herbicide injury symptoms, ranging from stunting and leaf cupping in response to the dicamba treatments, to foliar necrosis in response to lactofen. Based on the results from this research, it seems clear that soybean thrips are more attracted to soybean that are not exhibiting signs of herbicide injury. Given that a similar response occurred in response to injury from both lactofen and dicamba, it may also be a response of this species to avoid any areas that have previously been treated with pesticides. Huckaba and Coble (1990) also reported inconsistent responses of soybean thrips to various herbicide treatments. For example, at certain time intervals after application, naptalam plus dinoseb reduced thrip populations on soybean while at other times it did not.

Response of green cloverworm (*Hypena scabra*)

There was not a consistent response of green cloverworm to any herbicide treatment or application timing (Figure 4), although green cloverworm populations were reduced three and four weeks after V3 applications and 4 weeks after R1 applications.

Green cloverworm populations often varied significantly from week to week even in response to the same treatment. Buckelew et al. (2000) also observed that there was no consistent response of green cloverworm populations to soybean varieties or weed management systems.

Response of Japanese beetle (*Popillia japonica*)

As illustrated in Figure 5, virtually no Japanese beetle were present in the first three weeks following the V3 applications. This is likely a reflection of the short life cycle of this species in Missouri (Potter and Held 2002, Rice 2019). Once Japanese beetles emerged and reached detectable levels, 1/10th the labeled use rate of dicamba resulted in fewer beetles present 4 and 5 WAA after V3 applications. Additionally, Japanese beetle numbers were reduced compared to the non-treated control 4 WAA of dicamba at 1/100th the labeled rate and with lactofen.

Few discernable differences in Japanese beetle populations were observed between the herbicide treatments and the non-treated control following R1 injury applications (Figure 5). Similar to other insect species, Japanese beetle often chose to avoid areas that had been treated with $\geq 1/100^{\text{th}}$ the labeled rate of dicamba compared to non-injured control areas. For example, the 1/10th rate reduced Japanese beetle visitation of injured soybean through four WAA while the 1/100th rate caused reductions two and three WAA. Given that Japanese beetle are known to feed on numerous plant species (Fleming 1972, Turnipseed and Kogan 1976, Potter and Held 2002), it is likely that the reductions in available soybean biomass in response to these sub-lethal rates of dicamba were enough to force the beetle towards other vegetative areas in search of more food sources than were available in the severely injured areas. Potter and Held (2002)

reported that Japanese beetle do not appear to choose host plants based on criteria of foliar toughness, plant nutrients, or protein-binding capacity. Therefore, the response of Japanese beetle in this study is believed to be driven by the species' search for food quantity requirements where sufficient soybean biomass was available. Where soybean biomass was greatest in the non-treated control, soybean treated with lactofen, or soybean treated with $\leq 1/1,000^{\text{th}}$ rates of dicamba, there were few preferences exhibited by Japanese beetle.

Response of family *Cicadellidae*

Numerous *Cicadellidae* species were collected throughout the course of this experiment. Among the most common species found were *Empoasca fabae*, *Norvellina seminuda*, and *Agallia constricta*. In most cases, leafhopper species did not seem to have a preference for herbicide-treated soybean compared to the non-treated control in the week following V3 injury applications (Figure 6). Although there were some differences in leafhopper populations in response to a few of the herbicide treatments, there did not seem to be a consistent response of leafhopper species to any herbicide or application timing. These results suggest that *Cicadellidae* visitation to soybean is not influenced by herbicide injury. For example, although *Cicadellidae* species found in this study such as *E. fabae* are known to be leaf-sucking insects (Poos 1932, Turnipseed and Kogan 1976), the physical alterations observed on soybean leaf tissue following dicamba injury do not seem to have a large impact on the species' herbivory preferences.

Response of family *Pentatomidae*

Numerous members of the Pentatomidae family were found during this experiment such as green stink bug (*Chinavia hilaris*), brown stink bug (*Euschistus*

servus), brown marmorated stink bug (*Halyomorpha halys*), and spined soldier bug (*Podisus maculiventris*). Stink bug species are known to feed on pods and stems of soybean, and therefore their populations are more abundant in the latter parts of the growing season and/or once the crop has reached the reproductive stages of growth (McPherson et al. 1993, Depieri and Panizzi 2011, Rice et al. 2014). Following the V3 injury applications, little preference was given to the non-treated control areas over soybean treated with herbicides, with the exception of the 1/10th rate of dicamba three and four WAA as well as the 1/100th rate of dicamba three WAA, where visitation was reduced (Figure 7). Similar trends were observed following R1 herbicide injury applications in that visitation was reduced two weeks after the 1/100th rate of dicamba and four weeks after the 1/10th rate of dicamba, but overall few differences were observed.

Previous research has shown that the number of pods per plant are not reduced by sublethal doses of dicamba up to 1/100th the labeled rate when applied in the vegetative stages of soybean growth (Kelley et al. 2005, Solomon and Bradley 2014). Given that stink bug species commonly feed on soybean pods, it is reasonable to expect that their response to these early-season dicamba injury applications had minimal influence on feeding behavior. In contrast, few treatment differences were observed following R1 injury applications where the number of pods per plant is typically reduced following injury by 1/200th the labeled rate of dicamba or higher (Solomon and Bradley 2014). This suggests that sting bug species feeding preferences may not be associated with soybean pod number alone.

Species diversity response

Although the results from these experiments indicate that overall species visitation was often reduced in response to the highest rates of dicamba, few differences in insect diversity were observed in response to the herbicide treatments. The only diversity index scores that were different from the non-treated control occurred three, four, and five weeks after the V3 application of 1/10th the labeled rate of dicamba (Figure 8). No differences in insect diversity were observed between herbicide treatments and the non-treated control within the same week following the R1 applications. These results indicate that although an individual species may exhibit a habitat preference among herbicide-treated areas, another species linked to that species' behavior is likely to have a similar response. For example, if a phytophagous species shows a feeding preference for soybean injured by a given herbicide treatment, a predator of that initial species will likely follow, regardless of habitat choice. Therefore, the response of many predacious species is likely linked to phytophagous pests, ultimately causing insect diversity levels to remain the same.

Yield response

Soybean yield was not affected by lactofen or the 1/10,000th of the labeled rate of dicamba when applied at either the V3 or R1 stage of soybean growth (Table 2.2). Dicamba at 1/1000th (0.56 g ae ha⁻¹) of the labeled rate resulted in an approximate 6% yield reduction following application at R1 but not at the V3 stage of growth. These results are consistent with the findings from two previous meta-analyses conducted on the yield response of non-DR soybean to dicamba (Egan et al. 2014a; Kniss 2018). For example, in an analysis of 11 previously published field studies, Kniss (2018) reported

that a dose of 0.9 g ae ha⁻¹ would result in an approximate 5% soybean yield loss when applications were made at the early reproductive stages. Lastly, all rates of dicamba $\leq 1/100^{\text{th}}$ the labeled rate caused substantial yield reductions when applied at either growth stage. Kniss also reported that exposure of non-DR soybean to 56 g dicamba ha⁻¹ is likely to cause yield reductions greater than 48% regardless of the application timing (Kniss 2018). In this research, 56 g dicamba ha⁻¹ applied at either the V3 or R1 growth stages resulted in a 46% and 85% yield reduction, respectively.

DISCUSSION

The most common changes observed in insect presence in this research were reduced visitation after applications of dicamba at 1/10th or 1/100th the labeled rate compared to the non-treated control at the same sample collection timing. This response is likely related to the reduction in soybean growth and biomass observed following treatment with the higher rates of dicamba. Although exact responses vary by species, the criteria for habitat selection is likely linked to the amount of soybean available for habitat and/or food source. Limitations of food sources will likely force herbivorous species to seek out additional or alternate flora, consequently forcing predacious species to follow these herbivores in search of their food source. This is similar to the responses observed by Bohnenblust et al. (2016), who reported that when reductions in pollinator species occurred, their response was likely linked to reductions in available pollen-producing plant species caused by dicamba injury. The study went on to suggest that pollinator species would then be required to travel further in order to find sufficient pollen levels, thus reducing visitation of pollinator species on a localized level. With regard to specific insect species, such as bean leaf beetle, their response in this study is

likely linked to the reduction in soybean plant height caused by the lower rates of dicamba. However, the reason(s) for the observed increases in bean leaf beetles following R1 applications of dicamba at 1/1000th and 1/10,000th the labeled rate is unclear and warrants further research. Species diversity levels were often unaffected by dicamba injury throughout the course of this study. Although the total number of insects present were often reduced following severe cases of dicamba injury, the proportion of species present within those affected areas was rarely altered when compared to adjacent non-injured control areas.

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Table 2.1. Site description, planting information, and herbicide injury application dates.

County	Year	Soil Type	OM%	pH	CEC	Previous Years' Crop	Planting Date	Herbicide Injury Application Dates
Buchanan	2018	Marshall silt loam & Lamoni silty clay loam	3.4	6.4	16	Corn	4/30/2018	V3: June 1, 2018 R1: June 28, 2018
Boone	2018	Mexico silt loam	2.5	7	15.8	Corn	5/10/2018	V3: June 5, 2018 R1: July 2, 2018
Knox	2018	Putnam silt loam	1.8	5.8	12.6	Corn	5/1/2018	V3: May 31, 2018 R1: June 29, 2018
Moniteau	2018	Maplewood silt loam	2.3	6.1	11.4	Soybean	5/9/2018	V3: June 5, 2018 R1: July 2, 2018
Warren	2019	Mexico silt loam	2.6	6	10.3	Corn	5/13/2019	V3: June 18, 2019 R1: July 17, 2019
Jackson	2019	Arisburg silt loam & Higginsville silt loam	3.1	6.2	17.4	Soybean	6/3/2019	V3: June 28, 2019 R1: July 19, 2019
Scott	2019	Adler silt loam & Memphis silt loam	1.6	5.1	10.9	Corn	6/11/2019	V3: July 1, 2019 R1: July 23, 2019

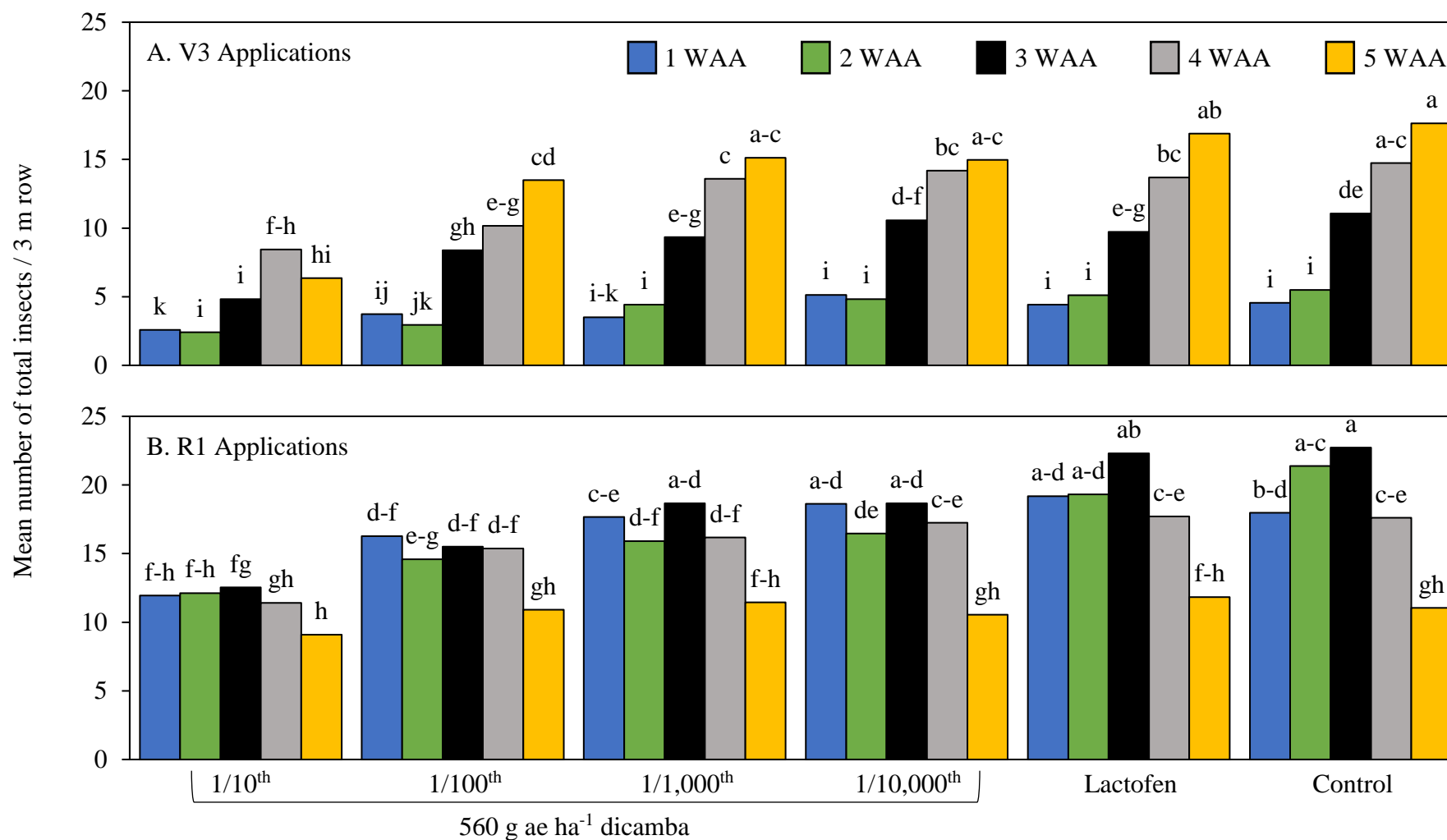


Figure 1. Mean number of insect species collected following V3 (A) and R1 (B) herbicide injury applications. Results are combined across 7 site-years. Means followed by the same letter within and application timing are not different, $P < 0.05$.

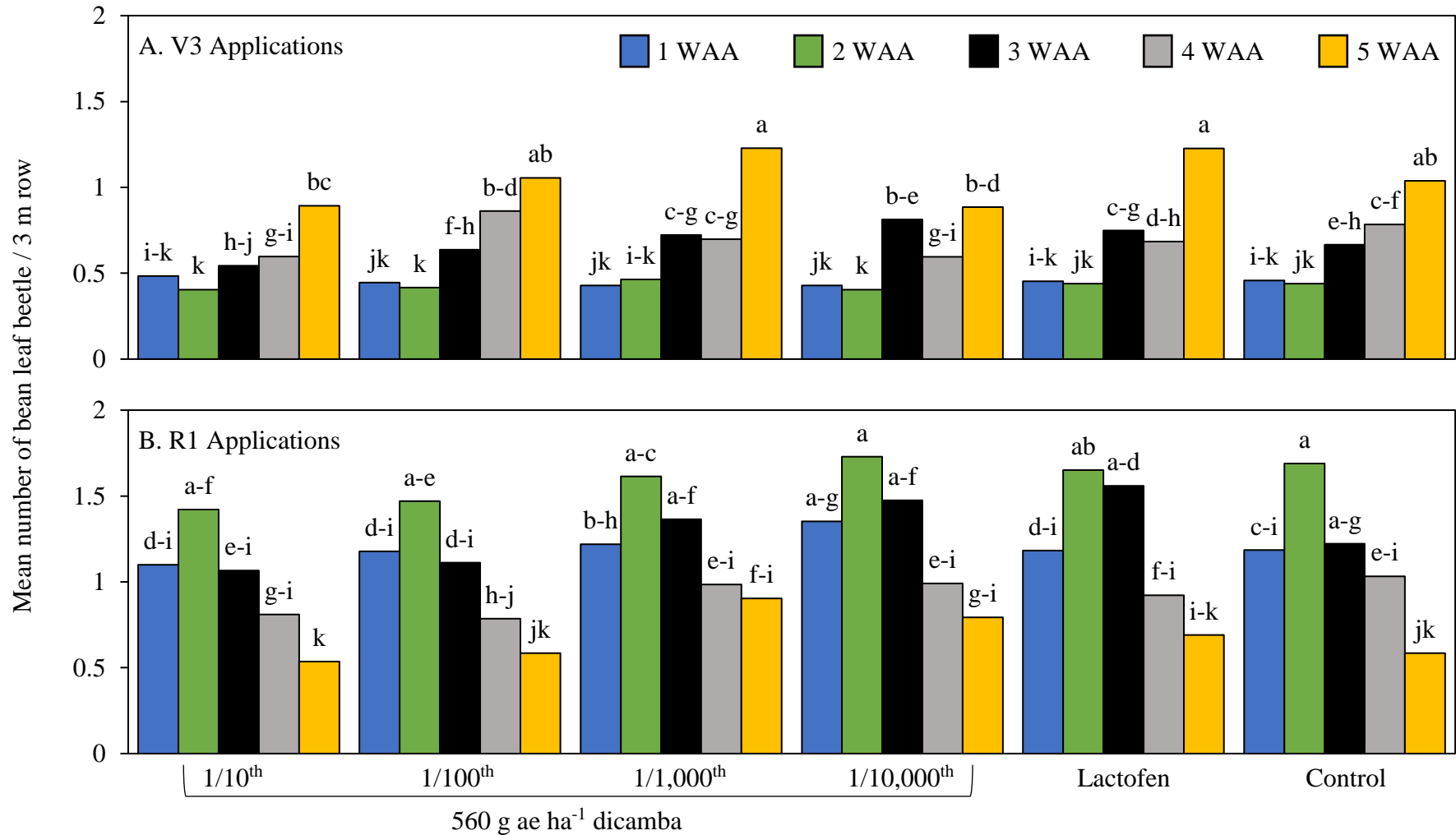


Figure 2. Mean number of bean leaf beetle collected following V3 (A) and R1 (B) herbicide injury applications. Results are combined across 7 site-years. Means followed by the same letter within and application timing are not different, $P < 0.05$.

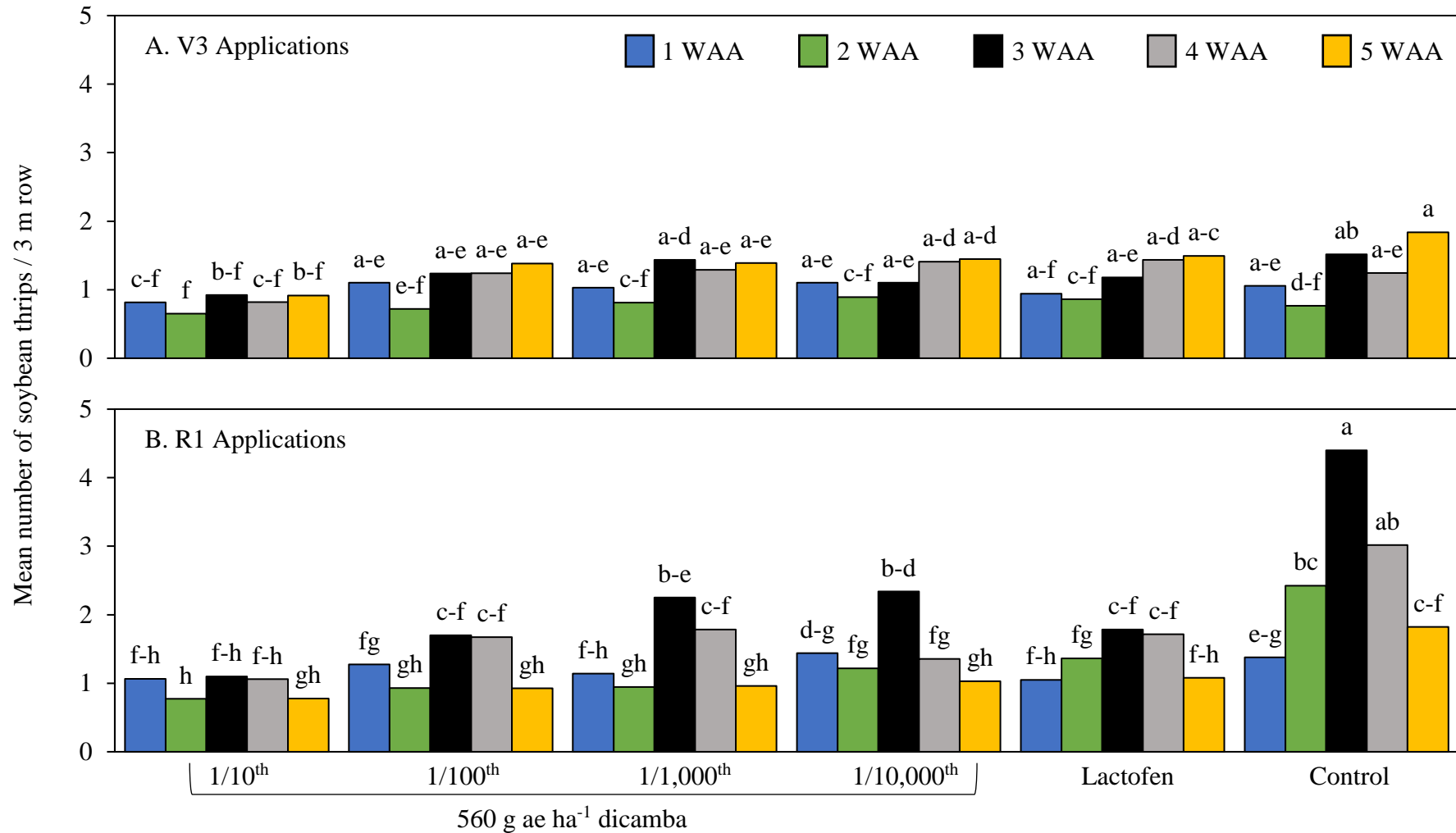


Figure 3. Mean number of soybean thrips collected following V3 (A) and R1 (B) herbicide injury applications. Results are combined across 7 site-years. Means followed by the same letter within and application timing are not different, $P < 0.05$.

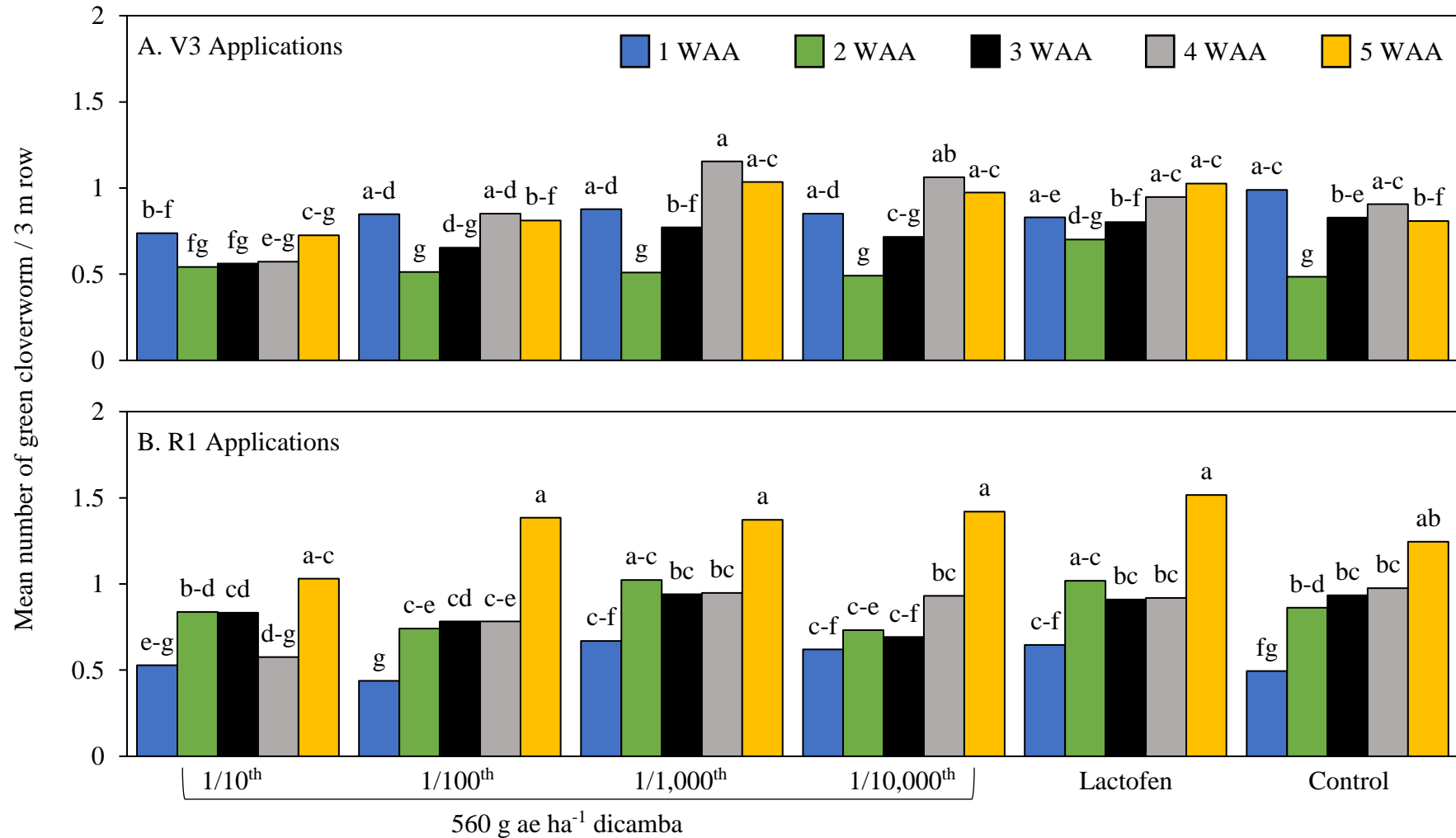


Figure 4. Mean number of green cloverworm collected following V3 (A) and R1 (B) herbicide injury applications. Results are combined across 7 site-years. Means followed by the same letter within and application timing are not different, $P < 0.05$.

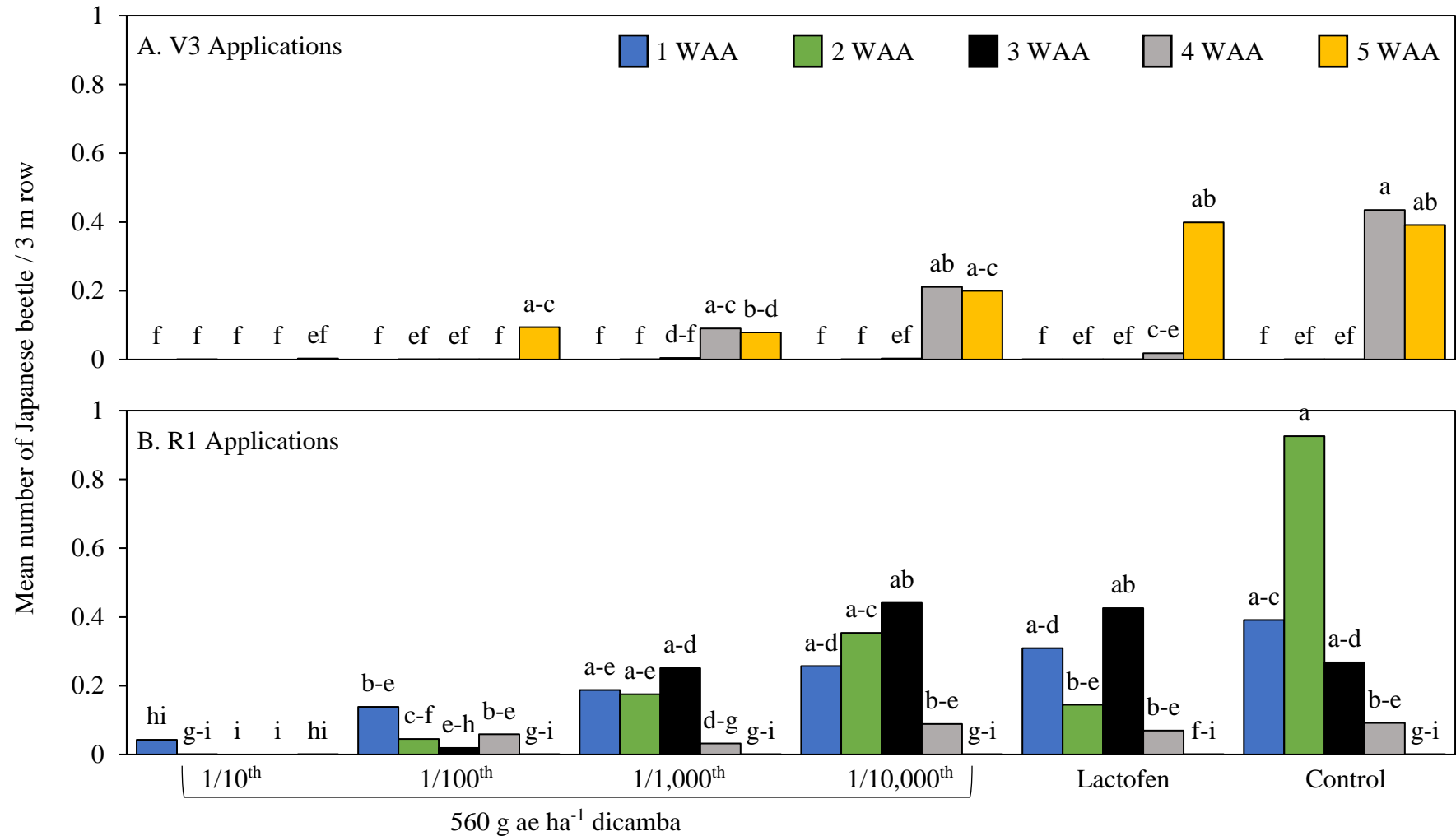


Figure 5. Mean number of Japanese beetle collected following V3 (A) and R1 (B) herbicide injury applications. Results are combined across 7 site-years. Means followed by the same letter within and application timing are not different, $P < 0.05$.

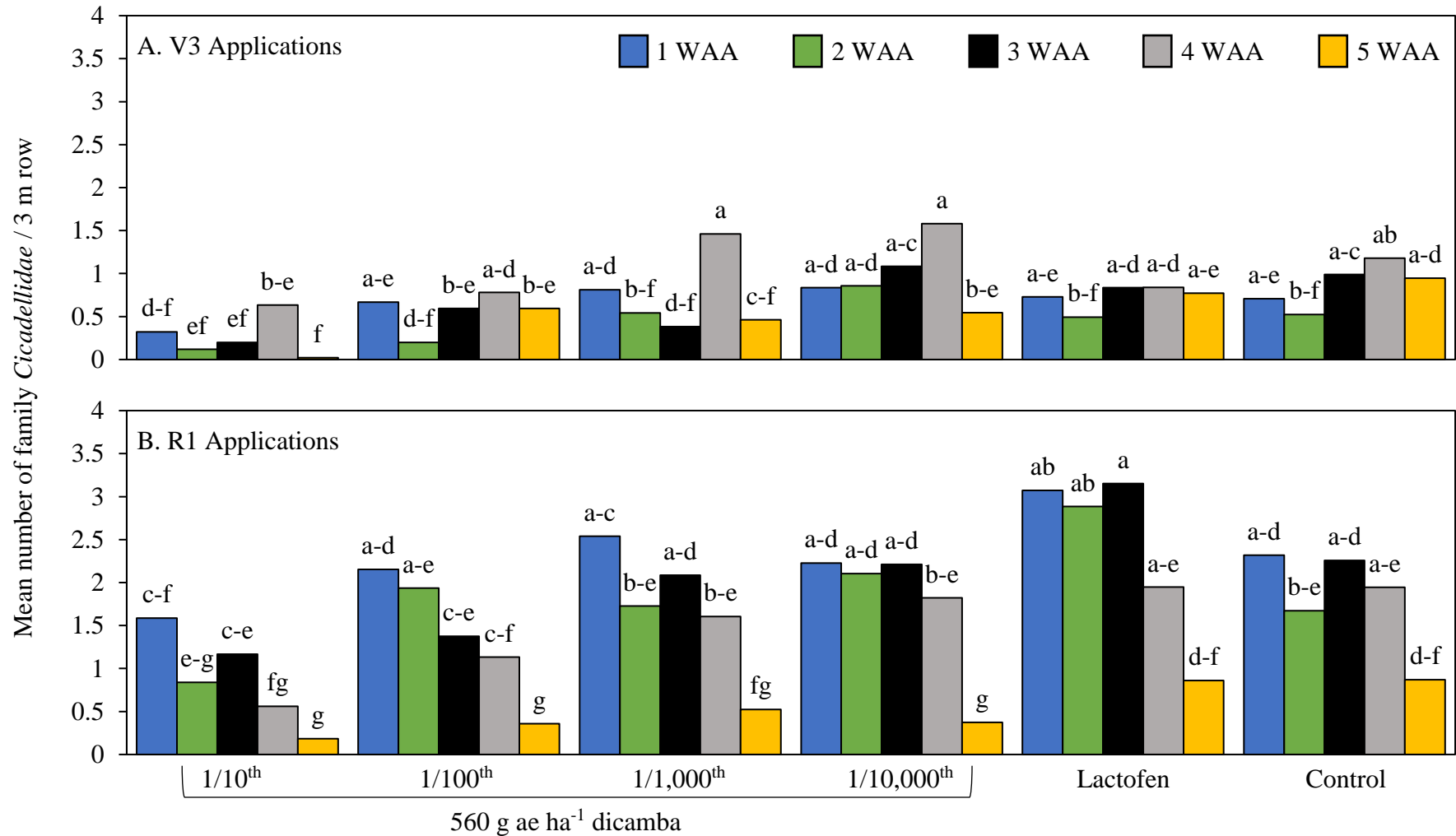


Figure 6. Mean number of insects in family *Cicadellidae* collected following V3 (A) and R1 (B) herbicide injury applications. Results are combined across 7 site-years. Means followed by the same letter within and application timing are not different, $P < 0.05$.

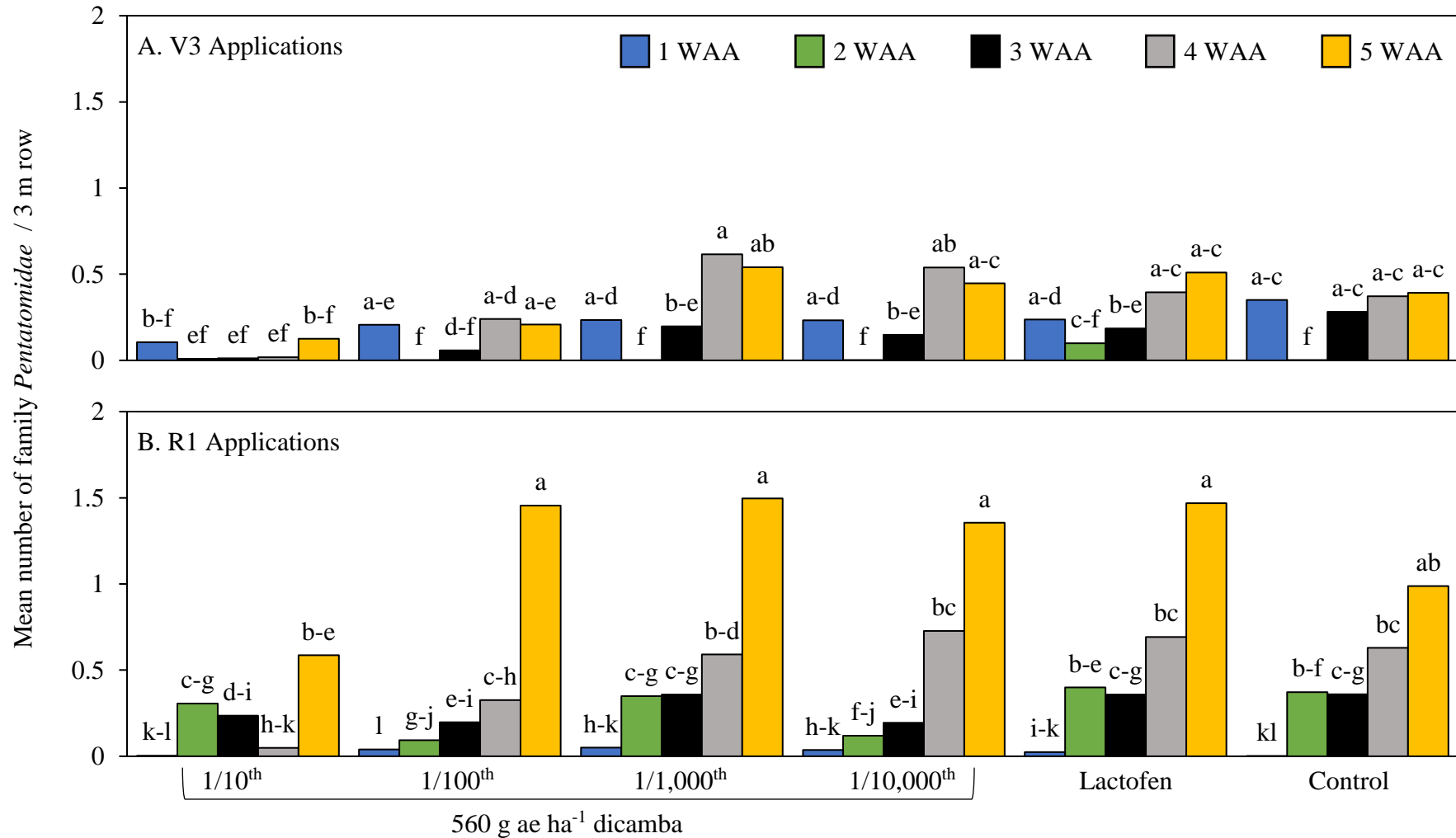


Figure 7. Mean number of insects in family *Pentatomidae* collected following V3 (A) and R1 (B) herbicide injury applications. Results are combined across 7 site-years. Means followed by the same letter within and application timing are not different, $P < 0.05$.

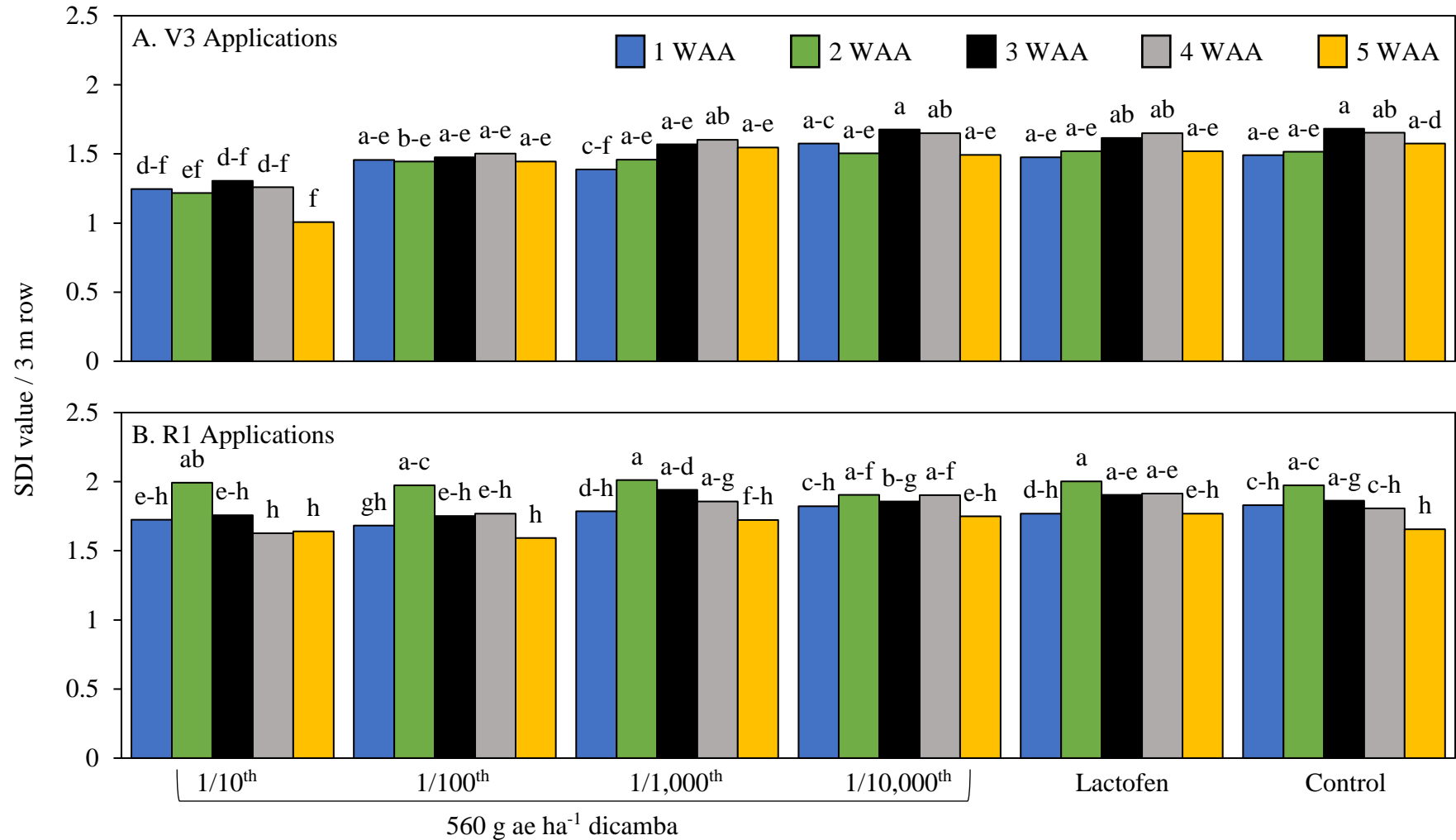


Figure 8. Shannon Diversity Index (SDI) values following V3 (A) and R1 (B) herbicide injury applications. Results are combined across 7 site-years. Means followed by the same letter within and application timing are not different, $P < 0.05$.

Table 2.2. Soybean yield response to V3 and R1 herbicide injury applications.

	----- 560 g ae ha ⁻¹ dicamba -----				Lactofen	Control
	1/10th	1/100th	1/1,000th	1/10,000th		
	----- ^c Soybean yield (kg ha ⁻¹) -----					
V3 Applications	1,835 d	3,194 b	3,282 ab	3,321 ab	3,351 ab	3,367 a
R1 Applications	495 e	2,612 c	3,189 b	3,343 ab	3,234 ab	3,367 a
P-value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

^aMeans followed by the same letter are not different, P<0.05.

^bResults are combined across 7 site-years.

^cYield adjusted to 13% moisture.