

PEST MANAGEMENT OF JAPANESE BEETLE (COLEOPTERA:  
SCARABAEIDAE) AND A STUDY OF STINK BUG (HEMIPTERA:  
PENTATOMIDAE) INJURY ON PRIMOCANE-BEARING CANEBERRIES IN  
SOUTHWEST VIRGINIA

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**ABSTRACT**

Field experiments (2007-2009) and laboratory bioassays (2009) tested the efficacy of insecticides with short pre-harvest intervals, caneberry cultivar susceptibility, and geranium toxicity for reducing Japanese beetle (JB) activity on primocane-bearing caneberries. Deltamethrin, chlorantraniliprole, bifenthrin, lime-alum, and thyme oil reduced JB activity in the field. Deltamethrin, chlorantraniliprole, acetamiprid, an azadirachtin and pyrethrin mixture, an azadirachtin and neem oil extract mixture, and an extract of *Chenopodium ambrosioides* reduced JB activity during the bioassays.

'Prelude' had significantly more JB than 'Anne', 'Caroline', 'Heritage', 'Dinkum', or 'Himbo Top' and 'Prime-Jan' had significantly more JB than 'Prime-Jim'. Compared to certain cultivars, 'Heritage', 'Caroline', 'Himbo Top', and 'Prime-Jan' had higher percentages of injured fruit and 'Autumn Bliss', 'Heritage', and 'Caroline' produced greater marketable and overall yields. 'Prime-Jan' produced more overall yield than 'Prime-Jim'; marketable yields from both blackberry cultivars were similar. Defoliation was significantly less for 'Dinkum', 'Caroline', 'Heritage', and 'Anne' than for 'Prelude' in 2008 and significantly less for 'Caroline' and 'Anne' than 'Prelude' or 'Fall Gold' in 2009.

In field tests, previous consumption of geraniums lessened raspberry defoliation by JB. Bioassays indicated that JB activity was only reduced if JB were continually exposed to geranium. Therefore, the efficacy of geranium as a trap crop for JB may be limited.

The stink bug species within the caneberries were identified (2008-2009) and *Euschistus servus* (Say) made up 48.1 % of the overall species composition. Stink bug injury to ripening raspberries was identified as small holes between drupelets; stink bug excretions also ruined fruit.

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## TABLE OF CONTENTS

ABSTRACT.....	ii
ACKNOWLEDGMENTS .....	iii
TABLE OF CONTENTS.....	iv
LIST OF FIGURES .....	vii
LIST OF TABLES .....	xii
CHAPTER 1: Introduction and Literature Review.....	1
<b>Primocane-bearing raspberry and blackberries.....</b>	<b>1</b>
Description of Caneberries.....	1
History and Development .....	2
Primocane-bearing Caneberries .....	3
Caneberry Arthropod Pest Management .....	5
<b>Japanese beetle.....</b>	<b>6</b>
History.....	6
Adult Description.....	7
Life History and Biology .....	7
Significance.....	9
Significance in Caneberries.....	10
Integrated Pest Management in Caneberries .....	12
<b>Stink bugs (Hemiptera: Pentatomidae) .....</b>	<b>15</b>
History.....	15
Significance.....	16
Reports of Stink Bugs in Caneberries .....	19
Pest Management of Stink Bugs .....	21
<b>References Cited .....</b>	<b>24</b>
CHAPTER 2: Efficacy of Insecticides with Short Pre-Harvest Intervals against Japanese Beetle, <i>Popillia japonica</i> Newman (Coleoptera: Scarabaeidae) Infestation and Fruit and Foliar Injury of Primocane-Bearing Caneberries .....	37
<b>Abstract .....</b>	<b>37</b>
<b>Introduction .....</b>	<b>38</b>
<b>Materials and Methods .....</b>	<b>40</b>
Field Experiments .....	40
2007 Treatment Applications .....	40
2008 Treatment Applications .....	41
2009 Treatment Applications .....	41
Japanese Beetle Counts 2007- 2009.....	41
Harvest 2007-2009 .....	42
Defoliation Study 2007 .....	43
Defoliation Study 2008-2009 .....	44
Laboratory Experiments.....	46
<b>Results.....</b>	<b>47</b>
Field Experiments .....	47
Raspberries 2007 .....	47
Blackberries 2007.....	49
Raspberries 2008.....	50

Blackberries 2008.....	51
Raspberries 2009.....	52
Laboratory Experiments.....	54
Japanese Beetles on Treated Leaves.....	54
Mortality.....	55
Defoliation.....	56
<b>Discussion .....</b>	<b>56</b>
<b>References Cited .....</b>	<b>62</b>
<b>CHAPTER 3: Susceptibility of Primocane-bearing Raspberry and Blackberry Cultivars to Japanese Beetle, <i>Popillia japonica</i> Newman (Coleoptera: Scarabaeidae), Infestations and Fruit and Foliar Injury .....</b>	
<b>Abstract .....</b>	<b>95</b>
<b>Introduction .....</b>	<b>96</b>
<b>Materials and Methods .....</b>	<b>98</b>
Japanese Beetle Counts.....	99
Harvest.....	99
Defoliation Studies.....	100
Defoliation Study 2007 .....	100
Defoliation Study 2008-2009 .....	101
<b>Results.....</b>	<b>102</b>
Japanese Beetle Counts.....	102
Harvest.....	104
Defoliation Studies.....	107
2007 Estimation of Defoliation .....	107
2008 Total Calculated Defoliation .....	107
2009 Total Calculated Defoliation in Raspberries.....	108
<b>Discussion .....</b>	<b>108</b>
Cultivar Susceptibility to Japanese Beetle Infestations and Plant Injury .....	108
Raspberries.....	109
Blackberries .....	112
Japanese Beetle Host Selection and Caneberry Cultivar Susceptibility.....	113
Summary.....	117
<b>References Cited .....</b>	<b>119</b>
<b>CHAPTER 4: Effects of Geranium (<i>Pelargonium × hortorum</i>) on Japanese Beetle, <i>Popillia japonica</i> Newman (Coleoptera: Scarabaeidae), in Primocane-Bearing Raspberries.....</b>	
<b>Abstract .....</b>	<b>161</b>
<b>Introduction .....</b>	<b>161</b>
<b>Materials and Methods .....</b>	<b>166</b>
2007 Field Experiments (Cage to Bag).....	166
2009 Laboratory Experiments.....	167
Raspberry and Geranium.....	167
Raspberry or Geranium.....	168
<b>Results.....</b>	<b>169</b>
2007 Field Experiments (Cage to Bag).....	169
2009 Laboratory Experiments.....	170
Raspberry and Geranium.....	170

Raspberry or Geranium .....	170
<b>Discussion .....</b>	<b>170</b>
<b>References Cited .....</b>	<b>175</b>
<b>CHAPTER 5: A Survey of the Stink Bug (Hemiptera: Pentatomidae) Species Present in a Primocane-bearing Raspberry Planting in Southwest Virginia and Identification of Stink Bug Injury to Raspberries .....</b>	
<b>Abstract .....</b>	<b>189</b>
<b>Introduction .....</b>	<b>190</b>
<b>Materials and Methods .....</b>	<b>193</b>
Species Survey .....	193
Identification of Stink Bug Damage to Raspberries .....	194
2008 Field Experiments.....	194
2009 Potted Raspberry Experiments .....	195
<b>Results and Discussion .....</b>	<b>195</b>
<b>References Cited .....</b>	<b>202</b>

## LIST OF FIGURES

Figure 1.1 NAPIS generated map of the distribution of Japanese beetle, <i>Popillia japonica</i> , in the United States as of 12/31/2009. ....	33
Figure 1.2 An adult Japanese beetle, <i>Popillia japonica</i> . A metallic green pronotum, coppery abdomen, and five visible tufts of white hair distinguish this beetle species from other scarabs. 34	
Figure 1.3 Skeletonized raspberry leaf. Japanese beetles feed on plant tissue in-between veins resulting in this skeletonized appearance.....	34
Figure 2.1 Mean number $\pm$ SE of Japanese beetle per 2.0 m raspberry plot that were treated approximately weekly (6 $\times$ ) with selected insecticides at Kentland Farm, Montgomery Co., VA in 2007. Only dates with significant differences include letters above the values and values followed by the same letter are not significantly different (Tukey's HSD ( $\alpha = 0.05$ ) following ( $\sqrt{x} + 0.5$ ) transformation). ....	69
Figure 2.2 Mean cumulative numbers $\pm$ SE of Japanese beetles per 2.0 m of raspberries that were treated approximately weekly (6 $\times$ ) with selected insecticides at Kentland Farm, Montgomery Co., VA in 2007. Values followed by the same letter are not significantly different Tukey's HSD ( $\alpha = 0.05$ ) following ( $\sqrt{x} + 0.5$ ) transformation) ( $F = 12.28$ ; $df = 5$ ; $P < 0.0001$ )..	70
Figure 2.3 Image of a raspberry plant showing white residue build-up after weekly applications (6 $\times$ ) of lime-alum at 1120.9 g lime and 350.3 g alum per acre. ....	71
Figure 2.4 Mean cumulative percent defoliation $\pm$ SE of 'Autumn Bliss' and 'Fall Gold' raspberries that were treated approximately weekly (6 $\times$ ) with selected insecticides at Kentland Farm, Montgomery Co., VA in 2007. The treatment source of variation was not significant according to ANOVA ( $F = 1.71$ ; $df = 5$ ; $P = 0.1851$ ). ....	73
Figure 2.5 Mean number $\pm$ SE of Japanese beetle per 2.0 m blackberry plot that were treated approximately weekly (6 $\times$ ) with selected insecticides at Kentland Farm, Montgomery Co., VA in 2007. There were only significant differences between treatments on 5 July with values followed by the same letter not being significantly different (Tukey's HSD ( $\alpha = 0.05$ ) following ( $\sqrt{x} + 0.5$ ) transformation).....	74
Figure 2.6 Mean cumulative numbers $\pm$ SE of Japanese beetles per 2.0 m blackberry plot treated approximately weekly (6 $\times$ ) with selected insecticides at Kentland Farm, Montgomery Co., VA in 2007. Treatment means labeled by the same letter are not significantly different according to Tukey's HSD ( $\alpha = 0.10$ ) ( $F = 3.10$ ; $df = 5$ ; $P = 0.0501$ ); data was ( $\sqrt{x} + 0.5$ ) transformed. ....	75
Figure 2.7 Mean percent defoliation $\pm$ SE after Japanese beetles were bagged onto individual canes of 'Prime-Jim' blackberries that were treated approximately weekly (6 $\times$ ) with selected insecticides at Kentland Farm, Montgomery Co., VA in 2007. Only dates with significant differences include letters above the values and values followed by the same letter are not significantly different (Tukey's HSD ( $\alpha = 0.05$ ) following (ArcSine ( $\sqrt{x}$ )) transformation). ....	77
Figure 2.8 Mean cumulative percent defoliation $\pm$ SE of 'Prime-Jim' blackberries that were treated approximately weekly (6 $\times$ ) with selected insecticides at Kentland Farm, Montgomery Co., VA in 2007. Values followed by the same letter are not significantly different (Tukey's HSD, $\alpha = 0.05$ ) ( $F = 4.34$ ; $df = 5$ ; $P = 0.0091$ ). ....	78

Figure 2.9 Mean number  $\pm$  SE of Japanese beetles per 1.2 m raspberry plot that were treated approximately weekly (7  $\times$ ) with selected insecticides at Kentland Farm, Montgomery Co., VA in 2008. Only dates with significant differences include letters above the values and values followed by the same letter are not significantly different (Tukey's HSD ( $\alpha = 0.05$ ) following ( $\sqrt{x} + 0.5$ ) transformation). ..... 79

Figure 2.10 Mean cumulative numbers  $\pm$  SE of Japanese beetles per 1.2 m raspberry plot treated approximately weekly (7  $\times$ ) with selected insecticides at Kentland Farm, Montgomery Co., VA in 2008. Values followed by the same letter are not significantly different (Tukey's HSD, ( $\alpha = 0.05$ ) following ( $\sqrt{x} + 0.5$ ) transformation) ( $F = 2.77$ ;  $df = 4$ ;  $P = 0.0469$ )..... 80

Figure 2.11 Mean cumulative percent defoliation  $\pm$  SE of primocane-bearing raspberries treated approximately weekly (7  $\times$ ) with selected insecticides at Kentland Farm, Montgomery Co., VA in 2008. Treatment means followed by the same letter are not significantly different (Tukey's HSD ( $\alpha = 0.05$ );  $F = 3.57$ ;  $df = 4$ ;  $P = 0.0069$ ) following ArcSine ( $\sqrt{x}$ ) transformation..... 82

Figure 2.12 Mean number  $\pm$  SE of Japanese beetle per 1.2 m blackberry plot that were treated approximately weekly (7  $\times$ ) with selected insecticides at Kentland Farm, Montgomery Co., VA in 2008. There were only significant differences between treatments on 15 August with values followed by the same letter not being significantly different (Tukey's HSD ( $\alpha = 0.05$ ) following ( $\sqrt{x} + 0.5$ ) transformation)..... 83

Figure 2.13 Mean cumulative numbers  $\pm$  SE of Japanese beetles per 1.2 m blackberry plot treated approximately weekly (7  $\times$ ) with selected insecticides at Kentland Farm, Montgomery Co., VA in 2008. Treatment means followed by the same letter are not significantly different (Tukey's HSD ( $\alpha = 0.10$ );  $F = 4.24$ ;  $df = 5$ ;  $P = 0.0693$ ) following ( $\sqrt{x} + 0.5$ ) transformation. .. 84

Figure 2.14 The mean percent defoliation  $\pm$  SE of primocane-bearing blackberries treated approximately weekly (7  $\times$ ) with selected insecticides at Kentland Farm, Montgomery Co., VA in 2008. The treatment source of variation was not significant according to ANOVA ( $\alpha = 0.05$ ) following an ArcSine ( $\sqrt{x}$ ) transformation ( $F = 0.20$ ;  $df = 5$ ;  $P = 0.9639$ )..... 86

Figure 2.15 Mean number  $\pm$  SE of Japanese beetle per 1.2 m raspberry plot that were treated approximately weekly (6  $\times$ ) with selected insecticides at Kentland Farm, Montgomery Co., VA in 2009. Only dates with significant differences include letters above the values and values followed by the same letter are not significantly different (Tukey's HSD ( $\alpha = 0.05$ ) following ( $\sqrt{x} + 0.5$ ) transformation). ..... 87

Figure 2.16 Mean cumulative numbers  $\pm$  SE of Japanese beetles counted per 1.2 m raspberry plot treated approximately weekly (6  $\times$ ) with selected insecticides at Kentland Farm, Montgomery Co., VA in 2009. Values followed by the same letter for each date are not significantly different (Tukey's HSD ( $\alpha = 0.05$ );  $F = 7.92$ ;  $df = 5$ ;  $P < 0.0001$ ) following ( $\sqrt{x} + 0.5$ ) transformation..... 88

Figure 2.17 The mean percent defoliation  $\pm$  SE of only the injured leaves of primocane-bearing raspberries treated approximately weekly (6  $\times$ ) with selected insecticides at Kentland Farm, Montgomery Co., VA in 2009. Values followed by the same letter are not significantly different (Tukey's HSD ( $\alpha = 0.05$ );  $F = 8.57$ ;  $df = 5$ ;  $P < 0.0001$ ) following ArcSine ( $\sqrt{x}$ ) transformation. 90

Figure 2.18 The mean percent of Japanese beetles on treated 'Prelude' leaves after 6, 18, 24, and 48 hrs exposure. Values followed by a different letter are significantly different (Tukey's HSD,  $\alpha = 0.05$ )..... 92



Figure 2.19 The mean percent mortality of Japanese beetles after 6, 18, 24, and 48 hrs exposure to six pesticides. Values followed by the same letter are not significantly different (Tukey's HSD, $\alpha=0.05$ following an ArcSine ( $\sqrt{x}$ ) transformation).....	93
Figure 2.20 Mean percent defoliations of 'Prelude' raspberry per treated leaf in a 2009 lab experiment. Values followed by the same letter are not significantly different (Tukey's HSD ( $\alpha=0.05$ ); $F = 6.25$ ; $df = 6$ ; $P < 0.0001$ ) following ArcSine ( $\sqrt{x}$ ) transformation.....	94
Figure 3.1 Mean number of Japanese beetles by date per 2.0 m plots in eight raspberry cultivars in 2007. Significant differences between cultivar means were only found on 17 and 20 July and values on those dates followed by the same letter are not significantly different (Tukey's HSD ( $\alpha = 0.05$ ) following [ $\sqrt{(x + 0.5)}$ ] transformation).....	128
Figure 3.2 Mean total number of Japanese beetles counted per 2.0 m in eight raspberry cultivars in 2007. Values followed by the same letter are not significantly different according to Tukey's HSD after [ $\sqrt{(x + 0.5)}$ ] transformation ( $F = 4.65$ ; $df = 7$ ; $P = 0.0009$ ). .....	129
Figure 3.3 Mean number of Japanese beetles by date per 2.0 m plots in 'Prime-Jan' or 'Prime-Jim' blackberries in 2007. Significant differences between cultivar means were only found on 12, 17, and 20 July and values on those dates followed by the same letter are not significantly different (Student's t-test ( $\alpha = 0.05$ ) following [ $\sqrt{(x + 0.5)}$ ] transformation). .....	130
Figure 3.4 Mean total number of Japanese beetles counted per 2.0 m in 'Prime-Jan' or 'Prime-Jim' blackberries in 2007. Values are significantly different (Student's t-test ( $F = 11.82$ ; $df = 1$ ; $P = 0.0049$ ) following [ $\sqrt{(x + 0.5)}$ ] transformation). .....	131
Figure 3.5 Mean number of Japanese beetles by date per 1.2 m plots of eight raspberry cultivars in 2008. Significant differences between cultivar means were only found on 15 August and values followed by the same letter are not significantly different on that date (Tukey's HSD ( $\alpha = 0.05$ ) following [ $\sqrt{(x + 0.5)}$ ] transformation). .....	132
Figure 3.6 Mean total number of Japanese beetles counted per 1.2 m treated raspberry plot in 2008. Values followed by the same letter are not significantly different (Tukey's HSD ( $F = 3.21$ ; $df = 7$ ; $P = 0.0126$ ) following [ $\sqrt{(x + 0.5)}$ ] transformation).....	133
Figure 3.7 Mean number of Japanese beetles by date per 1.2 m plots in 'Prime-Jan' or 'Prime-Jim' blackberries in 2008. Only dates with significant differences include letters above the values and values followed by the same letter are not significantly different (Student's t-test ( $\alpha = 0.05$ ) following [ $\sqrt{(x + 0.5)}$ ] transformation) .....	134
Figure 3.8 Mean total number of Japanese beetle counted per 1.2 m in 'Prime-Jan' or 'Prime-Jim' blackberries in 2008. Values are significantly different (Student's t-test ( $F = 27.26$ ; $df = 1$ ; $P = 0.0034$ ) following [ $\sqrt{(x + 0.5)}$ ] transformation). .....	135
Figure 3.9 Mean number of Japanese beetles by date per 1.2 m plots of eight raspberry cultivars in 2009. Only dates with significant differences include letters above the values and values followed by the same letter are not significantly different (Tukey's HSD ( $\alpha = 0.05$ ) following [ $\sqrt{(x + 0.5)}$ ] transformation).....	136
Figure 3.10 Mean total number of Japanese beetles counted per 1.2 m of eight raspberry cultivars in 2009. Values followed by the same letter are not significantly different (Tukey's HSD ( $F = 4.82$ ; $df = 7$ ; $P = 0.0004$ ) following [ $\sqrt{(x + 0.5)}$ ] transformation). .....	137

Figure 3.11 The estimated year total defoliation from Japanese beetles of eight raspberry cultivars in 2007. The cultivar source of variation was not significant according to ANOVA ( $F = 1.71$ ;  $df = 1$ ;  $P = 0.5457$ )..... 157

Figure 3.12 The mean percent defoliation of eight primocane-bearing raspberry plants per leaf including undamaged leaves in 2008. The percents defoliation followed by the same letter are not significantly different (Tukey's HSD, ( $F = 3.85$ ;  $df = 7$ ;  $P = 0.0004$ ) following [ArcSine ( $\sqrt{x}$ )] transformation)..... 158

Figure 3.13 The mean percent defoliation of 'Prime-Jan' and 'Prime-Jim' blackberries including undamaged leaves in 2008. The cultivar source of variation was not significant according to ANOVA ( $F = 1.54$ ;  $df = 1$ ;  $P = 0.2159$ ) following [ArcSine ( $\sqrt{x}$ )] transformation). ..... 159

Figure 3.14 The mean percent defoliation of eight primocane-bearing raspberry plants per leaf including only damaged leaves in 2009. Values followed by the same letter are not significantly different (Tukey's HSD, ( $F = 4.34$ ;  $df = 7$ ;  $P < 0.0001$ ) following [ArcSine ( $\sqrt{x}$ )] transformation). ..... 160

Figure 4.1 Nylon mesh, wood, and Plexiglas® cage designed to hold potted plants and Japanese beetles. .... 178

Figure 4.2 Japanese beetles were caged with either a raspberry or geranium plant and then bagged onto a raspberry plant. The percent of active beetles after being bagged on the raspberries were compared; significant difference between treatments was only found on 19 July (Student's t-test ( $\alpha = 0.05$ ))...... 179

Figure 4.3 The mean percentage of four trials observing the percent of active Japanese beetles after exposure to either a raspberry or a geranium plant and then a raspberry plant. The treatment source of variation was not significant according to ANOVA ( $\alpha = 0.05$ ;  $F = 1.68$ ;  $df = 1$ ;  $P = 0.3240$ ) after ArcSine ( $\sqrt{x}$ ) transformation. .... 180

Figure 4.4 Japanese beetles were caged with either a raspberry plant or a geranium plant followed by exposure to raspberry alone. The percent of living beetles from each treatment were compared; significant difference between treatments was only found on 19 July (Student's t-test ( $\alpha = 0.05$ )). ..... 181

Figure 4.5 The mean percentage of four trials observing the percent of living Japanese beetles after exposure to either a raspberry or a geranium plant followed by exposure to a raspberry plant. The treatment source of variation was significant (Student's t-test,  $\alpha = 0.10$ ;  $F = 4.73$ ;  $df = 1$ ;  $P = 0.0953$ ). ..... 182

Figure 4.6 Japanese beetles were caged with either a raspberry plant or a geranium plant and then removed and bagged onto a raspberry cane. There was significant difference between the treatments only on 19 July (Student's t-test ( $\alpha = 0.05$ )). ..... 183

Figure 4.7 The mean percentage of four trials observing the percent defoliation of raspberry leaves after Japanese beetles were exposed to either a raspberry or a geranium plant followed by exposure to a raspberry plant. The defoliation values are significantly different (Student's t-test,  $\alpha = 0.05$ ;  $F = 4.49$ ;  $df = 1$ ;  $P = 0.0422$ ). ..... 184

Figure 4.8 Two Japanese beetles were exposed to a 'Prelude' raspberry leaf and a geranium flower. After 24 hrs the percent of the beetles on each plant was recorded. The treatment source

of variation was not significant according to ANOVA ( $\alpha = 0.05$ ;  $F = 0.46$ ;  $df = 1$ ;  $P = 0.5055$ ).  
..... 185

Figure 4.9 Two Japanese beetles were exposed to a 'Prelude' raspberry leaf and a geranium flower and after 48 hrs the percent of the beetles on each plant was recorded. The percent of Japanese beetles on raspberries was significantly higher than the number of Japanese beetles on geranium (Student's t-test,  $\alpha = 0.05$ ;  $F = 6.55$ ;  $df = 1$ ;  $P = 0.0197$ ). ..... 186

Figure 4.10 Two Japanese beetles were exposed to a geranium flower along with a raspberry leaf or to a raspberry leaf only. After 48 hrs the percent defoliation of the raspberry leaves were compared and the values were significantly different (Student's t-test ( $\alpha = 0.05$ ;  $F = 4.43$ ;  $df = 1$ ;  $P = 0.0444$ ); data was ArcSine ( $\sqrt{x}$ ) transformed). ..... 187

Figure 4.11 Defoliations from Japanese beetle exposed to either a geranium flower or a 'Prelude' raspberry leaf and then exposed to a fresh raspberry leaf. The defoliations of the raspberry leaves were compared and the treatment source of variation was not significant according to ANOVA ( $\alpha = 0.05$ ;  $F = 2.37$ ;  $df = 1$ ;  $P = 0.1353$ ) after an ArcSine ( $\sqrt{x}$ ) transformation. .... 188

Figure 5.1 A brown stink bug (*Euschistus servus*) feeding on a vein of the underside of a raspberry leaf. .... 209

Figure 5.2 A brown stink bug (*Euschistus servus*) feeding on the sepal of a raspberry plant.. 210

Figure 5.3 Brown stink bugs (*Euschistus servus*) feeding in-between the drupelets of unripe red raspberries. .... 211

Figure 5.4 Green stink bug (*Acrosternum hilare*) feeding in-between the drupelets of a 'Fall Gold' raspberry..... 212

Figure 5.5 Enlarged image of stink bug damage on a red raspberry. Injury is seen as holes in between the drupelets and this caved-in drupelet. .... 213

Figure 5.6 Enlarged image of stink bug frass on a red raspberry. Berries with stink bug frass have a bad taste making them unmarketable. .... 214

## LIST OF TABLES

Table 1.1 Insecticides registered for Japanese beetle control on raspberries in Virginia, 2010. ..	35
Table 1.2 Insecticides registered for stink bug control on raspberries in Virginia, 2010. ....	36
Table 2.1 Treatment applied to 2.0 m sections of primocane-bearing raspberries in 2007. ....	66
Table 2.2 Treatments applied to 2.0 m sections of primocane-bearing blackberries in 2007. ....	66
Table 2.3 Treatments applied to 1.2 m sections of primocane-bearing raspberries in 2008. ....	67
Table 2.4 Treatments applied to 1.2 m sections of primocane-bearing blackberries in 2008. ....	67
Table 2.5 Treatments applied to 1.2 m sections of primocane-bearing raspberries in 2009. ....	68
Table 2.6 Treatments applied to 'Prelude' raspberry leaves during a bioassay conducted in 2009. .....	68
Table 2.7 Mean cumulative yields $\pm$ SE per m row of primocane-bearing raspberries that were treated approximately weekly (6 $\times$ ) with selected insecticides at Kentland Farm, Montgomery Co., VA in 2007. Yields harvested on individual dates are not included due to significant differences not being found. ....	72
Table 2.8 The means $\pm$ SE of marketable yields on 21 and 31 August and the mean cumulative yields $\pm$ SE per m row of primocane-bearing blackberries that were treated approximately weekly (6 $\times$ ) with selected insecticides at Kentland Farm, Montgomery Co., VA in 2007. Individual dates not listed for marketable or unmarketable yields did not have significant differences between treatments.....	76
Table 2.9 The means $\pm$ SE of unmarketable yields on 2 August and the mean cumulative yields $\pm$ SE per m row of primocane-bearing raspberries that were treated approximately weekly (7 $\times$ ) with selected insecticides at Kentland Farm, Montgomery Co., VA in 2008. Individual dates not listed for marketable or unmarketable yields did not have significant differences between treatments.....	81
Table 2.10 Mean cumulative yields $\pm$ SE per m row of primocane-bearing blackberries that were treated approximately weekly (7 $\times$ ) with selected insecticides at Kentland Farm, Montgomery Co., VA in 2008. Individual dates are not listed for 2008 blackberry marketable or unmarketable yields because significant differences were not present. ....	85
Table 2.11 The means $\pm$ SE of marketable yields on 10 August, of unmarketable yields on 24 and 31 August, and the mean cumulative yields $\pm$ SE per m row of primocane-bearing raspberries that were treated approximately weekly (6 $\times$ ) with selected insecticides at Kentland Farm, Montgomery Co., VA in 2009. Individual dates not listed for marketable or unmarketable yields did not have significant differences between treatments.....	89
Table 2.12 Cumulative numbers of Japanese beetles counted and percent unmarketable fruit yield weighed in the untreated control plots in a raspberry and blackberry planting at Kentland Farm, Montgomery Co., VA from 2007-2009.....	91
Table 3.1 Sample dates in which Japanese beetles were counted and berries were harvested within 2.0 m plots in 2007 and 1.2 m plots in 2008-2009. Blackberries were not sampled on dates or year followed by (*). ....	127

Table 3.2 Mean marketable yield per m row of primocane-bearing raspberries per cultivar on individual dates 2007. Dates where significant differences were present are highlighted. ....	138
Table 3.3 Summary of significant differences in marketable yields among raspberry cultivars in 2007.....	139
Table 3.4 Mean unmarketable yield per m row of eight raspberry cultivars on individual dates in 2007. Dates where significant differences were present are highlighted.....	140
Table 3.5 Summary of significant differences in unmarketable yields among raspberry cultivars in 2007. ....	141
Table 3.6 Mean total grams of raspberries that were marketable and unmarketable. Also listed are the total yields harvested and total percent of the fruit that was unmarketable for 2007. ....	142
Table 3.7 Mean marketable yield per m row of eight raspberry cultivars on individual dates in 2007. Dates where significant differences were present are highlighted.....	143
Table 3.8 Summary of significant differences in marketable yields among blackberry cultivars in 2007.....	143
Table 3.9 Mean unmarketable yield per m row of ‘Prime-Jan’ and ‘Prime-Jim’ blackberries on individual dates in 2007. Dates where significant differences were present are highlighted. ....	144
Table 3.10 Summary of significant differences in unmarketable yields among blackberry cultivars in 2007.....	144
Table 3.11 Mean total grams of blackberries that were marketable and unmarketable. Also listed are the total yields harvested and total percent of the fruit that was unmarketable for 2007. ....	145
Table 3.12 Mean marketable yield per m row of eight raspberry cultivars on individual dates in 2008. Dates where significant differences were present are highlighted.....	146
Table 3.13 Summary of significant differences in marketable yields among raspberry cultivars in 2008.....	146
Table 3.14 Mean unmarketable yield per m row of eight raspberry cultivars on individual dates in 2008. Dates in which significant differences were present are highlighted. ....	147
Table 3.15 Summary of significant differences in unmarketable yields among raspberry cultivars in 2008. ....	147
Table 3.16 Mean total grams of raspberries that were marketable and unmarketable. Also listed are the total yields harvested and total percent of the fruit that was unmarketable for 2008. ....	148
Table 3.17 Mean marketable yield per m row of two blackberry cultivars on individual dates in 2008.....	149
Table 3.18 Mean unmarketable yield per m row of two blackberry cultivars on individual dates in 2008. Dates in which significant differences were present are highlighted. ....	150
Table 3.19 Summary of significant differences in unmarketable yields among blackberry cultivars in 2008.....	150
Table 3.20 Mean total grams of blackberries that were marketable and unmarketable. Also listed are the total yields harvested and total percent of the fruit that was unmarketable for 2008. ....	151

Table 3.21 Mean marketable yield per m row of eight raspberry cultivars on individual dates in 2009. Dates in which significant differences were present are highlighted. ....	152
Table 3.22 Summary of significant differences in marketable yields among raspberry cultivars in 2009.....	153
Table 3.23 Mean unmarketable yield per m row of eight raspberry cultivars on individual dates in 2009. Dates in which significant differences were present are highlighted. ....	154
Table 3.24 Summary of significant differences in unmarketable yields among raspberry cultivars in 2009. ....	155
Table 3.25 Mean total grams of raspberries that were marketable and unmarketable. Also listed are the total yields harvested and total percent of the fruit that was unmarketable for 2009. ....	156
Table 5.1 The number and percentage of each stink bug species collected in 2008 from primocane-bearing raspberries.....	206
Table 5.2 The number and percentage of each stink bug species collected in 2009 from primocane-bearing raspberries.....	207
Table 5.3 Stink bug species collected from 2008 and 2009 were added together to get an overall stink bug species composition of the two years. The percentage of the overall species composition is also listed. A species is considered minor if it was only collected once in the two years. ....	208

## **CHAPTER 1: Introduction and Literature Review**

### **Primocane-bearing Caneberries and Management of Japanese beetle (Coleoptera: Scarabaeidae) and Stink Bug (Hemiptera: Pentatomidae)**

#### **Primocane-bearing raspberry and blackberries**

##### **Description of Caneberries**

Caneberries (raspberries and blackberries) are a group of fruits in the genus *Rubus* that grow on leafy canes in temperate regions of the world (Wada and Ou 2002). *Rubus* is a member of the Rosaceae family of flowering plants that also contains several other significant horticultural crops such as apples, peaches, strawberries and roses (Pritts and Handley 1989). Both raspberries and blackberries have perennial roots (Bowling 2000, Funt et al. 2000). The berries themselves are an aggregate fruit that consist of clusters of smaller fruits called drupelets, which aggregate to form the final berry (Pritts and Handley 1989, Demchak et al. 2010). Raspberries and blackberries differ in that, when harvested, the receptacles of the raspberries stay attached to the cane, whereas the blackberry receptacles are removed with the fruit (Poling 1996, Bowling 2000).

Raspberries may be red, yellow, purple, or black in color, whereas blackberries are true to their name and are black (Bowling 2000, Fernandez and Krewer 2008, Demchak et al. 2010). There are three types of growth habits in caneberries including erect, semi-erect, and trailing and two types of fruiting habits, floricanne-fruiting and primocane-fruiting. Another classification difference is either canes being thorny or thornless.

## History and Development

Today's commercial raspberries, *Rubus idaeus* L., are a hybrid of the European native raspberry, *Rubus idaeus vulgates* Arrh., the native red raspberry of North America and eastern Asia, *R. idaeus strigosus* Michx., and the black raspberry of eastern North America, *R. occidentalis* L. (Shoemaker 1987, Pritts and Handley 1989, Galletta and Himelrick 1990, Lim and Knight 2000). Yellow fruit occurs in both red and black raspberry species as a recessive mutation that results in a nonfunctioning pigment gene (Hancock 2008). Purple raspberry cultivars were developed by hybridizing red and black raspberry species (Bowling 2000). Commercial raspberries were present in the United States during the mid-19<sup>th</sup> century and by 1880, around 2,000 acres were in production (Pritts and Handley 1989). In 2006, the demand for raspberries continued with raspberries ranked the third most popular berry in the United States (Pollack and Perez 2006). Currently, red raspberry cultivars are better adapted for commercial production whereas the highly perishable, reduced-yield, yellow and purple cultivars are generally not commercially suitable (Fernandez and Krewer 2008). However, yellow and purple raspberries are popular in home gardens for their preferred taste (Demchak et al. 2010). The blackberries we know today have been domesticated from many wild European and American species (Pritts and Handley 1989, Galletta and Himelrick 1990). Native American cultivars spread and hybridized as pioneers cleared forest and land for agricultural purposes. Blackberries began to be cultivated in America about 1850-1860 and by 1867, 18 native cultivars were available. Today there are many blackberry cultivars available with new cultivars still being developed (Clark and Finn 2008).



## **Primocane-bearing Caneberries**

Most commonly known caneberry types are floricanes-fruiting cultivars with perennial crowns and roots that produce biennial shoots (Demchak et al. 2005). The term primocane was proposed by L. H. Bailey for the new or first year cane growth, and the term floricanes was given to the second year canes that were present the previous year (Shoemaker 1987). Primocanes in floricanes-fruiting cultivars produce vegetative growth and lateral shoots during the first season (Demchak et al. 2005, Demchak et al. 2010). After a dormant winter season, the second year's growth consists of floricanes leafing out, flowering, fruiting, and dying. For optimal yields, the dead floricanes are pruned away and the tops of the remaining canes are removed to ensure all resources benefit the remaining canes.

Primocane-fruiting cultivars produce primocanes that flower and fruit during the first year's growth of the canes (Shoemaker 1987, Demchak et al. 2005). In summer, primocanes produce flowers and fruits on the upper part of the new canes (Shoemaker 1987). These fruiting structures may extend down one-third to one-half the length of the canes (Bushway et al. 2008). Fruit is present from late July through the first killing frost (Demchak et al. 2005, Demchak et al. 2010). Since fruit is borne on the current season's growth, selection of dead canes during pruning is not needed; all canes can be mowed (Shoemaker 1987, Galletta and Himelrick 1990, Demchak et al. 2010). However, bases of these primocanes survive and will fruit the next year if not pruned away (Demchak et al. 2010). Therefore, if an earlier floricanes crop is desired during the next fruiting season, the top portion of these now floricanes can be selected and pruned the same as the floricanes fruiting cultivars.

To prune primocane-fruiting caneberries for a single late season crop, all canes are mowed to a height of 1 to 2 inches in late winter or early spring (Pritts and Handley 1989,

Demchak et al. 2010). New canes will grow each year and fruit in late summer. Some cultivars are capable of growing without a supporting trellis and when floricanes are removed yearly, trellising typically is not used (Galletta and Himelrick 1990, Fernandez and Krewer 2008). The yield of primocane-bearing cultivars is less than that of summer cropping cultivars (i.e., floricanes-bearing cultivars); however, primocane cultivars are economically appealing due to the reduced cost of pruning and trellising, the elimination of winter injury to overwintering floricanes, and the provided extension of the fruiting season (Galletta and Himelrick 1990).

For many years primocane-bearing red raspberries were recommended only for home gardens (Shoemaker 1987). The situation changed in 1969 when Cornell University's New York State Agricultural Experimental Station released 'Heritage'. This cultivar, along with the newer primocane-bearing cultivars, were developed from floricanes fruiting cultivars that bloomed and formed fruit on the distal ends of growth stunted primocanes (Galletta and Himelrick 1990). This growth was stunted with summer stress conditions and genetic manipulations. To aid with winter hardiness of these early primocane fruiting cultivars, genes from the arctic raspberry, *Rubus arcticus*, were incorporated.

There are three cultivars of primocane fruiting blackberries: 'Prime-Jan', 'Prime-Jim', and 'Prime-Ark 45,' all of which are thorny, erect plants (Clark 2008, 2010). Primocane-fruiting blackberry breeding started in the early 1990s at the University of Arkansas with 'Prime-Jan' and 'Prime-Jim' being the first primocane-fruiting cultivars developed in 2004. These caneberries were only recommended for home gardens or small commercial trials due to a trend of greatly reduced fruit set, size, and quality on primocanes after the plants were exposed to summer temperatures above 29 °C (Clark et al. 2005, Fernandez and Krewer 2008, Clark 2010). Therefore, on 'Prime-Jim' and 'Prime-Jan' there can be significant reductions in yield and

quality of fruit when cultivated in warmer climates. In 2009, 'Prime Ark 45' was released (Clark 2010). This primocane-fruiting blackberry produces a larger berry and has better flavor than 'Prime-Jim' or 'Prime-Jan'. The yield is also greater for 'Prime-Ark 45' than 'Prime-Jim'. However, this cultivar has the same issue of reduced fruit set and quality on primocanes after exposure to temperatures exceeding 29 °C. Research on primocane-bearing blackberries is underway to reduce the limitations present in today's cultivars (Clark 2008).

### **Caneberry Arthropod Pest Management**

Caneberries are susceptible to a variety of arthropod pests that affect the different parts of the plant including roots, crowns, fruit, foliage and canes (Funt et al. 2000). Since most arthropod pests of caneberries are generalist feeders that move into the planting from surrounding areas, the edge rows are greatly affected (Demchak et al. 2010). When insect pests are detected, integrating available pest control practices can aid in keeping populations below an economically damaging level (Pfeiffer et al. 2009).

Pest control in caneberries can include a combination of the following: cultural control (i.e. selecting a favorable location for production, the use of tunnels, variety or cultivar selection or sanitation), monitoring for pests or damage, biological control, and chemical control (Pfeiffer et al. 2009, Demchak et al. 2010). When utilizing chemical control in caneberries, some important aspects to consider are the degree of control desired, the appearance of the harvested fruit, the chemical's cross resistance with previously used chemicals, the chemical's effect on beneficial and targeted pests, and the desired pre-harvest and re-entry intervals. Since caneberries are not as highly produced in the United States as other commodities, the number of

registered insecticides is limited. Therefore, there is a continuing demand for new, effective chemical controls in caneberries.

## **Japanese beetle**

### **History**

Japanese beetle (JB), *Popillia japonica* Newman, (Coleoptera: Scarabaeidae), is an introduced beetle that was first discovered near Riverton, New Jersey in 1916 (Fleming 1972, Potter and Held 2002). Before its introduction to North America, JB was only found on the main islands of the Japanese archipelago, where it was considered a minor agricultural pest (Potter and Held 2002). However, once in North America, this scarab increased in numbers, dispersed quickly, and became a major pest. The rapid spread can be attributed to favorable conditions for its reproduction: the abundant host plants present, the favorable climate, and the lack of natural enemies (Oliver et al. 2005). Even though this introduced pest has thrived in eastern North America, JB populations also have had periods of decline, attributed to droughts (Fleming 1972).

Since 1916, JB has become established in all states east of the Mississippi, except for Florida and in localized areas in Montana, Wisconsin, Minnesota, Oklahoma, South Dakota, Iowa, Arkansas, Kansas, and Nebraska (Figure 1.1) (NAPIS et al. 2009). JB was first discovered in Virginia in 1928, south of Washington D. C. (Fleming 1972) and because JB can travel up to 11.9 km/yr (Allsopp 1996), it was not long before populations had spread throughout the state. Today, JB is a commonly known pest throughout Virginia, and much of the eastern United States.

## **Adult Description**

The first recorded description of the adult JB was by Edward Newman (1842):

*Popilla japonica*. Coppery gold-green; antennae pitchy, with a black apex: legs gold-green, or coppery gold-green, with the tarsi black: elytra testaceous, with the suture and margins nigro-aeneous, puncto-striate: terminal segment with 2 white pilose spots. Length .45 inch, breadth .275 inch. Japan.

However, the adult *Popillia japonica* today is described as:

[A] brightly colored oval insect, varying in length from 8 to 11 mm and in width from 5 to 7 mm. The female is usually larger than the males. The body is metallic green. It has three pairs of legs on the thorax which are dark coppery green, varying slightly in hue. The coppery brown elytra, do not cover the abdomen completely which exposes a row of five lateral spots of white hairs on each side of the abdomen (Figure 1.2) (Fleming 1972).

## **Life History and Biology**

Japanese beetle is univoltine in most of the United States where it is established (Funt et al. 2000). In a few instances when temperatures are cooler in New York and New England there have been cases of semivoltinism (Potter and Held 2002). Like other Coleoptera, JB are holometabolous insects that have an egg, larval, pupal, and adult stage. The timing of each stage is variable across North America due to diverse weather conditions.

In Virginia, adults start emerging from the soil during the last week of May or the first week of June (Fleming 1972). Males usually emerge a few days before females allowing insemination of the females as they emerge from the soil (Potter and Held 2002). The adult

populations increase and reach a peak usually during the second week of July. Adult densities slowly decline throughout August.

Life expectancy of an adult JB varies with climatic conditions, but generally an adult lives 30-45 days (Funt et al. 2000). During exposure to higher temperatures, the adult's life expectancy is shorter whereas cooler temperatures lead to a longer adult life.

Females alternate between feeding and ovipositing in soils throughout their lifetime with a typical female ovipositing 12 or more times and depositing 40-60 eggs (Fleming 1972). After the initial insemination, females oviposit an average of 20 eggs and then fly to a host plant to feed and remate (Potter and Held 2002). When desired host plants are close to the site of oviposition, females stay in the same vicinity (Potter and Held 2002). Others will travel in search of a suitable site with moderate to high soil moisture, moderate soil texture, sunlight, and short grass cover for oviposition.

Eggs, when exposed to favorable conditions, hatch within 10-14 days after oviposition (Potter and Held 2002). Scarab eggs and younger instars are especially vulnerable to desiccation (Fleming 1972). Drier years therefore can dramatically reduce JB populations the following year. First instars develop within 2-3 weeks after eclosion (Potter and Held 2002). The second instar is present within another 3-4 weeks and by mid-September most grubs are third instar larvae. The third instar white grubs feed throughout October and develop into fully grown C-shaped grubs.

Since white grubs are susceptible to freezing, in late autumn, mature grubs move down into the soil (Potter and Held 2002). While freezing temperatures are present, the grubs can be found overwintering at depths of 5-25 cm below the surface. When temperatures rise above 10 °C the larvae start moving upward in the soil. After grubs feed for 4-8 weeks in the spring, they

move further down into the soil to form a cell for pupation. The prepupal stage lasts around 10 days followed by the 7-17 day pupal stage. This pupal stage is followed by a teneral adult stage that lasts 2-14 days. Soon afterwards, the adults emerge from the soil and the cycle repeats.

## **Significance**

Japanese beetle is highly polyphagous, feeding on almost 300 species of wild and cultivated plants (Fleming 1972, Potter and Held 2002). Adults damage foliage, fruits and flowers in 79 plant families particularly in the Aceraceae, Anacardiaceae, Ericaceae, Fagaceae, Poaceae, Hippocastanaceae, Juglandaceae, Lauraceae, Leguminosae, Lilaceae, Lythraceae, Malvaceae, Onagraceae, Plantanaceae, Polygonaceae, Rosaceae, Salicaceae, Tiliaceae, Ulmaceae, and Vitaceae (Potter and Held 2002). Being a gregarious insect, one feeding JB can attract many other adults to feed on the same plant (Fleming 1972). Because adult JB's are highly polyphagous, mobile, gregarious, and relatively long lived compared to other insect pests, the beetle is an important pest where established. Also, the economic importance of JB is apparent when considering the more than \$450 million spent annually to control this pest (Potter and Held 2002).

Adult JB's mouth parts move laterally in a forward and backwards movement resulting in a rasping of the plant tissue (Fleming 1972). Adults feed from the top of plants and work their way downward. In most plants, damage from JB can be identified by the plant having a lacelike skeleton appearance (skeletonized) (Figure 1.3). Skeletonization is a result of feeding on tissue in-between the veins of the plant (Fleming 1972). The degree of damage on any plant is influenced by multiple factors such as the plant's texture and thickness, surrounding host plants, adjacent larval breeding sites, other feeding JB, exposure to light intensity, and the plant's response to feeding (Ladd 1987).

The larvae of JB, or annual white grubs, are also considered an important pest. They are the most destructive pest of turf-grass in the eastern United States (Potter and Held 2002). These grubs can also cause significant injury through feeding on root systems of other economically important plants.

Due to the impact this pest has on many plant species, in 1920 Federal Quarantine 48 was implemented to regulate the interstate movement of all agricultural products (Fleming 1972).

This quarantine prevented the artificial spread of JB through agricultural products. However, JB populations continued to spread through adults attaching themselves to trains, cars, trucks, ships and airplanes. The 1920 federal quarantine was in place until 1978 (Oliver et al. 2005).

Today, the U.S. Domestic Japanese Beetle Harmonization Plan, adopted on August 19, 1998 by the National Plant Board, restricts the movement of JB infested plants and turf grasses, especially to non-infested areas (Haun et al. 1998). The Animal and Plant Health Inspection Service also regulates airport facilities during the JB flight season to slow the spread of JB to non-infested or quarantined areas (USDA 2004).

### **Significance in Caneberries**

Japanese beetle adults are abundant in July and August when multiple cultivars of caneberries are fruiting (Demchak et al. 2010). On caneberries, JB are gregarious feeders of the fruit and foliage (Ellis et al. 1991). Feeding on fruit leaves irregular hollows in the berries or the entire berry can be consumed (Funt et al. 2000). The injured fruit is susceptible to fruit rots which can then be mechanically transferred from berry to berry and plant to plant by the beetles (Bushway et al. 2008). This transmission leads to previously uninjured fruit becoming



unmarketable due to disease. The adults prefer to feed on fruit that is exposed to sunlight with red raspberries being preferred over black raspberries (Funt et al. 2000).

Japanese beetle also causes indirect injury to caneberry plants through skeletonizing the leaves. Beetles start feeding at the upper portions of the caneberries and move downward as plant material is consumed (Demchak et al. 2010). When JBs are active and populations are high, foliage exposed to sunlight may be completely defoliated (Pritts and Handley 1989, Funt et al. 2000).

In a laboratory feeding response study, JB feeding preference was greater on foliage of *Rubus idaeus* over foliage and floral samples from 42 other host plants (Ladd 1987). Fleming (1972) listed plants that were fed upon by JB, ranking them on a 1 to 4 scale, with plants ranked 4 being the most heavily fed upon. The leaves of red raspberry were given a 3; whereas, both *R. argutus* and *R. cuneifolius* (blackberries) were rated a 2.

Raspberry fruit production is affected by the current year foliar functionality (Sullivan et al. 1994, Raworth and Clements 1996). Therefore, severe defoliation by JB can be economically important to the primocane-bearing cultivars. However, a raspberry plant's resiliency to defoliation was observed by Raworth and Clements (1996). In 'Willamette,' a floricanefruiting cultivar of red raspberry, primocane defoliation at 25 %, 50 %, and 75 % during the month of August resulted in 26 % fruit loss the following year (Raworth and Clements 1996). Since the defoliation of 25 and 75 % had the same affect on yield reduction, the plant displayed resiliency to defoliation up to 75 %. The most yield loss (55 %) for the following year was observed when 100 % of the primocanes were defoliated. Therefore, in a floricanefruiting cultivar of red raspberry, primocane defoliation of 25 % defoliation resulted in significant yield loss but the following year's crop was not completely lost. This study in a floricanefruiting cultivar of red

raspberry brings up the question: What effect does the previous year's defoliation have on the next year's yield in primocane-bearing cultivars?

The previous examples display the importance of JB as a pest in caneberries. Since JB are present when multiple caneberry cultivars are being harvested, are injurious directly and indirectly to the fruit, defoliate the canes, and prefer the raspberry foliage over other host plants, control of this pest is warranted.

### **Integrated Pest Management in Caneberries**

The biology of JB can make pest management challenging (Potter and Held 2002). The adults are highly mobile, gregarious, polyphagous feeders, and controlling the grubs or adults in any location does not assure their control elsewhere. However, due to the destructive habits of this pest, many different management techniques have been studied. JB management includes trapping, cultural control, biological control, and chemical control.

Adults are attracted to a variety of floral and fruit-like volatile oils. Effective JB traps contain phenethyl propionate, eugenol and geraniol (3:7:3 mixture) mixed with the synthetic sex pheromone, japonilure (Potter and Held 2002). The number of beetles found in these traps increases when captures are removed daily. These traps have not provided significant control in areas where used due to "trap spillover" onto surrounding plants (Switzer et al. 2009). Because of the "spillover" effect, females are attracted to the area of the trap but not necessarily to the trap itself. Once the volatile stimulus is found, females will land, which can either be on the trap or on a surrounding plant. For this reason, studies have shown an increase in damage in areas where these traps are present (Potter and Held 2002). Therefore, traps are sufficient for JB sampling and monitoring but not effective for control.

Cultural control is the deliberate alteration of a specific crop production practice to reduce or avoid injury to crops by pest populations (Kogan 1986). Cultural control of JB can include planting host plants with lower preference, surrounding a crop with non-preferred hosts, intercropping with a trap crop, providing refuge for biological agents, controlling weedy hosts, crop rotation, adjusting the timing of planting or of harvest (Ferro 2007), and regulatory control (already mentioned above) .

Biological control agents for JB include natural enemies (predators and parasitoids) and diseases. There are a few established natural enemies of JB in North America (Potter and Held 2002). Two tephritid wasps that are widely distributed, *Tiphia vernalis* Rohwer (Oliver et al. 2005) and *T. popilliavora* Rohwer, are known to parasitize the grub stage (Krombein 1948). *Istocheta aldrichi* Mesnil, a tachinid fly, parasitizes newly emerged adults (Fleming 1986). There are also several generalist predators that are known to kill JB eggs and young larvae including, ants, staphylinids and carabids. If turf appearance is not an issue, moles, skunks and raccoons also prey on the grubs. Various birds feed on grubs and adults as well.

*Paenibacillus* (formally *Bacillus*) *popilliae* and *P. lentimorbus* or milky spore disease, are two bacteria used for JB population suppression (Hanula and Andreadis 1988). Milky disease kills the grubs through spores being ingested (Black 1968) and then germinating and spreading throughout the body causing eventual death (Weeden et al. 2007). Grubs that fall victim to these bacteria are milky-white from infection. The disease can then multiply and be a source of control for years after establishment.

Another bacterium used for JB control is *Bacillus thuringiensis* (Bt), a soil-dwelling bacterium that is used mainly for grub suppression (USDA and APHIS 2004). There are different strains of Bt containing crystal proteins that are toxic to JB. Bt strains that have the

Cry8Da and Cry8Db proteins are toxic against both adults and larvae; Bt containing the Cry8Ca protein is toxic to only larvae (Yamaguchi et al. 2008).

The following entomopathogenic nematodes are effective biological control agents for grubs: *Steinernema glaseri* (Steiner), *Heterorhabditis bacteriophora* (Poinar), and *Steinernema kushidai* (Mamiya) (Potter and Held 2002). These nematodes only affect the grubs, are costly, have limited availability, and have a short shelf life. For these reasons, nematodes are not commonly used for JB control.

Because JB adults can damage caneberry fruit, many commercial growers will protect plants during harvest with insecticide applications (Ellis et al. 2004). While fruit is present, growers prefer to apply chemicals with a short pre-harvest interval to accommodate the high frequency of harvesting throughout the season. This preference limits the selection of insecticides. Table 1.1 lists insecticides that are registered for JB control in 2010 on raspberries in Virginia according to [www.greenbook.net](http://www.greenbook.net) and [www.cdms.net](http://www.cdms.net).

Scientific studies that strengthen the understanding of JB and caneberry biology or that improve the integration, use, and effectiveness of different pest management techniques are beneficial for caneberry growers. The objectives of the research presented in this thesis are: 1) To evaluate the efficacy of various insecticides for controlling JB in caneberries; 2) To evaluate the susceptibility of different cultivars of primocane-bearing raspberries to JB and their feeding injury; and 3) To evaluate the potential geranium (*Pelargonium × hortorum*) as a control method for JB.

## Stink bugs (Hemiptera: Pentatomidae)

### History

Stink bugs (Hemiptera: Pentatomidae) have been documented since the end of the eighteenth century, when Carolus Linnaeus, Charles De Geer, and Johan Christian pioneered studies on this complex of true bugs (McPherson 1982). In the 1800's, Thomas Say and Philip Uhler were the main taxonomists to identify Pentatomidae to species. T. Uhler, with the help of C. Stål, C. J. B. Amyot, J. G. A. Serville, A. M. F. J. Palisot de Beauvois, and W. S. Dallas, comprised a list of North American Pentatomidae. This list was later added to by many others (Van Duzee 1904, Banks 1910, Van Duzee 1916, 1917, McPherson 1982, Froeschner 1988, McPherson and McPherson 2000). McPherson (1982) provides the most complete summary and updated Pentatomidae list, key, and biology of stink bugs in northeastern America. However, the most updated keys and biology of economically important stink bugs in North America is summarized by J. E. McPherson and R. M. McPherson (2000) in *Stink Bugs of Economic Importance in America North of Mexico*.

The complex of insects referred to as stink bugs, family Pentatomidae, are members of the order Hemiptera, and suborder Heteroptera. Pentatomidae are distinguished from other Hemipterans by their shield shape, five segmented antennae (Triplehorn and Johnson 2005), and well-developed scutellum which can cover the entire abdomen (McPherson and McPherson 2000). This family, which can be found worldwide, contains five subfamilies: Asopinae, Discocephalinae, Edessinae, Pentatominae, and Podopinae (Triplehorn and Johnson 2005). Most stink bugs are phytophagous with the exception of Asopinae which are predacious. The subfamilies that are mainly phytophagous (Discocephalinae, Edessinae, Pentatominae, and Podopinae) differ from Asopinae in that the basal segment of the beak is slender and lies

between parallel bucculae; the basal segment of Asopine's beak is shorter and thicker with only the base lying between the bucculae (Triplehorn and Johnson 2005). Within Pentatomidae, Pentatominae is the only subfamily of major economic importance in North America and includes roughly 40 genera and 180 species (McPherson and McPherson 2000). Most of these species live in grassy or herbaceous habitats and all species are diurnal (McPherson 1982).

### **Significance**

The stink bug complex feeds on a wide range of fruits, vegetables, nuts, and grains though inserting proboscises into stems, petioles, foliage, flowers, fruits, and seeds (McPherson and McPherson 2000). For most stink bug species, all stages except for the first instar (Froeschner 1988), obtain their food by piercing plant tissues with their mandibular and maxillary stylets (proboscis) and extracting plant fluids (Panizzi et al. 2000). Newly-hatched first instars remain on and feed on the egg mass from which they emerged (Buchner 1965). Fruiting structures are preferred over other parts of the plant (Spangler et al. 1993, McPherson and McPherson 2000) and the immature fruits are preferred over the more mature fruiting structures (Schuh and Slater 1995).

Resulting stink bug injury is normally identified by minute discolored spots on the plant along with a stylet sheath at the feeding location (Schuh and Slater 1995, McPherson and McPherson 2000). Feeding causes injury through the loss of plant fluids, the injection of digestive enzymes, the deformation and abortion of seed and fruiting structures, the tendency of pathogenic and decay organisms to colonize around the feeding location, and delayed plant maturity (McPherson and McPherson 2000). Injury to younger fruit structures can result in shriveling, decrease of size, or abortion, whereas injury to more mature fruit and seeds causes

less severe damage. Other stink bug injury includes: foliar retention, early plant maturation, abnormal plant growth, and reduction of fruit size and weight. The time and location of feeding is important to a stink bug's impact on a crop. However, heavy feeding on any stage can have a significant magnitude on the crop's marketable yield (Panizzi et al. 2000).

Because stink bugs are normally grouped together as a pest complex, or are combined with other Heteropterans, economic loss from a specific stink bug species is impossible to assess accurately (McPherson and McPherson 2000). The producer, processor, or consumer can decide the tolerable amount of stink bug damage accepted. This accepted amount depends on crop quality, availability, and yield reductions. Any infestation may be tolerated until an economic injury level is reached before treatment is implemented.

The economic importance of stink bugs is based on the species in question and the hosts attacked (Froeschner 1988). According to Kamminga et al. (2009b), the most economically important stink bug species in the upper southern and mid-Atlantic United States include: the green stink bug, *Acrosternum hilare* (Say), *Euschistus quadrator* (Rolston), brown stink bug, *E. servus* (Say), dusky stink bug, *E. tristigma* (Say), brown marmorated stink bug, *Halyomorpha halys* (Stål), harlequin bug, *Murgantia histrionica* (Hahn), southern green stink bug, *Nezara viridula* (Linnaeus), rice stink bug, *Oebalus pugnax* (Fab.), redbanded stink bug, *Piezodorus guildinii* (Westwood), redshouldered stink bug, *Thyanta accerra* McAtee, and *Thyanta custator* (Fab.). These and other damaging species are mainly weed feeders that can move into cultivated crops, cause serious damage, and then move to another host (McPherson and McPherson 2000, Panizzi et al. 2000).

## **Life History and Biology**

Adult Pentatomidae and some nymphs hibernate through the winter in the protection of crevices in plants, in leaf litter, and in other ground covers (McPherson 1982, Froeschner 1988, McPherson and McPherson 2000). In warmer areas, some species may stay active during the winter (McPherson and McPherson 2000). In the spring, adults emerge with warming temperatures to begin mating and feeding (McPherson 1982). To gain nutrition for reproduction, the adults locate a preferred fruiting host (Todd 1989). This host will also act as a food source for their offspring. Eggs are laid in tight clusters of a few to several dozen on leaves, twigs, fruiting structures, and flower heads (Froeschner 1988). The females of most stink bug species abandon eggs after oviposition, but a few have been documented to guard the eggs and young nymphs (Schuh and Slater 1995). The barrel-shaped eggs hatch generally within one week after oviposition and the newly emerged nymphs often cling to the empty egg shells (Froeschner 1988).

The first instars are present for about a week and are usually gregarious, inactive, and non-plant-feeding (Froeschner 1988, Schuh and Slater 1995, McPherson and McPherson 2000). Even though these first instars do not feed on plant material (Schuh and Slater 1995), they have been reported to feed on the hatched egg shells (Buchner 1965). After the first molt, some species begin to disperse in search of food, while other species stay in clusters through the third molt (McPherson and McPherson 2000).

Pentatomoids have five nymphal instars in which the phytophagous species feed on plant material in the second through fifth stages (McPherson 1982). Instars one through five take place within a month-and-a-half and wing pads develop on the fourth and fifth instars. Fifth instars molt into adults (Froeschner 1988). In the northern United States, most pentatomid



species have one generation per year and in the southern portions of North America, up to five generations per year have been documented for some species (McPherson and McPherson 2000). Froeschner (1988) reports that adult stink bugs can be found somewhere within North America or Canada year round.

### **Reports of Stink Bugs in Caneberries**

Stink bugs are occasionally mentioned as being pests of commercial caneberries or are grouped together with other Hemiptera as pests of caneberries (Spangler et al. 1993). Across the United States, McPherson and McPherson (2000) listed the following *Rubus* species as hosts of stink bugs: American dewberry (*Rubus flagellaris* Willdenow), red raspberry (*Rubus idaeus* L.), and cut-leaf blackberry (*Rubus laciniatus* Willdenow). Along with these *Rubus* species, 12 other rosaceous plant species including apple, pear, plum, peach, and cherry, were reported hosts of stink bugs. The following are reports of pentatomids being present in caneberry plantings or actually being injurious to the caneberries.

The juniper plant bug, *Chlorochroa uhleri* Stål, was said to damage currants and blackberries in Schenectady County New York when present in abundance (Felt 1916). Also in New York, stink bugs were reported to be occasionally or moderately abundant in caneberry plantings (Spangler et al. 1993). The adults in this study were more abundant on canes with ripe berries in late August 1989. For the duration of this three-year study, stink bug numbers were higher in August and September on primocane-bearing 'Heritage' during the fall fruit ripening stage. The stink bug densities were lower during the summer/ floricanne fruiting stage.

On the west coast of the United States, the stink bugs, *Elasmotethus cruciatus* (Say), *Euschistus conspersus* Uhler and *Rhytidolomia ligata* (Say) are reported contaminants of

caneberries harvested by a mechanical harvester (Kieffer et al. 1983). When these berries were harvested, not only did the actual insect bodies contaminate the product, the stink bug's foul secretions also negatively impacted the marketability of the fruit. In Washington, McGhee (1997) found few stink bugs to be present on blackberry when studying this complex that were present on native vegetation surrounding fruit trees.

Other pentatomids that have previously been found on raspberries in the United States include: *A. hilare* (Knowlton and Harmston 1940, Furth 1974), *Banasa dimiata* (Say) (Furth 1974), *Coenus delius* (Say) (Stoner 1922), *Corimelaena pulicaria* (Germar) (Riley 1869, Saunders 1873, Forbes 1884, Osborn 1892, Van Duzee 1894, Lugger 1900, Slingerland and Crosby 1914, Procter 1946), *Cosmopepla lintneriana* (Thomas) (Lugger 1900, Stoner 1920, Tonks 1953, Furth 1974), *Dendrocoris humeralis* (Uhler) (Furth 1974), *E. servus* (Stoner 1922, Furth 1974), *E. tristigumus* (Say) (Stoner 1922, Furth 1974, McPherson and Mohlenbrock 1976), *E. variolarius* (Palisot) (Townsend 1890, Kirkaldy 1909) *Galgupha atra* Amyot and Serville (Fyles 1907), *Mormidea lugens* (Fabricius) (Hussy 1922, Furth 1974), *Sciocoris microphthalmus* Flor (Osborn and Drake 1922), and *Thyanta calceata* (Say) (Furth 1974).

There have also been international reports of stink bugs on caneberries. In Queensland, Australia, Coombs and Khan (1998) found that when both adults and nymphal *Plautia affinis* Dallas fed on raspberries for two weeks, the fruit was more distorted, discolored, and had reduced firmness compared to the control. Other plant-feeding stink bugs associated with raspberries in Queensland were *N. viridula*, *Cuspicona simplex* Walker, and *Piezodorus hybneri* [*Piezodorus hybneri*] (Gmelin). In Turkey, Kaya and Kovanci (2004) reported the species, *Acrosternum heegeri* Fieber, *Carpocoris purpureipennis* (De Geer), *Dolycoris baccarum* (L.), *Mustha spinulosa* (Lefebvre), *N. viridula*, and *Palomena viridissima* (Poda) to be harmful to

raspberry. The other species present within this raspberry planting included: *Eurydema ornatum* [*Eurydema ventralis*] (Linnaeus), *Eysarcoris inconspicuous* (Herrich-Schaffer)= *Eysarcoris ventralis* (Westwood) and *Graphosoma lineatum* (Linnaeus) (Kaya and Kovanci 2004). Also in Turkey, *N. viridula* was identified as being harmful to blackberries (Cetin et al. 2006). This same stink bug species was found to have preferences for different raspberry cultivars in China (YanLin et al. 2008). Egg masses of *D. baccarum* (L.) were found on raspberries in Norway (Conradi-Larsen and Soemme 1973) and egg masses of the predatory stink bug *Podisus nigrolimbatus* Spin were found on raspberries in Chile (Pairoa 1944).

Not only are caneberries hosts of stink bugs worldwide, there are also reports of the fruit being injured by these pests. Further investigations need to be made on the importance of this pest complex to caneberry cropping systems in Virginia. Once there is a better understanding of the impact stink bugs have on caneberries, control tactics can be implemented where needed.

### **Pest Management of Stink Bugs**

Because stink bugs prefer plant reproductive structures (fruits and seeds), stink bug management is important at this time in the crop cycle. Management, however, can be complex because stink bugs: 1) move from host to host in search of mature fruits and seeds; 2) move from older plants to feed on younger plants throughout the season; 3) have multiple generations per year allowing them to be present throughout the growing season; and 4) travel from wild hosts to cultivated plants depending on which is preferred at the time (McPherson and McPherson 2000).

With the feeding habits and biology of most stink bug species being similar, these insects are often treated as a pest complex instead of individual species (McPherson and McPherson 2000). Understanding the population biology of major pest species helps determine when

damaging stages are present and need to be managed. Many cultivated crops can sustain minor damage or even escape injury from stink bug feeding depending on phenology. Injury can also be minimized through implementing a balanced IPM program into a system (Panizzi et al. 2000). Stink bug IPM programs can include cultural control, biological control, and/or chemical control.

Cultural control could include using resistant crop varieties, planting at a time that would avoid high stink bug populations or at a time that avoids stink bug contact with a certain plant developmental stage, planting trap crops, removing wild hosts from an area, planting hosts that are not preferred near the main crop, spacing and tilling rows, planting hosts that attract natural enemies, and implementing clean culture (McPherson and McPherson 2000, Panizzi et al. 2000). Cultural control in the form of trap cropping was utilized to suppress populations of *Nezara viridula* (L.) and other stink bugs in soybeans (Panizzi et al. 2000). Also, stink bug resistant varieties of soybeans and pecans have been utilized as a form of cultural control for this pest complex.

Biological control of stink bugs includes control with parasitoids, predators, and entomopathogens. The primary natural enemies of stink bugs are hymenopteran and dipteran parasitoids (McPherson and McPherson 2000, Panizzi et al. 2000). In a survey of stink bug egg parasitoids in southeastern Virginia, Koppel et al. (2009) found *Telenomus podisi* Ashmead, *Trissolcus basalis* Wollaston, *Trissolcus edessae* Fouts, and *Trissolcus euschisti* Ashmead to parasitize the eggs of various stink bug species. In addition, adult stink bugs are parasitized by the tachinid flies *Cylindromyia* spp. and *Trichopoda pennipes* Fab. (Tillman 2008).

Some reported predators of stink bugs and eggs include: the red imported fire ant, *Solenopsis invicta* Buren, grasshoppers (Todd 1989), Argentine ant, *Linepithema humile* (Mayr), common pillbug, *Armadillidium vulgare* (Latreille), and the spined soldier bug, *Podisus maculiventris*

(Say) (McPherson and McPherson 2000). Other predators include birds and other arthropods not listed above.

Entomopathogens also contribute to natural stink bug control. One example is the fungus *Sporotrichum globuliferum* Spegazzini, which attacks both *Thyanta* spp. and *Oebalus pugnax pugnax* (McPherson and McPherson 2000). Pervez et al. (2008) studied four *Steinernema* spp. of entomopathogenic nematodes for control of *Nezara viridula* and found *S. seemae* to be the most virulent.

Chemical control of the stink bugs should only be incorporated into an IPM program when necessary. Many crops escape major stink bug injury or sustain little damage because of various biotic (parasitoids, predators, entomopathogens) and abiotic (temperature extremes, humidity, habitat availability) factors (Panizzi et al. 2000). Therefore, chemical control is only warranted when populations reach an economic threshold. Using economic injury levels or economic threshold levels and applying chemicals at the recommended rates, allows acceptable control while preserving many natural enemies and potentially reducing the number of pesticide applications. Identifying the species and life stages present also allows for more thorough control because the susceptibility to insecticides can differ for these groups (Kamminga et al. 2009c). The insecticides registered in Virginia for stink bug control in raspberries in 2010 according to [www.greenbook.net](http://www.greenbook.net) and [www.cdms.net](http://www.cdms.net) are listed in Table 1.2.

By studying stink bugs on caneberries, we can better understand the impact these pests have on raspberries and blackberries to improve management techniques. The objectives of the research presented in this thesis that consider stink bugs are: 1) To identify the species present within a southwest Virginia raspberry planting and 2) To identify and document stink bug injury on raspberries.

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## Tables and Figures

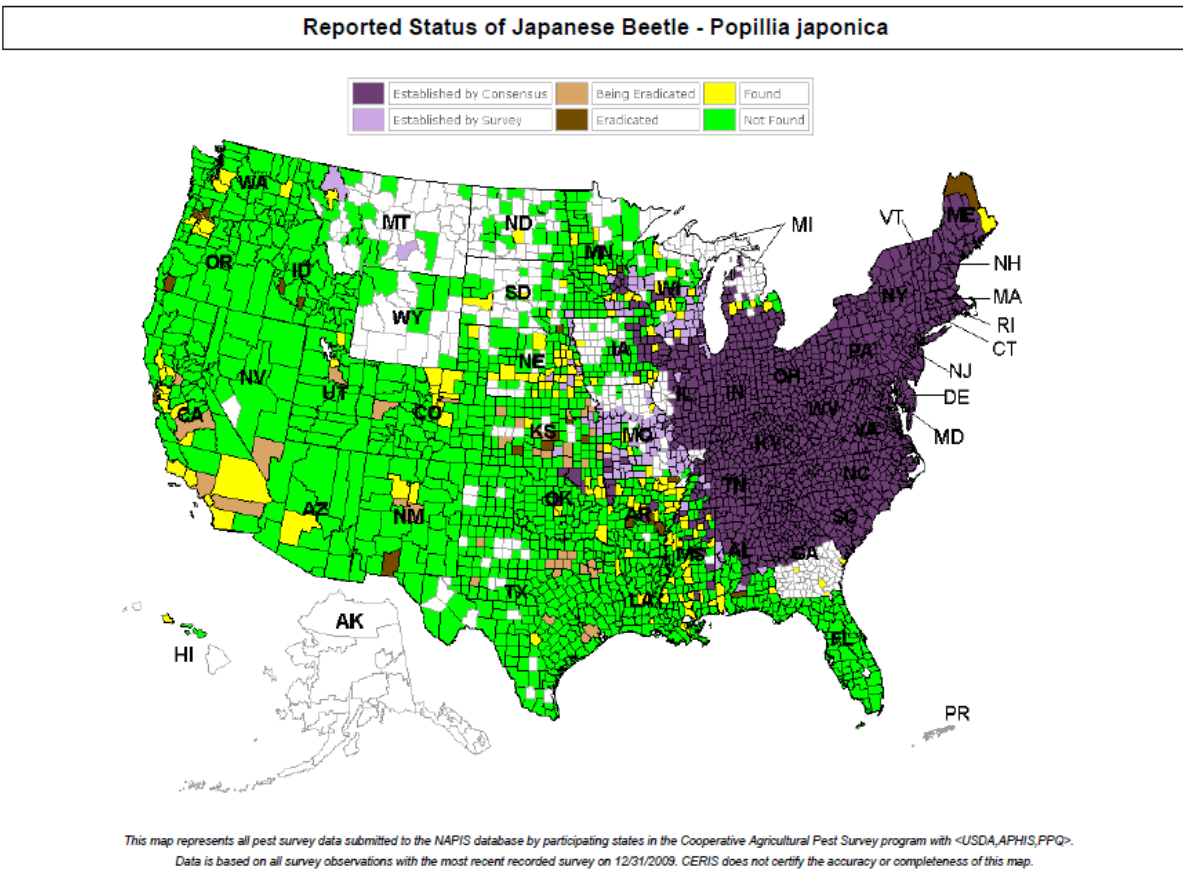


Figure 1.1 NAPIS generated map of the distribution of Japanese beetle, *Popillia japonica*, in the United States as of 12/31/2009.



Figure 1.2 An adult Japanese beetle, *Popillia japonica*. A metallic green pronotum, coppery abdomen, and five visible tufts of white hair distinguish this beetle species from other scarabs.



Figure 1.3 Skeletonized raspberry leaf. Japanese beetles feed on plant tissue in-between veins resulting in this skeletonized appearance.



Table 1.1 Insecticides registered for Japanese beetle control on raspberries in Virginia, 2010.

<b>Product Name</b>	<b>Manufacturer</b>	<b>Active Ingredient (AI)</b>	<b>REI * (hrs)</b>	<b>PHI** (days)</b>	<b>Rate/Acre per Application</b>
<b>Actara</b>	Syngenta Crop Protection, Inc.	thiamethoxam	12	3	3.0 oz.
<b>Alias 4F</b>	MANA	imidacloprid	12	7	8-16 oz.
<b>Asana XL</b>	DuPont	esfenvalerate	12	7	4.8-9.6 oz.
<b>Assail 30 SG</b>	United Phosphorus Inc.	acetamiprid	12	1	4.5-5.3 oz.
<b>Assail 70 WP</b>	United Phosphorus Inc.	acetamiprid	12	1	1.9-2.3 oz.
<b>Aza-Direct</b>	Gowan Co.	azadirachtin	4	0	16-56 oz.
<b>Brigade 2EC</b>	FMC	bifenthrin	12	3	3.2-6.4 oz.
<b>Carbaryl 4L</b>	Drexel	carbaryl	12	7	32-64 oz.
<b>Danitol 2.4 EC</b>	Valent	fenpropathrin	24	3	10.7-26 oz.
<b>Ecozin Plus 1.2 ME</b>	AMVAC	azadirachtin	4	0	15-30 oz.
<b>EverGreen EC</b>	MGK	pyrethrins	12	When dry	2-16 oz.
<b>Fanfare 2 EC</b>	MANA	bifenthrin	12	3	3.2-6.4 oz.
<b>Fyfanon</b>	Helena	malathion	12	1	24 oz.
<b>M-Pede</b>	Dow AgroSciences LLC	K <sup>+</sup> salts of fatty acids	12	0	62.5-125 oz.
<b>Malathion 57 EC</b>	Loveland Products, Inc	malathion	12	1	24 fl. oz.
<b>Mustang Max EC</b>	FMC	zeta-cypermethrin	12	1	4 oz.
<b>Pasada 1.6 F</b>	MANA	imidacloprid	12	3	8 oz.
<b>PyGanic EC 1.4 II</b>	MGK Co.	pyrethrins	12	0	16-32 oz.
<b>PyGanic EC 5.0 II</b>	MGK Co.	pyrethrins	12	0	4.5-18 oz.
<b>Sevin 4F</b>	Bayer CropScience	carbaryl	12	7	32-64 oz.
<b>Sevin 80S</b>	Bayer CropScience	carbaryl	12	7	20-40 oz.
<b>Sevin XLR Plus</b>	Bayer CropScience	carbaryl	12	7	32-64 fl. oz.
<b>* Re-entry interval    ** Pre-Harvest Interval</b>					

Table 1.2 Insecticides registered for stink bug control on raspberries in Virginia, 2010.

<b>Product Name</b>	<b>Manufacturer</b>	<b>Active Ingredient</b>	<b>REI* (hrs)</b>	<b>PHI** (days)</b>	<b>Rate/Acre per Application</b>
<b>Actara</b>	Syngenta	thiamethoxam	12	3	3.0 oz.
<b>Alias 4F</b>	MANA	imidacloprid	12	7	8-16 oz.
<b>Asana XL</b>	DuPont	esfenvalerate	12	7	4.8-9.6 oz.
<b>Actara</b>	Syngenta	thiamethoxam	12	3	3 oz.
<b>Aza-Direct</b>	Gowan Co.	azadirachtin	4	0	16-56 oz.
<b>Brigade 2EC</b>	FMC	bifenthrin	12	3	3.2-6.4 oz.
<b>Brigade WSB</b>	FMC	bifenthrin	12	3	8.0-16.0 oz
<b>Danitol 2.4 EC</b>	Valent	fenpropathrin	24	3	10.7-26 oz.
<b>Ecozin Plus 1.2 ME</b>	AMVAC	azadirachtin	4	0	15-30 oz.
<b>EverGreen EC 60-6</b>	MGK	piperonyl butoxide; pyrethrins	12	When dry	2-16 oz.
<b>Fanfare 2 EC</b>	MANA	bifenthrin	12	3	3.2-6.4 oz.
<b>M-Pede</b>	Dow AgroSciences	K <sup>+</sup> salts of fatty acids	12	0	62.5-125 oz.
<b>Mustang Max EC</b>	FMC	zeta-cypermethrin	12	1	4 oz.
<b>Pasada 1.6 F</b>	MANA	imidacloprid	12	3	8 oz.
<b>PyGanic EC 1.4 II</b>	MGK Co.	pyrethrins	12	0	16-32 oz.
<b>PyGanic EC 5.0 II</b>	MGK Co.	pyrethrins	12	0	4.5-18 oz.
<b>* Re-entry interval    ** Pre-harvest interval</b>					

## **CHAPTER 2: Efficacy of Insecticides with Short Pre-Harvest Intervals against Japanese Beetle, *Popillia japonica* Newman (Coleoptera: Scarabaeidae) Infestation and Fruit and Foliar Injury of Primocane-Bearing Caneberries**

### **Abstract**

Field experiments (2007-2009) and laboratory bioassays (2009) were conducted to test various insecticides with short pre-harvest intervals for control of Japanese beetle (JB), *Popillia japonica* Newman, in primocane-bearing caneberries (blackberries and raspberries). In field efficacy tests, JB pest pressure was relatively low; however, significant control of JB was observed when using the pyrethroid deltamethrin. Deltamethrin prevented infestation, promoted greater marketable yield in the blackberries, and reduced defoliation. Also, compared to the control ( $47.1 \pm 2.0$  %), the percentage of injured blackberries was reduced almost by half with use of deltamethrin ( $26.4 \pm 4.2$  %) in 2008. Deltamethrin also increased mortality and decreased defoliation in the bioassays. Another pyrethroid, bifenthrin, reduced JB activity on blackberries, but not significantly different from the control. Acetamiprid, a neonicotinoid, displayed insecticidal activity and reduced defoliation in the laboratory, but increased JB presence in the field. Chlorantraniliprole significantly reduced raspberry defoliation in the field and laboratory. There was also a trend of chlorantraniliprole reducing JB infestation, but not significantly compared to the untreated control. The botanical and inorganic insecticides that showed promise for JB control were lime-alum, thyme oil, and the extract of *Chenopodium ambrosioides*. Out of the considered formulations of azadirachtin, JB activity was only reduced in the laboratory; the azadirachtin/neem oil extract mixture significantly reduced JB defoliation and the azadirachtin/pyrethrin mixture significantly increased JB mortality. More informed treatment recommendations can now be made to caneberry growers looking for an effective JB control that also offers short pre-harvest intervals.

## Introduction

Japanese beetle (JB), *Popillia japonica* Newman (Coleoptera: Scarabaeidae), has become a significant pest of fruit and garden crops, ornamental plants, shrubs, vines and trees, and turf grasses since being discovered in New Jersey in 1916 (Fleming 1972, Potter and Held 2002). Today in the United States, JB is established in all states east of the Mississippi, except for Florida, and is established in localized areas in Montana, Wisconsin, Minnesota, Oklahoma, South Dakota, Iowa, Arkansas, Kansas, and Nebraska (NAPIS et al. 2009). In these established areas, the adults are highly mobile, traveling up to 11.9 km/yr (Allsopp 1996), and are highly polyphagous, feeding on over 300 host plant species from 79 different plant families (Potter and Held 2002). The adults are gregarious feeders and when one adult feeds, the damaged plant material releases volatiles attracting other adults to the same site (Loughrin et al. 1996). The larval or grub stages of JB are also pests causing damage to turf grasses and other plant root systems.

Caneberries, raspberries and blackberries, are aggregate fruits in the genus *Rubus*, family Rosaceae, that grow on leafy canes in temperate regions of the world (Pritts and Handley 1989, Ravai 1996, Wada and Ou 2002). Primocane-fruiting caneberries, produce canes that flower and fruit during the first year growth of the canes (Shoemaker 1987, Demchak et al. 2005). Fruit is present on these cultivars in July and August when adult JB are most abundant (Demchak et al. 2010). Effective management of JB is therefore important in primocane-bearing caneberry production. The need for JB management is also heightened because adults have a preference for raspberries over other host plants (Fleming 1972, Ladd 1987). On caneberries, JB are gregarious feeders on the fruit and foliage (Ellis et al. 1991), skeletonizing the leaves and chewing irregular hollows in the berries (Funt et al. 2000). Moreover, injured fruit is more

susceptible to fruit rot pathogens, which can then be transferred to uninjured fruit, making them unmarketable (Bushway et al. 2008). Through skeletonizing the leaves, JB injury lessens foliar function, which could affect fruit production in primocane-bearing red raspberries (Sullivan et al. 1994).

To prevent JB from causing economic damage to caneberries, an integrated pest management (IPM) program should be implemented. One tactic of JB remedial IPM is chemical control. Chemical control of JB in caneberries is complicated because of the previously mentioned JB characteristics. The short postharvest life of caneberries also causes difficulties in chemical control. Because raspberries and blackberries perish within several days of ripening, chemical controls that allow treated berries to be harvested sooner are preferred. In order to be used when fruit is present on plants, insecticides should have a minimal pre-harvest interval (PHI) of 2-3 days for immature ripening berries and 0-1 day for ripened berries. The obstacle of using a pesticide with a longer PHI is demonstrated when considering a commonly used pesticide for JB adults, carbaryl (Potter and Held 2002). Although carbaryl is effective at controlling JB, its 7 d PHI in raspberries and blackberries could result in treated berries perishing before harvest. These obstacles of JB chemical control in caneberries emphasize the need for new pesticide options with a short PHI.

The objectives of this study were to evaluate the efficacy, antifeedant activity, and toxicity of pesticides with short pre-harvest intervals against JB in primocane-bearing raspberries and blackberries. A better understanding of the available chemicals will improve recommendations for JB management in primocane-fruiting caneberry production.

## **Materials and Methods**

All field experiments were conducted in a 2005 planting of primocane-bearing raspberries and blackberries at Kentland Farm (College of Agriculture and Life Sciences, Virginia Tech), Montgomery County, VA. This planting is on an elevated site above the New River in southwestern Virginia (37° 12.417'N, 80° 35.513'W, 616 m elev.). The caneberry planting consists of six raised bed rows containing 11 raspberry cultivars adjacent to seven raised bed rows containing two blackberry cultivars. Each cultivar plot is 6.2 m long and 1.0 m wide; rows are 3.0 m apart. This caneberry planting is bordered by an apple orchard, forest and pasture. Eight primocane raspberry cultivars were used in these experiments including: 'Anne', 'Autumn Bliss', 'Caroline', 'Dinkum', 'Fall Gold', 'Heritage', 'Himbo Top', and 'Prelude'. The two cultivars of blackberries included 'Prime-Jim' and 'Prime-Jan'.

### **Field Experiments**

#### ***2007 Treatment Applications***

On 19 and 27 June, and 4, 9, 18, and 27 July 2007 the treatments in Table 2.1 were applied to 2.0 m sections of row to each of the eight raspberry cultivars. The treatments were applied in a completely randomized design with eight replications per treatment. On the same dates, the treatments listed in Table 2.2 were applied to the blackberries ('Prime-Jim' and 'Prime-Jan') in 2.0 m sections of row in a completely randomized design with 4 replications. The treatments applied to both the raspberries and blackberries were compared with untreated, 2.0 m control plots. All treatments were applied as foliar sprays using a CO<sub>2</sub> backpack sprayer equipped with 28008VS stainless steel spray tips and calibrated to deliver 80 GPa at 0.28 MPa.

### ***2008 Treatment Applications***

On 25 June, 3, 10, 17, 24, and 31 July, and 22 August 2008, the treatments listed in Table 2.3 were applied to 1.2 m sections of row in each of the eight raspberry cultivars. The treatments were applied in a completely randomized design with eight replications per treatment. The same cultivars used in the 2007 experiments were used in 2008. Also, on all dates listed above for 2008, except for 22 August, the treatments listed in Table 2.4 were applied to the blackberries in a completely randomized design. Four replications of 1.2 m treated and untreated control plots were compared for the blackberries. All treatments were applied as described for the 2007 experiment.

### ***2009 Treatment Applications***

On 24 June, 2, 9, 16, 23, and 31 July, the treatments provided in Table 2.5 were applied to 1.2 m sections of raspberry rows, with 16 replications in a balanced incomplete blocking design. In that season's experiment, each of the six rows acted as plots which had two treatments randomly applied to eight cultivar subplots within the row/plot. The treatment effects were determined using a full factorial design which incorporates both the treatment and subplot effects on the response. The raspberry cultivars used in 2007 and 2008 were again employed; the blackberries were not included. As in previous years, the treatments were applied using a CO<sub>2</sub>-powered backpack sprayer.

### ***Japanese Beetle Counts 2007- 2009***

Japanese beetles were counted within 2.0 m rows per each plot of raspberries and blackberries in 2007 and within 1.2 m rows in 2008. In 2009, JB counts were taken in 1.2 m plots of the raspberries only. Plants were carefully examined in an attempt to count all JB

present in the designated area of each plot. In 2007, sample dates were 26 and 29 June, 2, 5, 9, 12, 17, and 20 July, and 14 August. In 2008, JB counts were made on the following dates: 18, 25, and 28 July, 1, 4, 7, 11, and 18 August. In 2009, JB counts were obtained on 8, 15, and 22 July, and 5 August. The treated plot size in 2008 and 2009 was reduced to 1.2 m, and all JB present within this area were counted.

JB count data from each of the three years were collected and analyzed separately by date and by seasonal totals. Data were  $\sqrt{x} + 0.5$  transformed when not following a normal distribution. Both transformed and untransformed data were analyzed with analysis of variance (ANOVA) followed by a Tukey's HSD mean comparison ( $\alpha = 0.05$ ) (JMP 1989)(SAS Institute 1989).

### ***Harvest 2007-2009***

In order to determine if the pesticides used affected marketable, unmarketable, or percent unmarketable yield, raspberries and blackberries were harvested and weighed. Berries were picked within the treated and control 2.0 m sections in 2007 and within 1.2 m sections in 2008 and 2009. All ripened berries were harvested within each cultivar-treatment plot each season. These berries were divided into marketable and unmarketable yields and then weighed in grams. The weights were recorded to the closest, even, tenth of a gram (e.g. 93.11 grams was recorded as 93.2 grams). A berry was considered marketable if it did not contain injury of any kind; whereas unmarketable berries were injured by chewing or sucking insect feeding, sunburn, drupelet malformation, or mold. If any berries were unmarketable because of being overripe, the berries were discarded and not weighed. The same person did a final quality evaluation after berries were pre-separated into marketable or unmarketable containers by others.



Yield data were collected twice a week starting 14 August through 29 September 2007 in the raspberries and 21 August through 11 September 2007 in the blackberries. In 2008, the berries were harvested on 22, 26, and 29 August, 2, 12, 16, 19, and 30 September. The berries were harvested twice a week 6 August through 3 September 2009 and once a week on 10 September and 17 September 2009.

Yield data were analyzed by harvest date, as a seasonal total, and as a seasonal total percent unmarketable for each of the three years observed. The marketable and unmarketable data were  $\sqrt{x} + 0.5$  transformed when not following a normal distribution. When the proportion of unmarketable yield data did not follow a normal distribution, it was subjected to an ArcSine ( $\sqrt{x}$ ) transformation. Both transformed and untransformed raspberry data were analyzed with ANOVA followed by a Tukey's HSD mean comparison ( $\alpha = 0.05$ ) (SAS Institute 1989). Blackberry data was analyzed with ANOVA followed by a t-test mean comparison ( $\alpha = 0.05$ ) (SAS Institute 1989).

### ***Defoliation Study 2007***

To test the toxicity, antifeedant, and deterrent qualities of the insecticide treatments, JB were bagged onto canes within each treated plot and the resulting defoliation was analyzed. The raspberry and blackberry cultivars used for the bagging experiment, 'Fall Gold,' 'Autumn Bliss,' and 'Prime-Jim,' were chosen based on a 2006 study in which these cultivars were most preferred by JB (Maxey et al. 2006). In total, there were 20 bags applied in the raspberries with each of the four chemical treatments and untreated control plots having four replications (bags). Two of these replications were applied to each of the treated 'Fall Gold' and 'Autumn Bliss' plots. In the blackberries, 12 plots of 'Prime-Jim' were used. There were a total of 24 bags

applied in 'Prime-Jim' with each of the five chemical treatments and untreated control plots having four replications.

Each bag, containing five beetles, was placed over the top 18-30 cm of randomly chosen canes within the 2.0 m sections of treated or control plots. Before the bags were applied, the defoliation of the leaves was estimated based on a 0-100 scale. A score of "0" was given to leaves without any injury and a score of "100" given to leaves with only the veins present. The same individual estimated the defoliation throughout the entire study.

Defoliation from each leaflet within the top 18-30 cm of the cane was recorded starting with the apical-most leaf and working down the cane. The bag was then placed over the cane, with the bottom of the bag being at the level of the most basal leaf for which the defoliation was recorded. A medium sized binder clip was then clipped around the bag and cane to secure the bag on the plant. After 48 hrs, the bags were removed and the defoliation was recorded as described above. The comparison of defoliation from before and after JB application resulted in an estimate of foliar injury by the beetles.

The percent defoliation was analyzed by date and as a seasonal total to estimate which treatments prevented the beetles from injuring the foliage as compared with the control. Results that did not follow a normal distribution were subjected to an ArcSine ( $\sqrt{x}$ ) transformation. Both transformed and untransformed data were analyzed with ANOVA followed by a t-test mean comparison ( $\alpha = 0.05$ ) (SAS Institute 1989).

### ***Defoliation Study 2008-2009***

In order to incorporate more cultivars of raspberries and blackberries and to gain a more accurate estimate of defoliation, the defoliation study in 2008 contained different procedures than the 2007 study. Due to low JB pressure in 2008, beetles were not bagged onto plants and

percent defoliation was only analyzed as a seasonal total instead of a weekly percent defoliation as in 2007. From each of the 1.2 m treated plots described above under '2008 Treatment Application,' 35 leaves (injured and uninjured) were randomly selected and digitally photographed. All treated cultivars of blackberries and raspberries were included. The digital images, 35 for each treatment-cultivar plot, were analyzed using the computer program, ImageJ (Rasband 2009), which was downloaded from: <http://rsbweb.nih.gov/ij/download.html>. The estimated total area of each leaf was measured followed by the injured area being measured. All measurements were taken through ImageJ counting the number of pixels within the selected area of the image. The injured area was then divided by the total area to obtain the percent defoliation which was compared and analyzed.

Because the degree of foliar injury was low in 2008, the treatments' potential to control defoliation was masked through all results being lowered by the zero values. Therefore, in the 2009 defoliation study, only injured leaves were considered. This experimental procedure was implemented to better clarify treatment differences. At the end of the field season, digital images were taken of 20 arbitrarily selected injured raspberry leaves within each 1.2 m treated plot. All treated plots, 96 in total, were analyzed. In 2009, percent defoliation was calculated using the same procedure as in 2008.

The proportion of defoliation data for 2008 and 2009 were analyzed as seasonal totals. Data that did not follow a normal distribution were subjected to an ArcSine ( $\sqrt{x}$ ) transformation. Both transformed and untransformed raspberry data were analyzed using ANOVA followed by a Tukey's HSD mean comparison ( $\alpha = 0.05$ ) (SAS Institute 1989). The 2008 blackberry defoliation data were analyzed using ANOVA followed by a t-test mean comparison ( $\alpha = 0.05$ ) (SAS Institute 1989).

## Laboratory Experiments

Japanese beetle adults were collected 27 July 2009 from Japanese beetle traps baited with Tanglefoot® Japanese beetle bait and lure (japonilure). A water source was provided within the traps so the beetles did not die from desiccation before collection. Trapped beetles were in the trap for at most 48 hrs. Only living, active beetles were used for the experiments.

All bioassays took place from 27 to 29 July 2009, inside a room with artificial lighting and a constant temperature of 22 °C. The bioassays consisted of 25 replications for each of the six treatments (Table 2.6). The ‘Prelude’ raspberry leaves were dipped into each of the treatments that were mixed to rates labeled for field use. ‘Prelude’ leaves were used because a 2008 defoliation study suggested this cultivar incurred higher defoliation by JB than the other cultivars present in the field (Maxey et al. 2008). Shortly after being dipped in the treatments, one leaf was placed in an aerated Tupperware® container (16.3 x 17.8 x 9.7 cm) with one Japanese beetle and a moistened cotton ball for 48 hrs. The control treatments contained untreated leaves and moistened cotton balls. There were two trials in the JB presence and mortality bioassays; trial one contained 12 leaves and trial two contained 13 leaves treated with each treatment. The mean responses of the two trials were then analyzed to observe any treatment effects.

The percent defoliation was calculated by taking digital images of the leaves before and after JB exposure and analyzing the images using ImageJ as described above. The number of beetles on the leaves after 6, 18, 24 and 48 hrs was recorded to test if the treatment had an effect on the number of JB on the leaves. Also, to assess toxicity, the number of beetles alive after 6, 18, 24, and 48 hrs was recorded. These data were then used to calculate the percent of JB that were on treated leaves and the percent of JB that were killed after exposure to the chemicals.

The percent defoliation, percent mortality, and the percent of beetles on treated leaves were then analyzed. Any data that did not follow a normal distribution were transformed (ArcSine ( $\sqrt{x}$ )). Both transformed and untransformed data were subjected to ANOVA, followed by a Tukey's HSD mean comparison ( $\alpha = 0.05$ ) (SAS Institute 1989).

## **Results**

### **Field Experiments**

#### ***Raspberries 2007***

##### **Japanese Beetle Counts**

There were significant differences on 26 and 29 June, 2, 5, 9, 12, 17, and 20 July 2007 in the number of JB within the 2.0 m treated and untreated control sections (Figure 2.1). The control and plants treated with metaflumizone or lime-alum had significantly fewer JB present on 26 June than plants that were treated with honey-milk. On 29 June, deltamethrin and lime-alum had significantly fewer JB than honey-milk. Lime-alum also had significantly fewer JB than honey-milk on 2 July. On 5 July, there were significantly fewer JB on plants treated with deltamethrin or lime-alum than with honey-milk or azadirachtin (Aza-Direct). There were also significantly fewer beetles present on plants treated with lime-alum than with metaflumizone. On 9 July, there were significantly fewer JB on lime-alum than on honey-milk or in the control. On 12 July, there were significantly fewer JB on lime-alum and deltamethrin than on the control or honey-milk. Significantly fewer JB were also counted on lime-alum than within azadirachtin (Aza-Direct) or metaflumizone. On 17 July, deltamethrin, lime-alum, and metaflumizone had

significantly fewer JB than the control. On 20 July, lime-alum had fewer JB present than the control or metaflumizone.

When all JB counts were added together and the cumulative mean counts for each treatment were compared, deltamethrin and lime-alum had significantly fewer JB than the control, honey-milk, or metaflumizone ( $F = 12.28$ ;  $df = 5$ ;  $P < 0.0001$ ) (Figure 2.2). Lime-alum also had significantly fewer beetles than azadirachtin (Aza-Direct). Even though the lime-alum treatment reduced the number of JB present, there were residues left after application (Figure 2.3), the berries had an alum taste, and spray nozzles frequently became clogged, all of which made this treatment undesirable.

### **Harvest: Yield per Treatment**

There were no significant differences in the marketable or unmarketable yield between all treatments applied to the raspberries in 2007. The mean cumulative yields of marketable ( $F = 1.95$ ;  $df = 5$ ;  $P = 0.1106$ ) and unmarketable fruit ( $F = 0.60$ ;  $df = 5$ ;  $P = 0.7019$ ) and the percent unmarketable fruit ( $F = 1.47$ ;  $df = 5$ ;  $P = 0.2260$ ) were not significantly different either (Table 2.7).

### **Estimation of Defoliation**

For the JB bagging defoliation studies in the raspberries, there were no significant differences between treatments on any of the five dates considered. There were also no significant differences among the treatments after the average defoliation for the season was calculated ( $F = 1.71$ ;  $df = 5$ ;  $P = 0.1851$ ) (Figure 2.4).

## ***Blackberries 2007***

### **Japanese Beetle Counts**

In the 2007 blackberries, there were only significant differences in the number of JB on 5 and 12 July (Figure 2.5). On 5 July, there were fewer JB on deltamethrin treated plants than on those treated with thyme oil. On 12 July, bifenthrin had fewer JB present than the control or capsaicin. When the cumulative mean counts of each treatment were compared ( $\alpha = 0.10$ ), deltamethrin had significantly fewer JB present than the control or capsaicin ( $F = 3.10$ ;  $df = 5$ ;  $P = 0.0501$ ) (Figure 2.6).

### **Harvest: Yield per Treatment**

There were significant differences in the marketable blackberry yields produced between treatments on 21 and 31 August 2007 (Table 2.8). On 21 August, there was significantly greater marketable yield from deltamethrin than from potassium bicarbonate, thyme oil, and the control. On 31 August there was significantly greater marketable yield from deltamethrin than from potassium bicarbonate, thyme oil, and the control. Also on this date, capsaicin had significantly greater marketable yields than potassium bicarbonate and the control and bifenthrin had significantly greater marketable yields than the control. The cumulative marketable blackberry yield in 2007 was significantly greater for deltamethrin than all other insecticides or the control ( $F = 12.13$ ;  $df = 5$ ;  $P = 0.0002$ ) (Table 2.8). There were no significant differences on any date in the unmarketable yield. There were no significant differences in the total unmarketable ( $F = 0.62$ ;  $df = 5$ ;  $P = 0.6892$ ). When comparing cumulative percent unmarketable blackberry yields ( $\alpha = 0.10$ ), deltamethrin had significantly less injury than potassium bicarbonate ( $F = 2.46$ ;  $df = 5$ ;  $P = 0.0938$ ).

## **Estimation of Defoliation**

In the blackberries, JB were bagged onto 'Prime-Jim' on five dates, two of which revealed significant differences (Figure 2.7). On 20 July, there was significantly more defoliation in the control and capsaicin than in deltamethrin. On the other date that contained differences (14 August) there was significantly greater defoliation on capsaicin than on bifenthrin and deltamethrin. The control plot and potassium bicarbonate had significantly more defoliation than deltamethrin. When the blackberry defoliation levels were averaged from all dates in 2007 there was significantly greater defoliation in plants treated with capsaicin and in the control than in thyme oil and deltamethrin ( $F = 4.34$ ;  $df = 5$ ;  $P = 0.0091$ ) (Figure 2.8).

## ***Raspberries 2008***

### **Japanese Beetle Counts**

In 2008, there were three dates with significant differences in JB numbers in the raspberries; 18 and 28 July and 7 August (Figure 2.9). On 18 July there were significantly fewer JB present both on deltamethrin and azadirachtin (Neemix 4.5) than in the control. There were also significantly fewer JB on deltamethrin than the control on 28 July. On 7 August, there were significantly fewer JB on deltamethrin than azadirachtin (Neemix 4.5). When all JB counts in raspberries from 2008 were added together and the mean counts were compared, deltamethrin had significantly fewer JB present in 2008 than the control ( $F = 2.77$ ;  $df = 4$ ;  $P = 0.0469$ ) (Figure 2.10).



## **Harvest: Yield per Treatment**

In 2008, the raspberries did not have significant differences on any date in the marketable yield among treatments. However, there were differences in the cumulative marketable yield ( $\alpha = 0.10$ ); deltamethrin had significantly greater marketable yield than the neem oil extract ( $F = 2.49$ ;  $df = 4$ ;  $P = 0.0661$ ) (Table 2.9). One date, 2 September, did have significant differences in unmarketable yield between the treatments (Table 2.10). On this date there was significantly greater unmarketable yield for deltamethrin and azadirachtin (Neemix 4.5) than there was from the neem oil extract. There was also significantly greater unmarketable yield for azadirachtin (Neemix 4.5) than from chlorantraniliprole on 2 September. There were significant differences in the total unmarketable raspberry yield ( $\alpha = 0.10$ ) between treatments in 2008 with deltamethrin having greater cumulative unmarketable yield than the neem oil extract ( $F = 2.16$ ;  $df = 4$ ;  $P = 0.0991$ ). Significant differences between treatments were not present in the total percent unmarketable yield for 2008 ( $F = 0.70$ ;  $df = 4$ ;  $P = 0.5955$ ) (Table 2.9).

## **Total Calculated Defoliation**

Defoliation was measured at the end of the harvest season in 2008 and chlorantraniliprole had significantly less seasonal defoliation than the untreated control ( $F = 3.57$ ;  $df = 4$ ;  $P = 0.0069$ ) (Figure 2.11).

## ***Blackberries 2008***

### **Japanese Beetle Counts**

In the 2008 blackberries, 15 August was the only date in which significant differences between treatments were observed in the number of JB present (Figure 2.12). On this date, there

were significantly fewer JB present on deltamethrin than on the rosemary and peppermint oil combination. When comparing the mean cumulative number of JB per treatment in the 2008 blackberries ( $\alpha = 0.10$ ), bifenthrin had significantly fewer JB present than the rosemary and peppermint oils mixture ( $F = 4.24$ ;  $df = 5$ ;  $P = 0.0693$ ) (Figure 2.13).

### **Harvest: Yield per Treatment**

Significant differences were not present on any date for marketable or unmarketable yield in the 2008 blackberry trials. Significant differences were also not observed in the total marketable ( $F = 1.69$ ;  $df = 5$ ;  $P = 0.2104$ ) or in the total percent unmarketable yields ( $F = 0.89$ ;  $df = 5$ ;  $P = 0.5198$ ) (Table 2.10). There were significant differences between treatments in the amounts of unmarketable yields with the rosemary and peppermint oils mixture having less unmarketable yield than the control ( $F = 3.20$ ;  $df = 5$ ;  $P = 0.0459$ ).

### **Defoliation**

There was no significant treatment effect on blackberry defoliation in 2008 ( $F = 0.20$ ;  $df = 5$ ;  $P = 0.9639$ ) (Figure 2.14).

## ***Raspberries 2009***

### **Japanese Beetle Counts**

There were two days within the 2009 raspberry trials with a significant treatment effect on the number of JB between treatments, 8 and 22 July (Figure 2.15). On 8 July, there were significantly more JB present on plots treated with acetamiprid than with chlorantraniliprole and the combination of two azadirachtin oil products (Neemix 4.5 and Trilogy). On 22 July, acetamiprid again had significantly more JB than chlorantraniliprole and the azadirachtin and

neem oil extract combination, but also had more JB than the control, the azadirachtin and pyrethrin combination and deltamethrin. Acetamiprid had significantly more JB present than all other insecticides and the control when comparing the cumulative means of JB present for each treatment in 2009 ( $F = 7.92$ ;  $df = 5$ ;  $P < 0.0001$ ) (Figure 2.16).

### **Harvest: Yield per Treatment**

On 10 August 2009, there was significantly higher marketable yield from deltamethrin than from chlorantraniliprole or the control (Table 2.11). All other dates in 2009 had no significant differences in marketable yield. The total marketable yield also did not contain significant differences ( $F = 0.71$ ;  $df = 5$ ;  $P = 0.6182$ ). And even though there were no significant differences in the total unmarketable yield ( $F = 0.76$ ;  $df = 5$ ;  $P = 0.5865$ ) or in the total percent unmarketable yield ( $F = 0.42$ ;  $df = 5$ ;  $P = 0.8354$ ), there were significant differences on 24 and 31 August in unmarketable yield (Table 2.11). On 24 August, the azadirachtin and pyrethrin combination had significantly greater unmarketable yield than chlorantraniliprole. On 31 August, chlorantraniliprole, deltamethrin, and the control had significantly greater unmarketable yield than the azadirachtin and pyrethrin combination.

### **Defoliation**

When the mean percent defoliations of treated raspberry leaves were compared in 2009, significant differences were present (Figure 2.17). Significantly reduced defoliation was present in plants treated with chlorantraniliprole and deltamethrin compared to the azadirachtin and pyrethrin combination, the azadirachtin and neem oil extract combination, and the untreated control ( $F = 8.57$ ;  $df = 5$ ;  $P < 0.0001$ ).

## Laboratory Experiments

### *Japanese Beetles on Treated Leaves*

The percentages of JB on individually insecticide-treated leaves were compared after 6, 18, 24, and 48 hrs (Figure 2.18). After six hrs, the percentage of beetles on deltamethrin treated leaves was significantly less than leaves treated with the *Chenopodium* extract, the azadirachtin and neem oil extract combination, chlorantraniliprole, and the control. Also, significantly fewer JB were on leaves treated with acetamiprid than on the azadirachtin and neem oil extract combination, and the control. The azadirachtin and pyrethrin combination had significantly fewer JB present than the control.

After 18 hrs, significantly fewer beetles were on deltamethrin than *Chenopodium* extract, the azadirachtin and neem oil extract combination, the azadirachtin and pyrethrin combination, chlorantraniliprole, and the control (Figure 2.18). The leaves treated with acetamiprid had significantly fewer JB present than the azadirachtin and neem oil extract combination, chlorantraniliprole, and the control. The azadirachtin and pyrethrin combination had significantly fewer JB than the azadirachtin and neem oil extract combination, chlorantraniliprole, and the control. Also, the *Chenopodium* extract had significantly fewer JB than chlorantraniliprole and the control.

After 24 hrs, 4.2 percent of the beetles were on deltamethrin-treated leaves (Figure 2.18). There were significantly fewer JB on leaves treated with deltamethrin than on leaves treated with *Chenopodium* extract, the azadirachtin and neem oil extract combination, the azadirachtin and pyrethrin combination, chlorantraniliprole, or on the control. Significantly fewer JB were present on acetamiprid than on the azadirachtin and neem oil extract combination,

chlorantraniliprole or on the control. Also, significantly fewer JB were on leaves treated with the *Chenopodium* extract than on the azadirachtin and neem oil extract combination, chlorantraniliprole, and on the control.

The last observation was taken after the beetles were exposed to the treated leaves for 48 hrs. After this time, there were significantly fewer JB on deltamethrin than the control, the azadirachtin combination, chlorantraniliprole, the *Chenopodium* extract, or the azadirachtin and pyrethrin combination. Acetamiprid had significantly fewer JB present compared to the control, the azadirachtin and neem oil extract combination, or chlorantraniliprole. The *Chenopodium* extract also had fewer JB than the control.

### ***Mortality***

The percent of dead JB after 6, 18, 24, and 48 hrs was observed and the results are displayed in Figure 2.19. After six hrs, there was no significant treatment effect. After 18 hrs, there was significantly higher mortality from acetamiprid than from chlorantraniliprole, the azadirachtin and pyrethrin combination, the *Chenopodium* extract, the azadirachtin and neem oil extract combination, or the control. There was also significantly higher mortality in deltamethrin than in the *Chenopodium* extract, the azadirachtin and neem oil extract combination, or the control. Chlorantraniliprole and the azadirachtin and pyrethrin combination both had a higher percent mortality than the azadirachtin and neem oil extract combination and the control.

After 24 hrs, acetamiprid caused significantly higher mortality than chlorantraniliprole, the azadirachtin and pyrethrin combination, the *Chenopodium* extract, the azadirachtin and neem oil extract combination, or the control. Deltamethrin caused significantly higher mortality than chlorantraniliprole, the *Chenopodium* extract, the azadirachtin and neem oil extract combination,

or the control whereas the azadirachtin and pyrethrin combination only had a significantly higher mortality than the control.

After 48 hrs, acetamiprid imposed significantly higher mortality than chlorantraniliprole, the *Chenopodium* extract, the azadirachtin and neem oil extract combination or the control. Significantly more JB that were exposed to deltamethrin died than JB exposed to the *Chenopodium* extract, the azadirachtin and neem oil extract combination, or the control. The mortality was significantly higher for the azadirachtin and pyrethrin combination than the control.

### ***Defoliation***

After each treated leaf was exposed to JB for 48 hrs, the resulting percent defoliation was recorded (Figure 2.20). The control leaves had the highest defoliation ( $10.0 \pm 2.0$  %). Defoliation was significantly less defoliation than the control on leaves treated with the azadirachtin and neem oil extract combination, chlorantraniliprole, acetamiprid, deltamethrin, and the *Chenopodium* extract ( $F = 6.25$ ;  $df = 6$ ;  $P < 0.0001$ ).

### **Discussion**

The efficacy of insecticides with short pre-harvest intervals was tested for control of JB in caneberries. Although a wide array of insecticides were considered, significant JB control was minimal. Over the experiment, JB densities were relatively low ranging in the untreated control plots from 4.8 JB/m row/d in 2007 to 1.1 JB/m row/d in 2009 (Table 2.12). Thus, the amount of JB injury observed on the caneberries, especially in 2008 and 2009, is not typical injury that would exist under greater JB pressure. Due to the reduced JB pest pressure, the effect of insecticide treatments was rarely significantly different from the untreated controls.

The only treatment that continually exhibited significant JB control in the field and laboratory was the pyrethroid deltamethrin. In field experiments, deltamethrin prevented JB from being on caneberries (Figures 2.2, 2.6, and 2.10), promoted greater marketable yield (Table 2.8), and reduced defoliation (Figures 2.8 and 2.17). Also, compared to the control ( $47.1 \pm 2.0$  %), the percentage of injured blackberries was reduced almost by half with use of deltamethrin ( $26.4 \pm 4.2$  %) in 2008 (Table 2.8). In the bioassays, this treatment increased mortality (Figure 2.19), reduced the number of JB present on treated leaves (Figure 2.18), and reduced defoliation (Figure 2.20). Another pyrethroid, bifenthrin reduced JB activity on blackberries but not significantly different from the control plots (Figures 2.6, 2.8, and 2.13). On crapemyrtles, Pettis et al. (2005) showed that bifenthrin reduced JB defoliation. Baulmer and Potter (2007) found that 19 d old deltamethrin and bifenthrin residues exhibited nearly 100% JB knockdown and defoliation reduction of linden by 97.9 % (deltamethrin) and 94.5 % (bifenthrin). During my experiments, the oldest residues that JB were exposed to were approximately a week old. The residual effect observed by Baulmer and Potter support that both pyrethroids would reduce JB activity with fewer than weekly applications. In choice tests, Baulmer and Potter (2007) concluded that both pyrethroids would provide effective control without total spray coverage. Thus, treating caneberry foliage should provide sufficient protection for berries that ripen after treatment application. This trait increases appeal in this cropping system since ripened berries are only on the plants for a short period of time. Overall, the detected control with these pyrethroids while JB pressure was low, along with other reports of their effectiveness (Pettis et al. 2005, Baumler and Potter 2007), confirms that deltamethrin and bifenthrin are valuable JB controls.

The neonicotinoid, acetamiprid, works through mortality after contact and ingestion. During the bioassays, this treatment displayed insecticidal activity (Figure 2.19), reduced the number of JB present on treated leaves (Figure 2.18), and decreased defoliation (Figure 2.20). In the field however, JB numbers were the highest on acetamiprid treated plants (Figures 2.15 and 2.16). Acetamiprid has been reported to penetrate fruit and leaf tissue (Hoffmann et al. 2010) so effectiveness could occur after consumption of the treated plant material. However, infestation and injury were not reduced in the field, suggesting that acetamiprid may not prevent the initial feeding that releases JB attracting volatiles (Loughrin 1996). An increased JB population could therefore result in aggregation and more severe injury. Further tests need to be conducted testing the rate in which acetamiprid reduces JB activity in the field.

Japanese beetle activity was also reduced by chlorantraniliprole and in 2008 and 2009 the plants treated with this treatment had reduced defoliation compared to the untreated control (Figures 2.11 and 2.17). Plants treated with this treatment also experienced less infestation in 2008 and 2009 (Figures 2.10 and 2.16). Also in the bioassays, chlorantraniliprole increased JB mortality (Figure 2.19) and decreased defoliation (Figure 2.20) compared to untreated leaves. Chlorantraniliprole has been proven to be effective against JB (DuPont 2007, Williams 2008). Also, chlorantraniliprole does not kill honey bees or other beneficial insects or mammals, which makes this insecticide it an ideal alternative to pyrethroids (DuPont 2007). This treatment also rapidly ceases insect feeding on treated plant material resulting in less overall injury (Hannig et al. 2009). The sooner JB quit feeding on plant material, the less aggregation pheromones are released (Loughrin et al. 1996). Therefore, this treatment which quickly prevents JB feeding while being less harmful to beneficials is ideal.



Multiple botanical and inorganic insecticides with short pre-harvest intervals were tested for JB control including azadirachtin, neem oil extract, a honey and milk combination, thyme oil, capsaicin, potassium bicarbonate, lime-alum, a rosemary oil and peppermint oil combination, an oxymatrine and prosuler combination, a vegetable, thyme, tagetes, and wintergreen oil mixture, and a *Chenopodium* extract. The botanical insecticides that were ineffective or did not display controlling qualities include: the honey and milk combination, capsaicin, the rosemary and peppermint oil combination, potassium bicarbonate, the oxymatrine and prosuler combination, and the vegetable, thyme, tagetes, and wintergreen oil mixture. Most of these treatments are marketed to homeowners or small scale operations and may provide enough control at this level. However, these treatments did not reduce JB activity under low population pressure and are not recommended for commercial use.

Different formulations of azadirachtin and/or neem oil extract were tested during this three-year study (Aza-Direct, Neemix 4.5, Trilogy, a Neemix 4.5 and Trilogy combination, and an azadirachtin and pyrethrin combination). Azadirachtin acts as an antifeedant and repellent towards JB adults. In a defoliation bioassay (Figure 2.20), azadirachtin (Neemix 4.5/ Trilogy) significantly reduced defoliation compared to an untreated control. Also in the laboratory, the pyrethrin mixture increased JB mortality (Figure 2.19) but was not as effective as deltamethrin or acetamiprid. Still, this mixture offers an effective botanical insecticide that combines the antifeedant and repellent qualities of azadirachtin with the insecticidal properties of pyrethrins. Laboratory studies by others confirm that azadirachtin prevents injury by JB (Held et al. 2001, Baumler and Potter 2007). In field trials, no azadirachtin formulation stood out from the others or reduced overall JB activity. Conversely, protection found in other field studies indicates that azadirachtin is effective in reducing JB activity even under greater JB pressure (Gupta and

Krischik 2007, Vitullo and Sadof 2007). Japanese beetle activity on raspberries may not be reduced by azadirachtin since raspberries are a preferred host (Fleming 1972, Ladd 1987). If the attractive qualities of raspberries were not negated by azadirachtin's antifeedant and repellent characteristics, control would not be observed. Yet again, a larger JB population would aid better understanding azadirachtin's effect on JB.

Other notable botanical or inorganic insecticides that were evaluated include lime-alum, thyme oil, and the extract of *Chenopodium ambrosioides*. Numbers were significantly reduced on raspberries treated with lime-alum (Figure 2.2) but this treatment had unwanted side-effects (Figure 2.3). When lime-alum was recommended for JB control application in 1938, instructions included applying this treatment once and reapplying only when new growth was present (Britton and Johnson 1938). Following spray recommendations would in itself reduce the side-effects observed on raspberry. However, since new growth of raspberries occurs terminally where fruit is present, the berries are still vulnerable to becoming ruined with reapplication. A possible explication of why fewer JB were present on the lime-alum treated plants is that the treatment had an undesirable smell, taste, or appearance. Another explanation is that the residue blocked visual and/or olfactory cues that make raspberries desirable. Further studies should be conducted to better understand the effectiveness of this insecticide.

Thyme oil lessened JB defoliation (Figure 2.8), but did not prevent JB infestation or prevent blackberry injury. This essential oil has been shown to be toxic to other beetles (Righi et al. 2008, Demirel et al. 2009) but the insecticidal properties have yet to be tested against JB. Mortality could explain the reduced activity on blackberry. Fewer JB were present on leaves treated with an extract of *Chenopodium ambrosioides* during a bioassay (Figure 2.18) but this treatment did not increase mortality (Figure 2.19). Defoliation was also reduced by this

treatment (Figure 2.20). Field tests were not conducted with this treatment so it is not known whether the absence from treated plants in the laboratory will translate into reduced JB activity in the field.

In conclusion, multiple synthetic, botanical, and inorganic insecticides with short pre-harvest intervals were tested for JB control on raspberries and blackberries. The low JB populations complicated the field efficacy trials. However, both deltamethrin and bifenthrin continually reduced JB activity on caneberries and are recommended for this cropping system. The residual effects of these treatments also indicate that JB control would be possible with fewer than weekly applications (Baumler and Potter 2007). Acetamiprid displayed insecticidal activity but did not lessen JB activity in the field. Control with this neonicotinoid may not occur until after too much injury is present, making it an unsuitable pesticide for the aggregating JB. Chlorantraniliprole significantly reduced defoliation and numerically reduced JB infestation. The reduced injury is explained by insects ceasing feeding shortly after ingestion of this treatment (Hannig et al. 2009). Through decreasing the release of feeding induced volatiles, this treatment decreases JB activity on treated plants. Azadirachtin did not provide conclusive control of JB in my field experiment, but control has been observed by others (Held et al. 2001, Baumler and Potter 2007, Gupta and Krischik 2007, Vitullo and Sadof 2007). The other botanical and inorganic insecticides that showed promise for JB control were lime-alum, thyme oil, and the extract of *Chenopodium ambrosioides*.

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## Tables and Figures

Table 2.1 Treatment applied to 2.0 m sections of primocane-bearing raspberries in 2007.

Active Ingredient	Trade name (Formulation)	Manufacturer	AI (% in product)	Material rate per Hectare (g or ml/ha)
Honey and Milk	Honey/Milk	Homemade	12.5 % honey & 8.7 % milk	876.9 ml honey & 9,353.9 ml milk
Azadirachtin	Aza-Direct	Gowan Co.	1.2 %	2,338.4 ml
Deltamethrin	Battalion (0.2 EC)	Arysta Lifescience N.A. Corp.	2.9 %	876.9 ml
Lime and Alum	Lime-Alum	Homemade	1.2 % lime & 0.4 % alum	1,120.9 g lime & 350.3 g alum
Metaflumizone	Alverde (240 SC) plus Penetrator Plus (non-ionic surfactant)	BASF	22.2 %	1,169.2 ml
Control	N/A	N/A	N/A	N/A

Table 2.2 Treatments applied to 2.0 m sections of primocane-bearing blackberries in 2007.

Active Ingredient	Trade name (Formulation)	Manufacturer	AI (% in product)	Material rate per Hectare (g or L/ha)
Thyme oil	Proud (3)	Bio Huma Netics, Inc	5.6 %	2,338.5 ml
Potassium bicarbonate	Agricure (85)	H & I Agritech, Inc.	0.51%	5604.3 g
Deltamethrin	Battalion (0.2 EC)	Arysta Lifescience N.A. Corp.	2.9 %	876.9 ml
Bifenthrin	Capture (2 EC)	FMC Corporation	25.10%	467.7 ml
Capsaicin	Hot Pepper Wax	Bonide Products, Inc	0.00018%	43,870.0 ml
Control	N/A	N/A	N/A	N/A



Table 2.3 Treatments applied to 1.2 m sections of primocane-bearing raspberries in 2008.

Active Ingredient	Trade name (Formulation)	Manufacturer	AI (% in product)	Material rate per Hectare (g or L/ ha)
Deltamethrin	Battalion (0.2 EC)	Arysta Lifescience N.A. Corp.	2.9 %	876.9 ml
Rynaxypyr/ Chlorantraniliprole	Altacor (35 WD)	DuPont	35.0%	315.2 g
Azadirachtin	Neemix (4.5)	Certis USA, LLC	4.50%	1,169.2 ml
Clarified hydrophobic extract of neem oil	Trilogy	Certis USA, LLC	70.0%	4677.0 ml
Control	N/A	N/A	N/A	N/A

Table 2.4 Treatments applied to 1.2 m sections of primocane-bearing blackberries in 2008.

Active Ingredient	Trade name (Formulation)	Manufacturer	AI (% in product)	Material rate per Hectare (g or L/ ha)
Rosemary oil & peppermint oil	Ecotrol (EC)	EcoSmart technologies	10.0 % Rosemary oil & 2.0 % Peppermint oil	3,507.7 ml
Oxymatrine & Prosuler	Moi-201	Kingbo Biotech Co.	0.6%	1,461.6 ml
Vegetable, thyme, tagetes, & wintergreen oil	Bug oil	Plant Impact	2.0%	11,692.4 ml
Deltamethrin	Battalion (0.2 EC)	Arysta Lifescience N.A. Corp.	2.9 %	876.9 ml
Bifenthrin	Capture (2EC)	FMC Corporation	25.1%	467.7 ml
Control	N/A	N/A	N/A	N/A

Table 2.5 Treatments applied to 1.2 m sections of primocane-bearing raspberries in 2009.

Active Ingredient	Trade name (Formulation)	Manufacturer	AI (% in product)	Material rate per Hectare (g or L/ha)
Deltamethrin	Battalion (0.2 EC)	Arysta Lifescience N.A. Corp.	2.9 %	876.9 ml
Rynaxypyr/ Chlorantraniliprole	Altacor (35 WD)	DuPont	35.00%	315.2 g
Azadirachtin & Clarified hydrophobic extract of neem oil	Neemix (4.5) & Trilogy	Certis USA, LLC	3.6 % Neemix & 14.0 % Trilogy	1,892.7 ml Neemix & 473.2 ml Trilogy
Azadirachtin & Pyrethrins	MGK (EC)	McLaughlin Gormley King Co.	1.2% azadirachtin & 1.4% pyrethrins	2,338.5 ml
Acetamiprid	Assail (30 SG)	United Phosphorous Inc.	30.0%	150.3 g
Control	N/A	N/A	N/A	N/A

Table 2.6 Treatments applied to 'Prelude' raspberry leaves during a bioassay conducted in 2009.

Active Ingredient	Trade name (Formulation)	Manufacturer	AI (% in product)	Material rate per Hectare (g or L/ha)
Deltamethrin	Battalion (0.2 EC)	Arysta Lifescience N.A. Corp.	2.9 %	876.9 ml
Rynaxypyr/ Chlorantraniliprole	Altacor (35 WD)	DuPont	35.00%	315.2 g
Azadirachtin & Clarified hydrophobic extract of neem oil	Neemix (4.5) & Trilogy	Certis USA, LLC	3.6 % Neemix & 14.0 % Trilogy	1,892.7 ml Neemix & 473.2 ml Trilogy
Azadirachtin & Pyrethrins	MGK (EC)	McLaughlin Gormley King Co.	1.2% Azadirachtin & 1.4% Pyrethrins	2,338.5 ml
Acetamiprid	Assail (30 SG)	United Phosphorous Inc.	30.0%	150.3 g
Chenopodium ambrosioides	Requiem (25 EC)	AgraQuest Inc.	25%	7,017.8 ml
Control	N/A	N/A	N/A	N/A

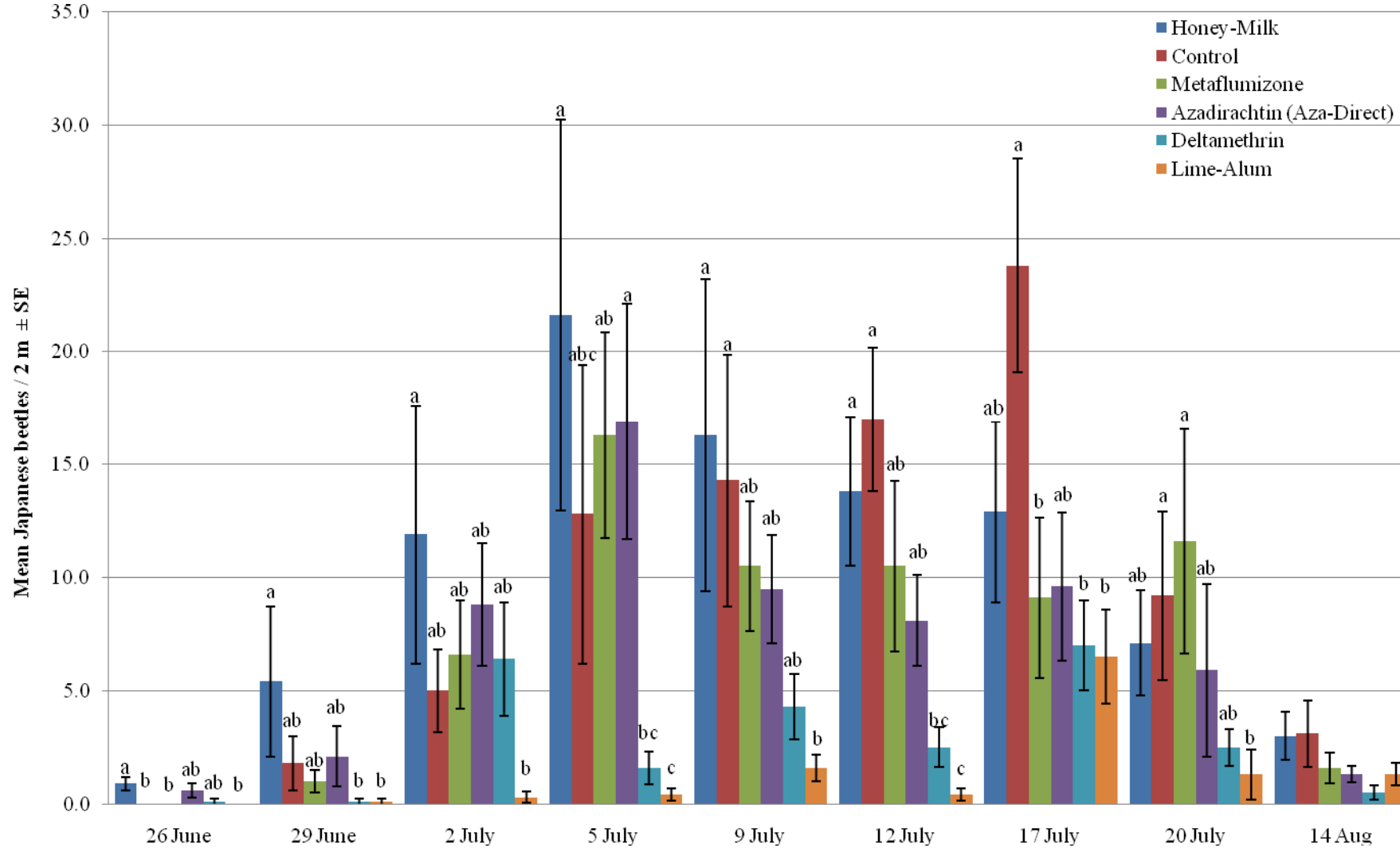


Figure 2.1 Mean number  $\pm$  SE of Japanese beetle per 2.0 m raspberry plot that were treated approximately weekly (6  $\times$ ) with selected insecticides at Kentland Farm, Montgomery Co., VA in 2007. Only dates with significant differences include letters above the values and values followed by the same letter are not significantly different (Tukey's HSD ( $\alpha = 0.05$ ) following ( $\sqrt{x + 0.5}$ ) transformation).

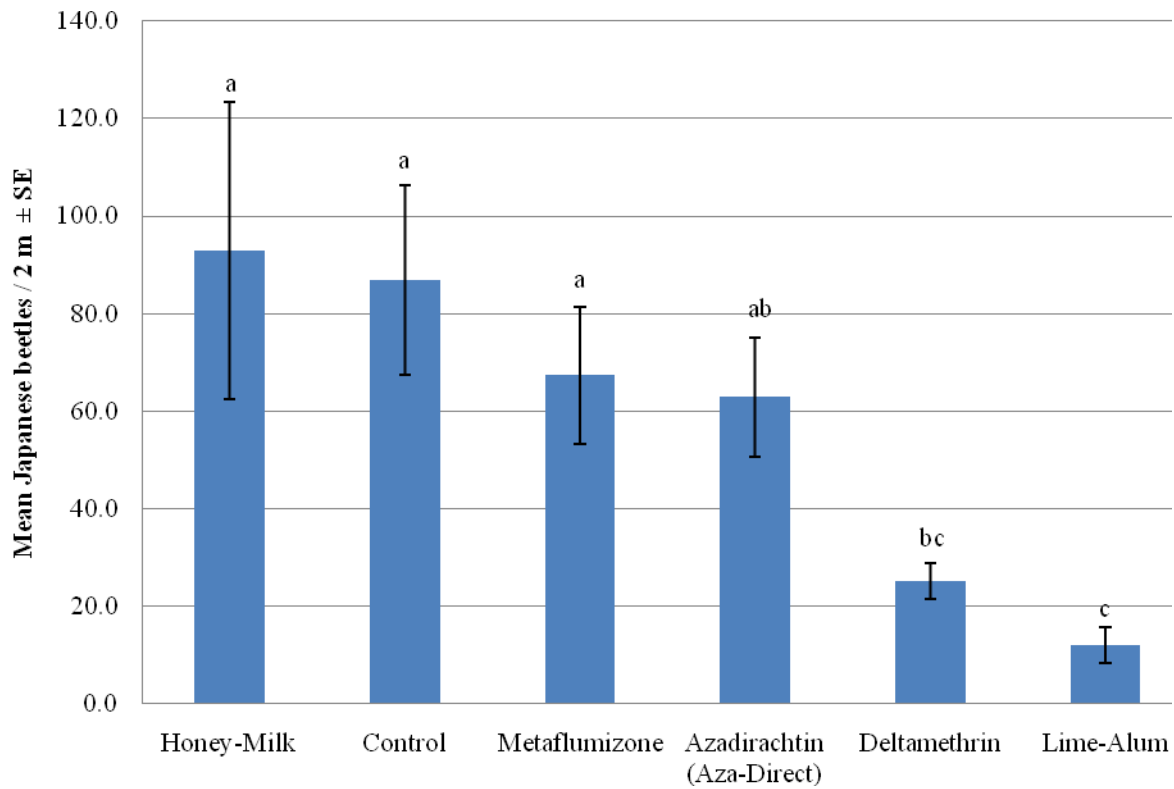


Figure 2.2 Mean cumulative numbers  $\pm$  SE of Japanese beetles per 2.0 m of raspberries that were treated approximately weekly (6  $\times$ ) with selected insecticides at Kentland Farm, Montgomery Co., VA in 2007. Values followed by the same letter are not significantly different Tukey's HSD ( $\alpha = 0.05$ ) following ( $\sqrt{x + 0.5}$ ) transformation) ( $F = 12.28$ ;  $df = 5$ ;  $P < 0.0001$ ).



Figure 2.3 Image of a raspberry plant showing white residue build-up after weekly applications (6 ×) of lime-alum at 1120.9 g lime and 350.3 g alum per acre.

Table 2.7 Mean cumulative yields  $\pm$  SE per m row of primocane-bearing raspberries that were treated approximately weekly (6  $\times$ ) with selected insecticides at Kentland Farm, Montgomery Co., VA in 2007. Yields harvested on individual dates are not included due to significant differences not being found.

Treatment	Yield (g/m) $\pm$ SE		Cumulative % Unmarketable $\pm$ SE ***
	Cumulative Marketable *	Cumulative Unmarketable**	
Metaflumizone	1175.3 $\pm$ 108.0	267.5 $\pm$ 47.8	18.4 $\pm$ 2.6
Azadirachtin (Aza-Direct)	1139.5 $\pm$ 150.4	254.8 $\pm$ 40.8	18.3 $\pm$ 1.5
Deltamethrin	1594.8 $\pm$ 149.6	307.0 $\pm$ 57.1	15.7 $\pm$ 1.4
Control	1277.8 $\pm$ 178.4	304.8 $\pm$ 70.4	18.3 $\pm$ 1.9
Honey Milk	1245 $\pm$ 205.5	312.5 $\pm$ 43.3	20.8 $\pm$ 1.5
Lime-Alum	1216.7 $\pm$ 90.1	290.1 $\pm$ 32.3	19.3 $\pm$ 1.8

\* Values did not have a significant treatment source of variation ( $P > 0.05$ ) according to ANOVA ( $F = 1.95$ ;  $df = 5$ ;  $P = 0.1106$ ).

\*\* Values did not have a significant treatment source of variation ( $P > 0.05$ ) according to ANOVA following ( $\sqrt{x + 0.5}$ ) transformation ( $F = 0.60$ ;  $df = 5$ ;  $P = 0.7019$ ).

\*\*\* Values did not have a significant treatment source of variation ( $P > 0.05$ ) according to ANOVA ( $F = 1.47$ ;  $df = 5$ ;  $P = 0.2260$ ).

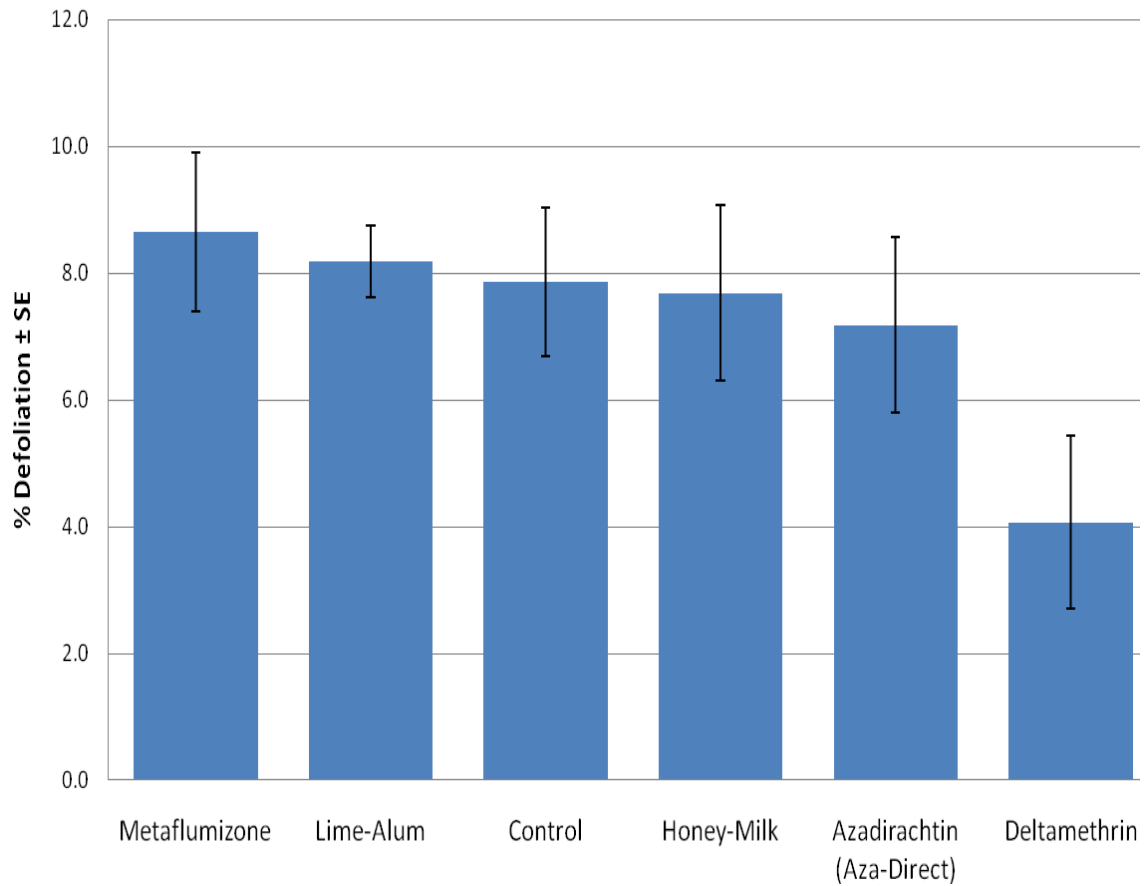


Figure 2.4 Mean cumulative percent defoliation  $\pm$  SE of 'Autumn Bliss' and 'Fall Gold' raspberries that were treated approximately weekly (6  $\times$ ) with selected insecticides at Kentland Farm, Montgomery Co., VA in 2007. The treatment source of variation was not significant according to ANOVA ( $F = 1.71$ ;  $df = 5$ ;  $P = 0.1851$ ).

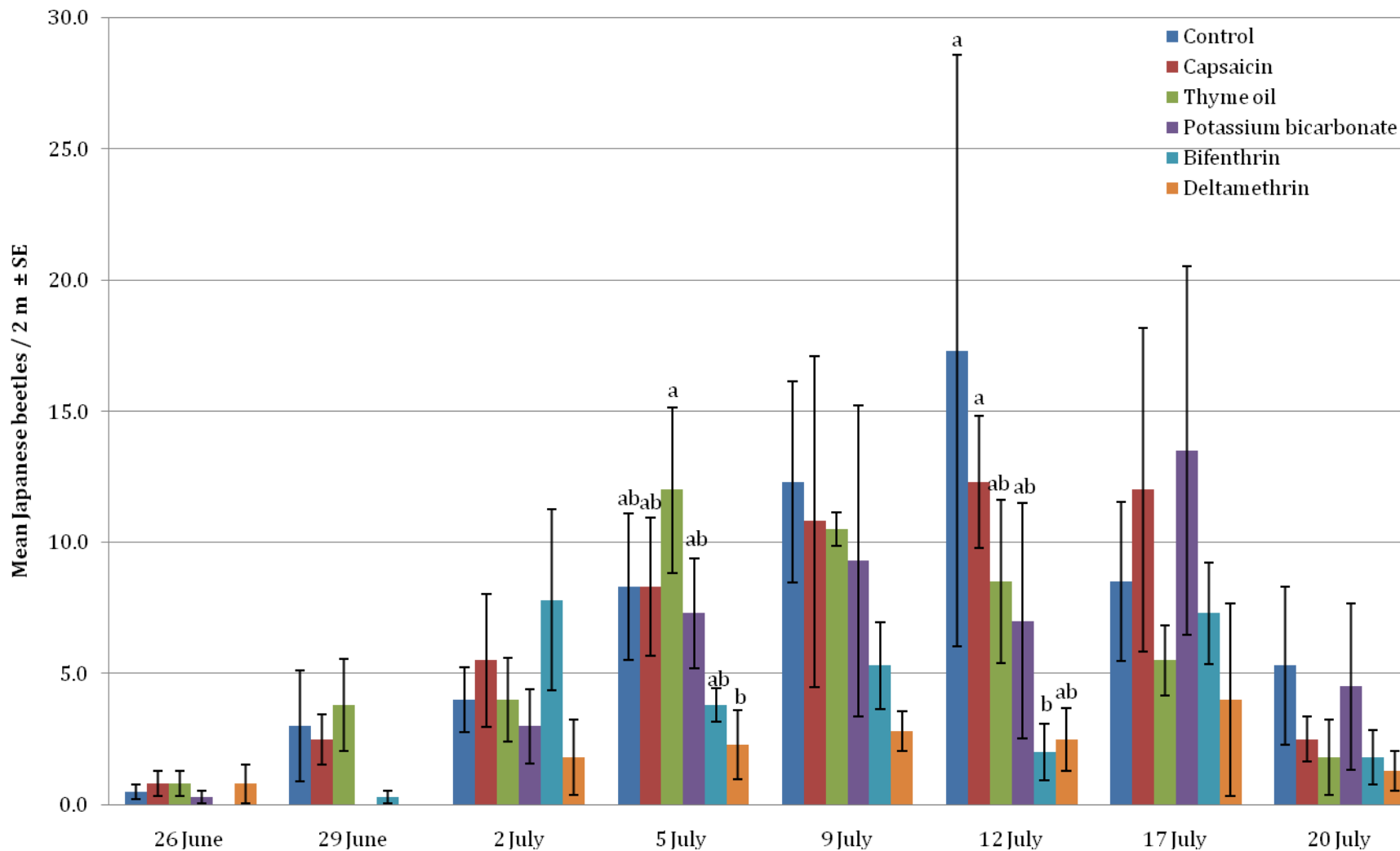


Figure 2.5 Mean number  $\pm$  SE of Japanese beetle per 2.0 m blackberry plot that were treated approximately weekly (6  $\times$ ) with selected insecticides at Kentland Farm, Montgomery Co., VA in 2007. There were only significant differences between treatments on 5 July with values followed by the same letter not being significantly different (Tukey's HSD ( $\alpha = 0.05$ ) following ( $\sqrt{x + 0.5}$ ) transformation).



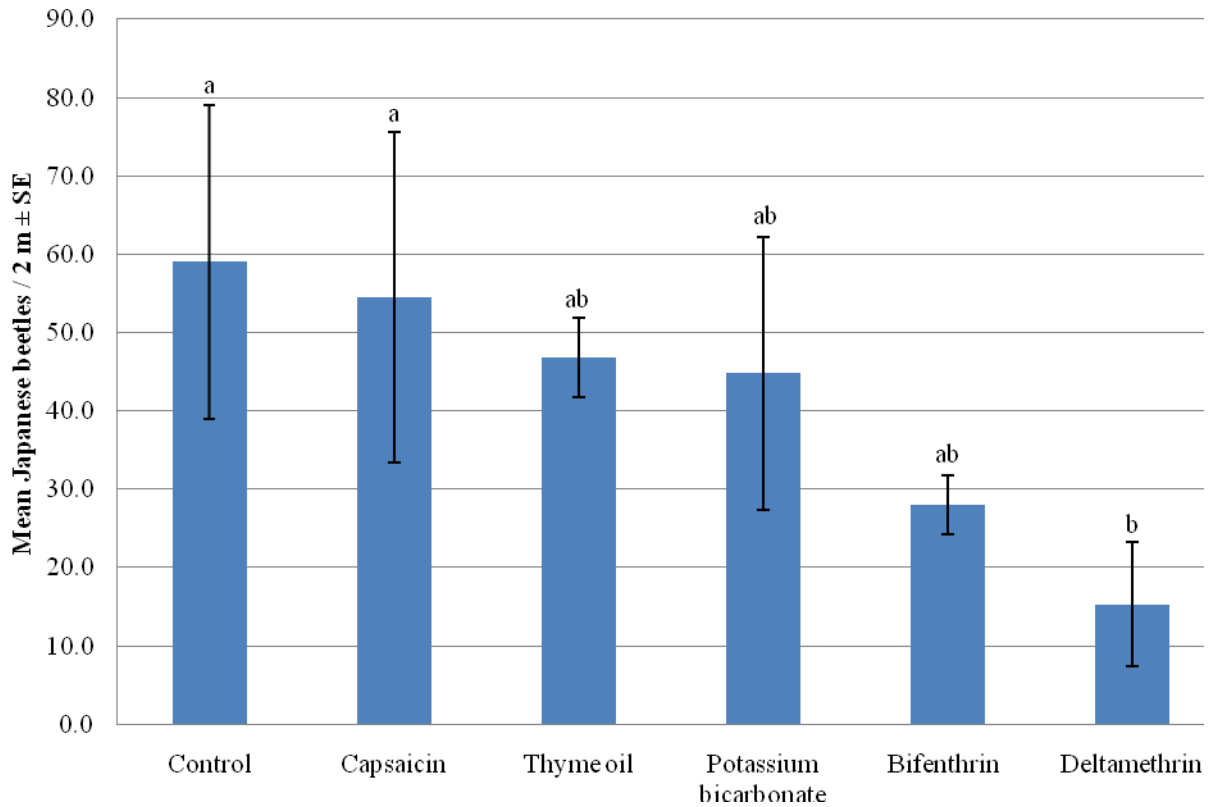


Figure 2.6 Mean cumulative numbers  $\pm$  SE of Japanese beetles per 2.0 m blackberry plot treated approximately weekly (6  $\times$ ) with selected insecticides at Kentland Farm, Montgomery Co., VA in 2007. Treatment means labeled by the same letter are not significantly different according to Tukey's HSD ( $\alpha = 0.10$ ) ( $F = 3.10$ ;  $df = 5$ ;  $P = 0.0501$ ); data was  $(\sqrt{x + 0.5})$  transformed.

Table 2.8 The means  $\pm$  SE of marketable yields on 21 and 31 August and the mean cumulative yields  $\pm$  SE per m row of primocane-bearing blackberries that were treated approximately weekly (6  $\times$ ) with selected insecticides at Kentland Farm, Montgomery Co., VA in 2007. Individual dates not listed for marketable or unmarketable yields did not have significant differences between treatments.

Treatment	Yield (g/m) $\pm$ SE				Cumulative % Unmarketable $\pm$ SE ****
	Marketable 21-Aug *	Marketable 31-Aug *	Cumulative Marketable **	Cumulative Unmarketable ***	
Potassium bicarbonate	47.5 $\pm$ 21.6 b	39.5 $\pm$ 15.5 cd	234.0 $\pm$ 29.7 b	305.0 $\pm$ 56.5	50.6 $\pm$ 4.5 a
Deltamethrin	170.5 $\pm$ 32.7 a	116.5 $\pm$ 11.1 a	734.5 $\pm$ 49.4 a	262.5 $\pm$ 42.5	26.4 $\pm$ 4.2 b
Bifenthrin	59.0 $\pm$ 21.0 ab	76.0 $\pm$ 11.8 abc	458.0 $\pm$ 19.3 b	385.5 $\pm$ 89.5	38.1 $\pm$ 7.4 ab
Control	32.0 $\pm$ 15.8 b	37.5 $\pm$ 15.7 d	271.5 $\pm$ 44.4 b	277.0 $\pm$ 56.4	47.1 $\pm$ 2.0 ab
Capsaicin	85.5 $\pm$ 29.0 ab	87.0 $\pm$ 16.4 ab	462.0 $\pm$ 40.9 b	437.0 $\pm$ 119.6	39.7 $\pm$ 6.5 ab
Thyme oil	34.5 $\pm$ 13.2 b	46.0 $\pm$ 10.0 bcd	288.5 $\pm$ 7.6 b	240.0 $\pm$ 25.3	43.7 $\pm$ 3.6 ab

\* Data in same column followed by the same letter are not significantly different (Tukey's HSD ( $\alpha = 0.05$ ) following ( $\sqrt{x + 0.5}$ ) transformation).

\*\* Data in the same column followed by the same letter are not significantly different (Tukey's HSD ( $\alpha = 0.05$ ) ( $F = 12.13$ ;  $df = 5$ ;  $P = 0.0002$ ).

\*\*\* Values did not have a significant treatment source of variation ( $P > 0.05$ ) according to ANOVA ( $F = 0.62$ ;  $df = 5$ ;  $P = 0.6892$ ).

\*\*\*\* Data in the same column followed by the same letter are not significantly different (Tukey's HSD ( $\alpha=0.10$ ) ( $F = 2.46$ ;  $df = 5$ ;  $P = 0.0938$ ).

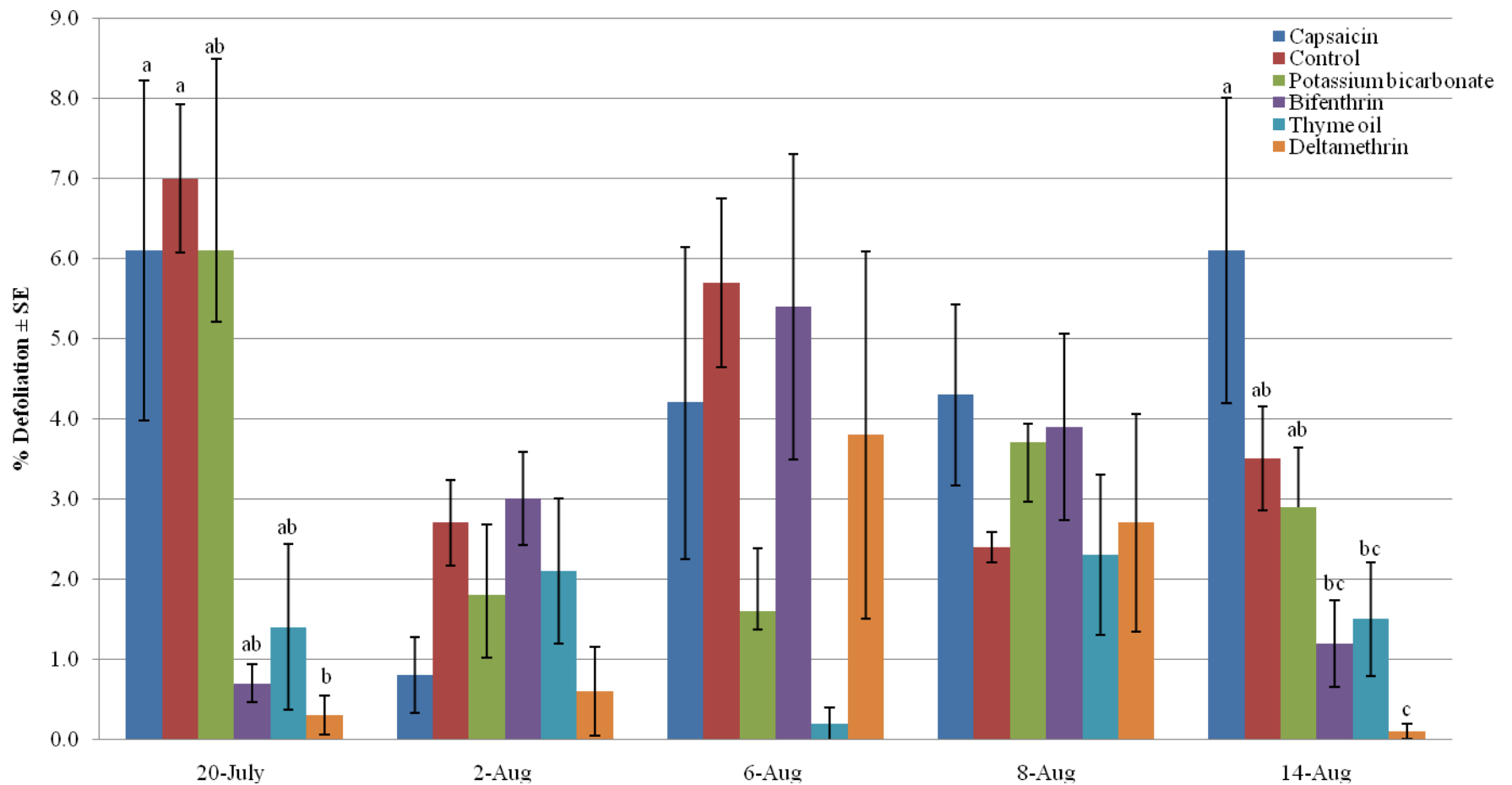


Figure 2.7 Mean percent defoliation  $\pm$  SE after Japanese beetles were bagged onto individual canes of 'Prime-Jim' blackberries that were treated approximately weekly (6  $\times$ ) with selected insecticides at Kentland Farm, Montgomery Co., VA in 2007. Only dates with significant differences include letters above the values and values followed by the same letter are not significantly different (Tukey's HSD ( $\alpha = 0.05$ ) following (ArcSine ( $\sqrt{x}$ )) transformation).

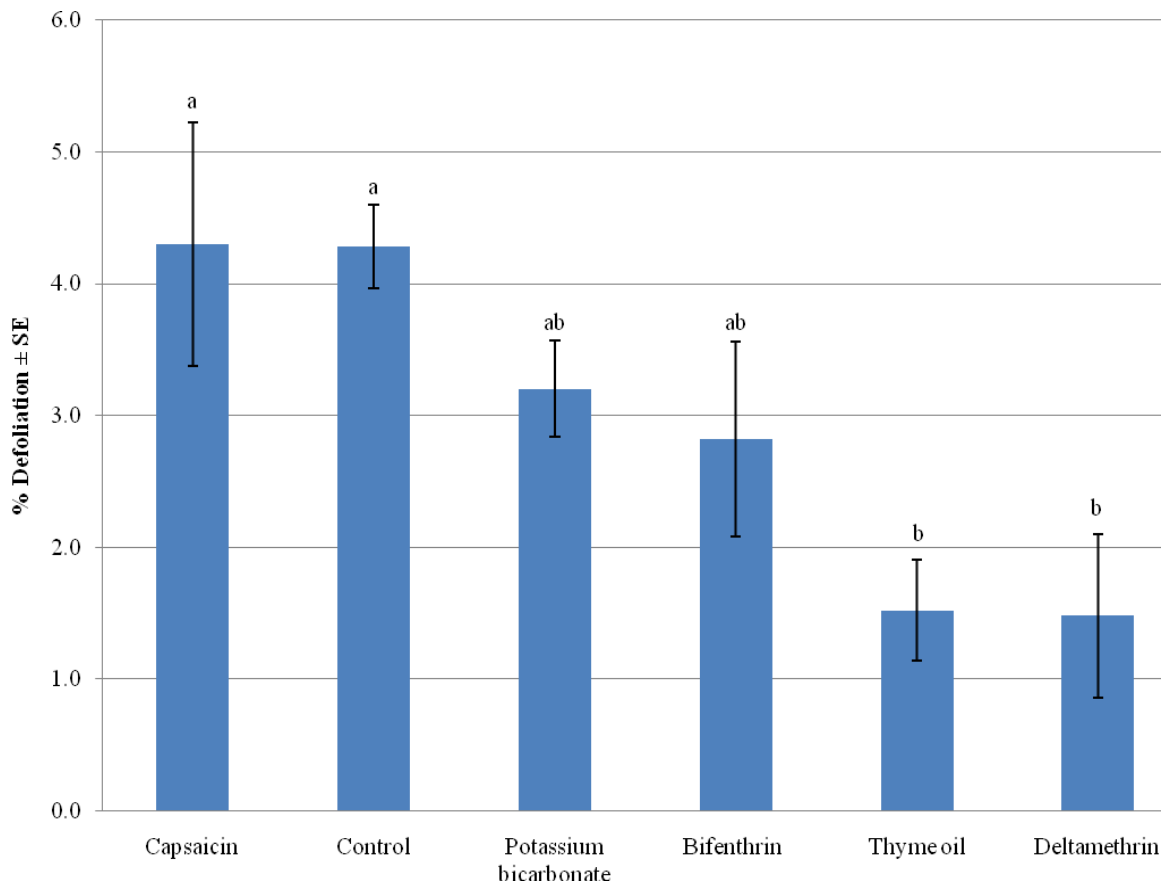


Figure 2.8 Mean cumulative percent defoliation  $\pm$  SE of 'Prime-Jim' blackberries that were treated approximately weekly (6  $\times$ ) with selected insecticides at Kentland Farm, Montgomery Co., VA in 2007. Values followed by the same letter are not significantly different (Tukey's HSD,  $\alpha = 0.05$ ) ( $F = 4.34$ ;  $df = 5$ ;  $P = 0.0091$ ).

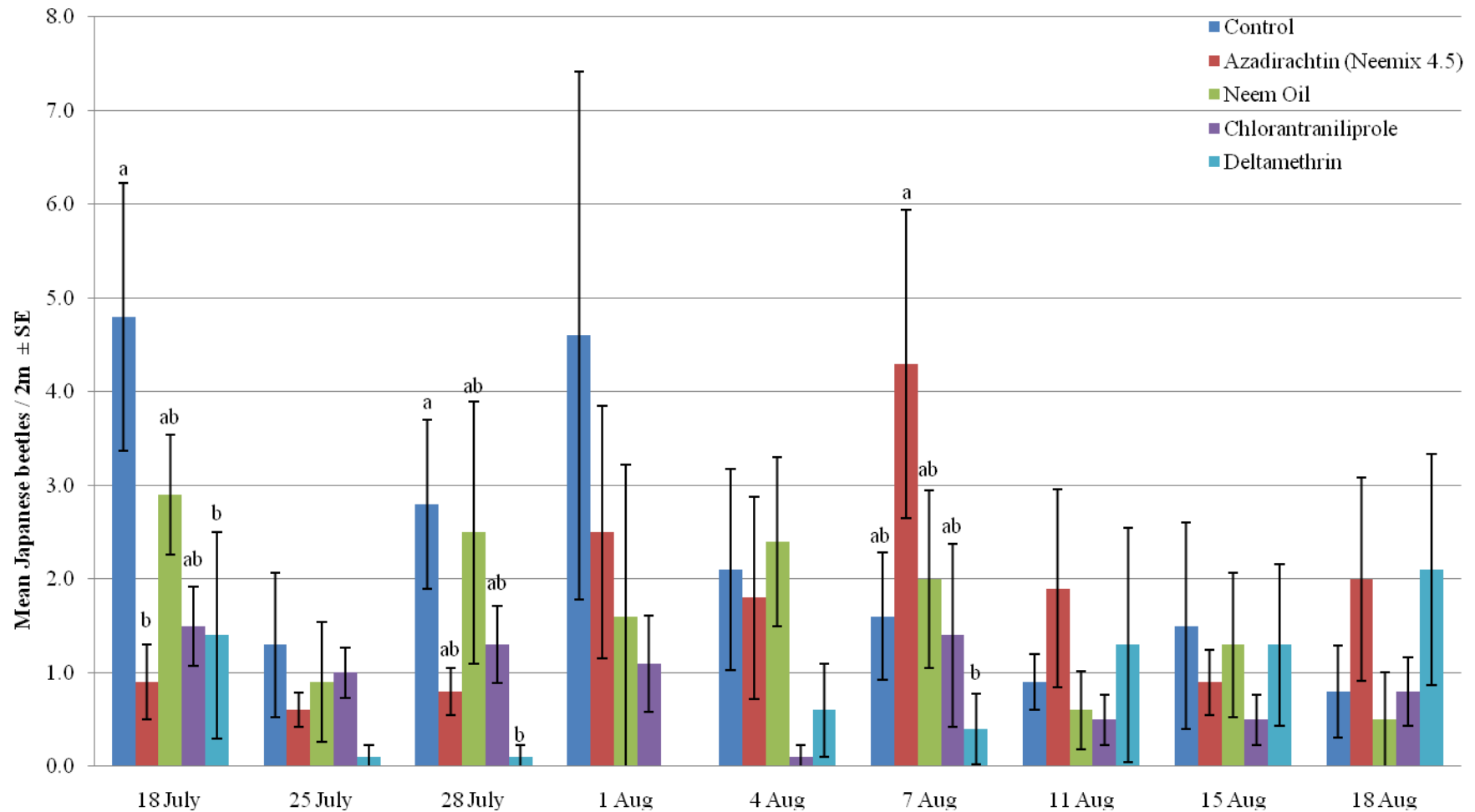


Figure 2.9 Mean number  $\pm$  SE of Japanese beetles per 1.2 m raspberry plot that were treated approximately weekly (7  $\times$ ) with selected insecticides at Kentland Farm, Montgomery Co., VA in 2008. Only dates with significant differences include letters above the values and values followed by the same letter are not significantly different (Tukey's HSD ( $\alpha = 0.05$ ) following  $(\sqrt{x + 0.5})$  transformation).

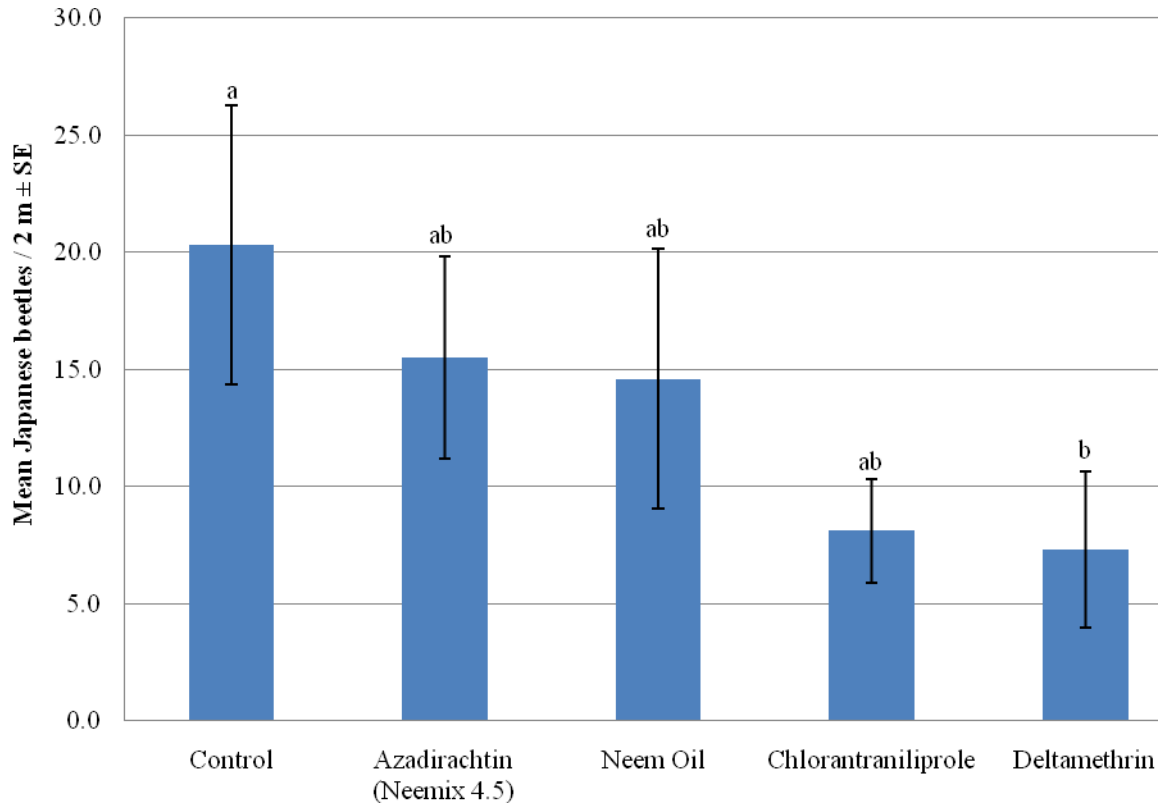


Figure 2.10 Mean cumulative numbers  $\pm$  SE of Japanese beetles per 1.2 m raspberry plot treated approximately weekly (7  $\times$ ) with selected insecticides at Kentland Farm, Montgomery Co., VA in 2008. Values followed by the same letter are not significantly different (Tukey's HSD, ( $\alpha = 0.05$ ) following  $(\sqrt{x + 0.5})$  transformation) ( $F = 2.77$ ;  $df = 4$ ;  $P = 0.0469$ ).

Table 2.9 The means  $\pm$  SE of unmarketable yields on 2 August and the mean cumulative yields  $\pm$  SE per m row of primocane-bearing raspberries that were treated approximately weekly (7  $\times$ ) with selected insecticides at Kentland Farm, Montgomery Co., VA in 2008. Individual dates not listed for marketable or unmarketable yields did not have significant differences between treatments.

Treatment	Yield (g/m) $\pm$ SE			Cumulative % Unmarketable $\pm$ SE ****
	Cumulative Marketable *	Unmarketable 2-Sept **	Cumulative Unmarketable***	
Deltamethrin	1048.4 $\pm$ 138.1 a	38.7 $\pm$ 8.9 ab	186.0 $\pm$ 45.6 a	22.0 $\pm$ 2.5
Control	814.8 $\pm$ 140.5 ab	27.9 $\pm$ 14.7 abc	156.7 $\pm$ 47.3 ab	23.6 $\pm$ 1.4
Azadirachtin (Neemix)	931.1 $\pm$ 83.0 ab	42.4 $\pm$ 9.0 a	161.9 $\pm$ 36.4 ab	22.5 $\pm$ 2.0
Chlorantraniliprole	791.2 $\pm$ 124.2 ab	19.7 $\pm$ 5.3 bc	132.2 $\pm$ 33.5 ab	21.3 $\pm$ 1.3
Neem oil extract	708.0 $\pm$ 95.5 b	12.3 $\pm$ 9.9 c	105.0 $\pm$ 29.7 b	19.6 $\pm$ 1.0

\* Values followed by the same letter are not significantly different (Tukey's HSD ( $\alpha=0.10$ )) ( $F = 2.49$ ;  $df = 4$ ;  $P = 0.0661$ ).

\*\* Values followed by the same letter are not significantly different (Tukey's HSD ( $\alpha = 0.05$ )).

\*\*\* Values followed by the same letter are not significantly different (Tukey's HSD ( $\alpha=0.10$ )) ( $F = 2.16$ ;  $df = 4$ ;  $P = 0.0991$ ).

\*\*\*\* Values followed by the same letter are not significantly different (Tukey's HSD ( $\alpha=0.10$ )) ( $F = 0.70$ ;  $df = 4$ ;  $P = 0.5955$ ).

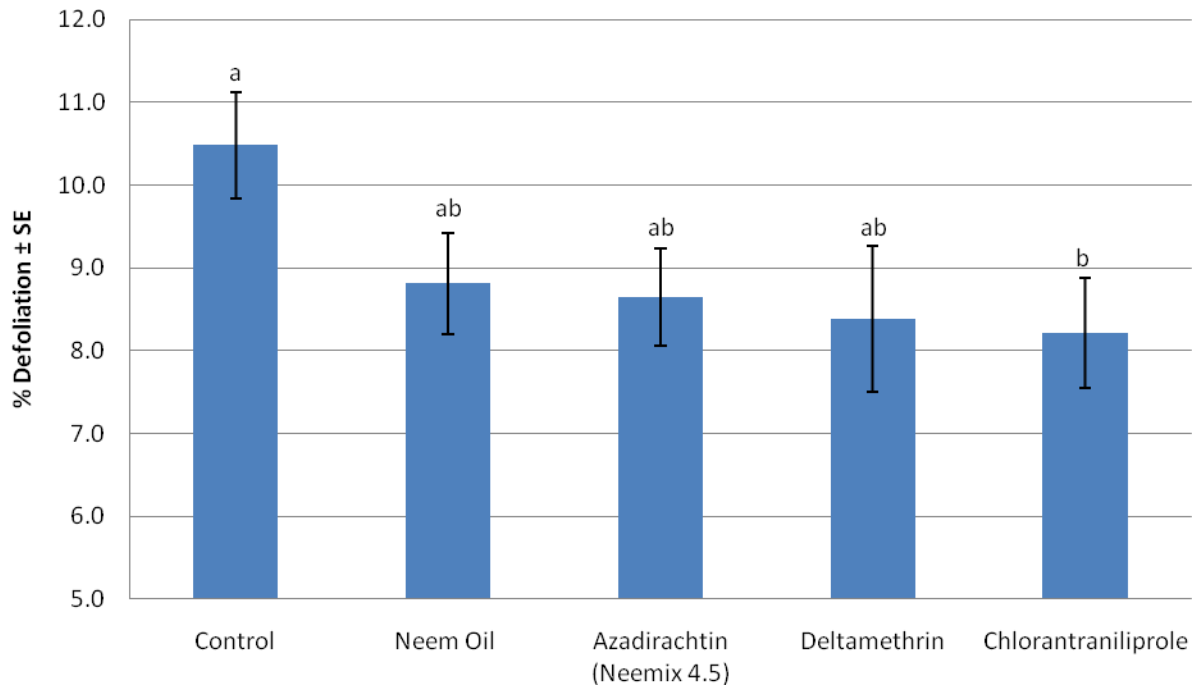


Figure 2.11 Mean cumulative percent defoliation  $\pm$  SE of primocane-bearing raspberries treated approximately weekly (7  $\times$ ) with selected insecticides at Kentland Farm, Montgomery Co., VA in 2008. Treatment means followed by the same letter are not significantly different (Tukey's HSD ( $\alpha = 0.05$ );  $F = 3.57$ ;  $df = 4$ ;  $P = 0.0069$ ) following ArcSine ( $\sqrt{x}$ ) transformation.



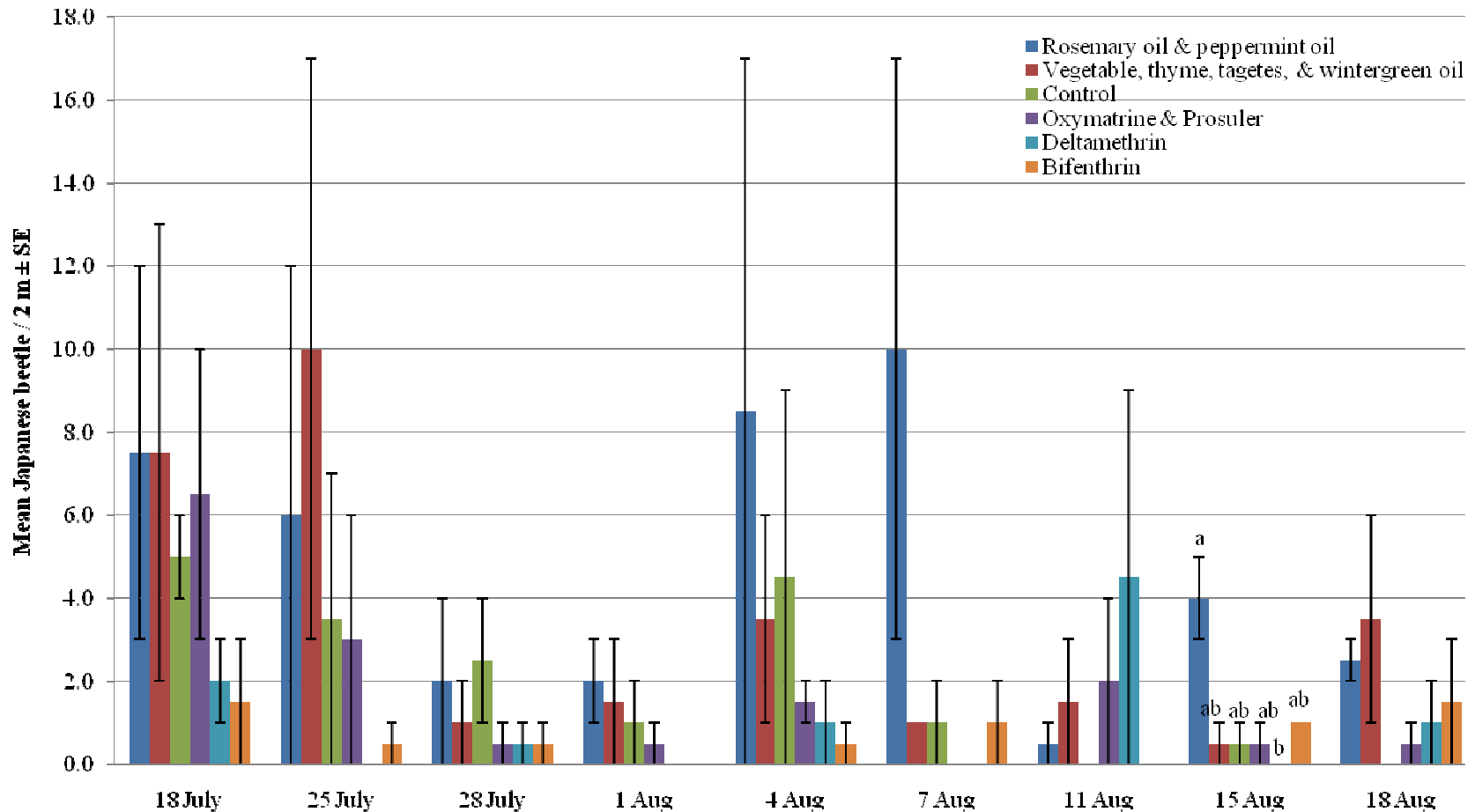


Figure 2.12 Mean number  $\pm$  SE of Japanese beetle per 1.2 m blackberry plot that were treated approximately weekly (7  $\times$ ) with selected insecticides at Kentland Farm, Montgomery Co., VA in 2008. There were only significant differences between treatments on 15 August with values followed by the same letter not being significantly different (Tukey's HSD ( $\alpha = 0.05$ ) following ( $\sqrt{x + 0.5}$ ) transformation).

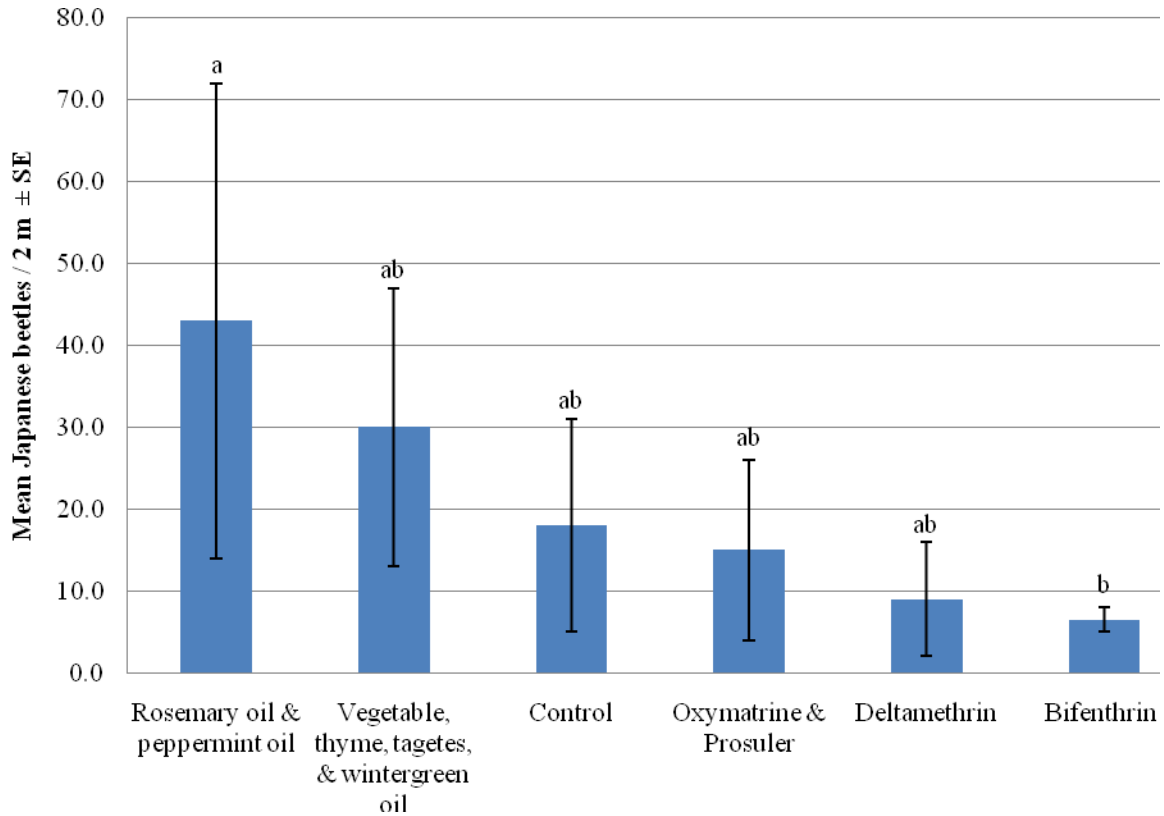


Figure 2.13 Mean cumulative numbers  $\pm$  SE of Japanese beetles per 1.2 m blackberry plot treated approximately weekly (7  $\times$ ) with selected insecticides at Kentland Farm, Montgomery Co., VA in 2008. Treatment means followed by the same letter are not significantly different (Tukey's HSD ( $\alpha = 0.10$ );  $F = 4.24$ ;  $df = 5$ ;  $P = 0.0693$ ) following ( $\sqrt{x + 0.5}$ ) transformation.

Table 2.10 Mean cumulative yields  $\pm$  SE per m row of primocane-bearing blackberries that were treated approximately weekly (7  $\times$ ) with selected insecticides at Kentland Farm, Montgomery Co., VA in 2008. Individual dates are not listed for 2008 blackberry marketable or unmarketable yields because significant differences were not present.

Treatment	Yield (g/m) $\pm$ SE		Cumulative % Unmarketable $\pm$ SE ***
	Cumulative Marketable *	Cumulative Unmarketable **	
Deltamethrin	253.3 $\pm$ 73.0	56.9 $\pm$ 37.0 ab	20.9 $\pm$ 11.6
Vegetable, thyme, tagetes, & wintergreen oils	291.7 $\pm$ 28.0	124.7 $\pm$ 39.8 ab	27.3 $\pm$ 5.4
Bifenthrin	312.5 $\pm$ 29.0	133.3 $\pm$ 51.8 ab	26.7 $\pm$ 8.6
Control	399.4 $\pm$ 49.2	150.3 $\pm$ 39.8 a	25.6 $\pm$ 4.5
Rosemary oil & peppermint oil	202.1 $\pm$ 37.0	41.3 $\pm$ 7.6 b	17.9 $\pm$ 5.2
Oxymatrine & prosuler	276.7 $\pm$ 31.9	134 $\pm$ 61.6 ab	27.1 $\pm$ 7.5

\*Values did not have a significant treatment source of variation according to ANOVA ( $\alpha = 0.05$ ) following ( $\sqrt{x + 0.5}$ ) transformation ( $F = 1.69$ ;  $df = 5$ ;  $P = 0.2104$ ).

\*\* Values followed by the same letter are not significantly different according to ANOVA ( $\alpha = 0.05$ ) following ( $\sqrt{x + 0.5}$ ) transformation ( $F = 3.20$ ;  $df = 5$ ;  $P = 0.0459$ ).

\*\*\* Values did not have a significant treatment source of variation according to ANOVA ( $\alpha = 0.05$ ) ( $F = 0.89$ ;  $df = 5$ ;  $P = 0.5198$ ).

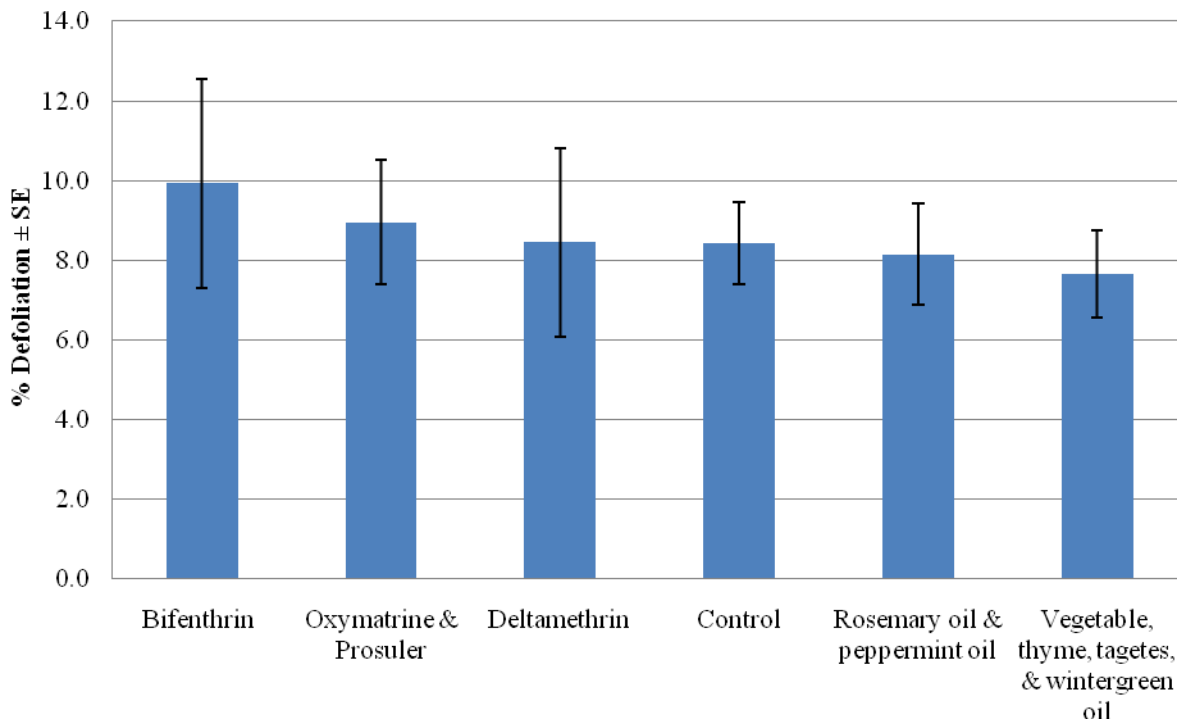


Figure 2.14 The mean percent defoliation  $\pm$  SE of primocane-bearing blackberries treated approximately weekly (7  $\times$ ) with selected insecticides at Kentland Farm, Montgomery Co., VA in 2008. The treatment source of variation was not significant according to ANOVA ( $\alpha = 0.05$ ) following an ArcSine ( $\sqrt{x}$ ) transformation ( $F = 0.20$ ;  $df = 5$ ;  $P = 0.9639$ ).

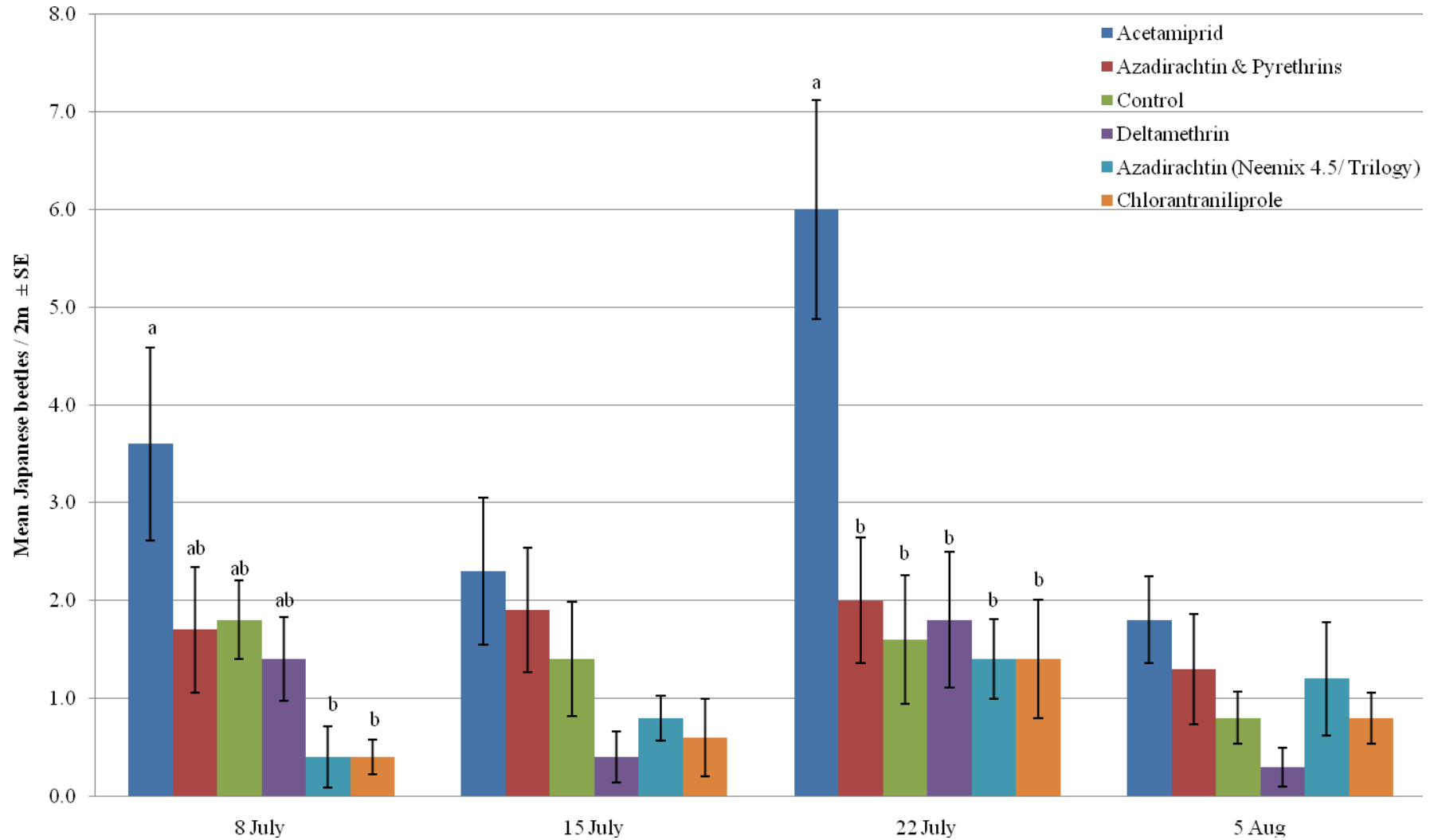


Figure 2.15 Mean number  $\pm$  SE of Japanese beetle per 1.2 m raspberry plot that were treated approximately weekly (6  $\times$ ) with selected insecticides at Kentland Farm, Montgomery Co., VA in 2009. Only dates with significant differences include letters above the values and values followed by the same letter are not significantly different (Tukey's HSD ( $\alpha = 0.05$ ) following  $(\sqrt{x + 0.5})$  transformation).

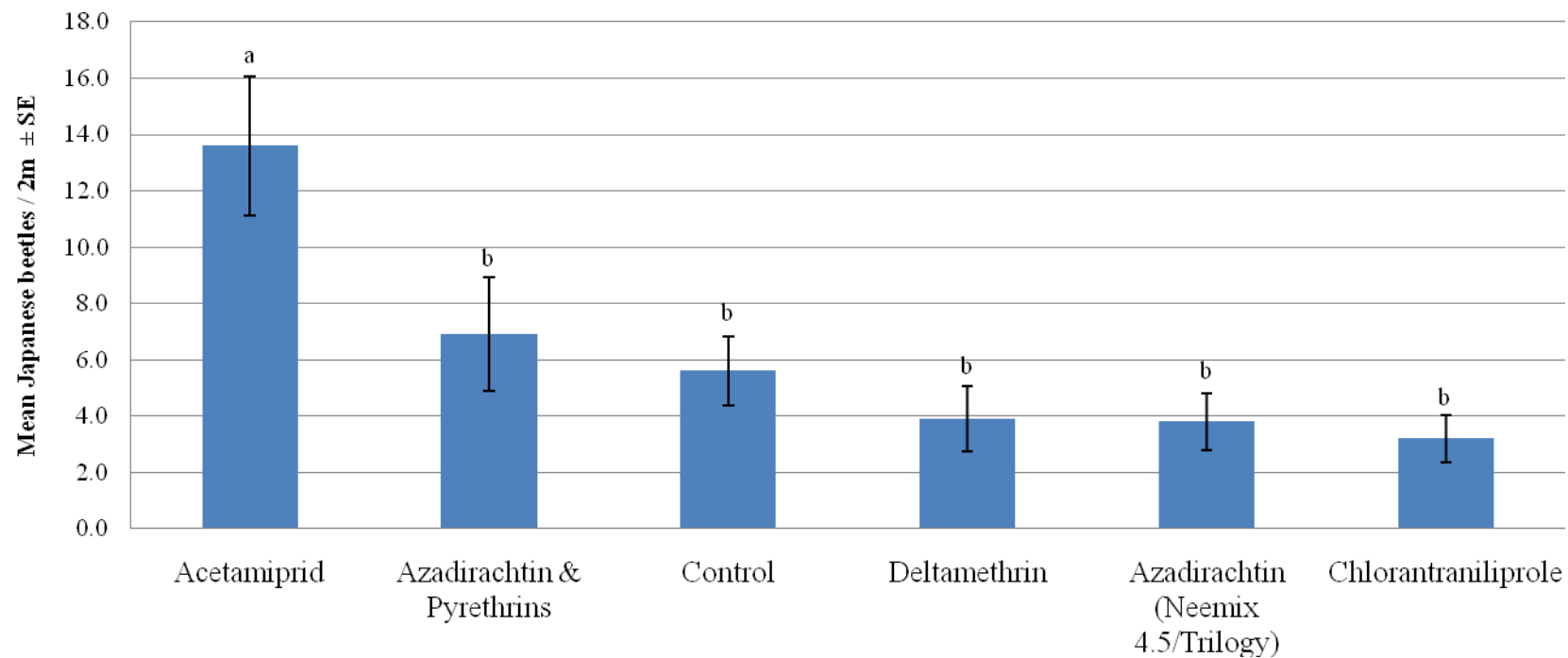


Figure 2.16 Mean cumulative numbers  $\pm$  SE of Japanese beetles counted per 1.2 m raspberry plot treated approximately weekly (6  $\times$ ) with selected insecticides at Kentland Farm, Montgomery Co., VA in 2009. Values followed by the same letter for each date are not significantly different (Tukey's HSD ( $\alpha = 0.05$ );  $F = 7.92$ ;  $df = 5$ ;  $P < 0.0001$ ) following ( $\sqrt{x + 0.5}$ ) transformation.

Table 2.11 The means  $\pm$  SE of marketable yields on 10 August, of unmarketable yields on 24 and 31 August, and the mean cumulative yields  $\pm$  SE per m row of primocane-bearing raspberries that were treated approximately weekly (6  $\times$ ) with selected insecticides at Kentland Farm, Montgomery Co., VA in 2009. Individual dates not listed for marketable or unmarketable yields did not have significant differences between treatments.

Treatment	Marketable Yield (g/m) $\pm$ SE		Unmarketable Yield (g/m) $\pm$ SE			Cumulative % Unmarketable $\pm$ SE ****
	10-Aug *	Cumulative Marketable **	24- Aug *	31-Aug *	Cumulative Unmarketable ***	
Chlorantraniliprole	122.9 $\pm$ 21.3 b	1600.1 $\pm$ 122.0	22.9 $\pm$ 2.8 b	27.4 $\pm$ 5.3 a	244.4 $\pm$ 24.4	15.9 $\pm$ 1.0
Acetamiprid	130.1 $\pm$ 12.8 ab	1675.6 $\pm$ 114.4	39.2 $\pm$ 6.8 ab	15.5 $\pm$ 1.8 ab	252.1 $\pm$ 22.5	15.2 $\pm$ 0.7
Deltamethrin	190.1 $\pm$ 24.9 a	1894.5 $\pm$ 142.7	30.9 $\pm$ 4.7 ab	26.3 $\pm$ 4.0 a	274.2 $\pm$ 28.0	14.7 $\pm$ 1.0
Control	116.1 $\pm$ 14.8 b	1746.2 $\pm$ 120.8	37.7 $\pm$ 5.1 ab	29.5 $\pm$ 5.0 a	287.9 $\pm$ 33.1	16.3 $\pm$ 1.4
Azadirachtin & Pyrethrins	130.2 $\pm$ 19.6 ab	1903.2 $\pm$ 149.2	49.2 $\pm$ 6.5 a	10.2 $\pm$ 1.9 b	314.1 $\pm$ 39.8	15.8 $\pm$ 1.0
Azadirachtin & Neem oil extract	123.3 $\pm$ 13.0 ab	1769.3 $\pm$ 152.1	32.7 $\pm$ 4.1 ab	17.9 $\pm$ 2.6 ab	265.7 $\pm$ 33.6	15.2 $\pm$ 1.1

\* Values in the same column followed by the same letter are not significantly different (Tukey's HSD ( $\alpha = 0.05$ ) following ( $\sqrt{x + 0.5}$ ) transformation).

\*\* Values did not have a significant treatment source of variation according to ANOVA ( $\alpha = 0.05$ ) ( $F = 0.71$ ;  $df = 5$ ;  $P = 0.6182$ ).

\*\*\* Values did not have a significant treatment source of variation according to ANOVA ( $\alpha = 0.05$ ) ( $F = 0.76$ ;  $df = 5$ ;  $P = 0.5865$ ).

\*\*\*\* Values did not have a significant treatment source of variation according to ANOVA ( $\alpha = 0.05$ ) ( $F = 0.42$ ;  $df = 5$ ;  $P = 0.8354$ ).

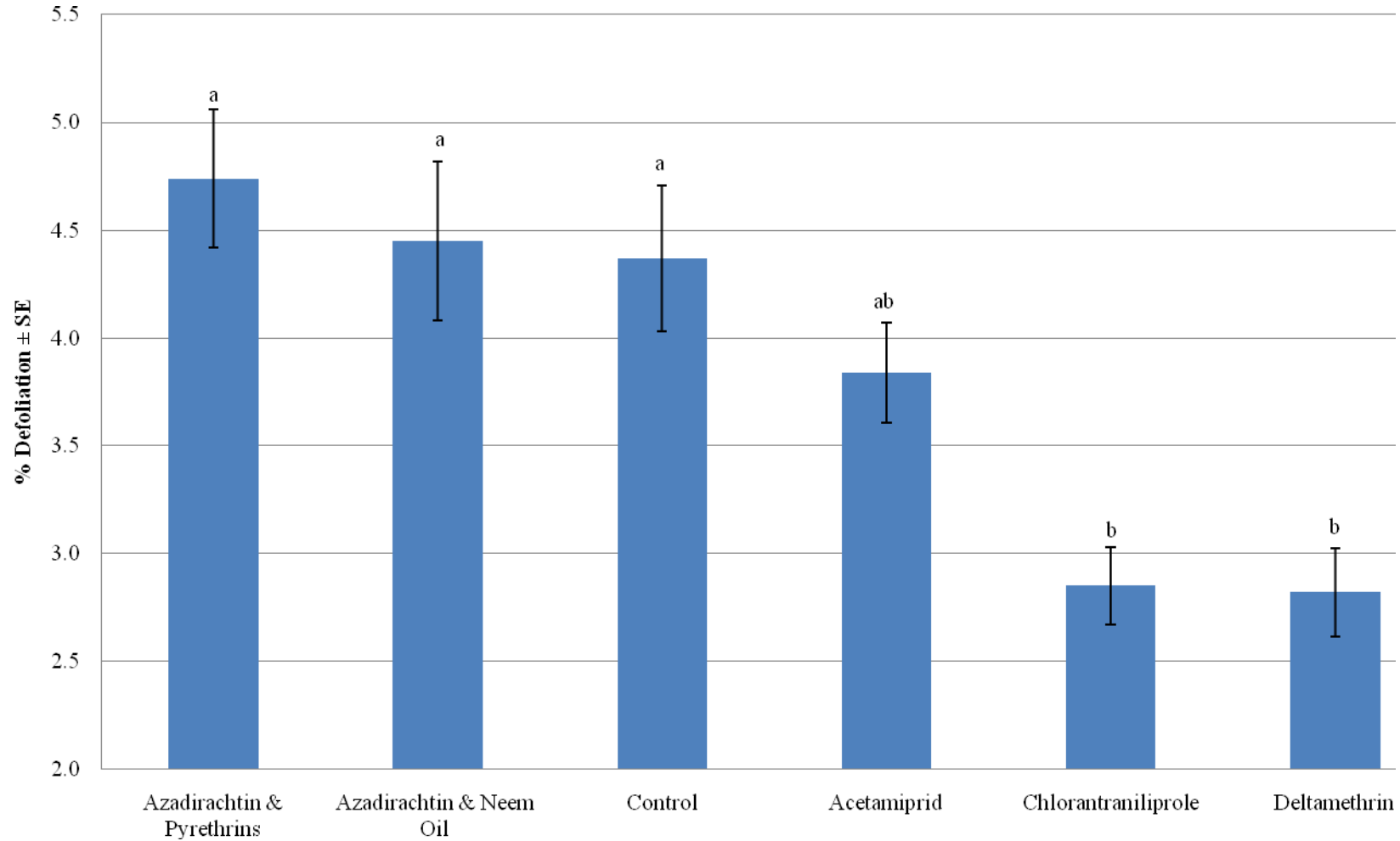


Figure 2.17 The mean percent defoliation  $\pm$  SE of only the injured leaves of primocane-bearing raspberries treated approximately weekly (6  $\times$ ) with selected insecticides at Kentland Farm, Montgomery Co., VA in 2009. Values followed by the same letter are not significantly different (Tukey's HSD ( $\alpha= 0.05$ );  $F = 8.57$ ;  $df = 5$ ;  $P < 0.0001$ ) following ArcSine ( $\sqrt{x}$ ) transformation.



Table 2.12 Cumulative numbers of Japanese beetles counted and percent unmarketable fruit yield weighed in the untreated control plots in a raspberry and blackberry planting at Kentland Farm, Montgomery Co., VA from 2007-2009.

	2007 Raspberry	2007 Blackberry	2008 Raspberry	2008 Blackberry	2009 Raspberry
Total no. JB/m row	695.0	236.0	160.0	78.0	88.0
Average no. JB per m	43.4	29.5	16.7	16.3	4.6
No. of times counted	9	8	9	9	4
Average no. JB/m/day	4.8	3.7	1.9	1.8	1.1
Percent unmarketable fruit yield	18.3	47.1	23.6	25.6	16.3

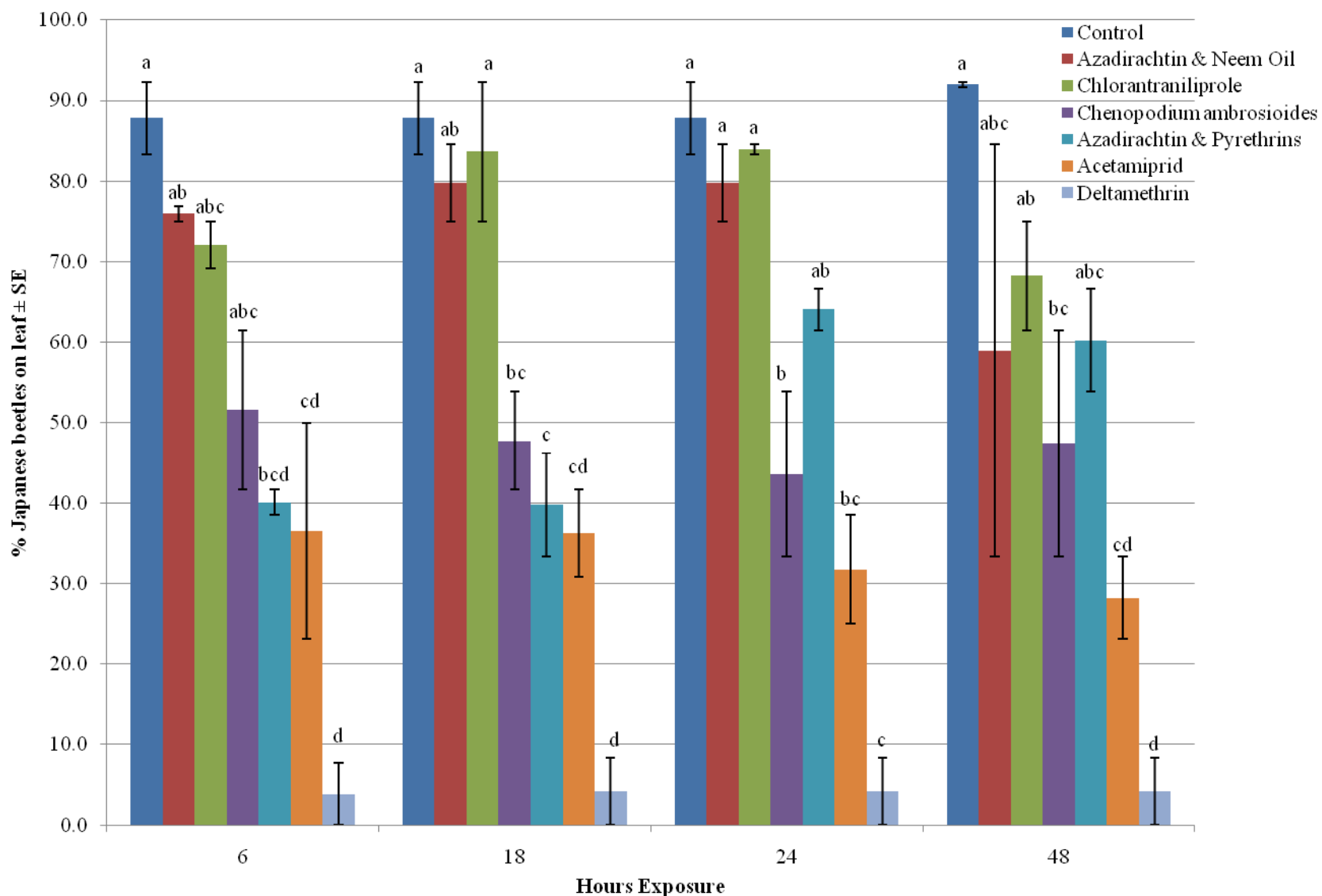


Figure 2.18 The mean percent of Japanese beetles on treated 'Prelude' leaves after 6, 18, 24, and 48 hrs exposure. Values followed by a different letter are significantly different (Tukey's HSD,  $\alpha=0.05$ ).

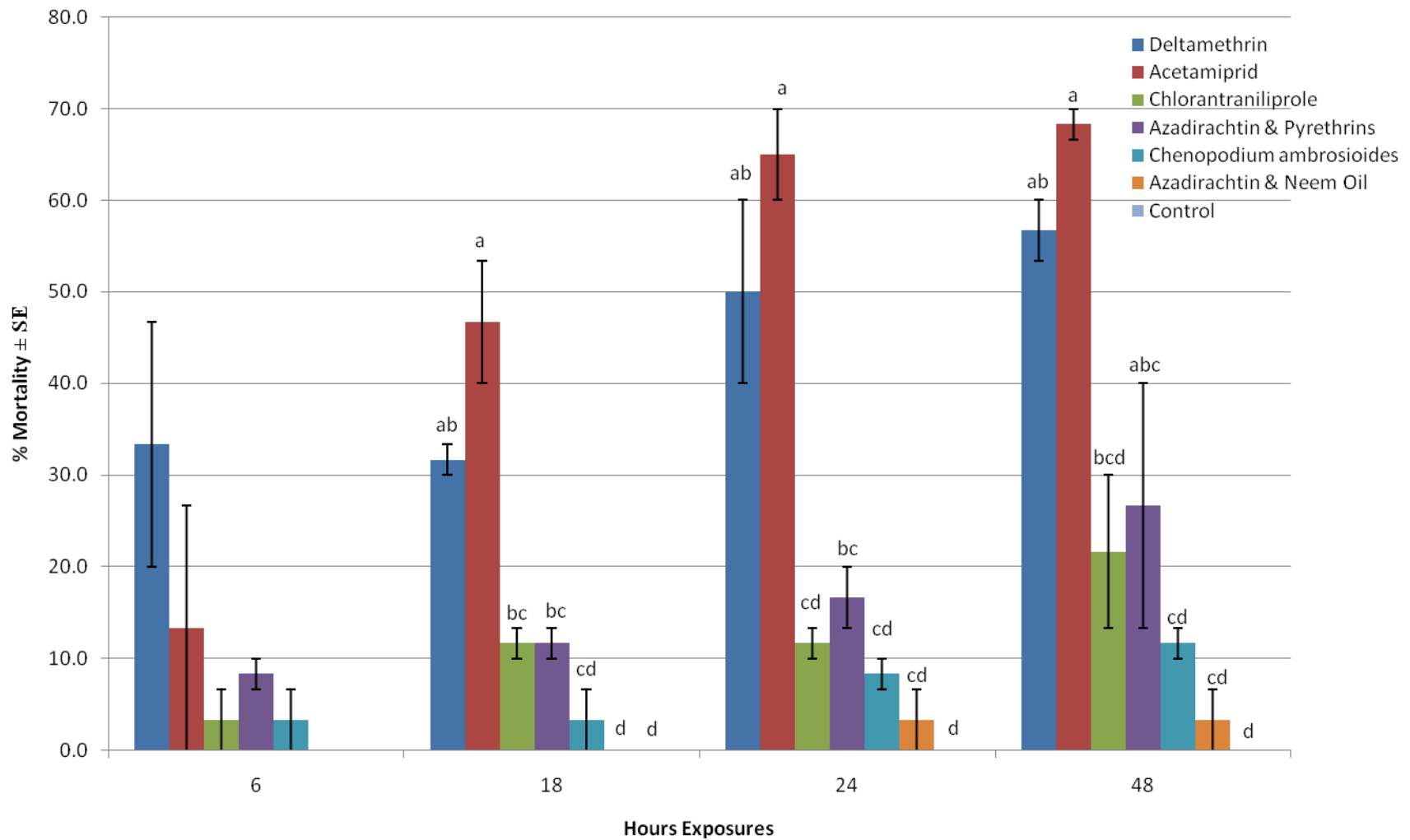


Figure 2.19 The mean percent mortality of Japanese beetles after 6, 18, 24, and 48 hrs exposure to six pesticides. Values followed by the same letter are not significantly different (Tukey's HSD,  $\alpha=0.05$  following an ArcSine ( $\sqrt{x}$ ) transformation).

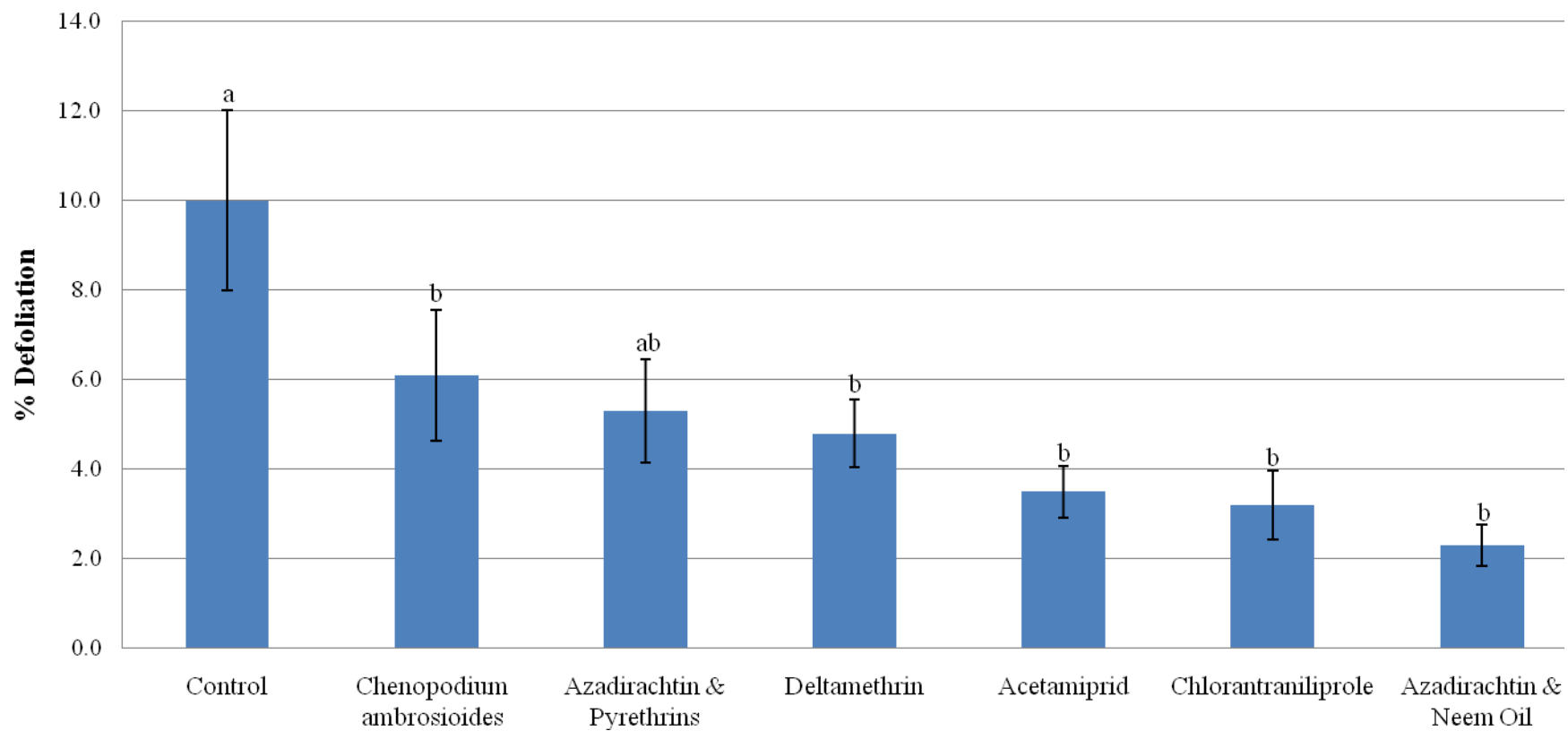


Figure 2.20 Mean percent defoliations of 'Prelude' raspberry per treated leaf in a 2009 lab experiment. Values followed by the same letter are not significantly different (Tukey's HSD ( $\alpha=0.05$ );  $F=6.25$ ;  $df=6$ ;  $P<0.0001$ ) following ArcSine ( $\sqrt{x}$ ) transformation.

### **CHAPTER 3: Susceptibility of Primocane-bearing Raspberry and Blackberry Cultivars to Japanese Beetle, *Popillia japonica* Newman (Coleoptera: Scarabaeidae), Infestations and Fruit and Foliar Injury**

#### **Abstract**

The Japanese beetle (JB), *Popillia japonica* Newman is an introduced pest species that feeds on over 300 plant species including primocane-bearing raspberries and blackberries. The goal of this study was to determine the relative susceptibility of several primocane-bearing raspberry and blackberry cultivars to JB infestation, fruit injury, and defoliation. During the 2007-2009 seasons 'Prelude' raspberries had more JB than 'Anne', 'Heritage', 'Caroline', 'Dinkum', or 'Himbo Top'. 'Heritage' raspberries were more susceptible to fruit injury than 'Prelude', 'Himbo Top', 'Fall Gold', or 'Autumn Bliss' in 2007. Also in 2007, 'Caroline' had a higher percentage of injured fruit than 'Autumn Bliss'. 'Heritage'; 'Autumn Bliss' had the highest overall yields in 2007. In 2008, 'Autumn Bliss', 'Heritage', and 'Caroline' had greater marketable yields compared to some other cultivars considered. 'Autumn Bliss' also had the highest amount of overall yield in 2008. In 2009, 'Caroline' had greater fruit injury than 'Autumn Bliss', 'Prelude', 'Fall Gold', 'Heritage', or 'Dinkum'. Also in 2009, 'Himbo Top' and 'Anne' had a higher percentage of injured fruit than 'Autumn Bliss', 'Dinkum', 'Prelude', and 'Fall Gold'. When comparing the raspberry cultivars' percent defoliations, in 2008, 'Prelude', had more seasonal defoliation than 'Anne', 'Heritage', 'Caroline' and 'Dinkum'. In 2009, 'Prelude' and 'Fall Gold' had more seasonal defoliation than 'Caroline' or 'Anne'. For the blackberries, in 2007 and 2008, 'Prime-Jim' blackberries had more JB than 'Prime-Jan' but 'Prime-Jan' blackberries were more susceptible to fruit injury than 'Prime-Jim'. Overall marketable yields from both blackberry cultivars were similar. More studies need to be conducted correlating the factor(s) responsible for each cultivar's susceptibility to JB and injury.

## Introduction

Japanese beetle (JB), *Popillia japonica* Newman, is an introduced pest species that feeds on over 300 plant species within 79 different plant families in the United States (Potter and Held 2002). Rosaceae, which contains caneberries, are among this pest's wide array of hosts (Pritts and Handley 1989). Adults are gregarious feeders and are highly mobile traveling up to 11.9 km/yr (Allsopp 1996). Severe injury can occur from the adults aggregating on plants after attractive feeding induced volatiles are released (Loughrin et al. 1996b).

Caneberries, in the genus *Rubus* (raspberries and blackberries), grow on leafy canes in temperate regions of the world (Ravai 1996, Wada and Ou 2002). There are two fruiting types of caneberries; primocane-fruiting and floricanes-fruiting. Primocane-fruiting caneberries, unlike floricanes-fruiting cultivars, produce primocanes that flower and fruit during the first year's growth of the canes (Shoemaker 1987, Demchak et al. 2005). Floricanes-fruiting cultivars only produce fruit on the second year's growth. There are monetary advantages in growing primocane-bearing cultivars including: reduced maintenance since selective pruning of dead floricanes is not needed, a trellising system is not required, and the extended fruiting season provides fruit after the floricanes-bearing cultivars have fruited (Bushway et al. 2008, Demchak et al. 2010). For this study, only primocane-fruiting cultivars are considered.

Japanese beetles feed on the foliage and fruit of caneberries (Ellis et al. 1991), skeletonizing the leaves and chewing irregular hollows in the berries (Funt et al. 2000). After the fruit is injured, it becomes more susceptible to fruit rots that are transferrable to uninjured fruit (Bushway et al. 2008). Through skeletonizing the leaves, JB injury potentially lessens foliar functionality affecting fruit production in primocane-bearing red raspberries (Sullivan et al.

1994). Two factors that increase JB injury to caneberries include a preference for raspberries (Fleming 1972, Ladd 1987) and the presence of adults while fruiting (Demchak et al. 2010).

In 1987, Ladd found JB feeding to be greater on foliage of red raspberry, *Rubus idaeus*, than on foliage and floral samples from 42 other JB favored host plants. Preference was also noted by Fleming (1972), who ranked plants that were fed upon by JB on a 1 to 4 scale, with plants ranked 4 being the most heavily fed upon. The leaves of red raspberry were given a 3. Because JB are injurious to raspberries and blackberries in direct and indirect ways and because JB prefer red raspberries, planting a caneberry cultivar that is less susceptible to JB injury is ideal for optimal plant quality and yield.

Cultivar selection is a preventive Integrated Pest Management (IPM) approach that can be implemented into cropping systems to prevent injury (Koul et al. 2004). This tactic is accomplished through choosing a cultivar within a plant species that is less susceptible to a certain pest or injury. While JB are highly polyphagous, multiple field and laboratory experiments have demonstrated differing susceptibilities within cultivars of the same plant species to JB. Species that have differing susceptibilities to JB include maple (*Acer* spp.) (Loughrin et al. 1997, Rowe et al. 2002), birch (*Betula* sp.) (Ranney and Walgenbach 1992, Santamour 2001, Gu et al. 2008), soybeans (*Glycine* sp.) (Coon 1946, Hammond and Cooper 1989), crape myrtle (*Lagerstroemia* spp.) (Pettis et al. 2004), apples (*Malus* sp.) (Langford and Cory 1948, Fleming 1986, Ranney and Walgenbach 1992, Spicer et al. 1995, Fulcher et al. 1998, Potter et al. 1998, Hogmire and Miller 2005), cherries, plums, and peaches (*Prunus* spp.) (Fleming 1976, Ranney and Walgenbach 1992, Patton et al. 1997a), roses (*Rosa* spp.) (Potter et al. 1998, Held and Potter 2004a, Tiddens and Cloyd 2006), linden/basswood (*Tilia* spp.) (Fleming 1976, Potter et al. 1998, Miller and Ware 1999), elms (*Ulmus* spp.) (Miller et al. 1999,

Miller et al. 2001, Paluch et al. 2006), grapes (*Vitis* spp.) (Langford and Cory 1948, Gu and Pomper 2008), and corn (*Zea mays*) (Langford et al. 1944).

Because there are multiple plant species with differing susceptibilities to JB among cultivars, there could also be differing JB susceptibility within primocane-bearing caneberry cultivars. I investigated this by observing the number of JB present, measuring marketable and injured yields, and calculating the percent defoliation for each of the cultivars observed. Through identifying and utilizing caneberry cultivars that are less susceptible to JB infestation, and fruit and foliar injury, the need for chemical control could be reduced.

### **Materials and Methods**

All field experiments were conducted in a 2005 planting of primocane-bearing raspberries and blackberries at Kentland Farm (College of Agriculture and Life Sciences, Virginia Tech), Montgomery County. This planting is on an elevated site above the New River in southwestern Virginia (37° 12.417'N, 80° 35.513'W, 615.7 m elev.). Plantings consisted of six raised bed rows containing 11 raspberry cultivars adjacent to seven raised bed rows containing two blackberry cultivars. Each cultivar plot is 6.2 m long and 1.0 m wide. Rows are 3.0 m apart and were bordered by an apple orchard, forest, and pasture. Eight primocane-bearing raspberry cultivars were considered including 'Anne', 'Autumn Bliss', 'Caroline', 'Dinkum', 'Fall Gold', 'Heritage', 'Himbo Top', and 'Prelude'. Rows one through six contained plots of these eight cultivars, planted using a randomized complete block design, blocked by row. The two cultivars of blackberries, 'Prime-Jim' and 'Prime-Jan,' were planted in a completely randomized design. In the years observed, there were also insecticide efficacy experiments conducted in the selected raspberry and blackberry cultivars (see Chapter 2).



## Japanese Beetle Counts

All JB were counted within a 2.0 m section of each raspberry and blackberry experimental plot in 2007 and within a 1.2 m section of each raspberry and blackberry plot in 2008. In 2009, JB numbers were observed within a 1.2 m plot section of each raspberry plot. Dates in which JB were counted are listed in Table 3.1. Japanese beetle count data were analyzed separately by date and by year totals. Data were transformed [ $\sqrt{(x + 0.5)}$ ] when not following a normal distribution. Both transformed and untransformed raspberry data were analyzed with a 2-way analysis of variance (ANOVA); followed by a Tukey's Honestly Significant Difference (HSD) means comparison test ( $\alpha = 0.05$ ) (SAS Institute 1989). Blackberry were analyzed with 2-way analysis of variance (ANOVA) following a completely random design followed by a Student's t-test means comparison ( $\alpha = 0.05$ ) (SAS Institute 1989).

## Harvest

In order to detect cultivar differences in the marketable, unmarketable, total harvested or percent unmarketable yield, ripe raspberries and blackberries were harvested and weighed within 2.0 m sections in 2007 and within 1.2 m sections in 2008 and 2009 (raspberries only 2009). On each sample date listed in Table 3.1, berries within each cultivar plot were sorted into marketable and unmarketable categories and then weighed to the closest, even tenth of a gram (e.g. 93.11 g was recorded as 93.2 g). A berry was considered marketable if it did not contain injury whereas unmarketable berries were the berries that were injured in any way (i.e. chewing or sucking insect damage, sunburn, drupelet malformation or mold). Any berries that were overripe were discarded and not weighed because the marketable state of these berries before they became

overripe was unknown. If the berries were not harvested by the same person on a given date, one person did a final quality evaluation.

Yield data were analyzed by date, as a year total for both marketable and unmarketable fruit, as a total harvested yield, and as a seasonal total percent unmarketable for each of the three years observed. The marketable, unmarketable, and seasonal total data were transformed [ $\sqrt{(x + 0.5)}$ ] when not following a normal distribution and when the proportion unmarketable data did not follow a normal distribution, it was subjected to an ArcSine ( $\sqrt{x}$ ) transformation. Both transformed and untransformed raspberry data were analyzed with ANOVA followed by a Tukey's HSD mean comparison ( $\alpha = 0.05$ ) and the blackberry data were analyzed with a Student's t-test ( $\alpha = 0.05$ ) (SAS Institute 1989).

## **Defoliation Studies**

### ***Defoliation Study 2007***

To test the susceptibility of two raspberry cultivars to JB in 2007, JB were bagged onto canes within each cultivar plot and the resulting defoliation was analyzed. The raspberry cultivars used for the bagging experiment, 'Fall Gold', and 'Autumn Bliss', were chosen based on a 2006 study in which these cultivars had the most JB present (Maxey et al. 2006). In total, there were 24 bags applied in the raspberries with each of the cultivar plots containing two replications (bags). Two replications were applied in each of the 2.0 m 'Fall Gold' or 'Autumn Bliss' plots with a total of 12 replications for each cultivar being applied. The cultivar effects of blackberry defoliation were not compared. Each bag contained five beetles and was placed over

the top 18-30 cm of two randomly chosen canes within the selected 2.0 m sections of each cultivar.

The defoliation of each leaflet within the top 18-30 cm cane area, starting with the apical-most leaf and working downward, was estimated before applying the bags. The defoliation was estimated based on a 0-100 scale with a score of “0” being given to leaves without any injury and a score of “100” given to leaves with only the veins remaining. To reduce variability, the same individual (LM) estimated the defoliation throughout the entire study.

After the pre-bagging defoliation was recorded, a nylon mesh bag (20 cm × 27 cm) containing five JB was placed over the cane, with the bottom of the bag enclosing the most basal leaf for which defoliation had been recorded. A medium sized binder clip was then clipped around the bag and cane to secure the bag on the plant. After 48 hrs, the bags were removed and defoliation was estimated and recorded as above. The comparisons of defoliations from before and after JB exposure resulted in an estimate of the overall foliar injury by JB.

The proportion defoliation was analyzed by date and as a seasonal total to estimate the effect of the two cultivars on the amount of JB defoliation. Results that did not follow a normal distribution were subjected to ArcSine ( $\sqrt{x}$ ) transformation and both transformed and untransformed data means were compared with a Student’s t-test ( $\alpha = 0.05$ ) (SAS Institute 1989).

### ***Defoliation Study 2008-2009***

Eight raspberry cultivars (‘Anne’, ‘Autumn Bliss’, ‘Caroline’, ‘Dinkum’, ‘Fall Gold’, ‘Heritage’, ‘Himbo Top’, and ‘Prelude’) and both blackberry cultivars (‘Prime-Jan’ and ‘Prime-Jim’) were included in the defoliation study in 2008. To gain a more accurate estimate of defoliation by JB, 35 leaves (injured and uninjured) were randomly digitally photographed from

each of the six 1.2 m plots per cultivar (210 images per cultivar). The computer program Image J (Rasband 2009) [downloaded from: <http://rsbweb.nih.gov/ij/download.html>] was used to analyze the digital images. The total area of each leaf was estimated, and the injured area was measured. Due to low JB pressure in 2008, percent defoliation was only analyzed as a seasonal total instead of a weekly percent defoliation as in 2007.

Because the number of uninjured leaves was high in 2008, the resulting average percent defoliation for each treatment was low. Therefore, in the 2009 defoliation study, only injured leaves were considered. The same eight raspberry cultivars that were considered in 2008 were again considered in 2009. At the end of the field season, digital images were taken of 20 randomly chosen injured leaves from each of the six 1.2 m plots for each cultivar (120 leaves for each cultivar were observed). ImageJ was again utilized in 2009, using the same procedure as in 2008 to calculate the percent defoliation.

The proportion defoliation for 2008 and 2009 was analyzed as a seasonal total. Results that did not follow a normal distribution were subjected to an ArcSine ( $\sqrt{x}$ ) transformation. Both transformed and untransformed raspberry data were analyzed with ANOVA followed by a Tukey's HSD mean comparison ( $\alpha = 0.05$ ) (SAS Institute 1989). The blackberry data means compared with a Student's t-test ( $\alpha = 0.05$ ) (SAS Institute 1989).

## **Results**

### **Japanese Beetle Counts**

In 2007, there were significant differences in the number of JB present among the different primocane-bearing raspberries on 5, 17, and 20 July (Figure 3.1). On 5 July, 'Prelude' had significantly more JB present than 'Himbo Top'. On 17 July, there were significantly more JB present on 'Prelude' than on 'Caroline'. On 20 July, there were significantly more JB present

on 'Prelude' than on 'Fall Gold', 'Anne', 'Caroline', 'Heritage', 'Dinkum', and 'Himbo Top'. When all JB numbers were added and averaged for 2007, there were significantly more JB on 'Prelude' than on 'Anne', 'Caroline', 'Heritage', 'Dinkum', and 'Himbo Top' ( $F = 4.65$ ;  $df = 7$ ;  $P = 0.0009$ ) (Figure 3.2).

In the 2007 blackberries, there were significantly more JB on 'Prime-Jim' than on 'Prime Jan' on 12, 17, and 20 July (Figure 3.3). The overall average number of JB in 2007 was also significantly higher on 'Prime-Jim' than on 'Prime-Jan' ( $F = 11.82$ ;  $df = 1$ ;  $P = 0.0049$ ) (Figure 3.4).

In 2008, there were significant differences in JB counts between raspberry cultivars on 15 August (Figure 3.5). On this date, there were significantly more JB on 'Prelude' than on 'Anne', 'Caroline', 'Dinkum', and 'Himbo Top'. When all JB counts were added and averaged from the 2008 raspberry observations, there were more JB on 'Prelude' than on 'Caroline', 'Dinkum', and 'Himbo Top' ( $F = 3.21$ ;  $df = 7$ ;  $P = 0.0126$ ) (Figure 3.6).

In 2008, there were five dates with significant differences in the number of JB present on the two blackberry cultivars. On all dates where significant differences were observed (25 July and 1 and 4 August), there were more JB on 'Prime-Jim' than on 'Prime-Jan'. The year's overall average was also significantly higher for 'Prime-Jim' than 'Prime-Jan' ( $F = 27.26$ ;  $df = 1$ ;  $P = 0.0034$ ) (Figure 3.8).

In 2009, there were significant differences in JB counts between raspberries cultivars on 5 August (Figure 3.9). On this date, there were significantly more JB on 'Prelude' than on 'Himbo Top'. The mean total number of JB in 2009 was significantly higher for 'Prelude' than for 'Dinkum', 'Heritage', 'Caroline', and 'Himbo Top' and 'Fall Gold' had significantly more JB than 'Himbo Top' ( $F = 4.82$ ;  $df = 7$ ;  $P = 0.0004$ ) (Figure 3.10).

## Harvest

There were significant differences on nine dates in 2007 in the amount of marketable yields from the different raspberry cultivars harvested (Table 3.2). Table 3.3 summarizes these significant differences. When all raspberry yields were averaged to obtain a seasonal total mean of marketable berries, there was significantly greater marketable yield from ‘Autumn Bliss’ and ‘Heritage’ than from ‘Anne’ ( $F = 5.05$ ;  $df = 7$ ;  $P = 0.0005$ ) (Table 3.6). Also, the seasonal marketable yield from ‘Autumn Bliss’ was significantly greater than ‘Dinkum’ and ‘Fall Gold’.

There were also differences in the unmarketable raspberry yields in 2007 (Tables 3.4). The eleven dates with significant differences in unmarketable yields are summarized in Table 3.5. When the weights of the unmarketable berries were averaged as a seasonal mean, there was significantly greater unmarketable fruit from ‘Caroline’ and ‘Heritage’ than from ‘Anne’, ‘Autumn Bliss’, ‘Dinkum’, ‘Fall Gold’, ‘Himbo Top’, and ‘Prelude’ ( $F = 11.31$ ;  $df = 7$ ;  $P < 0.0001$ ) (Table 3.6). There was a significantly higher percent of unmarketable fruit from ‘Heritage’ than from ‘Autumn Bliss’, ‘Fall Gold’, ‘Himbo Top’, or ‘Prelude’ ( $F = 5.57$ ;  $df = 7$ ;  $P = 0.0002$ ) (Table 3.6). ‘Caroline’ also had a significantly greater percent injury than ‘Autumn bliss’ in 2007. When the total marketable and unmarketable yields were added to obtain a total yield from each cultivar, ‘Heritage’ had significantly higher overall yield than ‘Anne’, ‘Dinkum’, ‘Fall Gold’, or ‘Prelude’ ( $F = 6.06$ ;  $df = 7$ ;  $P = 0.0001$ ) (Table 3.6). ‘Autumn Bliss’ also had significantly more overall yield than ‘Anne’ or ‘Fall Gold’ and ‘Caroline’ had significantly more overall yield than ‘Anne’.

There were three dates in 2007 where there were significant differences in the amount of marketable blackberries between the cultivars studied (Tables 3.7). A summary of these

differences can be found in Table 3.8. The year's total marketable means were not significantly different in the 2007 blackberries ( $F = 0.00$ ;  $df = 1$ ;  $P = 0.9993$ ) (Table 3.11).

Significant differences in unmarketable yields were also found between the blackberry cultivars in 2007 (Table 3.9). On all dates with significant differences (21, 24, 28, and 31 August, and 4 and 11 September), there were significantly greater unmarketable yields harvested from 'Prime-Jan' than from 'Prime-Jim' (Table 3.10). This trend continued with the year's total unmarketable means ( $F = 71.19$ ;  $df = 1$ ;  $P < 0.0001$ ) and the year's total percent unmarketable yields ( $F = 40.14$ ;  $df = 1$ ;  $P < 0.0001$ ) being significantly greater for 'Prime-Jan' than for 'Prime-Jim' (Table 3.11). When all yields from each cultivar were added together, 'Prime-Jan' yielded significantly more overall yield than 'Prime-Jim' ( $F = 35.44$ ;  $df = 1$ ;  $P < 0.0001$ ) (Table 3.11).

In the 2008 raspberries, there were four dates on which there were significant differences among the cultivars in the amount of marketable yield (Table 3.12). The significant differences from these four dates are summarized in Table 3.13. There were also significant differences in the marketable yields between cultivars when comparing the seasonal total means ( $F = 5.93$ ;  $df = 7$ ;  $P = 0.0003$ ) (Table 3.16). Mean marketable fruit of 'Autumn Bliss' was significantly greater than those of 'Anne', 'Fall Gold', 'Himbo Top', and 'Prelude'. Also, 'Heritage' had a significantly greater mean of marketable yield than 'Anne' and 'Prelude' and 'Caroline' had greater marketable yield than 'Prelude'.

Significant differences between the different raspberry cultivars were observed on three dates in 2008 when comparing unmarketable yields (Table 3.14) which are summarized in Table 3.15. There were significant differences in the season's total mean of unmarketable fruit with 'Caroline' having more than 'Anne' ( $\alpha = 0.10$ ;  $F = 2.20$ ;  $df = 7$ ;  $P = 0.0649$ ). Significant differences were not present in the season's total mean percent of unmarketable fruit ( $F = 0.54$ ;

df = 7;  $P = 0.7979$ ) (Table 3.16). When all yields for the season from each cultivar were added together and compared, there was significantly more overall yield from ‘Autumn Bliss’ than from ‘Anne’ or ‘Prelude’ ( $F = 4.91$ ; df = 7;  $P = 0.0010$ ).

There were dates where differences in the amount of unmarketable blackberries were detected (Table 3.18) even though there were no significant differences on any date or as a year total in 2008 in the amount of marketable blackberries ( $F = 0.28$ ; df = 1;  $P = 0.6052$ ) (Tables 3.17 and 3.20). On 2, 12, and 19 September, ‘Prime-Jan’ had significantly greater unmarketable yield than ‘Prime-Jim’ (Table 3.19). ‘Prime-Jan’ also had significantly greater values than ‘Prime-Jim’ for the year’s total mean of unmarketable fruit ( $F = 17.65$ ; df = 1;  $P = 0.0012$ ) and for the year’s mean percent of unmarketable fruit ( $F = 21.00$ ; df = 1;  $P = 0.0006$ ) (Table 3.20). There were significantly more overall yields from ‘Prime-Jan’ than from ‘Prime-Jim’ in 2008 ( $\alpha = 0.10$ ;  $F = 3.44$ ; df = 1;  $P = 0.0883$ ) (Table 3.20).

There were ten dates in 2009 with significant differences between raspberry cultivars in the amount of marketable yield (Table 3.21) which are summarized in Table 3.22. When all yields were averaged, there was significantly greater marketable yield from ‘Heritage’ than from ‘Anne’, ‘Fall Gold’, or ‘Prelude’ ( $F = 2.51$ ; df = 7;  $P = 0.0276$ ) (Table 3.25). There were ten dates when there were significant differences in the unmarketable yields between the raspberry cultivars observed (Table 3.23). The significant differences seen on these ten dates are summarized in Table 3.24. When unmarketable yield from all dates harvested in 2009 were added and averaged, there was significantly greater unmarketable yield from ‘Caroline’ than from ‘Anne’, ‘Autumn Bliss’, ‘Dinkum’, ‘Fall Gold’ or ‘Prelude’ ( $F = 5.37$ ; df = 7;  $P = 0.0001$ ) (Table 3.25). There was also significantly greater total unmarketable yield from ‘Himbo Top’ than from ‘Autumn Bliss’, ‘Fall Gold’, or ‘Prelude’ and from ‘Heritage’ than from ‘Fall Gold’ or



'Prelude'. There was a significantly greater percent of unmarketable fruit from 'Caroline' than from 'Autumn Bliss', 'Dinkum', 'Fall Gold', 'Prelude', or 'Heritage' ( $F = 7.26$ ;  $df = 7$ ;  $P < 0.0001$ ) (Table 3.25). 'Anne' and 'Himbo Top' also had a significantly greater percentage of unmarketable fruit than did 'Autumn Bliss', 'Dinkum', 'Fall Gold', or 'Prelude'. When all yields, marketable and unmarketable were added and compared, 'Heritage' produced significantly more overall yield than 'Anne', 'Fall Gold', or 'Prelude' and 'Caroline' produced significantly more overall yield than 'Anne' or 'Prelude' ( $F = 2.76$ ;  $df = 7$ ;  $P = 0.0170$ ) (Table 3.25).

## **Defoliation Studies**

### ***2007 Estimation of Defoliation***

There was not a significant difference in seasonal defoliation between bagged 'Autumn bliss' and 'Fall Gold' foliage in 2007 ( $F = 1.71$ ;  $df = 1$ ;  $P = 0.5457$ ) (Figure 3.11).

### ***2008 Total Calculated Defoliation***

The injury sustained to leaves of each of the eight raspberry cultivars and two blackberry cultivars was calculated at the end of the season for 2008. Both injured and uninjured leaves were observed. There were significant differences between the raspberry cultivars ( $F = 3.85$ ;  $df = 7$ ;  $P = 0.0004$ ) (Figure 3.12), but not between the blackberry cultivars ( $F = 1.54$ ;  $df = 1$ ;  $P = 0.2159$ ) (Figure 3.13). In the raspberries, 'Prelude' had significantly more defoliation than, 'Anne', 'Heritage', 'Caroline', and 'Dinkum' (Figure 3.12).

### ***2009 Total Calculated Defoliation in Raspberries***

When the mean percent defoliation of injured raspberry leaves from eight cultivars was compared in 2009, differences were present (Figure 3.14). ‘Prelude’ and ‘Fall Gold’ experienced significantly more defoliation than ‘Caroline’ or ‘Anne’ ( $F = 4.34$ ;  $df = 7$ ;  $P < 0.0001$ ).

## **Discussion**

### **Cultivar Susceptibility to Japanese Beetle Infestations and Plant Injury**

Japanese beetles fed on all cultivars examined; however, there were significant differences among cultivars in the number of beetles present and in the fruit and foliar injury. JB defoliation has a distinct skeletonized appearance that makes it easy to distinguish (Fleming 1972, Potter and Held 2002, Bushway et al. 2008, Demchak et al. 2010). However, the reported defoliations could include injury from raspberry sawfly, *Monophadnoides geniculatus* Hartig, and eastern raspberry fruitworm, *Byturus rubi* Barber, which cause similar foliar injury to that of JB (Bushway et al. 2008, Demchak et al. 2010). Since JB populations were scarce or not present for most of the harvesting seasons, the yield data relates more to a cultivar’s susceptibility to overall pest injury and not just JB feeding injury. A cultivar’s marketable yields are discussed since greater marketable yield signifies the cultivars resistance to pests. Greater marketable yields also mean greater profit for the producer. The percentages of unmarketable yields are discussed because those values represent a cultivar’s susceptibility to injury. Also, the less percentage of unmarketable berries present, the less time is spent separating marketable and unmarketable berries. The total quantities harvested are also discussed because these values show a cultivar’s potential to produce greater yield if injurious factors were controlled.

## Raspberries

In all three seasons in which JB counts were made in the raspberries, the four cultivars with the highest number of JB present were ‘Prelude’, ‘Fall Gold’, ‘Anne’, and ‘Autumn Bliss’ and the four cultivars with the lowest number of JB present were ‘Dinkum’, ‘Heritage’, ‘Caroline’, and ‘Himbo Top’ (Figures 3.2, 3.6, and 3.10). However, significant differences in seasonal means were found in 2007 with ‘Prelude’ having significantly more JB present than ‘Anne’, ‘Caroline’, ‘Heritage’, ‘Dinkum’, and ‘Himbo Top’ (Figure 3.2). In 2008, ‘Prelude’ had significantly more JB present than ‘Caroline’, ‘Dinkum’, and ‘Himbo Top’ (Figure 3.6) and in 2009 ‘Prelude’ had significantly more JB present than ‘Heritage’, ‘Caroline’, or ‘Himbo Top’ (Figure 3.10). During the 2007-2009 seasons, ‘Prelude’ also had significantly less overall yield compared to ‘Heritage’ (2007 and 2009), ‘Autumn Bliss’ (2008), or ‘Caroline’ (2009) (Tables 3.6, 3.16, and 3.25). Weber et al. (2004) also found ‘Prelude’ to have less overall yield compared to ‘Autumn Bliss’ and ‘Heritage’ but similar yields to ‘Anne’. ‘Prelude’ also had significantly less marketable yield than ‘Autumn Bliss’, ‘Caroline’, and ‘Heritage’ in 2008 (Table 3.16) and significantly less marketable yield compared to ‘Heritage’ in 2009 (Table 3.25). When comparing the percents of unmarketable yields from the different varieties, ‘Prelude’ had significantly less injury than ‘Heritage’ in 2007 (Table 3.6), and in 2009, ‘Prelude’ had significantly less injury than ‘Anne’, ‘Caroline’, and ‘Himbo Top’ (Table 3.25). ‘Prelude’ experienced significantly more defoliation than ‘Anne’, ‘Heritage’, ‘Caroline’ and ‘Dinkum’ in 2008 (Figure 3.12) and significantly more defoliation than ‘Anne’ and ‘Caroline’ in 2009 (Figure 3.14), which could be a result of the greater JB infestation. Overall, this cultivar was more

susceptible to greater JB infestation, less overall and marketable yields, and increased foliar injury.

The yellow raspberry cultivars observed, 'Anne' and 'Fall Gold', varied in the number of JB present with 'Anne' having fewer JB present than 'Prelude' in 2007 (Figure 3.2) and 'Fall Gold' having more JB present than 'Himbo Top' in 2009 (Figure 3.10). Both yellow cultivars had significantly fewer marketable yields compared to 'Autumn Bliss' in 2007 and 2008 (Tables 3.6 and 3.16) and significantly fewer marketable yields compared to 'Heritage' in 2009 (Table 3.25). 'Anne' also had a higher percentage of injured fruit in 2009 (Table 3.25) and had the least overall yield out of any cultivar considered in all three seasons (Tables 3.6, 3.16, and 3.25). 'Anne' therefore, is all together an undesirable cultivar when wanting greater marketable yield. The other yellow cultivar that was harvested, 'Fall Gold', did not have as high of a percentage of injured berries as 'Anne' but did produce relatively low overall yields. The reduced-yield harvested from both yellow raspberry cultivars is common and these cultivars are mainly grown for their preferred taste (Fernandez and Krewer 2008, Demchak et al. 2010). 'Fall Gold' experienced greater defoliation than 'Caroline' or 'Anne' in 2009 (Figure 3.14) but had similar defoliation to all other cultivars in 2008 (Figure 3.12). 'Anne' on the other hand experienced less defoliation than 'Prelude' in 2008 (Figure 3.12) and less than 'Prelude' and 'Fall Gold' in 2009 (Figure 3.14). Overall, JB populations were similar on both cultivars, 'Fall Gold' is more susceptible to defoliation and 'Anne' is more susceptible to berry injury. This statement signifies that injuries caused by other pests were included in defoliation and yield measurements and/or that JB had a greater preference for 'Fall Gold' foliage and 'Anne' berries.

For all three seasons, 'Autumn Bliss', 'Heritage', and 'Caroline' had the largest amounts of marketable yields (Tables 3.6, 3.16, and 3.25). 'Autumn Bliss' also had some of the highest

overall yields (marketable + unmarketable) and the least percentages of unmarketable fruit for all three seasons. This cultivar is therefore appealing due to its relatively high yields along with the less needed time to separate marketable from unmarketable fruit. Relatively high numbers of JB were present on ‘Autumn Bliss’ throughout the three seasons (Figures 3.2, 3.6, and 3.10) but this pest pressure was not great enough to significantly lessen the marketable yields. Defoliation of ‘Autumn Bliss’ was similar to the other cultivars in both 2008 and 2009 (Figures 3.12 and 3.14). ‘Heritage’ and ‘Caroline’ also had higher overall yields compared to the other cultivars, but both cultivars also had relatively higher percents of injured fruit (Tables 3.6, 3.16, and 3.25). The greater amount of injured fruit cannot be directly related to JB feeding since JB infestation was low on these cultivars (Figures 3.2, 3.6, and 3.10). However, these cultivars’ low susceptibility to JB infestation did result in less defoliation of both cultivars in 2008 (Figure 3.12) and less defoliation to ‘Caroline’ in 2009 (Figure 3.13).

Both ‘Dinkum’ and ‘Himbo Top’ were relatively less susceptible to JB infestation compared to the other cultivars considered and had significantly less JB present than ‘Prelude’ during all three seasons (Figures 3.2, 3.6, and 3.10). Marketable yields and total yields were similar to the other cultivars for ‘Himbo Top’ across all three seasons, except for in 2008, when there was significantly greater marketable yield from ‘Autumn Bliss’ than ‘Himbo Top’ (Table 3.16). ‘Himbo Top’ had a lower percentage of injured fruit than ‘Heritage’ in 2007 and a higher percentage of injured fruit than ‘Fall Gold’, ‘Dinkum’, ‘Prelude’ and ‘Autumn Bliss’ in 2009 (Tables 3.6, 3.16, and 3.25). Therefore, the levels of fruit injury to ‘Himbo Top’ varied between seasons and multiple factors most likely responsible. The similar susceptibilities of ‘Dinkum’ and ‘Himbo Top’ to JB infestation resulted in similar defoliation of both cultivars in 2008 and 2009 (Figures 3.12 and 3.14). When considering ‘Dinkum’s’ yields, this cultivar had

significantly less marketable yield than ‘Autumn Bliss’ in 2007; a lesser amount of overall yield compared to ‘Heritage’ in 2007; and a lesser percentage of injured fruit than ‘Caroline’, ‘Himbo Top’, and ‘Anne’ in 2009 (Tables 3.6 and 3.25). Overall though, ‘Dinkum’ is an appealing raspberry cultivar that produces similar yields to the other cultivars and offers less JB susceptibility and less susceptibility to defoliation.

## **Blackberries**

‘Prime-Jan’ yielded similar cumulative marketable yields to ‘Prime-Jim’ in both years of blackberry harvest (Tables 3.11 and 3.20). The ‘Prime-Jim’ blackberries had a lower percent of injury but also produced fewer overall yields; whereas, the ‘Prime-Jan’ berries were more susceptible to injury, but the plants produced greater overall yields. There was no significant difference in the level of defoliation between ‘Prime-Jan’ and ‘Prime-Jim’ (Figure 3.13) even though there were more beetles present on ‘Prime-Jim’ than ‘Prime-Jan’ in both 2007 and 2008 (Figures 3.4 and 3.8); the greater JB infestation did not translate into greater defoliation or greater amounts of injured fruit. The levels of injury observed in the blackberries are most likely a result of another pest or factor. Conclusively, both cultivars produce similar amounts of marketable berries, but each cultivar has qualities that would appeal to different stake-holders. Clark (2008) also found ‘Prime-Jan’ to normally produce higher yields than ‘Prime-Jim’. Both cultivars are only recommended for home gardens or small commercial trials due to a trend of greatly reduced fruit set, size, and quality on primocanes after the plants were exposed to summer temperatures above 29 °C (Clark et al. 2005, Fernandez and Krewer 2008, Clark 2010). Research on primocane-bearing blackberries is ongoing in an effort to reduce the limitations present in today’s cultivars (Clark 2008).

## Japanese Beetle Host Selection and Caneberry Cultivar Susceptibility

The raspberry and blackberry cultivars evaluated have different characteristics that could explain their level of susceptibility to JB. Here, I propose explanations for the differing susceptibilities using past works completed. These studies on JB discuss the cues used by JB when choosing a host for infestation and ingestion. The characteristics of each cultivar were not compared through correlation to the different susceptibilities during my study so the following is only proposed overview of explanations for my findings.

The degree of feeding depends generally on the plants attractiveness and the abundance of JB in the area (Hawley and Metzger 1940). Since all cultivars were exposed to the same JB abundance in a given year, the focus here is on what attracts JB to a plant and stimulates feeding. Held and Potter (2002, 2004b) suggested that JB are attracted to a host plant through a combination of physical stimuli including visual cues, plant characteristics at the surface, and aromatic cues (naturally occurring and injury induced).

The visual cues used by JB when orientating to a host include height (Ladd and Klein 1982, Rowe and Potter 1996), color (Ladd and Klein 1986, Rowe et al. 2002, Held and Potter 2004a), floral size (Held and Potter 2004a), and the number of flowers present. On roses (*Rosa x hybrida* spp.), another plant group in Rosaceae along with caneberries, JB mainly land on the flowers (Held and Potter 2004a). It is proposed that this preference for the flowers is because reproductive structures display more dynamic and variable visual spectra than the foliage allowing JB to identify the preferred flowers and fruits (Held and Potter 2004a). Held and Potter (2004b) also proposed that JB prefer the flowers and fruits because these structures are located terminally on many plant species. Rowe and Potter (1996) found that the height of the host per se strongly affect the initial orientation and attack by JB. A preference for yellow and white

flowers over darker colored flowers was also found (Hawley and Metzger 1940, Held and Potter 2004a). In a study testing the effects of JB trap color, white traps caught more JB than did yellow, blue, green, arc yellow, signal green or saturn yellow traps (Ladd and Klein 1986). The size of the flower also influences where JB landed with larger yellow flowers being preferred over smaller ones (Held and Potter 2004a). Also in the same study, the number of open blooms influenced the number of JB present.

The raspberry cultivars used during my study grew at different heights, displayed variable numbers of flowers and fruits at one time, displayed differing shades of green in the foliage and differing shades of reds and yellows in the berries, and had different sizes of berries and flowers. Also, certain raspberry cultivars' berries ripened at different times meaning the fruit's darkness and color saturation varied at differing times of JB exposure (Perkins-Veazie 1992). Therefore, the height, color, and/or size could explain why JB favored to land on the 'Prelude' raspberries over the other cultivars present. For the three years of study, while the differences were not statistically significant, the pattern of the yellow raspberry cultivars numerically having more JB present than four of the six red raspberry cultivars was consistent (Figures 3.2, 3.6, and 3.10). The numeric differences are consistent with findings of Hawley and Metzger (1940) and Held and Potter (2004b) in roses, where yellow cultivars were preferred over darker cultivars. However, other variables such as sugar content or odor are most likely responsible for the differences in JB presence (Metzger et al. 1934). The blackberry cultivars were similar in height, similar in color to the human eye, and similar in ripening times, suggesting another factor (such as the amount of fruit or volatiles) attributed to JB landing on 'Prime-Jim' preferentially over 'Prime-Jan'.



Japanese beetle presence on a plant and the amount of plant material consumed is also related to the plant surface characteristics which can include the toughness of the plant material and the presence or absence of pubescence (Langford and Cory 1948, Miller and Ware 1999, Miller et al. 2001, Potter and Held 2002, Gu and Pomper 2008). JB prefer grape leaves that are thin, glossy, tender, and which have smooth and leathery tops and smooth or woolly undersides (Langford and Cory 1948, Fleming 1976, Gu and Pomper 2008). The less attractive grape qualities include young shoots and coarse and leathery leaf tops covered with a tomentum on the underside. Pubescence or tomentum have also been reported to deter JB from feeding in lindens (*Tilia* spp.) and elms (*Ulmus* spp.) (Miller and Ware 1999, Miller et al. 2001). However, Keathley and Potter (2008) found that leaf toughness was not used by JB when choosing between the following hosts: sassafras (*Sassafras albidum* (Nuttall)), cherry plum (*Prunus cerasifera* J.F. Ehrhart), Virginia creeper (*Parthenocissus quinquefolia* L.), littleleaf linden (*Tilia cordata* Miller), tuliptree (*Liriodendron tulipifera* L.), lilac (*Syringa vulgaris* L.), dogwood (*Cornus florida* L.) or Bradford callery pear (*Pyrus calleryana* Decaisne). Because different cultivars of raspberries and blackberries were studied, there were most likely differences in the plant material's toughness, the presence of pubescence, or that both could have favored JB presence or injury.

Keathley and Potter (2008) also found that secondary chemistry not mechanical defenses, such as those discussed above, are the determining factor in a JB's dietary range. Plant contents that have been reported to be related to a plant's susceptibility or preference by JB include hydroxycinnamic acid (Gu et al. 2008), geraniol (Smith 1924, Langford et al. 1943, Fleming 1976, Loughrin et al. 1996a, Patton et al. 1997a), eugenol (Smith 1924, Langford et al. 1943, Fleming 1976, Loughrin et al. 1996a, Patton et al. 1997a), caproic acid (Langford et al. 1943,

Fleming 1976), phenyl ethyl acetate (Langford and Gilbert 1949, Fleming 1976), phenyl ethyl alcohol (Langford et al. 1943, Fleming 1976), phenyl ethyl butyrate (Langford and Cory 1946, Fleming 1976), phenyl iso-valerate (Langford and Cory 1946, Fleming 1976), methyl cyclohexanepropionate (McGovern et al. 1970, Ladd 1971), phenylethyl propionate (Fleming 1976, McGovern and Ladd 1984), high concentration of sugars (sucrose, maltose, fructose, glucose, arabinose, xylose, raffinose) (Metzger et al. 1934, Hawley and Metzger 1940, Ladd 1986, Held and Potter 2004b), benzaldehyde (Loughrin et al. 1996a), citral (Richmond 1927, Loughrin et al. 1996a), linalool (Langford et al. 1943, Loughrin et al. 1996a, Loughrin et al. 1997), terpenes ((E)- $\alpha$ -ocimene, caryophyllene, germacrene D and (E,E)- $\alpha$ -farnesene)(Loughrin et al. 1996a), rutin (Patton et al. 1997b, Fulcher et al. 1998), phloridzin (Patton et al. 1997b, Fulcher et al. 1998), quercetin (Patton et al. 1997b, Fulcher et al. 1998), chlorogenic acid (Patton et al. 1997b, Fulcher et al. 1998), and lipids (Paluch et al. 2006). Many of these chemicals are olfactory stimulants that help JB orient to the plant whereas others listed are gustatory stimulants.

Japanese beetles orient to plant volatiles when choosing a host to infest or ingest (Loughrin et al. 1995, Loughrin et al. 1996b, Loughrin et al. 1996a, Rowe and Potter 1996, Loughrin et al. 1998, Potter et al. 1998, Potter and Held 2002, Keathley and Potter 2008). Loughrin et al. (1996a) proposed that after JB emerge from the soil, they are attracted to a host through many volatiles produced by plants, especially those with fruit or floral-like components (Hawley and Metzger 1940, Fleming 1972, Loughrin et al. 1998). Also, multiple blends of odors are more attractive to JB than less complex blends (Loughrin et al. 1998). After the JB is attracted to a plant there is an initial tasting. After this test, the beetle will continue feeding if phagostimulants are present. Through continued feeding, injury-induced volatiles are released.

The injury-induced chemicals are more complex than the naturally occurring volatiles making them more preferred by JB (Loughrin et al. 1996b). If after the initial tasting, the plant material contains deterrents or a component that is not preferred, JB will continue searching until a preferred host is found. Therefore, through continuing feeding on a preferred host, the beetle releases increasing amounts of injury-induced volatiles. These injury-induced volatiles are more complex and preferred by JB than the naturally occurring volatiles. The preference for injury-induced volatiles will in turn attract more beetles to a preferred host over other hosts that have not been fed upon (Loughrin et al. 1996b, Rowe and Potter 1996).

In conclusion, the caneberry cultivars that were more susceptible to JB infestation and ingestion were so because of the plant characteristics that JB found more favorable than others. My study is only an introductory study identifying the more and less susceptible caneberry cultivars. Further studies should be conducted to determine which of the above physical and chemical characteristics are responsible for JB infestation and consumption for a more complete understanding of the differing susceptibilities.

## **Summary**

The presence of more JB on ‘Prelude’, ‘Fall Gold’, ‘Anne’, and ‘Autumn Bliss’ over ‘Dinkum’, ‘Heritage’, ‘Caroline’, and ‘Himbo Top’ and the greater number of JB on ‘Prime-Jim’ versus ‘Prime-Jan’, are most likely due to the multiple JB stimulants present on each cultivar. These JB stimulants include physical and chemical characteristics, naturally occurring volatiles, and injury-induced volatiles. The preferred cultivars most likely possess a combination of these characteristics that make them more susceptible to JB infestation and consumption. The susceptibility of a cultivar to JB infestation related more to the reported defoliations than to the yield findings due to few JB being present during harvest. ‘Autumn Bliss’, ‘Heritage’, and

'Caroline' produced the highest marketable yields for all three seasons measured. The yellow cultivars, 'Anne' and 'Fall Gold' produced the least amounts of marketable yields which were to be expected (Fernandez and Krewer 2008, Demchak et al. 2010). 'Prelude' often had the lowest marketable yields of the red raspberries harvested. 'Himbo Top' and 'Dinkum' produced average marketable yields that were comparable to the other red raspberry cultivars harvested. Both cultivars of primocane-bearing blackberries yielded similar marketable yields. In 2008, 'Prelude', 'Autumn Bliss', and 'Himbo Top' were more susceptible to defoliation than 'Dinkum'. In 2009, 'Prelude' and 'Fall Gold' were more susceptible to defoliation than 'Caroline' or 'Anne'. My study only identified the primocane-bearing caneberry cultivars that were more susceptible to JB infestation, fruit injury, and defoliation and more studies need to be conducted to correlating the factor(s) responsible for each cultivar's susceptibility.

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## Tables and Figures

Table 3.1 Sample dates in which Japanese beetles were counted and berries were harvested within 2.0 m plots in 2007 and 1.2 m plots in 2008-2009. Blackberries were not sampled on dates or year followed by (\*).

2007	Dates Sampled
JB counts	26 and 29 Jun., 2, 5, 9, 12, 17, and 20 Jul., and 14* Aug.
Harvest	14*, 21, 24, 28, and 31 Jul., 4, 7, 11, 14*, 18*, 21*, 25*, and 28* Aug., and 2* Oct.
2008	
JB counts	18, 25, and 28 Jul., and 1, 4, 7, 11, 15, and 18 Aug.
Harvest	22, 26, and 29 Aug., 2, 12, 16*, 19, 23, and 30 Sept., and 7 Oct.
2009 *	
JB counts	8, 15, and 22 Jul. and 5 Aug.
Harvest	6, 10, 13, 17, 20, 24, 27, and 31 Aug. and 3, 10, and 17 Sept.

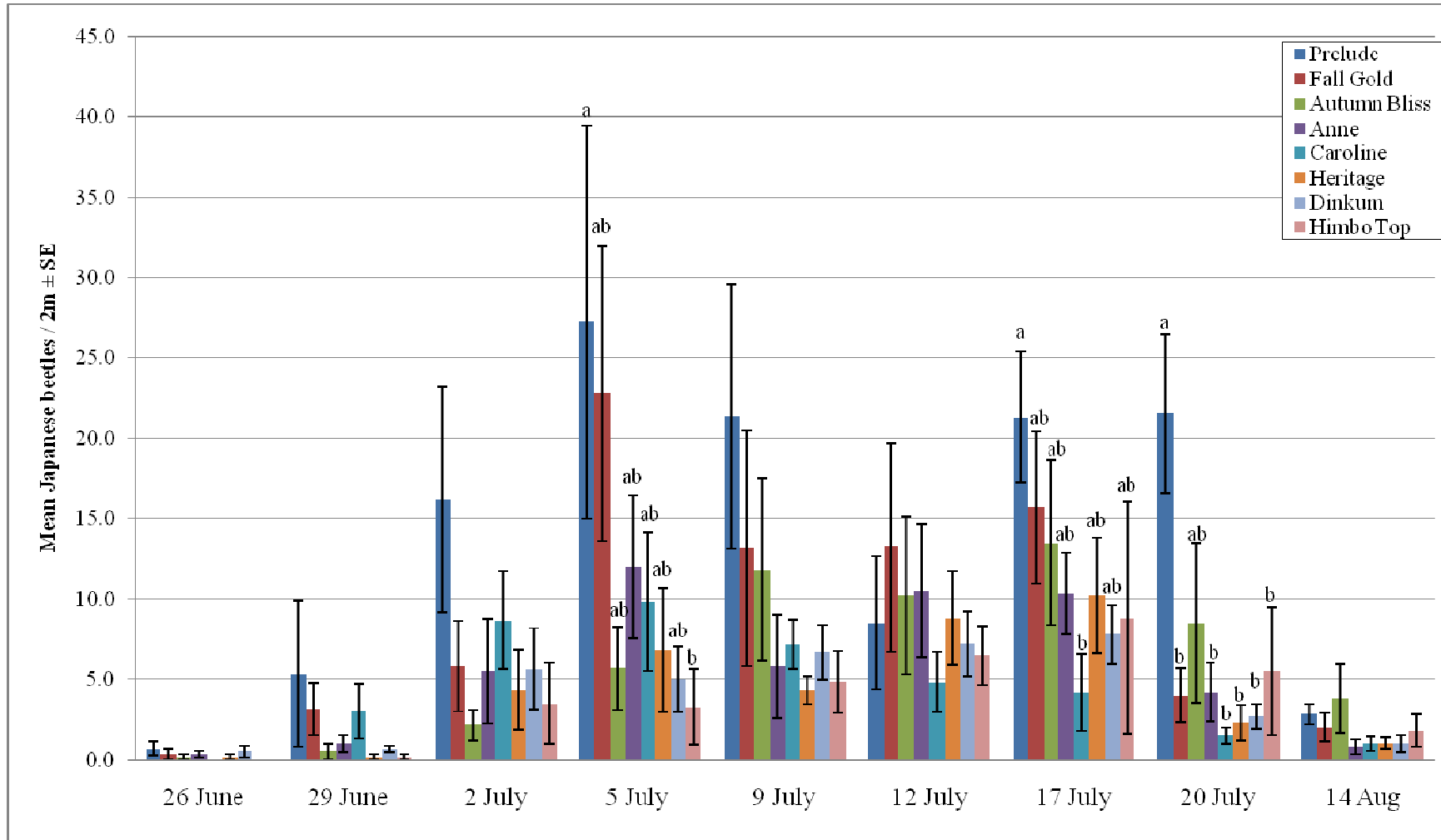


Figure 3.1 Mean number of Japanese beetles by date per 2.0 m plots in eight raspberry cultivars in 2007. Significant differences between cultivar means were only found on 17 and 20 July and values on those dates followed by the same letter are not significantly different (Tukey's HSD ( $\alpha = 0.05$ ) following  $[\sqrt{(x + 0.5)}]$  transformation).

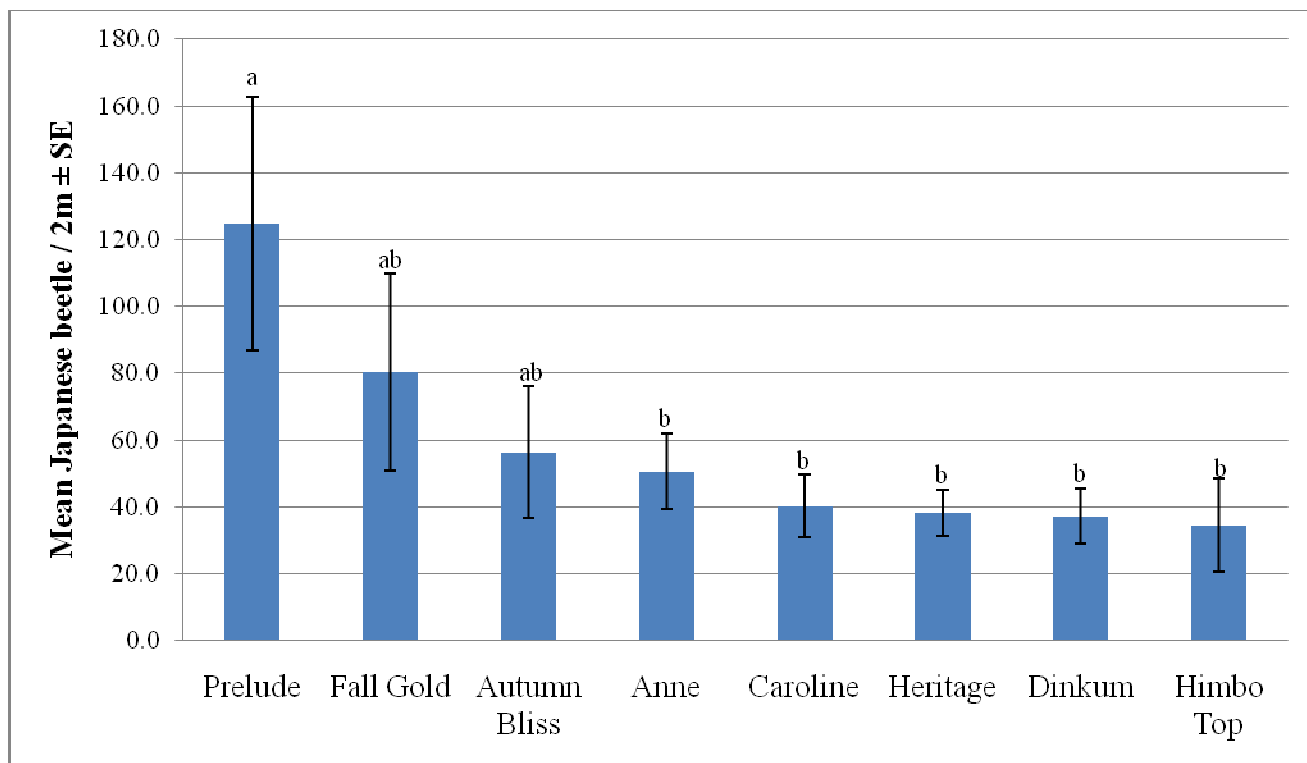


Figure 3.2 Mean total number of Japanese beetles counted per 2.0 m in eight raspberry cultivars in 2007. Values followed by the same letter are not significantly different according to Tukey's HSD after  $[\sqrt{(x + 0.5)}]$  transformation ( $F = 4.65$ ;  $df = 7$ ;  $P = 0.0009$ ).

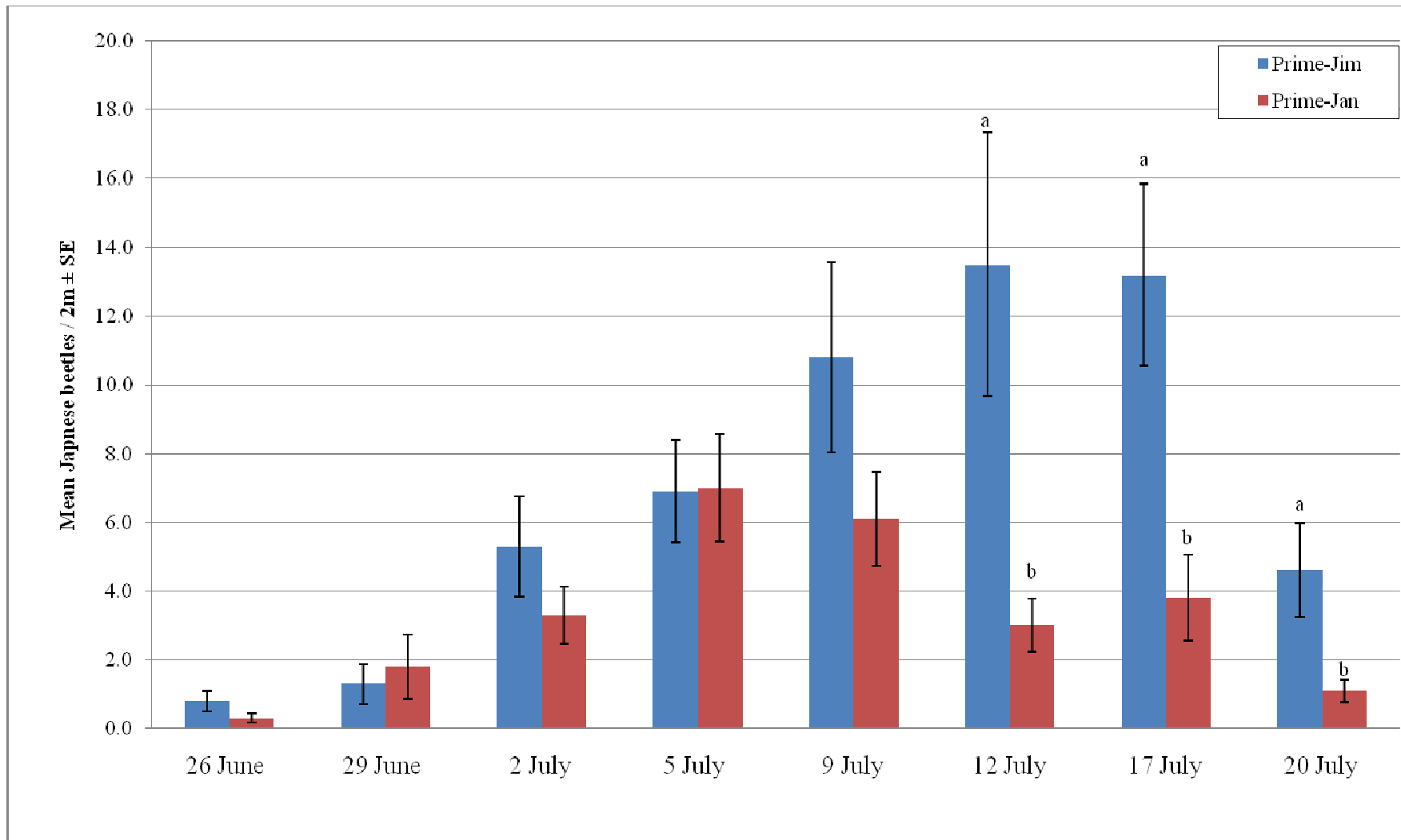


Figure 3.3 Mean number of Japanese beetles by date per 2.0 m plots in 'Prime-Jan' or 'Prime-Jim' blackberries in 2007. Significant differences between cultivar means were only found on 12, 17, and 20 July and values on those dates followed by the same letter are not significantly different (Student's t-test ( $\alpha = 0.05$ ) following  $[\sqrt{(x + 0.5)}]$  transformation).



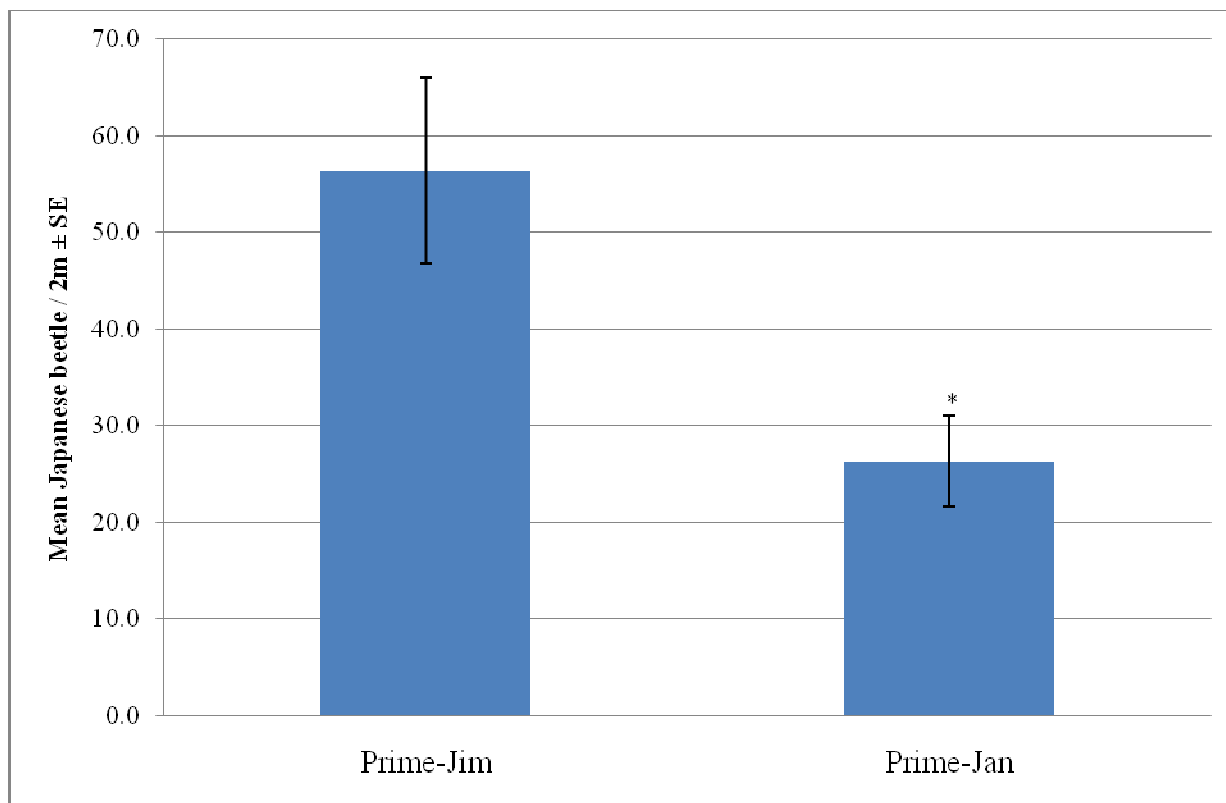


Figure 3.4 Mean total number of Japanese beetles counted per 2.0 m in 'Prime-Jan' or 'Prime-Jim' blackberries in 2007. Values are significantly different (Student's t-test ( $F = 11.82$ ;  $df = 1$ ;  $P = 0.0049$ ) following  $[\sqrt{(x + 0.5)}]$  transformation).

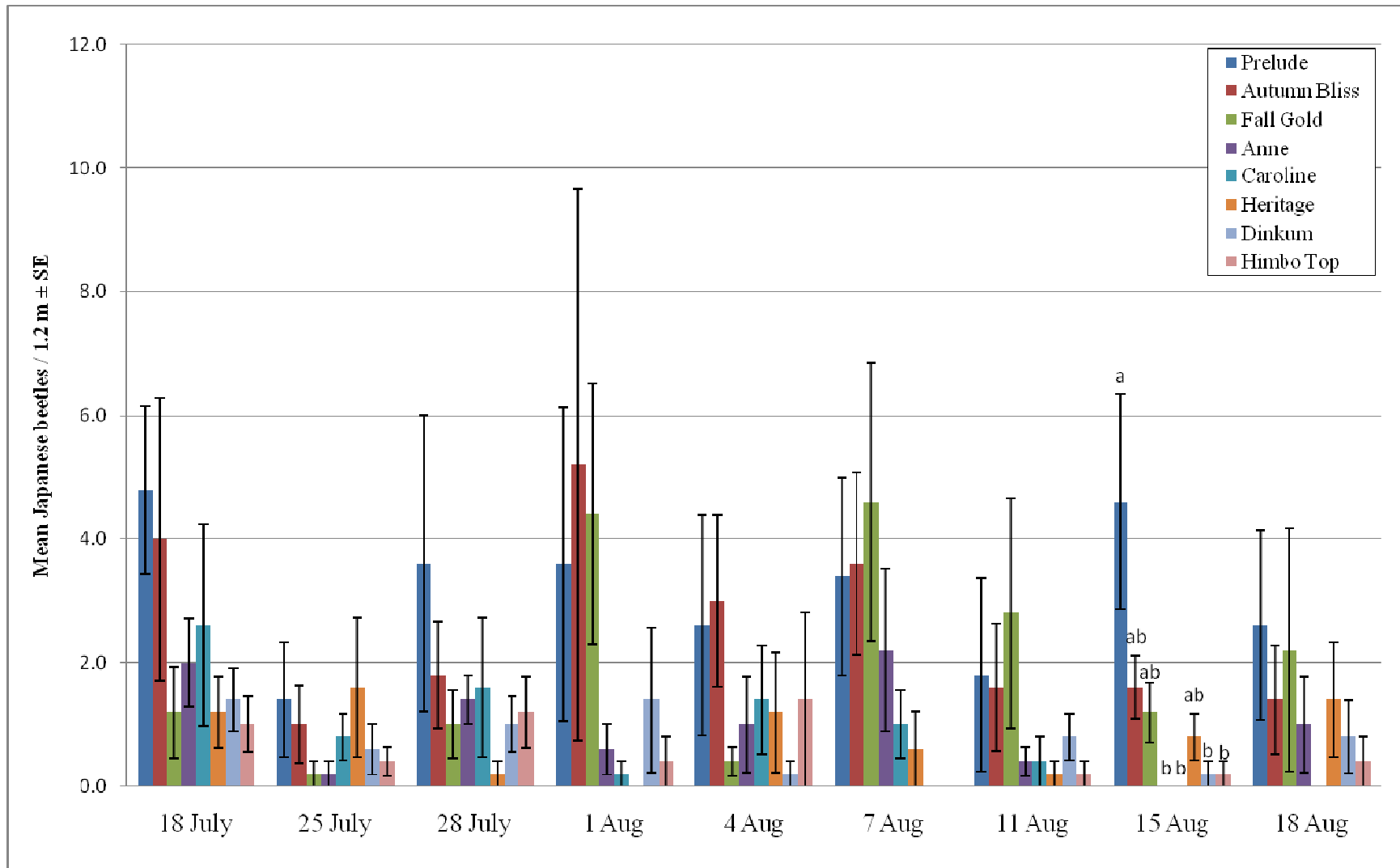


Figure 3.5 Mean number of Japanese beetles by date per 1.2 m plots of eight raspberry cultivars in 2008. Significant differences between cultivar means were only found on 15 August and values followed by the same letter are not significantly different on that date (Tukey's HSD ( $\alpha = 0.05$ ) following  $[\sqrt{(x + 0.5)}]$  transformation).

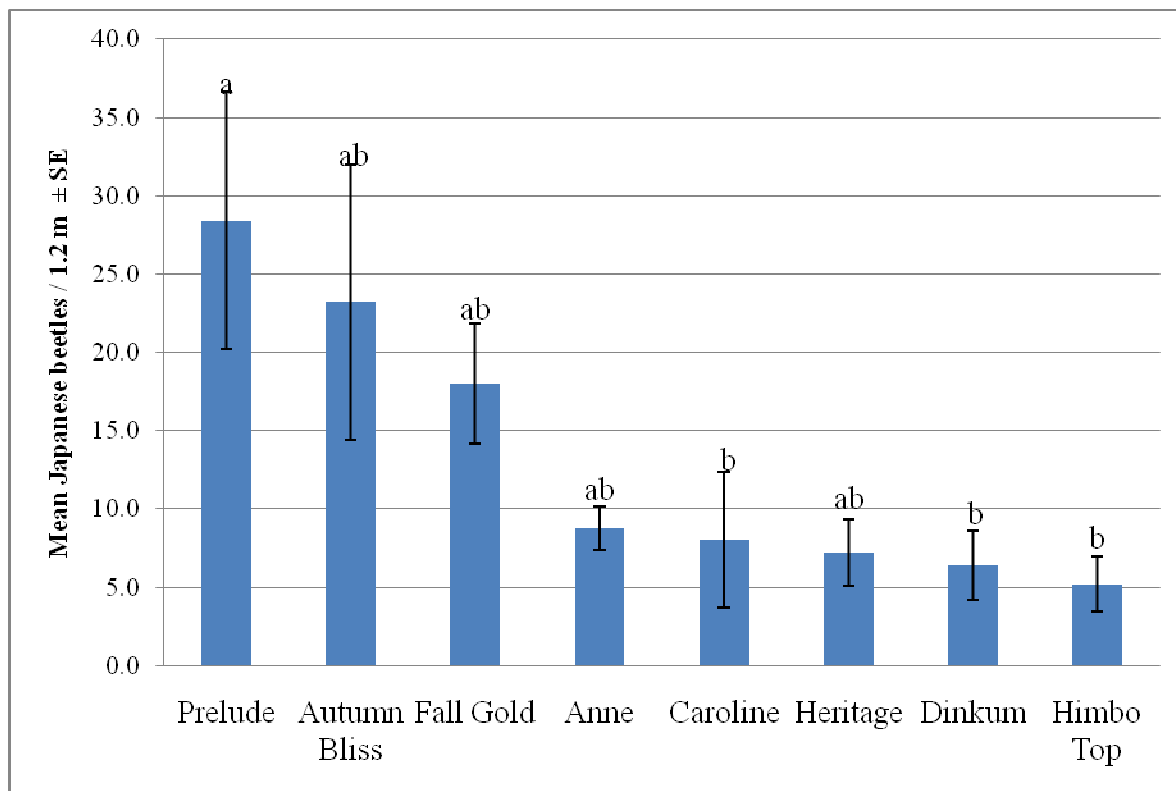


Figure 3.6 Mean total number of Japanese beetles counted per 1.2 m treated raspberry plot in 2008. Values followed by the same letter are not significantly different (Tukey's HSD ( $F = 3.21$ ;  $df = 7$ ;  $P = 0.0126$ ) following  $[\sqrt{(x + 0.5)}]$  transformation).

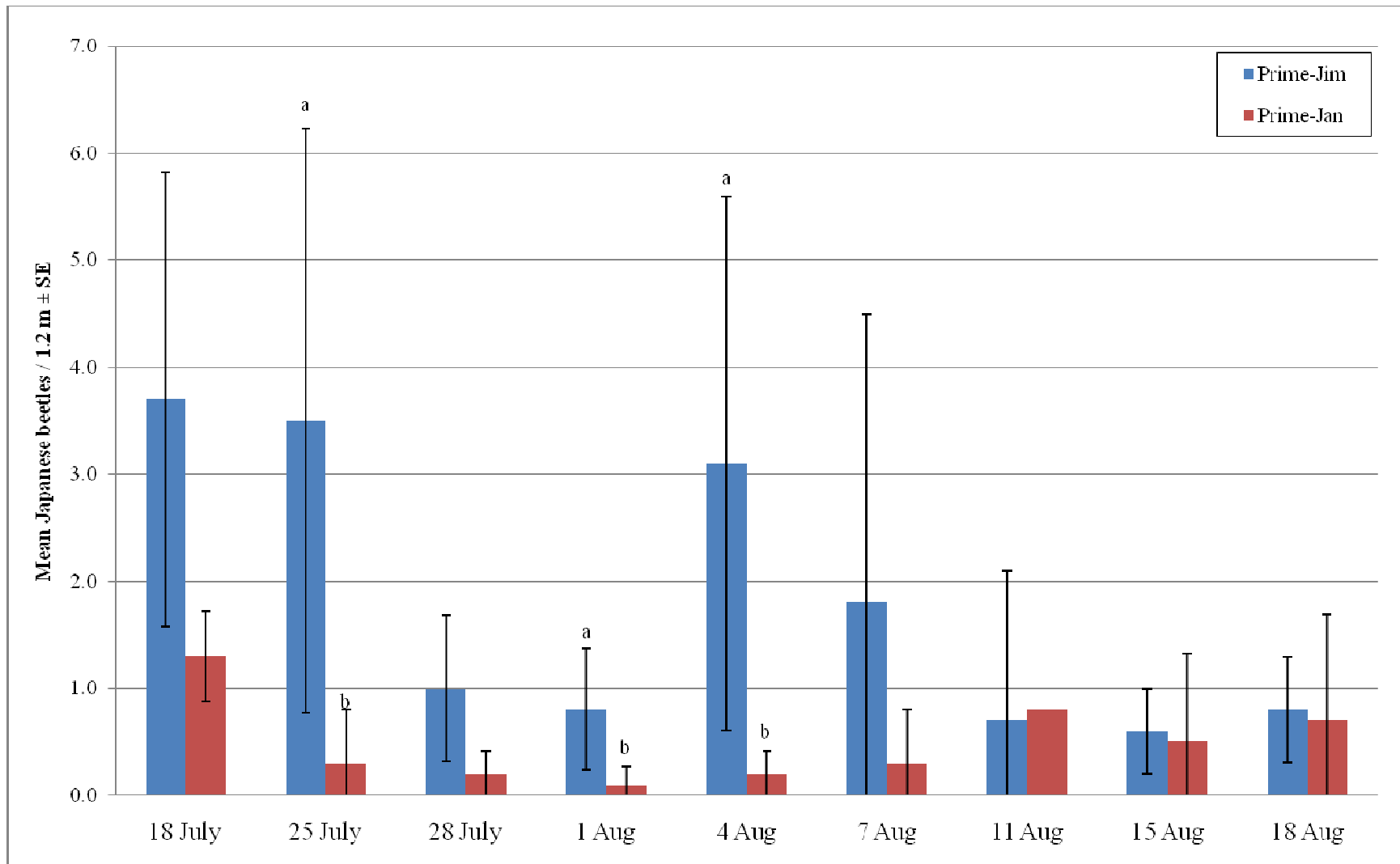


Figure 3.7 Mean number of Japanese beetles by date per 1.2 m plots in 'Prime-Jan' or 'Prime-Jim' blackberries in 2008. Only dates with significant differences include letters above the values and values followed by the same letter are not significantly different (Student's t-test ( $\alpha = 0.05$ ) following  $[\sqrt{(x + 0.5)}]$  transformation).

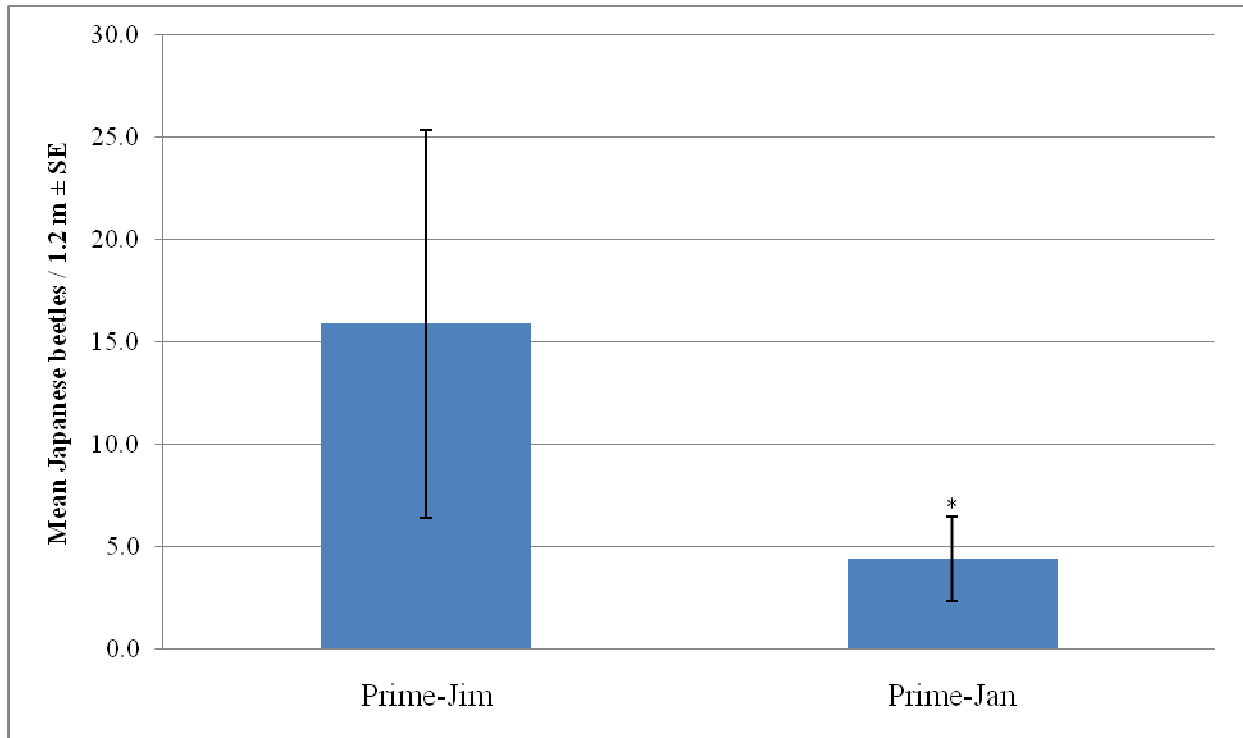


Figure 3.8 Mean total number of Japanese beetle counted per 1.2 m in 'Prime-Jan' or 'Prime-Jim' blackberries in 2008. Values are significantly different (Student's t-test ( $F = 27.26$ ;  $df = 1$ ;  $P = 0.0034$ ) following  $[\sqrt{(x + 0.5)}]$  transformation).

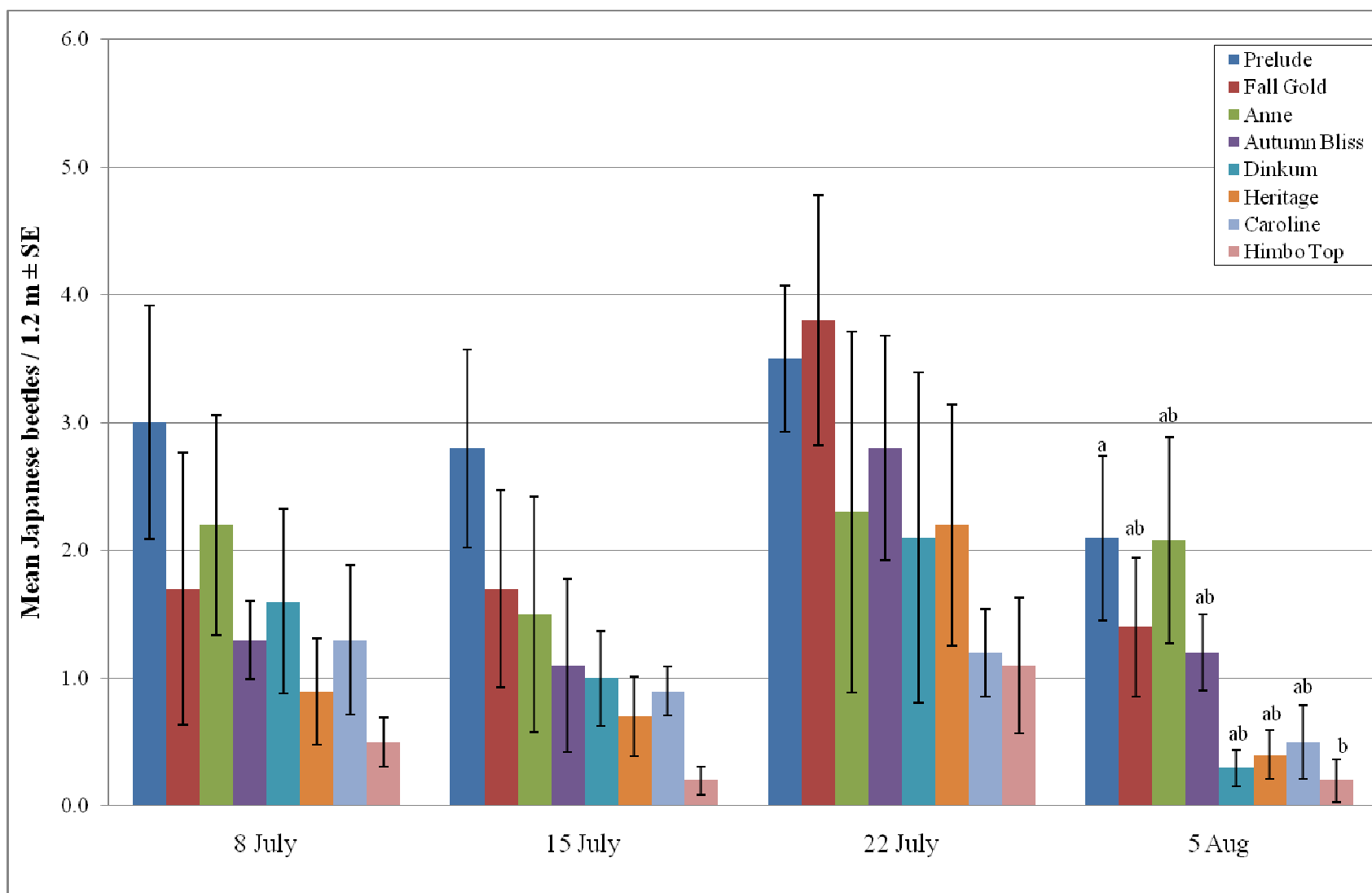


Figure 3.9 Mean number of Japanese beetles by date per 1.2 m plots of eight raspberry cultivars in 2009. Only dates with significant differences include letters above the values and values followed by the same letter are not significantly different (Tukey's HSD ( $\alpha = 0.05$ ) following  $[\sqrt{(x + 0.5)}]$  transformation).

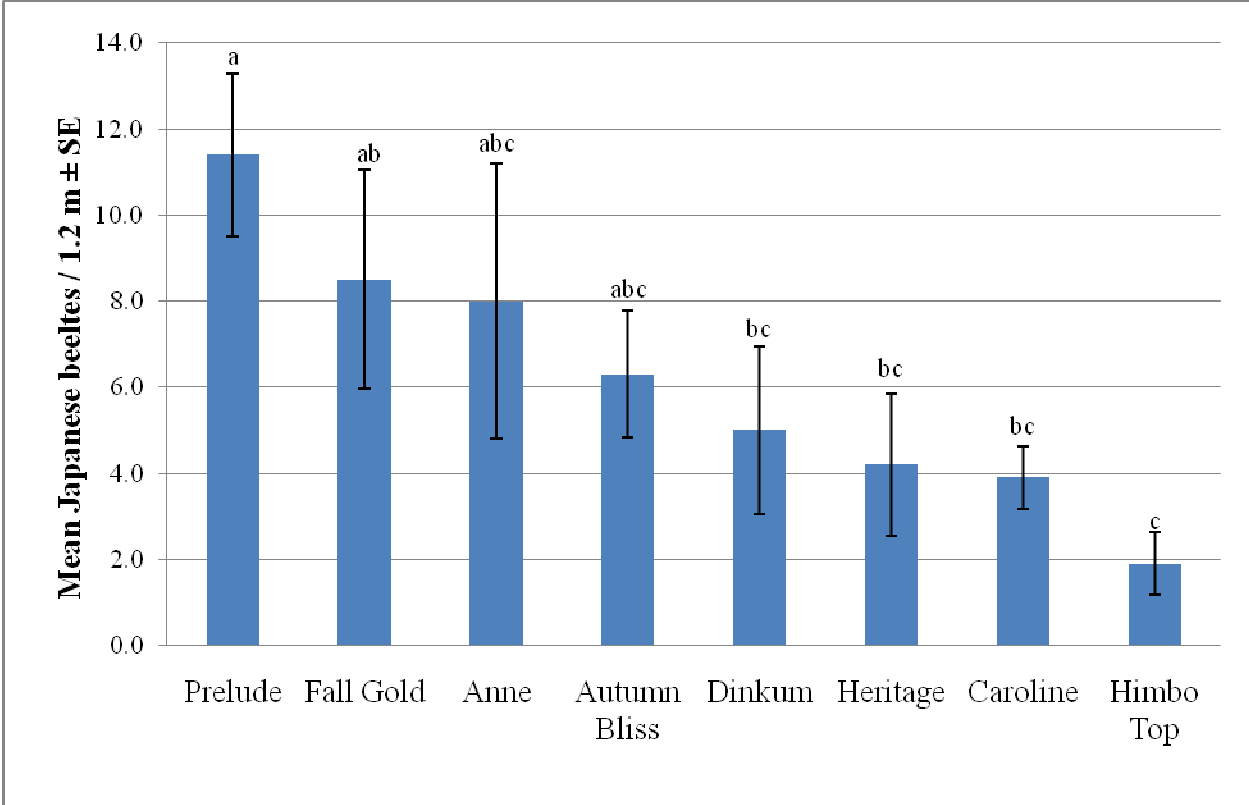


Figure 3.10 Mean total number of Japanese beetles counted per 1.2 m of eight raspberry cultivars in 2009. Values followed by the same letter are not significantly different (Tukey's HSD ( $F = 4.82$ ;  $df = 7$ ;  $P = 0.0004$ ) following  $[\sqrt{(x + 0.5)}]$  transformation).

Table 3.2 Mean marketable yield per m row of primocane-bearing raspberries per cultivar on individual dates 2007. Dates where significant differences were present are highlighted.

Cultivar	Marketable Yield (g/m)						
	14-Aug**	21-Aug**	24-Aug**	28-Aug**	31-Aug**	4-Sept*	7-Sept*
Anne	20.2 ± 10.5	50.7 ± 21.9	57.0 ± 21.7 b	79.2 ± 19.0 b	102.1 ± 31.1 b	95.2 ± 26.2 b	52.5 ± 13.0 b
Autumn Bliss	95.0 ± 24.8	133.5 ± 11.7	127.2 ± 27.4 ab	223.2 ± 14.7 a	161.2 ± 11.3 ab	221.2 ± 20.0 a	105.8 ± 9.0 a
Caroline	105.8 ± 35.8	167.8 ± 44.4	136.3 ± 21.2 ab	181.2 ± 30.7 ab	164.5 ± 21.4 ab	183 ± 28.0 ab	53.3 ± 8.6 b
Dinkum	26.0 ± 7.5	50.3 ± 11.8	93.0 ± 17.6 ab	112.3 ± 11.7 ab	98.0 ± 12.1 b	148.5 ± 23.0 ab	64.2 ± 5.4 ab
Fall Gold	25.7 ± 7.2	74.7 ± 15.6	95.3 ± 17.2 ab	119.0 ± 31.5 ab	121.5 ± 20.2 b	128.8 ± 17.9 ab	60.3 ± 11.6 ab
Heritage	47.3 ± 25.0	157.5 ± 58.1	181.2 ± 38.7 a	225.8 ± 40.0 a	233.3 ± 33.4 a	195.2 ± 26.6 ab	83.2 ± 16.0 ab
Himbo Top	22.5 ± 6.9	52.7 ± 12.1	79.3 ± 11.5 ab	156.7 ± 21.2 ab	110.2 ± 14.5 b	233.5 ± 37.1 a	77.8 ± 10.4 ab
Prelude	28.3 ± 16.5	58.5 ± 20.7	98.3 ± 33.4 ab	135.0 ± 45.2 ab	134.2 ± 23.8 ab	188.5 ± 23.0 ab	72.5 ± 9.7 ab
	11-Sept**	14-Sept*	18-Sept**	21-Sept**	25-Sept**	28-Sept*	2-Oct*
Anne	66.3 ± 5.0 bc	113.2 ± 14.9	54.2 ± 9.2 b	48.8 ± 8.1 b	77.0 ± 9.3 ab	48.2 ± 12.8	35.5 ± 5.8
Autumn Bliss	131.2 ± 12.6 a	151.8 ± 8.5	103.8 ± 13.0 a	93.7 ± 5.9 a	110.2 ± 19.1 a	35.0 ± 7.8	25.2 ± 9.2
Caroline	62.3 ± 10.9 c	134.5 ± 14.6	83.3 ± 6.6 ab	52.0 ± 7.5 b	57.8 ± 10.8 b	26.2 ± 8.0	36.0 ± 7.8
Dinkum	76.3 ± 13.0 abc	98.7 ± 13.1	75.0 ± 7.6 ab	57.5 ± 7.6 ab	68.8 ± 12.2 ab	38.8 ± 11.1	27.3 ± 6.3
Fall Gold	85.5 ± 16.6 abc	92.3 ± 14.1	58.2 ± 7.6 ab	48.0 ± 6.2 b	54.5 ± 12.0 b	29.0 ± 7.5	21.8 ± 6.5
Heritage	88.3 ± 13.0 abc	165.7 ± 19.0	99.5 ± 14.1 ab	54.3 ± 10.2 b	48.5 ± 10.0 b	22.8 ± 5.5	39.8 ± 5.3
Himbo Top	126.2 ± 13.2 ab	143.8 ± 31.0	86.2 ± 12.7 ab	74.8 ± 6.0 ab	84.8 ± 6.4 ab	36.7 ± 7.8	35.5 ± 3.6
Prelude	71.8 ± 7.3 abc	129.2 ± 17.6	66.0 ± 8.8 ab	52.8 ± 9.0 b	55.5 ± 8.3 b	42.2 ± 8.1	20.5 ± 2.4

\* Yields followed by the same letter on the same date are not significantly different (Tukey's HSD ( $\alpha = 0.05$ )).

\*\* Yields followed by the same letter on the same date are not significantly different (Tukey's HSD ( $\alpha = 0.05$ ) following  $[\sqrt{(x + 0.5)}]$  transformation).



Table 3.3 Summary of significant differences in marketable yields among raspberry cultivars in 2007.

<b>Date (2007)</b>	<b>Significant difference in marketable yield between:</b>
24 August	'Heritage' > 'Anne'
28 August	'Autumn Bliss' and 'Heritage' > 'Anne'
31 August	'Heritage' > Anne', 'Dinkum', 'Fall Gold', and 'Himbo Top'
4 September	'Autumn Bliss' and 'Himbo Top' > 'Anne'
7 September	'Autumn Bliss' > 'Anne' and 'Caroline'
11 September	'Autumn Bliss' > 'Anne' and 'Caroline' 'Himbo Top' > 'Caroline'
18 September	'Autumn Bliss' > 'Anne'
21 September	'Autumn Bliss' > 'Anne', 'Caroline', 'Fall Gold', 'Heritage', and 'Prelude'
25 September	'Autumn Bliss' > 'Caroline', 'Fall Gold', 'Heritage', and 'Prelude'

Table 3.4 Mean unmarketable yield per m row of eight raspberry cultivars on individual dates in 2007. Dates where significant differences were present are highlighted.

Cultivar	Unmarketable Yield (g/m)*						
	14-Aug	21-Aug	24-Aug	28-Aug	31-Aug	4-Sep	7-Sep
Anne	5.7 ± 2.2 c	17.0 ± 4.6 ab	15.0 ± 3.0 bc	29.2 ± 4.2 b	24.2 ± 4.7 bc	36.5 ± 11.7 bc	9.5 ± 2.9 abc
Autumn Bliss	9.0 ± 1.9 abc	27.2 ± 6.9 ab	19.3 ± 6.8 bc	44.5 ± 6.8 ab	20.7 ± 6.2 c	39.5 ± 5.4 abc	8.7 ± 2.6 abc
Caroline	24.5 ± 6.4 ab	52.5 ± 14.6 a	38.0 ± 7.3 ab	72.5 ± 22.3 ab	56.3 ± 5.9 ab	63.2 ± 9.9 ab	18.2 ± 5.0 a
Dinkum	8.3 ± 4.8 bc	22.3 ± 7.5 ab	19.5 ± 2.7 abc	31.7 ± 10.2 b	20.8 ± 4.8 bc	23.7 ± 4.6 c	6.0 ± 2.8 bc
Fall Gold	2.5 ± 0.8 c	10.8 ± 3.5 b	12.2 ± 3.0 c	69.7 ± 22.8 ab	16.0 ± 5.5 c	47.2 ± 12.4 abc	4.2 ± 1.5 c
Heritage	30.5 ± 10.4 a	54.2 ± 10.3 a	42.5 ± 5.6 a	102.7 ± 10.0 a	74.5 ± 19.3 a	74.0 ± 10.7 a	19.2 ± 6.7 ab
Himbo Top	2.2 ± 1.4 c	10.8 ± 2.4 b	16.5 ± 4.7 bc	19.2 ± 4.8 b	25.2 ± 4.3 bc	40.3 ± 8.4 abc	12.5 ± 3.3 abc
Prelude	1.8 ± 1.8 c	23.3 ± 9.0 ab	15.8 ± 2.7 bc	47.3 ± 9.6 ab	23.0 ± 2.4 bc	42.3 ± 8.0 abc	10.5 ± 3.0 abc
	11-Sep	14-Sep	18-Sep	21-Sep	25-Sep	28-Sep	2-Oct
Anne	8.3 ± 1.7 bc	13.8 ± 3.4 ab	7.8 ± 1.8 ab	13.5 ± 4.8 ab	13.8 ± 4.8	11.3 ± 4.2	6.2 ± 1.6 ab
Autumn Bliss	13.5 ± 1.3 abc	19.5 ± 3.0 ab	16.8 ± 2.8 ab	11.2 ± 3.1 ab	12.3 ± 4.1	6.7 ± 2.3	5.2 ± 1.9 ab
Caroline	25.3 ± 2.3 a	27.5 ± 6.7 ab	10.7 ± 4.2 ab	14.7 ± 2.8 ab	13.7 ± 3.5	6.7 ± 2.5	9.5 ± 2.9 ab
Dinkum	11.5 ± 2.8 abc	18.2 ± 4.5 ab	18.7 ± 6.5 ab	10.0 ± 2.4 ab	16.3 ± 3.1	7.3 ± 1.9	6.7 ± 2.5 ab
Fall Gold	5.8 ± 2.0 c	9.3 ± 2.4 b	3.2 ± 0.5 b	7.0 ± 1.6 ab	6.5 ± 1.9	3.3 ± 1.2	2.0 ± 0.6 b
Heritage	23.7 ± 3.8 ab	34.5 ± 8.3 ab	23.5 ± 3.9 a	15.2 ± 1.2 a	6.3 ± 1.3	6.7 ± 1.9	8.5 ± 3.0 ab
Himbo Top	25.7 ± 8.4 ab	39.1 ± 8.3 a	20.3 ± 5.4 a	17.0 ± 2.3 a	13.4 ± 5.2	7.0 ± 1.7	11.2 ± 2.3 a
Prelude	10.7 ± 3.3 abc	20.3 ± 4.9 ab	7.0 ± 0.6 ab	6.0 ± 2.1 b	5.2 ± 1.3	5.5 ± 2.4	4.7 ± 1.3 ab

\* Unmarketable yields in same column are not significantly different (Tukey's HSD ( $\alpha = 0.05$ ) following  $[\sqrt{(x + 0.5)}]$  transformation)

\*\* The total percent unmarketable values followed by the same letter are not significantly different (Tukey's HSD ( $\alpha = 0.05$ ))

Table 3.5 Summary of significant differences in unmarketable yields among raspberry cultivars in 2007.

<b>Date (2007)</b>	<b>Significant difference in unmarketable yield between:</b>
14 August	'Heritage' > 'Anne', 'Dinkum', 'Fall Gold', 'Himbo Top', and 'Prelude' 'Caroline' > 'Anne', 'Fall Gold', 'Prelude', and 'Himbo Top'
21 August	'Heritage' and 'Caroline' > 'Fall Gold' and 'Himbo Top'
24 August	'Heritage' > 'Anne', 'Autumn Bliss', 'Fall Gold', 'Himbo Top', and 'Prelude' 'Caroline' > 'Fall Gold'
28 August	'Heritage' > 'Anne', 'Dinkum' and 'Himbo Top'
31 August	'Heritage' > 'Anne', 'Autumn Bliss', 'Dinkum', 'Fall Gold', 'Himbo Top', and 'Prelude' 'Caroline' > 'Autumn Bliss' and 'Fall Gold'
4 September	'Heritage' > 'Anne' and 'Dinkum' 'Caroline' > 'Dinkum'
7 September	'Caroline' > 'Dinkum' and 'Fall Gold' 'Heritage' > 'Fall Gold'
11 September	'Caroline' > 'Anne' and 'Fall Gold' 'Heritage' and 'Himbo Top' > 'Fall Gold'
14 September	'Himbo Top' > 'Fall Gold'
18 September	'Heritage' and 'Himbo Top' > 'Fall Gold'
21 September	'Heritage' and 'Himbo Top' > 'Prelude'
2 October	'Himbo Top' > 'Fall Gold'

Table 3.6 Mean total grams of raspberries that were marketable and unmarketable. Also listed are the total yields harvested and total percent of the fruit that was unmarketable for 2007.

Cultivar	Yield (g/m)			
	Total Marketable *	Total Unmarketable **	Total Harvested ***	Total % Unmarketable ****
Anne	899.9 ± 150.7 c	211.8 ± 37.6 b	1111.8 ± 181.3 d	19.0 ± 1.6 abc
Autumn Bliss	1717.8 ± 49.9 a	254.0 ± 20.2 b	1971.8 ± 61.1 ab	12.8 ± 0.8 c
Caroline	1444.2 ± 155.3 abc	433.2 ± 54.8 a	1877.4 ± 198.1 abc	23.2 ± 1.7 ab
Dinkum	1034.8 ± 103.8 bc	221.0 ± 28.5 b	1255.9 ± 94.5 bcd	18.2 ± 2.6 abc
Fall Gold	1014.7 ± 142.7 bc	199.7 ± 38.6 b	1214.4 ± 172 cd	16.4 ± 1.6 bc
Heritage	1642.5 ± 209.2 ab	515.8 ± 37.0 a	2158.4 ± 244.2 a	24.5 ± 1.1 a
Himbo Top	1320.7 ± 96.6 abc	260.3 ± 18.4 b	1581 ± 109.9 abcd	16.6 ± 0.8 bc
Prelude	1124.0 ± 177.2 abc	219.6 ± 23.0 b	1343.6 ± 191 bcd	17.1 ± 2.1 bc

\* Values followed by the same letter in same column are not significantly different (Tukey's HSD ( $F = 5.05$ ;  $df = 7$ ;  $P = 0.0005$ )).

\*\* Values followed by the same letter in same column are not significantly different (Tukey's HSD ( $F = 11.31$ ;  $df = 7$ ;  $P < 0.0001$ )).

\*\*\* Values followed by the same letter in same column are not significantly different (Tukey's HSD ( $F = 6.06$ ;  $df = 7$ ;  $P = 0.0001$ )).

\*\*\*\* Values followed by the same letter in same column are not significantly different (Tukey's HSD ( $F = 5.57$ ;  $df = 7$ ;  $P = 0.0002$ )).

Table 3.7 Mean marketable yield per m row of eight raspberry cultivars on individual dates in 2007. Dates where significant differences were present are highlighted.

Cultivar	Marketable Yield (g/m) *						
	21-Aug	24-Aug	28-Aug	31-Aug	4-Sep	7-Sep	11-Sep
Prime-Jan	69.5 ± 12.6	29.1 ± 7.4 b	88.3 ± 16.4	81.8 ± 7.7 a	81.8 ± 13.8 a	29.6 ± 7.7	28.0 ± 6.5
Prime-Jim	73.3 ± 23.4	77.2 ± 19.6 a	124.3 ± 26.5	52.1 ± 12.4 a	44.6 ± 9.1 b	20.7 ± 5.5	15.9 ± 4.0

\* Values in the same column followed by the same letter are not significantly different (Student's t-test ( $\alpha = 0.05$ ) following  $[\sqrt{(x + 0.5)}]$  transformation).

Table 3.8 Summary of significant differences in marketable yields among blackberry cultivars in 2007.

Date (2007)	Significant difference in marketable yield between:
24 August	'Prime-Jim' > 'Prime-Jan'
31 August	'Prime-Jan' > 'Prime-Jim'
4 September	'Prime-Jan' > 'Prime-Jim'

Table 3.9 Mean unmarketable yield per m row of ‘Prime-Jan’ and ‘Prime-Jim’ blackberries on individual dates in 2007. Dates where significant differences were present are highlighted.

Unmarketable Yield (g/m) *							
Cultivar	21-Aug	24-Aug	28-Aug	31-Aug	4-Sep	7-Sep	11-Sep
Prime-Jan	112 ± 24.4 a	82.2 ± 16.6 a	162.2 ± 39.7 a	92.5 ± 8.1 a	38.8 ± 8.2 a	18.5 ± 6.5	14.2 ± 3.3 a
Prime-Jim	19.3 ± 5.1 b	30.0 ± 6.4 b	46.9 ± 18.3 b	7.5 ± 2.1 b	0.9 ± 0.4 b	6.3 ± 2.2	4.3 ± 1.8 b

\* Unmarketable yields in same column followed by the same letter are not significantly different (Student's t-test ( $\alpha = 0.05$ ) following  $[\sqrt{(x + 0.5)}$  transformation).

Table 3.10 Summary of significant differences in unmarketable yields among blackberry cultivars in 2007.

Date (2007)	Significant difference in unmarketable yield between:
21 August	‘Prime-Jan’ > ‘Prime-Jim’
24 August	‘Prime-Jan’ > ‘Prime-Jim’
28 August	‘Prime-Jan’ > ‘Prime-Jim’
31 August	‘Prime-Jan’ > ‘Prime-Jim’
4 September	‘Prime-Jan’ > ‘Prime-Jim’
11 September	‘Prime-Jan’ > ‘Prime-Jim’

Table 3.11 Mean total grams of blackberries that were marketable and unmarketable. Also listed are the total yields harvested and total percent of the fruit that was unmarketable for 2007.

Cultivar	Yields (g/m)			
	Total Marketable *	Total Unmarketable **	Total Harvested ***	Total % Unmarketable ****
Prime-Jan	204.0 ± 17.2	260.2 ± 33.2 a	464.2 ± 37.5 a	55.0 ± 3.3 a
Prime-Jim	204.0 ± 41.3	57.5 ± 9.3 b	261.5 ± 42.3 b	27.0 ± 4.4 b

\* Values are not significantly different (ANOVA ( $\alpha = 0.05$ ;  $F = 0.00$ ;  $df = 1$ ;  $P = 0.9993$ )).

\*\*Values followed by the same letter are not significantly different (Student's t-test ( $F = 71.19$ ;  $df = 1$ ;  $P < 0.0001$ ) following ( $\sqrt{x} + 0.5$ ) transformation).

\*\*\*Values followed by the same letter are not significantly different (Student's t-test ( $F = 35.44$ ;  $df = 1$ ;  $P < 0.0001$ ) following ( $\sqrt{x} + 0.5$ ) transformation).

\*\*\*\* Mean percent unmarketable yields followed by the same letter are not significantly different (Student's t-test ( $F = 40.14$ ;  $df = 1$ ;  $P < 0.0001$ ) following [ArcSine ( $\sqrt{x}$ )] transformation).

Table 3.12 Mean marketable yield per m row of eight raspberry cultivars on individual dates in 2008. Dates where significant differences were present are highlighted.

Cultivar	Marketable Yield (g/m)									
	22-Aug **	26-Aug **	29-Aug**	2-Sept**	12-Sept *	16-Sept **	19-Sept *	23-Sept **	30-Sept *	7-Oct *
Anne	26.3 ± 18.0 b	46.7 ± 24.3	44.7 ± 15.3 b	5.7 ± 3.7 d	151.3 ± 41.3	118.3 ± 34.2 ab	57.7 ± 10.5	54.7 ± 15.1	54.0 ± 11.1	38.7 ± 9.8
Autumn Bliss	100.7 ± 37.7 a	133.3 ± 31.6	184.3 ± 38.0 a	76.0 ± 7.9 a	261.3 ± 21.3	142.8 ± 22.0 ab	101.3 ± 16.2	118.0 ± 28.9	68.3 ± 13.7	37.0 ± 6.7
Caroline	76.3 ± 23.7 ab	85.7 ± 23.0	148.0 ± 34.2 ab	57.7 ± 16.4 ab	255.7 ± 38.9	105.8 ± 16.4 ab	109.7 ± 13.1	91.0 ± 18.9	105.0 ± 19.5	34.7 ± 5.5
Dinkum	64.3 ± 19.0 ab	116.7 ± 24.3	127.7 ± 27.7 ab	76.0 ± 9.3 a	202.7 ± 26.2	84.8 ± 6.6 ab	119.0 ± 21.8	55.3 ± 4.8	72.7 ± 11.3	45.0 ± 8.0
Fall Gold	24.7 ± 7.0 b	32.2 ± 19.9	48.3 ± 11.6 b	36.0 ± 8.7 abc	180.0 ± 30.4	81.7 ± 10.4 b	97.7 ± 17.7	75.7 ± 10.8	50.7 ± 12.1	28.3 ± 9.8
Heritage	54.0 ± 13.3 ab	99.7 ± 35.0	71.3 ± 7.0 ab	66.0 ± 2.7 a	242.3 ± 28.0	174.7 ± 14.0 a	125.7 ± 18.1	121.0 ± 18	100.7 ± 11.4	46.0 ± 10.3
Himbo Top	19.0 ± 7.6 b	40.0 ± 12.9	62.0 ± 32.3 b	12.7 ± 6.2 cd	143.0 ± 29.4	86.8 ± 23.6 b	119.7 ± 14.7	89.7 ± 17.7	87.0 ± 18.2	32.7 ± 6.3
Prelude	14.7 ± 8.8 b	46.7 ± 20.0	42.3 ± 13.2 b	23.7 ± 6.1 bcd	131.0 ± 19.9	81.0 ± 6.3 b	84.0 ± 16.0	63.0 ± 10.1	50.7 ± 13.3	28.7 ± 9.9

\* The cultivar source of variation was not significant (AVOVA ( $P > 0.05$ )).

\*\* Marketable yields in same column followed by the same letter are not significantly different (Tukey's HSD ( $\alpha = 0.05$ ) following  $[\sqrt{(x + 0.5)}]$  transformation).

Table 3.13 Summary of significant differences in marketable yields among raspberry cultivars in 2008.

Date (2008)	Significant difference in marketable yield between:
22 August	'Autumn Bliss' > 'Anne', 'Fall Gold', 'Himbo Top', and 'Prelude'
29 August	'Autumn Bliss' > 'Anne', 'Fall Gold', 'Himbo Top', and 'Prelude'
2 September	'Autumn Bliss', 'Dinkum', and 'Heritage' > 'Anne', 'Himbo Top', and 'Prelude' 'Caroline' > 'Anne' and 'Himbo Top' 'Fall Gold' > 'Anne'
16 September	'Heritage' > 'Fall Gold', 'Himbo Top', and 'Prelude'



Table 3.14 Mean unmarketable yield per m row of eight raspberry cultivars on individual dates in 2008. Dates in which significant differences were present are highlighted.

Cultivar	Unmarketable Yield (g/m)									
	22-Aug **	26-Aug **	29-Aug**	2-Sept*	12-Sept **	16-Sept *	19-Sept **	23-Sept **	30-Sept **	7-Oct **
Anne	4.7 ± 2.9 b	0.3 ± 0.3 b	13.0 ± 7.0	24.3 ± 14.2	40.3 ± 14.2	19.7 ± 4.0	10.3 ± 2.7	3.0 ± 1.0 b	27.3 ± 8.0	7.3 ± 2.1
Autumn Bliss	13.0 ± 3.4 ab	5.3 ± 2.3 ab	42.0 ± 8.5	70.7 ± 19.6	79.3 ± 13.1	30.7 ± 9.0	10.0 ± 2.4	5.7 ± 1.5 ab	34.0 ± 11.6	8.7 ± 2.1
Caroline	24.7 ± 7.7 a	22.8 ± 12.8 a	35.3 ± 7.8	48.7 ± 10.2	78.3 ± 20.9	23.7 ± 5.4	24.0 ± 10.0	16.0 ± 9.7 ab	31.7 ± 9.5	19.0 ± 6.8
Dinkum	8.7 ± 2.4 ab	18.7 ± 7.0 a	50.7 ± 19.3	42.3 ± 13.3	66.7 ± 14.1	42.7 ± 6.0	15.0 ± 5.9	6.7 ± 2.4 ab	19.7 ± 3.9	14.3 ± 3.7
Fall Gold	8.7 ± 4.3 ab	4.3 ± 2.7 ab	17.0 ± 4.4	31.7 ± 12.6	43.0 ± 13.8	29.2 ± 6.5	28.7 ± 8.2	24.7 ± 6.3 a	23.0 ± 8.9	18.0 ± 5.9
Heritage	15.7 ± 4.1 ab	11.3 ± 2.7 ab	37.7 ± 6.0	73.0 ± 15.9	88.7 ± 18.5	24.5 ± 4.4	10.3 ± 2.8	6.0 ± 1.8 ab	29.3 ± 5.3	14.0 ± 2.7
Himbo Top	2.0 ± 1.6 b	3.7 ± 2.5 ab	23.0 ± 8.5	42.3 ± 12.0	39.3 ± 15.0	19.2 ± 2.3	9.7 ± 2.7	8.0 ± 2.9 ab	33.3 ± 6.5	16.3 ± 2.5
Prelude	8.7 ± 5.8 ab	2.8 ± 1.3 ab	32.3 ± 9.4	42.3 ± 13.3	49.3 ± 26.9	17.5 ± 3.2	9.7 ± 4.8	3.0 ± 1.3 b	12.0 ± 4.3	6.3 ± 2.1

\* Unmarketable yields in a column followed by the same letter are not significantly different (Tukey's HSD ( $\alpha = 0.05$ )).

\*\* Unmarketable yields in a column followed by the same letter are not significantly different (Tukey's HSD ( $\alpha = 0.05$ ) following  $[\sqrt{(x + 0.5)}]$  transformation).

Table 3.15 Summary of significant differences in unmarketable yields among raspberry cultivars in 2008.

Date (2008)	Significant difference in marketable yield between:
22August	'Caroline' > 'Anne' and 'Himbo Top'
26 August	'Caroline' and 'Dinkum' > 'Anne'
23 September	'Fall Gold' > 'Anne' and 'Prelude'

Table 3.16 Mean total grams of raspberries that were marketable and unmarketable. Also listed are the total yields harvested and total percent of the fruit that was unmarketable for 2008.

Cultivar	Yields (g/m)			
	Total Marketable*	Total Unmarketable **	Total Harvested***	Total % Unmarketable ****
Anne	598.0 ± 155.0 cd	150.3 ± 50.0 b	748.3 ± 203.6 b	19.1 ± 2.2
Autumn Bliss	1223.2 ± 49.6 a	299.3 ± 3.2 ab	1522.5 ± 52.3 a	19.7 ± 0.5
Caroline	1069.5 ± 146.7 abc	324.2 ± 46.1 a	1393.7 ± 191.5 ab	23.2 ± 0.6
Dinkum	964.2 ± 126.3 abcd	285.3 ± 61.1 ab	1249.5 ± 182.1 ab	22.1 ± 2.4
Fall Gold	655.2 ± 110.4 bcd	228.2 ± 55.4 ab	883.3 ± 156.3 ab	24.2 ± 3.4
Heritage	1101.3 ± 102.2 ab	310.5 ± 46.1 ab	1411.8 ± 145.3 ab	21.7 ± 1.3
Himbo Top	692.5 ± 119.1 bcd	196.8 ± 34.1 ab	889.3 ± 148.7 ab	22.4 ± 1.9
Prelude	565.7 ± 81.5 d	184.0 ± 57.8 ab	749.7 ± 135.7 b	22.2 ± 3.5

\* Values in same column followed by the same letter are not significantly different (Tukey's HSD ( $\alpha = 0.05$ ;  $F = 5.93$ ;  $df = 7$ ;  $P = 0.0003$ )).

\*\* Values in same column followed by the same letter are not significantly different (Tukey's HSD ( $\alpha = 0.10$ ;  $F = 2.20$ ;  $df = 7$ ;  $P = 0.0649$ )).

\*\*\* Values in same column followed by the same letter are not significantly different (Tukey's HSD ( $\alpha = 0.05$ ;  $F = 4.91$ ;  $df = 7$ ;  $P = 0.0010$ )).

\*\*\*\*The cultivar source of variation was not significant (ANOVA ( $\alpha = 0.05$ ;  $F = 0.54$ ;  $df = 7$ ;  $P = 0.7979$ )).

Table 3.17 Mean marketable yield per m row of two blackberry cultivars on individual dates in 2008.

Marketable Yield (g/m)									
Cultivar	22-Aug **	26-Aug **	29-Aug **	2-Sept **	12-Sept *	19-Sept *	23-Sept **	30-Sept **	7-Oct **
Prime-Jan	26.1 ± 7.8	45.6 ± 17.5	20.8 ± 9.2	37.5 ± 11.2	51.0 ± 10.5	36.7 ± 8.3	17.4 ± 5.4	18.2 ± 5.1	30.7 ± 12.7
Prime-Jim	31.1 ± 9.4	46.3 ± 13.6	20.3 ± 3.7	38.5 ± 8.5	82.2 ± 17.1	43.8 ± 7.2	11.4 ± 3.5	13.6 ± 3.1	7.5 ± 2.8

\* The cultivar source of variation was not significant according to ANOVA ( $P > 0.05$ ).

\*\* The cultivar source of variation was not significant according to ANOVA ( $P > 0.05$ ) following  $[\sqrt{(x + 0.5)}]$  transformation).

Table 3.18 Mean unmarketable yield per m row of two blackberry cultivars on individual dates in 2008. Dates in which significant differences were present are highlighted.

Cultivar	Unmarketable Yield (g/m)								
	22-Aug*	26-Aug*	29-Aug*	2-Sep*	12-Sept **	19-Sept **	23-Sep*	30-Sep*	7-Oct*
Prime-Jan	18.2 ± 5.7	8.6 ± 3.0	4.9 ± 2.3	40.0 ± 10.1 a	43.8 ± 8.8 a	44.4 ± 12.9 a	5.6 ± 2.7	8.5 ± 2.6	6.8 ± 2.5
Prime-Jim	10.6 ± 3.9	5.4 ± 2.1	1.7 ± 0.6	14.2 ± 3.8 b	18.8 ± 3.6 b	14.6 ± 3.3 b	1.8 ± 0.6	4.7 ± 1.8	3.9 ± 1.3

\* Mean yield values in the same column followed by the same letter are not significantly different (Student's t-test ( $\alpha = 0.05$ )).

\*\* Mean yield values in the same column followed by the same letter are not significantly different (Student's t-test ( $\alpha = 0.05$ ) following [ $\sqrt{(x + 0.5)}$ ] transformation).

Table 3.19 Summary of significant differences in unmarketable yields among blackberry cultivars in 2008.

Date (2007)	Significant difference in unmarketable yield between:
2 September	'Prime-Jan' > 'Prime-Jim'
12 September	'Prime-Jan' > 'Prime-Jim'
19 September	'Prime-Jan' > 'Prime-Jim'

Table 3.20 Mean total grams of blackberries that were marketable and unmarketable. Also listed are the total yields harvested and total percent of the fruit that was unmarketable for 2008.

	Total Marketable *	Total Unmarketable *	Total Harvested **	Total % Unmarketable ***
<b>Cultivar</b>				
Prime-Jan	283.9 ± 35.6	180.7 ± 27.8 a	464.6 ± 58.3 a	38.4 ± 3.2 a
Prime-Jim	294.7 ± 21.3	75.6 ± 13.6 b	370.2 ± 27.1 b	19.8 ± 2.9 b

\* The cultivar source of variation was not significant according to ANOVA ( $\alpha = 0.05$ ;  $F = 0.28$ ;  $df = 1$ ;  $P = 0.6052$ ).

\*\* Values followed by the same letter are not significantly different (Student's t-test ( $\alpha = 0.05$ ;  $F = 17.65$ ;  $df = 1$ ;  $P = 0.0012$ )).

\*\*\* The cultivar source of variation was significant according to ANOVA ( $\alpha=0.10$ ;  $F = 3.44$ ;  $df = 1$ ;  $P = 0.0883$ ).

\*\*\* Values followed by the same letter are not significantly different (Student's t-test ( $\alpha = 0.05$ ;  $F = 21.00$ ;  $df = 1$ ;  $P = 0.0006$ ) following [ArcSine ( $\sqrt{x}$ )] transformation).

Table 3.21 Mean marketable yield per m row of eight raspberry cultivars on individual dates in 2009. Dates in which significant differences were present are highlighted.

Cultivar	Marketable Yield (g/m)					
	6-Aug **	10-Aug **	13 Aug **	17 -Aug**	20-Aug **	24 Aug *
Anne	56.5 ± 13.1 b	76.7 ± 16.3 c	108.6 ± 21.3 c	127.2 ± 25.8 b	162.1 ± 34.2	157.9 ± 21.7 c
Autumn Bliss	136.1 ± 23.0 a	235.8 ± 23.5 a	256.1 ± 16.6 a	265.7 ± 25.6 a	180.4 ± 18.0	172.9 ± 18.9 c
Caroline	76 ± 17.1 ab	165.1 ± 20.9 ab	202.1 ± 20.2 ab	191.5 ± 27.8 ab	187.1 ± 18.9	283.9 ± 29.1 ab
Dinkum	76.0 ± 14.6 ab	144 ± 18.9 abc	183.3 ± 29.0 abc	185.7 ± 18.8 ab	195.8 ± 18.2	211.3 ± 18.8 bc
Fall Gold	61.1 ± 11.1 b	118.1 ± 20.5 bc	171.9 ± 30.7 abc	201.0 ± 35.0 ab	171.1 ± 28.1	189.0 ± 27.9 c
Heritage	28.7 ± 4.7 b	110 ± 8.5 bc	138.6 ± 18.3 bc	202.1 ± 23.1 ab	250.3 ± 21.8	328.3 ± 30.5 a
Himbo Top	50.1 ± 13.2 b	112.1 ± 16 bc	131.9 ± 11.1 bc	151.5 ± 24.5 b	217.9 ± 26.7	239.4 ± 21.8 abc
Prelude	51.5 ± 8.6 b	121.9 ± 16.8 bc	141.9 ± 17.7 bc	152.4 ± 17.2 b	172.2 ± 20.1	177.8 ± 13.6 c
	27-Aug **	31-Aug **	3-Sept **	10-Sept **	17-Sept **	
Anne	105.0 ± 20.9 b	123.2 ± 24.0 b	78.1 ± 17.7 b	90.1 ± 21.1 b	30.0 ± 5.5 cd	
Autumn Bliss	87.8 ± 13.9 b	105.7 ± 11.1 b	59.6 ± 9.6 b	69.6 ± 15.4 b	20.6 ± 3.4 d	
Caroline	127.8 ± 14.0 ab	164.6 ± 22.8 ab	114.4 ± 20.6 ab	114.6 ± 18.0 ab	58.2 ± 11.1 abcd	
Dinkum	113.3 ± 10.2 ab	137.6 ± 15.7 ab	83.6 ± 7.4 b	123.8 ± 14.5 ab	77.9 ± 12.6 ab	
Fall Gold	123.1 ± 16.3 ab	108.8 ± 14.4 b	70.6 ± 9.1 b	80.3 ± 10.9 b	39.0 ± 6.5 bcd	
Heritage	179.7 ± 17.8 a	215.1 ± 21.8 a	169.9 ± 21.2 a	186.8 ± 28.9 a	113.3 ± 21.1 a	
Himbo Top	120.3 ± 17.7 ab	205.6 ± 26.9 a	107.8 ± 12.5 ab	161.8 ± 31.3 ab	67.5 ± 14.5 abc	
Prelude	93.1 ± 15.9 b	116.3 ± 16.6 b	104.9 ± 22.4 ab	70.0 ± 8.5 b	35.0 ± 5.3 bcd	

\* Values from the same date followed by the same letter are not significantly different (Tukey's HSD ( $\alpha = 0.05$ )).

\*\* Values from the same date followed by the same letter are not significantly different (Tukey's HSD ( $\alpha = 0.05$ ) following  $[\sqrt{(x + 0.5)}]$  transformation).

Table 3.22 Summary of significant differences in marketable yields among raspberry cultivars in 2009.

<b>Date (2008)</b>	<b>Significant difference in marketable yield between:</b>
6 August	'Autumn Bliss' > 'Anne', 'Fall Gold', 'Heritage', 'Himbo Top', and 'Prelude'
10 August	'Autumn Bliss' > 'Anne', 'Fall Gold', 'Heritage', 'Himbo Top', and 'Prelude' 'Caroline' > 'Anne'
13 August	'Autumn Bliss' > 'Anne', 'Heritage', 'Himbo Top', and 'Prelude' 'Caroline' > 'Anne'
17 August	'Autumn Bliss' > 'Anne', 'Himbo Top', and 'Prelude'
24 August	'Heritage' > 'Anne', 'Autumn Bliss', 'Dinkum', 'Fall Gold', and 'Prelude' 'Caroline' > 'Anne', 'Autumn Bliss', 'Fall Gold', and 'Prelude'
27 August	'Heritage' > 'Anne', 'Autumn Bliss', and 'Prelude'
31 August	'Heritage' and 'Himbo Top' > 'Anne', 'Autumn Bliss', 'Fall Gold', and 'Prelude'
3 September	'Heritage' > 'Anne', 'Autumn Bliss', 'Dinkum', and 'Fall Gold'
10 September	'Heritage' > 'Anne', 'Autumn Bliss', 'Fall Gold', and 'Prelude'
17 September	'Heritage' > 'Anne', 'Autumn Bliss', 'Fall Gold', and 'Prelude' 'Dinkum' > 'Anne' and 'Autumn Bliss' 'Himbo Top' > 'Autumn Bliss'

Table 3.23 Mean unmarketable yield per m row of eight raspberry cultivars on individual dates in 2009. Dates in which significant differences were present are highlighted.

Cultivar	Unmarketable Yield (g/m)*					
	6-Aug	10-Aug	13-Aug	17-Aug	20-Aug	24-Aug
Anne	18.6 ± 5.9	20.0 ± 5.0 b	15.6 ± 5.7 b	22.8 ± 5.3 bc	20.6 ± 4.3 bc	40 ± 8.4 ab
Autumn Bliss	15.6 ± 4.1	27.4 ± 4.4 ab	26.3 ± 4.4 ab	23.5 ± 3.9 bc	20.4 ± 2.3 bc	26.0 ± 6.2 b
Caroline	18.3 ± 4.2	41.8 ± 4.9 a	46.7 ± 6.5 a	48.3 ± 6.5 a	59.4 ± 9.2 a	40.7 ± 5.0 ab
Dinkum	16.5 ± 5.1	28.3 ± 6.3 ab	17.1 ± 2.1 ab	16.8 ± 2.9 bc	24.6 ± 3.1 bc	27.2 ± 3.5 ab
Fall Gold	11.1 ± 4.5	22.1 ± 3.2 ab	16.7 ± 3.9 b	19.0 ± 5.5 bc	13.6 ± 2.7 c	40.7 ± 8.1 ab
Heritage	9.0 ± 1.7	29.3 ± 3.8 ab	39.3 ± 5.7 ab	32.1 ± 3.8 ab	57.8 ± 8.6 a	33.9 ± 6.1 ab
Himbo Top	10.6 ± 2.3	26.9 ± 4.6 ab	51.6 ± 26.3 ab	21.9 ± 7.9 bc	31.4 ± 4.1 b	47.4 ± 6.1 a
Prelude	16.4 ± 2.9	26.1 ± 3.0 ab	20.3 ± 5.6 ab	11.0 ± 1.8 c	19.2 ± 2.2 bc	27.6 ± 4.1 ab
	27-Aug	31-Aug	3-Sep	10-Sep	17-Sep	
Anne	24.2 ± 6.6 ab	17.7 ± 4.0 b	11.9 ± 3.8 ab	42.9 ± 11.5 b	7.5 ± 2.1 bc	
Autumn Bliss	10.4 ± 2.1 bc	14.4 ± 2.9 bc	4.0 ± 1.2 b	50.7 ± 7.7 ab	6.7 ± 1.9 c	
Caroline	20.3 ± 4.4 abc	36.9 ± 7.1 a	19.7 ± 3.1 a	40.0 ± 6.3 ab	26.0 ± 5.4 a	
Dinkum	22.4 ± 4.2 ab	15.1 ± 2.7 bc	10.7 ± 1.6 ab	41.3 ± 6.9 ab	20.0 ± 4.0 ab	
Fall Gold	16.4 ± 2.5 abc	13.1 ± 3.2 bc	7.1 ± 1.2 b	40.3 ± 4.1 ab	5.3 ± 1.8 c	
Heritage	12.4 ± 1.8 abc	17.1 ± 2.7 b	10.0 ± 2.5 ab	68.1 ± 13.2 ab	27.1 ± 6.0 a	
Himbo Top	28.3 ± 7.0 a	20.7 ± 4.7 ab	10.1 ± 2.7 ab	81.1 ± 14.1 a	22.9 ± 4.3 a	
Prelude	6.7 ± 2.0 c	5.7 ± 1.6 c	7.4 ± 1.8 b	28.9 ± 4.9 b	16.0 ± 3.6 abc	

\* Values from the same date followed by the same letter are not significantly different (Tukey's HSD ( $\alpha = 0.05$ ) following  $[\sqrt{(x + 0.5)}]$  transformation).



Table 3.24 Summary of significant differences in unmarketable yields among raspberry cultivars in 2009.

<b>Date (2008)</b>	<b>Significant difference in unmarketable yield between:</b>
10 August	'Caroline' > 'Anne'
13 August	'Caroline' > 'Anne' and 'Fall Gold'
17 August	'Caroline' > 'Anne', 'Autumn Bliss', 'Dinkum', 'Fall Gold', 'Himbo Top', and 'Prelude' 'Heritage' > 'Prelude'
20 August	'Caroline' and 'Heritage' > 'Anne', 'Autumn Bliss', 'Dinkum', 'Fall Gold', 'Himbo Top', and 'Prelude' 'Himbo Top' > 'Fall Gold'
24 August	'Himbo Top' > 'Autumn Bliss'
27 August	'Himbo Top' > 'Autumn Bliss' and 'Prelude' 'Anne' and 'Dinkum' > 'Prelude'
31 August	'Caroline' > 'Anne', 'Autumn Bliss', 'Dinkum', 'Fall Gold', 'Heritage', and 'Prelude' 'Himbo Top' > 'Prelude'
3 September	'Caroline' > 'Autumn Bliss', 'Fall Gold', and 'Prelude'
10 September	'Himbo Top' > 'Anne' and 'Prelude'
17 September	'Caroline', 'Heritage', and 'Himbo Top' > 'Anne', 'Autumn Bliss', and 'Fall Gold' 'Dinkum' > 'Autumn Bliss' and 'Fall Gold'

Table 3.25 Mean total grams of raspberries that were marketable and unmarketable. Also listed are the total yields harvested and total percent of the fruit that was unmarketable for 2009.

Cultivar	Yields (g/m)			
	Total Marketable *	Total Unmarketable **	Total Harvested ***	Total % Unmarketable ****
Anne	1115.4 ± 186.9 b	241.7 ± 43.3 bcd	1357.2 ± 226.9 c	18.0 ± 1.2 ab
Autumn Bliss	1590.3 ± 101.5 ab	225.3 ± 18.2 cd	1815.6 ± 111.9 abc	12.5 ± 0.8 c
Caroline	1685.3 ± 167.4 ab	398.2 ± 38.1 a	2083.5 ± 196.9 ab	19.4 ± 1.2 a
Dinkum	1532.4 ± 120.3 ab	240.0 ± 23.6 bcd	1772.4 ± 140.7 abc	13.5 ± 0.6 c
Fall Gold	1333.9 ± 162.7 b	205.3 ± 22.8 d	1539.2 ± 184.1 bc	13.7 ± 0.5 c
Heritage	1922.9 ± 143.4 a	335.9 ± 33.7 abc	2258.8 ± 172.3 a	14.8 ± 0.7 bc
Himbo Top	1566 ± 124.5 ab	353.0 ± 29.2 ab	1919.0 ± 139.9 abc	18.8 ± 1.3 ab
Prelude	1236.9 ± 111.8 b	185.1 ± 11.8 d	1422.1 ± 118.4 c	13.4 ± 0.9 c

\* Mean values followed by the same letter are not significantly different (Tukey's HSD ( $\alpha = 0.05$ ;  $F = 2.51$ ;  $df = 7$ ;  $P = 0.0276$ )).

\*\* Mean values followed by the same letter are not significantly different (Tukey's HSD ( $\alpha = 0.05$ ;  $F = 5.37$ ;  $df = 7$ ;  $P = 0.0001$ ) following [ $\sqrt{(x + 0.5)}$ ] transformation).

\*\*\* Mean values followed by the same letter are not significantly different (Tukey's HSD ( $\alpha = 0.05$ ;  $F = 2.76$ ;  $df = 7$ ;  $P = 0.0170$ )).

\*\*\*\* Mean percent unmarketable yields followed by the same letter are not significantly different (Tukey's HSD ( $\alpha = 0.05$ ;  $F = 7.26$ ;  $df = 7$ ;  $P < 0.0001$ ) following [ArcSine ( $\sqrt{x}$ )] transformation).

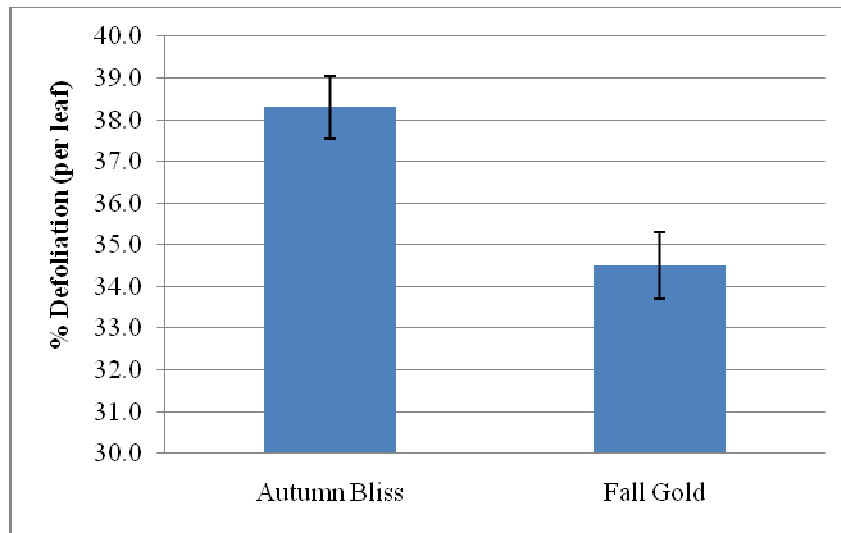


Figure 3.11 The estimated year total defoliation from Japanese beetles of eight raspberry cultivars in 2007. The cultivar source of variation was not significant according to ANOVA ( $F = 1.71$ ;  $df = 1$ ;  $P = 0.5457$ ).

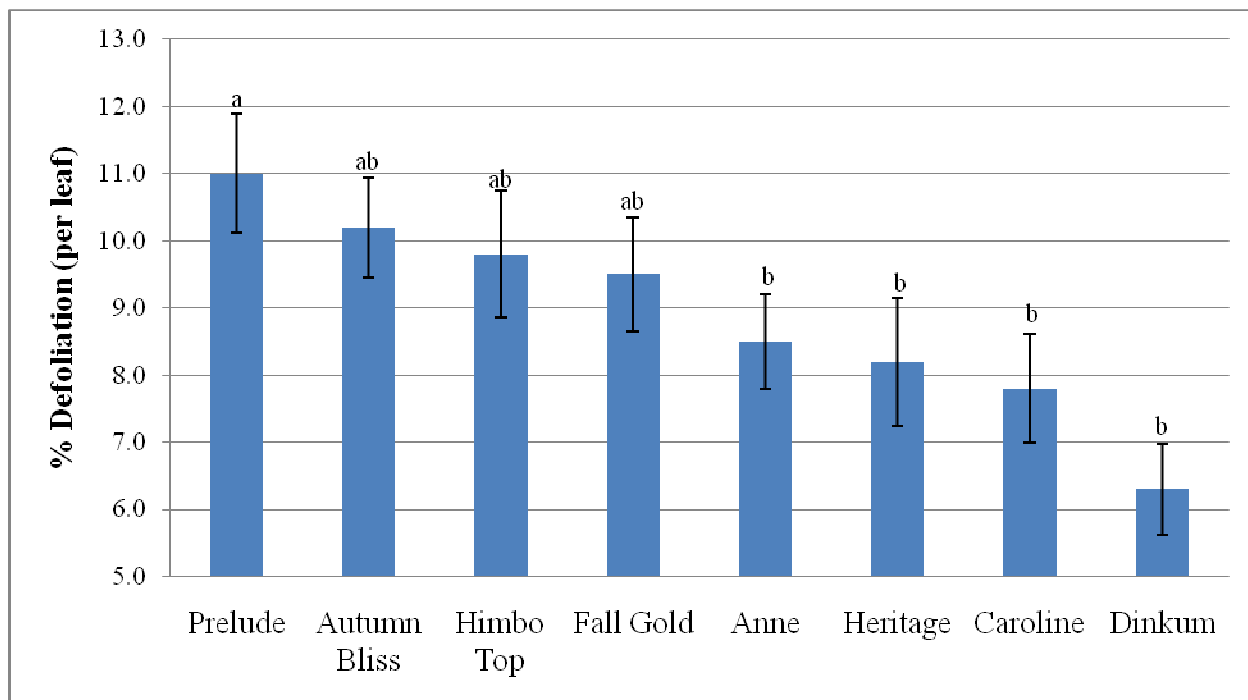


Figure 3.12 The mean percent defoliation of eight primocane-bearing raspberry plants per leaf including undamaged leaves in 2008. The percents defoliation followed by the same letter are not significantly different (Tukey's HSD, ( $F = 3.85$ ;  $df = 7$ ;  $P = 0.0004$ ) following [ArcSine ( $\sqrt{x}$ )] transformation).

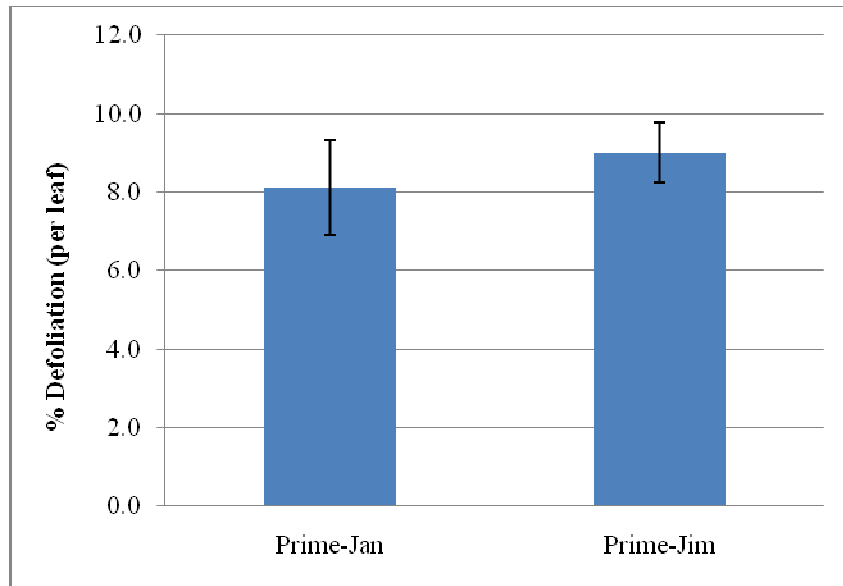


Figure3.13 The mean percent defoliation of 'Prime-Jan' and 'Prime-Jim' blackberries including undamaged leaves in 2008. The cultivar source of variation was not significant according to ANOVA ( $F = 1.54$ ;  $df = 1$ ;  $P = 0.2159$ ) following [ArcSine ( $\sqrt{x}$ )] transformation).

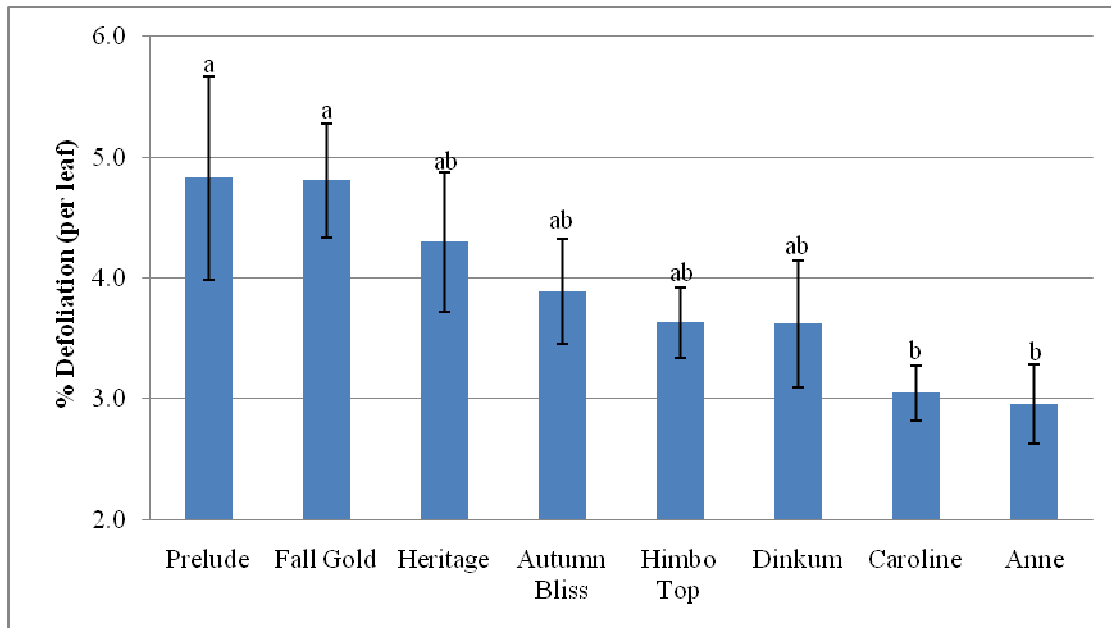


Figure 3.14 The mean percent defoliation of eight primocane-bearing raspberry plants per leaf including only damaged leaves in 2009. Values followed by the same letter are not significantly different (Tukey's HSD, ( $F = 4.34$ ;  $df = 7$ ;  $P < 0.0001$ ) following [ArcSine ( $\sqrt{x}$ )] transformation).

## **CHAPTER 4: Effects of Geranium (*Pelargonium × hortorum*) on Japanese Beetle, *Popillia japonica* Newman (Coleoptera: Scarabaeidae), in Primocane-Bearing Raspberries**

### **Abstract**

Preliminary tests were conducted to evaluate the potential of geraniums in controlling Japanese beetle (JB) in raspberries. Field tests found that previous consumption of geraniums lessened overall raspberry defoliation by JB. Previous exposure to geraniums also decreased JB activity and increased JB mortality on one date observed. Laboratory experiments indicated that previous exposure to geraniums did not prevent raspberry defoliation; however, continual exposure to geraniums lessened defoliation on raspberry. In a choice test, JB were observed more often on ‘Prelude’ raspberry leaves than to geranium petals. As a result, the efficacy of geranium as a trap crop for JB may be limited. Future field tests need to be conducted before geraniums are ruled out as an effective companion plant or trap crop in raspberries.

### **Introduction**

Japanese beetle (JB), *Popillia japonica* Newman, is an herbivore of over 300 plant species and 79 plant families in the United States (Fleming 1972, Potter and Held 2002). One of the preferred hosts of JB is red raspberry (*Rubus idaeus* L.) (Fleming 1972, Ladd 1987). On red raspberry, JB are gregarious feeders of the fruit and foliage (Ellis et al. 1991), skeletonizing the leaves and chewing irregular hollows in the berries (Funt et al. 2000). In Virginia, the demand for fresh fruit, including raspberries, is rising (Kaufman et al. 2000, Monson 2008). The major benefit of consuming raspberries is the natural nutrients they provide (Ravai 1996). These beneficial nutrients include carbohydrates, dietary fiber, vitamins, minerals, antioxidants, and ellagic acid (an anti-carcinogenic compound). Since raspberries are increasing in popularity

because of their natural benefits, it is important to explore new options in pest control that maintain the naturalness of the end product.

One control method that may reduce or eliminate the use of pesticides is companion planting (Cunningham 2000, Shelton and Badenes-Perez 2006). Companion planting is the interplanting of the desired crop with another plant species that is beneficial to the cash crop (Yepez 1984). Through companion planting, pest populations can be controlled by 1) attracting more pests to the companion plant than to the desired crop (acting as a trap crop), 2) through repelling the pest, 3) attracting natural enemies of the pest, 4) increasing the health of the plants, and/or 5) masking attractive volatiles of the desired crop (Yepez 1984, Shelton and Badenes-Perez 2006). Several plant species are reported to be good companion plants when targeting JB. Chives (*Allium schoenoprasum* L.), onions (*Allium cepa* L.), garlic (*Allium sativum* L.), tansy (*Tanacetum vulgare* L.), and rue (*Ruta graveolens* L.) are companion plants that are recommended to repel JB (Yepez 1984, Cunningham 2000). There are also companion plants that are recommended as trap crops which include geraniums (*Pelagonium* spp.), soybeans (*Glycine max*), smartweed (*Polygonum* spp.), zinnias (*Zinnia* spp.), roses (*Rosa* spp.), larkspur (*Delphinium* spp.) and four o'clocks (*Mirabilis* spp.). For a companion plant to act as a trap crop, the trap crop is naturally attractive for feeding and/or has spatial characteristics that attracts the pest to the trap crop instead of the crop with higher value (Shelton and Badenes-Perez 2006). Along with these characteristics, if the trap crop is also toxic, pests would die preventing them from injuring other crops.

Several host plants of JB have been reported as toxic to JB including, common horsechestnut, (*Aesculus hippocastanum* L.) (Potter and Held 2002); bottlebrush buckeye, (*Aesculus parviflora* Walt.) (Hawley and Metzger 1940, Fleming 1972, Potter and Held 2002);



larkspur, (*Delphinium* sp.) (Yepsen 1984); Carolina silverbell, (*Halesia carolina* L.) (Metzger 1933, Hawley and Metzger 1940); castor bean (*Ricinus communis* L.) (Landreth 1932, Hawley and Metzger 1940); and geranium (*Pelargonium* sp.) (Davis 1920, Ballou 1929, Fleming 1972, Potter and Held 2002). The toxicity of these plants was determined based on observations of numerous dead JB found on their backs below the plant in question (Hawley and Metzger 1940). If these toxic plants are also preferred by JB or placed in a location that increases attraction, they would be ideal trap crops (Shelton and Badenes-Perez 2006).

The most commonly documented toxic plant to JB is the geranium (*Pelargonium* spp.) (Davis 1920, Ballou 1929, Hawley and Metzger 1940, Fleming 1972, Held and Potter 2003). Species of geranium that are toxic to JB include zonal geranium, *Pelargonium* × *hortorum* L. H. Bailey, scarlet geranium, *P. inquinans* (L.) L'Héritier, horseshoe geranium, *P. zonale* (L.) L'Héritier, ivyleaf geranium, *P. peltatum* [L.] L'Héritier (Held and Potter 2003), and regal geranium, *P. × domesticum* L. H. Bailey (Fleming 1972). JB become paralyzed within 30 minutes to 4 hrs after consuming geranium petals (Held and Potter 2003, Flores 2010). Paralysis is first noticed in the metathoracic legs (Ballou 1929, Fleming 1972, Held and Potter 2003). Within 24 hrs, paralysis progresses anteriorly until all legs and the body are paralyzed (Ballou 1929, Hawley and Metzger 1940, Held and Potter 2003). When JB are completely paralyzed, the legs are rigid and they lie on their back or side for 12-16 hrs (Potter and Held 1999). JB that are still alive only move their heads when disturbed (Ballou 1929). Most paralyzed beetles in laboratory conditions survive and recover within 24 hrs (Ballou 1929), but time to recover ranges from 4-46 hrs (Held and Potter 2003). Paralyzed JB exposed to field conditions are vulnerable to predation or desiccation which decreases their chances of survival (Potter and Held 1999). The recovered JB do not exhibit food aversion learning and continue to choose geranium flowers

over other suitable hosts (Potter and Held 1999). Geraniums can cause multiple paralyses and a reduction in JB fecundity. Potter and Held (2002) suggest that repeated paralyses and recoveries are possible through the insect's biochemical defense system, which breaks down the paralyzing secondary compounds (Ahmad 1983). When JB are no longer allowed to consume geranium flowers after paralysis, 38 percent die within four days of being given another food source (Ballou 1929). When female JB were only given a diet of geranium for 2 weeks, around 60 percent died (Potter and Held 1999). Ballou (1929) found that geraniums are corrosive to the JB midgut. The midgut of the deceased beetles disintegrates within 24 hrs and the soft contents of the body cavity disintegrate within 48 hrs. The disintegration results in a strong putrid odor that can also be used to identify dead beetles.

Differing factors affect geranium's toxicity toward JB. For example, JB become paralyzed after feeding on the flowers of geranium, but not after consuming the leaves (Held and Potter 2003). Ballou (1929) reported a slightly higher mortality of JB that fed on red-flowered geranium over a pink-flowered plant. However, Held and Potter (2003) found that different colored geraniums were equally active toward JB paralysis with differences only in the time taken to become paralyzed (within five hrs of eating white flowers versus within eight hrs of consuming red or salmon flowers). Ballou (1929) also found a higher mortality in JB that were caged with a sun-exposed geranium versus JB that were caged with a shaded plant. Held and Potter (2003) report that this higher mortality from sun versus shade exposure was a result of JB losing their ability to thermoregulate while paralyzed. The higher mortality was not a result of the sun exposed geraniums producing more toxins. JB consumed an average of 72 percent of a petal before becoming paralyzed but the amount of flower tissue consumed did not relate to the rate of recovery (Held and Potter 2003). Previous exposure to geranium is another factor that

determines toxicity to JB (Potter and Held 1999). JB that had previously been paralyzed by geranium and continued to feed on geranium had lower incidences of paralysis after recovery. Geranium-conditioned beetles also consumed more than seven times as much plant material before becoming paralyzed compared to beetles that were newly introduced to the plant.

Several attempts have been made to identify the toxic component of geranium that is responsible for JB paralysis. Potter and Held (1999) first credited light-activated flavonoids or anacardic acids as the active compounds responsible for JB paralysis. However, they later found that detached flowers from shaded and sun-grown plants caused comparable paralysis in the laboratory discrediting the flavonoid hypothesis (Held and Potter 2003). The anacardic acid hypothesis was also disproven by JB not becoming paralyzed after consuming geranium leaves that contained anacardic acids. Held and Potter (2003) also ruled out other flower pigments as the toxic component because paralysis was induced equally by different colored geraniums and different geranium species. Ranger et al. (2011), recently identified the paralytic compound in zonal geraniums to be quisqualic acid. Quisqualic acid is an excitatory amino acid that mimics L-glutamic acid, a neurotransmitter in insect neuromuscular junctions. Through identifying the paralytic compound of geraniums, new botanical insecticides can be explored to provide an alternative control option for JB in raspberry and other crops.

Today there are few natural, pesticide-free, JB control methods available for raspberry growers. I tested the hypothesis that zonal geraniums can be used to control JB in red raspberries through conducting laboratory and field experiments. Utilizing geranium's toxicity toward JB, a pesticide free, consumer-friendly, JB control method in raspberry was explored.

## Materials and Methods

### 2007 Field Experiments (Cage to Bag)

In July and August 2007 at the Kentland Research Farm near Blacksburg, VA, JB adults were collected from Tanglefoot® JB traps baited with Tanglefoot® JB bait and lure. A water source was provided within the traps to prevent desiccation before collection. Only living, active beetles were placed into cages for the experiments. Cages were designed to hold beetles along with individually potted plants (Figure 4.1). A Petri dish with water was also provided in each cage. On 16 and 31 July and 3 and 11 August 2007, 25 JB were placed into each of two screened cages (0.6 × 0.6 × 1.0 m). One cage included a potted ‘Autumn Bliss’ raspberry plant that was two years old before potting. The other cage contained an annual zonal geranium plant (*Pelargonium × hortorum*). The JB were exposed to each treatment for 48 hrs.

After 48 hrs, the living JB were removed from each cage and divided into groups of five beetles. Each group of five was placed in a nylon mesh bag (20 × 27 cm). Whether the JB were active or inactive was recorded before being placed in the bag. A JB was considered active if it walked around freely. An inactive JB only moved when probed. Three mesh bags, containing five JB each for each treatment, were applied to an ‘Autumn Bliss’ raspberry plant in the field at the Kentland Research Farm on 19 and 3 July. On 6 and 14 August there were only enough living JB for two mesh bags per treatment. Before the bags were pinned onto the plant, the defoliation of each leaflet within the top 18-30 cm of the cane was estimated based on a 0-100 scale with a score of “0” being given to leaves without any injury and a score of “100” given to leaves with only the veins remaining. To reduce variability, the same individual (LM) estimated the defoliation throughout the entire study. The bags were then placed over the cane, with the bottom of the bag enclosing the most basal leaf for which defoliation had been recorded. A

medium sized binder clip was then clipped around the bag and cane to secure the bag and beetles on the plant. After 24 hrs, the bags were removed and defoliation was estimated and recorded as above. The comparison of defoliations before and after JB exposure resulted in an estimate of the overall foliar injury by JB. The number of JB living and active was also recorded.

The percent defoliation, the percentage of living JB, and the percentage of active JB were analyzed by date. An average of the four dates was also calculated to estimate an overall effect of the two treatments. Proportions that did not follow a normal distribution were subjected to an ArcSine ( $\sqrt{x}$ ) transformation and both transformed and untransformed data were analyzed with ANOVA followed by a Student's t-test mean comparison ( $\alpha = 0.05$ ) (SAS Institute 1989).

## **2009 Laboratory Experiments**

Japanese beetle adults were collected 27 July 2009 from Tanglefoot® JB traps baited with Tanglefoot® JB floral bait and sex lure. A water source was provided within the traps so the beetles did not die from desiccation before collection. Only living, active JB were used for the experiments. Laboratory experiments took place on 27-29 July 2009, inside a room with artificial lighting and a constant temperature of 22 °C.

### **Raspberry and Geranium**

Two JB were placed into 25 aerated Tupperware® containers (16.3 × 17.8 × 9.7 cm) containing a fresh 'Prelude' raspberry leaf, a red annual zonal geranium flower (*Pelargonium × hortorum*), and a moistened cotton ball. Two JB were also placed into each of 25 aerated Tupperware® containers with only a 'Prelude' raspberry leaf and a moistened cotton ball. The containers with the raspberry leaf and geranium flower were observed after 24 and 48 hrs to

count the number of JB on the raspberry leaves versus the geranium flower. After 48 hrs, digital images were taken of all 50 raspberry leaves (25 leaves in with flower plus the 25 leaves that were alone). These digital images were then analyzed with the software Image J (Rasband 2009) [downloaded from: <http://rsbweb.nih.gov/ij/download.html>] to calculate the area of defoliation. Both the total leaf area and injured area of each leaf was measured. The injured area was then divided by the total area to obtain a percent defoliation. The mean defoliations obtained from the raspberry leaves contained with the geranium flowers were compared to the defoliations of the raspberry leaves that were alone with JB.

The percent of JB on either the raspberry leaf or the geranium flower after 24 and 48 hrs were analyzed without transformation with a Student's t-test mean comparison ( $\alpha = 0.05$ ) (SAS Institute 1989). The percent defoliation data were subjected to an ArcSine ( $\sqrt{x}$ ) transformation because the raw data did not follow a normal distribution. The data were then analyzed with a Student's t-test mean comparison ( $\alpha = 0.05$ ) (SAS Institute 1989).

### **Raspberry or Geranium**

Individual JB were placed into 25 aerated Tupperware® containers (16.3 × 17.8 × 9.7 cm) along with a fresh 'Prelude' raspberry leaf and a moistened cotton ball. Twenty-five more JB were individually placed into Tupperware® containers with a zonal geranium flower (*Pelargonium × hortorum*) and a moistened cotton ball. After 24 hrs of exposure to each treatment (raspberry leaf or geranium flower), the JB were removed from their initial containers and placed into 50 fresh containers with 'Prelude' raspberry leaves and moistened cotton balls. After another 24 hrs, JB were removed and digital images were taken of the 50 leaves. The digital images were then used to calculate the percent defoliation with ImageJ. The defoliation

of the raspberry leaves injured by JB that were previously exposed to geranium flowers were compared to defoliations of raspberry leaves that were injured by JB previously exposed to raspberry leaves. The defoliation data did not follow a normal distribution and were therefore subjected to ArcSine ( $\sqrt{x}$ ) transformations and then analyzed with ANOVA. Means were then compared with a Student's t-test ( $\alpha = 0.05$ ) (SAS Institute 1989).

## Results

### 2007 Field Experiments (Cage to Bag)

There was only one date observed in which significant differences were found between the treatments in the percentages of active (Figure 4.2) and living (Figure 4.4) JB. On this date (19 July), more JB were active and alive after initially being caged with a raspberry plant versus initially being caged with a geranium plant. When the percentages of active JB were averaged from the four trials conducted observing each factor, significant differences were not found ( $F = 1.68$ ;  $df = 1$ ;  $P = 0.3240$ ) (Figure 4.3). There was a significant treatment effect in the average percentage of living JB after exposure to both treatments ( $\alpha = 0.10$ ;  $F = 4.73$ ;  $df = 1$ ;  $P = 0.0953$ ) (Figure 4.5).

The treatments (raspberry or geranium) had an effect on the percentage of foliage consumed on 19 July (Figure 4.6). On this date, there was less defoliation from JB that were caged with a geranium plant before being bagged onto the raspberry cane in the field. When the defoliations were averaged from the four trials, there was significantly less overall defoliation from JB that were initially caged with a geranium plant ( $F = 4.49$ ;  $df = 1$ ;  $P = 0.0422$ ) (Figure 4.7).

## 2009 Laboratory Experiments

### *Raspberry and Geranium*

There were significantly more JB present on the 'Prelude' raspberry leaf than there were on the geranium flower after the beetles were contained with both plant pieces after 48 hrs ( $F = 6.55$ ;  $df = 1$ ;  $P = 0.0197$ ) (Figure 4.9). Significant differences were not observed after 24 hrs ( $F = 0.46$ ;  $df = 1$ ;  $P = 0.5055$ ) (Figure 4.8). JB contained only with a raspberry leaf consumed a significantly higher percentage of foliage than JB that were contained with both a raspberry leaf and a geranium flower ( $F = 4.43$ ;  $df = 1$ ;  $P = 0.0444$ ) (Figure 4.10).

### *Raspberry or Geranium*

When JB were first placed in a container with either a raspberry leaf or a geranium flower for 24 hrs and then placed into a different container with a fresh raspberry leaf for 24 hrs, there were no significant differences in the defoliation of the last exposed raspberry leaves ( $F = 2.37$ ;  $df = 1$ ;  $P = 0.1353$ ) (Figure 4.11).

## Discussion

Previous exposure to geranium reduced JB defoliation in field trials (Figure 4.7) but not in a bioassay (Figure 4.11). These confounding results can be explained by the different conditions that were present for each study. Defoliation of 'Autumn bliss' leaves was observed in the field whereas 'Prelude' leaves were used in the lab. As stated in Chapter 3, 'Prelude' raspberries are often preferred by JB. Consequently, the lack of control exhibited in the laboratory could be a result of 'Prelude' being a highly preferred host. Another explanation is that JB were exposed to environmental stresses (heat, wind, rain, etc) in the field which added to



the stress caused by geranium intoxication to significantly lessen JB feeding. The controlled cooler temperature in the laboratory would not have stressed the JB as much as field conditions. Leaves used in the laboratory were also removed from the plant whereas leaves in the field experiments were attached. Therefore, the volatiles that were released in each trial were most likely different explaining the confounding results. The methods of obtaining the percent defoliation were also different for the two experiments. Defoliation was estimated in the field and measured in the laboratory. Therefore, the measured findings from the laboratory experiment could be more accurate.

Since previous exposure to zonal geraniums reduced JB defoliation to field planted raspberries, geraniums potentially can be used to reduce JB activity on raspberries. However, before geraniums could be applied as JB control, a method that ensures previous exposure to geraniums needs to be found. Insuring that JB are first exposed to geraniums could require a combination of factors including JB choosing geraniums over raspberries and/or placing the geraniums in a location that orients JB towards them over the raspberries. When JB were given the choice between a zonal geranium petal and a 'Prelude' raspberry leaf, significantly more beetles choose to be on the raspberry leaf after 48 hrs (Figure 4.9). This finding suggests that geraniums would not be a JB trap crop that works through attraction. However, further field tests need to be conducted before a definite preference is determined. Intact geranium and raspberry plant material could attract JB differently than did the removed plant material used in the laboratory. Also, using a raspberry cultivar that is less preferred by JB could allow them to choose geraniums as a host.

The placement of a plant acting as a trap crop is important in its effectiveness (Shelton and Badenes-Perez 2006). Thus, finding an effective placement of geraniums within a raspberry

field can increase the control exhibited. Ladd and Klein (1982) found that different formulations of JB attractants caught optimal JB depending on their height from the ground. This finding suggests that placing geraniums at differing heights from the ground could have different effects on the number of JB attracted to them. Also, height of the host *per se* strongly affects the initial orientation to the host plant and attack by JB (Rowe and Potter 1996). Rowe and Potter's findings suggest that if the geraniums were positioned higher than the raspberries, then more JB would choose the geraniums as hosts. It is cautioned however, that the presence of geraniums could increase the number of JB in raspberries as demonstrated in a companion planting experiment in roses (Held et al. 2003). Instead of repelling JB from the roses as intended, the presence of geraniums increased the number of JB on roses. However, in the referred experiment the geraniums were planted in the ground (not providing a physical barrier) and the spatial components were not manipulated to increase attractiveness. Therefore, the lack of control in roses could be a result of an ineffective placement of the geraniums when using them as a trap crop. These studies support that more field tests are needed in finding an effective position for geraniums as a trap crop in raspberries.

The level of JB control exhibited by geraniums in my experiments could be heightened or lessened under more natural conditions. For example, the cages and the bags used in the field experiments provided a level of shade that would not be naturally available. This shade would have aided in keeping their body temperature lower than if the beetles were paralyzed in the direct sun. Since geraniums cause JB to lose their ability to thermoregulate (Held and Potter 2003), the shade provided by the cages and bags could have prevented higher levels of desiccation. Another factor decreasing geranium's effectiveness in my trials is that JB were not allowed to fall to the ground. When JB are paralyzed, they land on the ground where they are

susceptible to predation (Potter and Held 1999) . The cages and bags therefore protected JB from predation. Also, after the JB recovered from paralysis they were not offered geranium as a food choice. JB that are previously paralyzed by geraniums recover to consume more geranium plant material than during the initial feeding (Potter and Held 1999). If giving a choice, the JB might have returned to feed on geraniums making them paralyzed again and lessening the amount of raspberry plant material consumed. Further tests also need to be conducted to see if geraniums would lessen JB injury to the berries. Since JB prefer berries and florescences over foliage (Potter and Held 2002), the decreased injury to the foliage might not translate into reduced injury to the berries. An example of the experimental design resulting in less JB activity on raspberry is seen when considering JB aggregation. JB aggregate on preferred food sources (Fleming 1972, Loughrin et al. 1996). The mesh bags used in the experiments prevented more than five beetles from aggregating on the raspberries. If JB were allowed to aggregate, more than five beetles could have defoliated the bagged leaves. Therefore, the bags potentially decreased the amount of injury sustained.

The toxic component of geraniums was recently identified and extracted (Ranger et al. 2011). This identification provides another avenue for JB control with geraniums. Application of quisqualic acid to raspberry as JB control would be logical since geraniums are toxic to JB (Davis 1920, Ballou 1929, Hawley and Metzger 1940, Fleming 1972, Held and Potter 2003). Also, since previous exposure to geraniums lessens the amount of raspberry defoliation, application of a cover spray containing quisqualic acid is justified. Another benefit of a cover spray being developed is that JB would be exposed to the toxic component. Then, both the placement of geranium plants and the preference for geraniums over raspberries would no longer

be important factors. Because the toxic component is plant derived, development of a treatment containing quisqualic acid would provide a more natural control method for JB in raspberries.

In conclusion, the tests conducted enhance our understandings in utilizing geraniums as JB control in raspberries. Field tests found that previous exposure to geraniums lessened the average raspberry defoliation. Also, defoliation was lessened by continual exposure to geraniums. Beetles did not choose to be on a geranium petal over a 'Prelude' raspberry leaf, but other choice tests using different raspberry cultivars are suggested. It is proposed that placement of the geraniums can be used to orient JB towards the geraniums instead of the raspberries. However, geranium's effectiveness as a trap crop needs elaboration. These preliminary findings can be used when developing more in-depth studies of geraniums as JB control and support the production of a treatment containing quisqualic acid. Once an effective control method is identified, more natural, consumer-friendly pest management strategies can be implemented.

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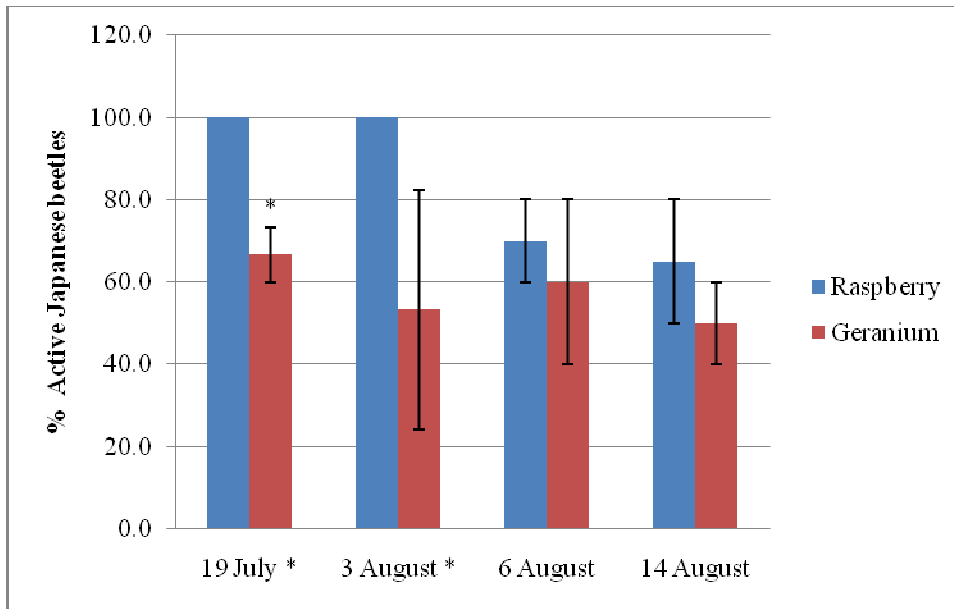
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## Figures



Figure 4.1 Nylon mesh, wood, and Plexiglas® cage designed to hold potted plants and Japanese beetles.





\* Data was ArcSine ( $\sqrt{x}$ ) transformed before being compared but values shown are the untransformed means.

Figure 4.2 Japanese beetles were caged with either a raspberry or geranium plant and then bagged onto a raspberry plant. The percent of active beetles after being bagged on the raspberries were compared; significant difference between treatments was only found on 19 July (Student's t-test ( $\alpha= 0.05$ )).

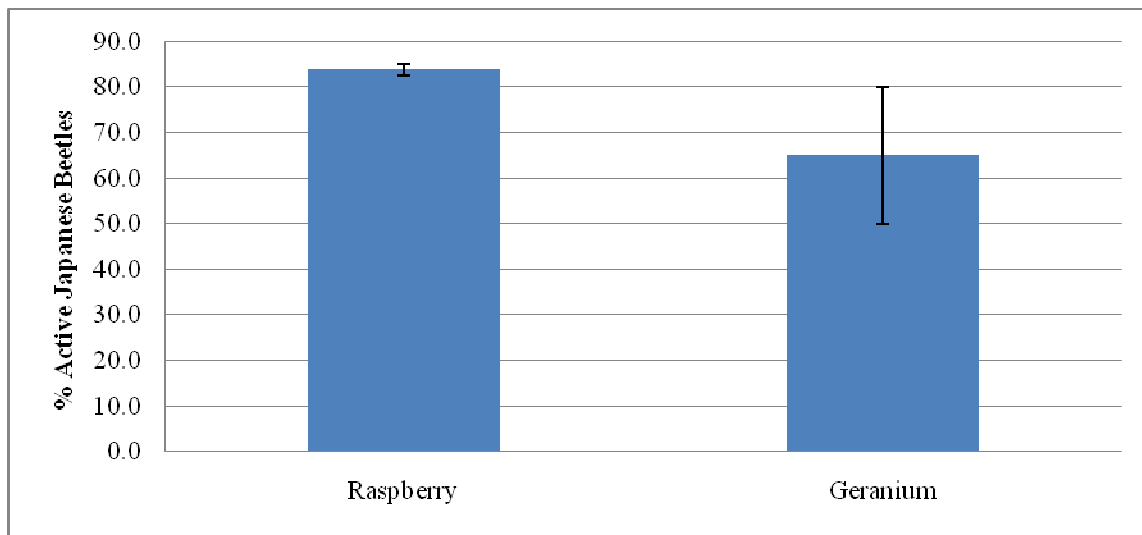
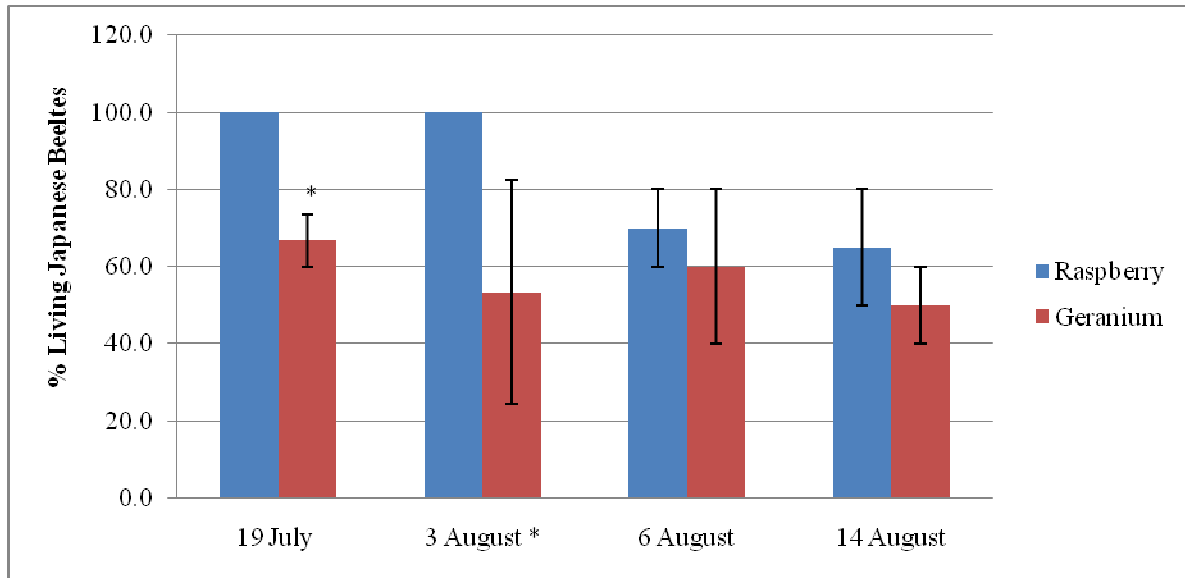


Figure 4.3 The mean percentage of four trials observing the percent of active Japanese beetles after exposure to either a raspberry or a geranium plant and then a raspberry plant. The treatment source of variation was not significant according to ANOVA ( $\alpha = 0.05$ ;  $F = 1.68$ ;  $df = 1$ ;  $P = 0.3240$ ) after ArcSine ( $\sqrt{x}$ ) transformation.



\* Data was ArcSine ( $\sqrt{x}$ ) transformed before being subjected to ANOVA but the values shown are the untransformed means.

Figure 4.4 Japanese beetles were caged with either a raspberry plant or a geranium plant followed by exposure to raspberry alone. The percent of living beetles from each treatment were compared; significant difference between treatments was only found on 19 July (Student's t-test ( $\alpha= 0.05$ )).

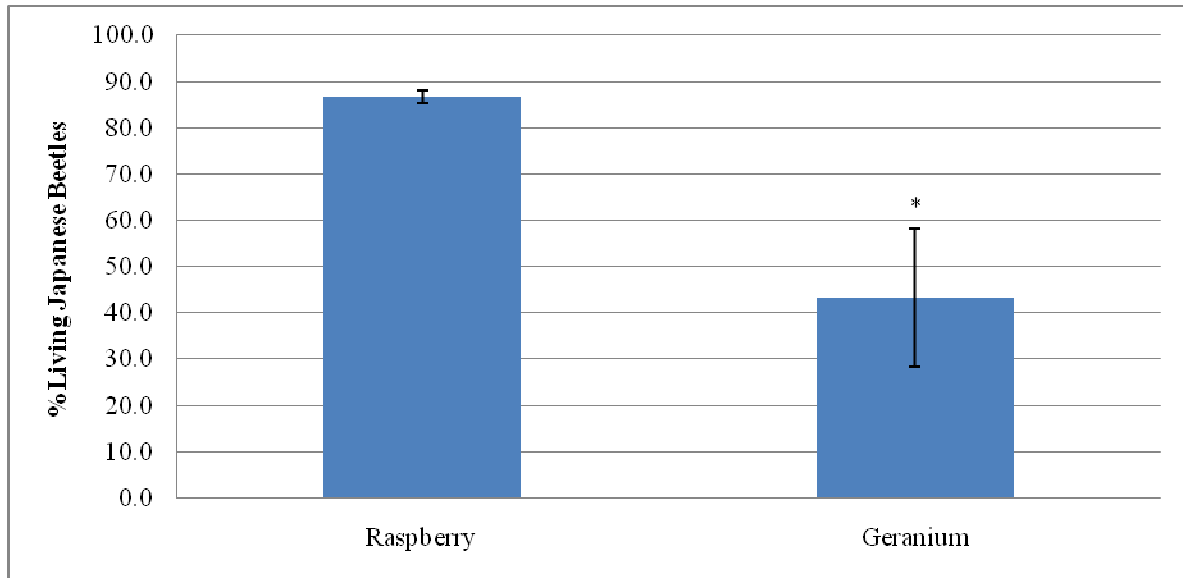
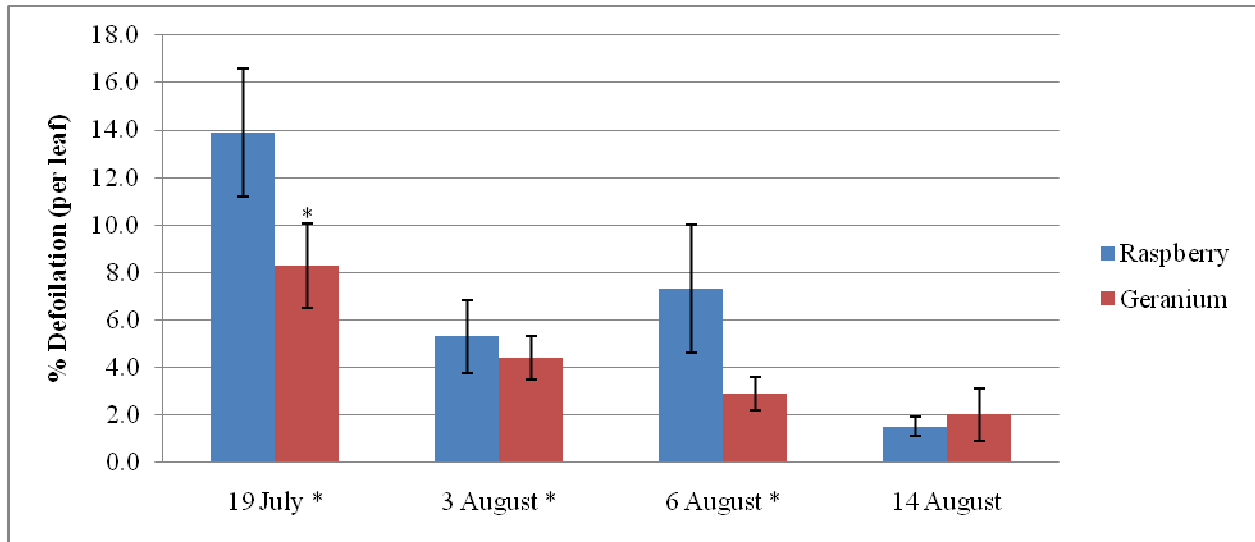


Figure 4.5 The mean percentage of four trials observing the percent of living Japanese beetles after exposure to either a raspberry or a geranium plant followed by exposure to a raspberry plant. The treatment source of variation was significant (Student's t-test,  $\alpha = 0.10$ ;  $F = 4.73$ ;  $df = 1$ ;  $P = 0.0953$ ).



\* Data was ArcSine ( $\sqrt{x}$ ) transformed.

Figure 4.6 Japanese beetles were caged with either a raspberry plant or a geranium plant and then removed and bagged onto a raspberry cane. There was significant difference between the treatments only on 19 July (Student's t-test ( $\alpha= 0.05$ )).

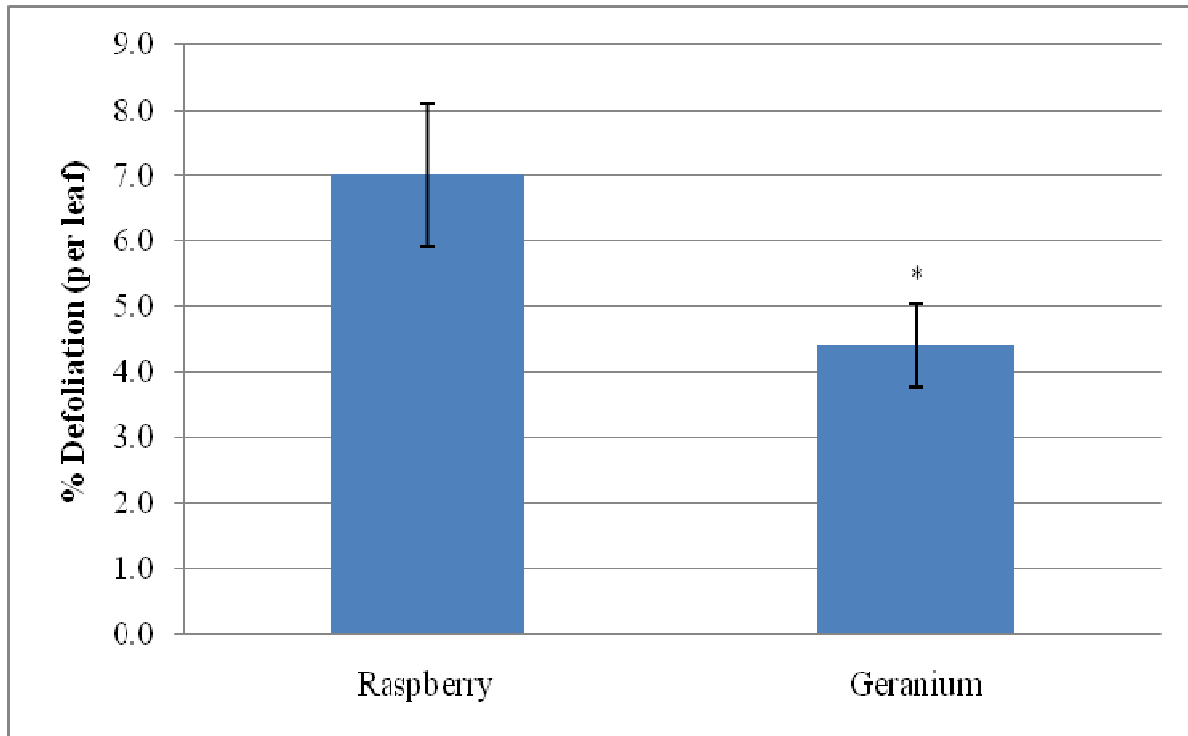


Figure 4.7 The mean percentage of four trials observing the percent defoliation of raspberry leaves after Japanese beetles were exposed to either a raspberry or a geranium plant followed by exposure to a raspberry plant. The defoliation values are significantly different (Student's t-test,  $\alpha= 0.05$ ;  $F = 4.49$ ;  $df =1$ ;  $P = 0.0422$ ).

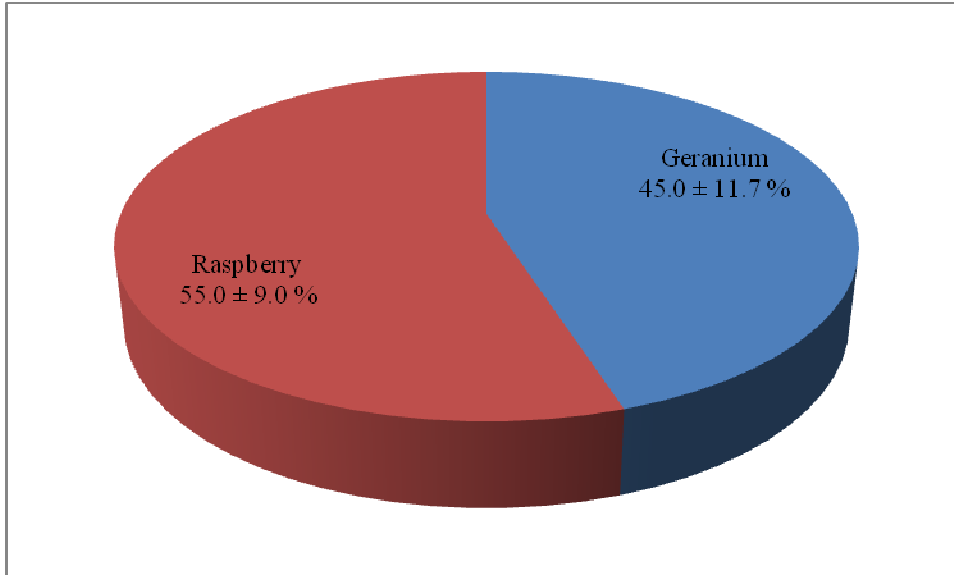


Figure 4.8 Two Japanese beetles were exposed to a 'Prelude' raspberry leaf and a geranium flower. After 24 hrs the percent of the beetles on each plant was recorded. The treatment source of variation was not significant according to ANOVA ( $\alpha = 0.05$ ;  $F = 0.46$ ;  $df = 1$ ;  $P = 0.5055$ ).

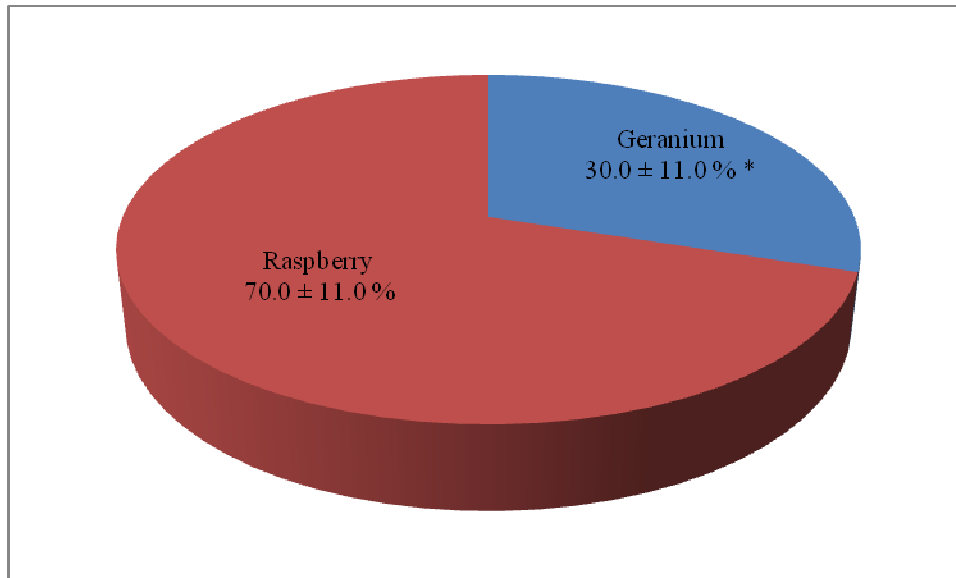


Figure 4.9 Two Japanese beetles were exposed to a 'Prelude' raspberry leaf and a geranium flower and after 48 hrs the percent of the beetles on each plant was recorded. The percent of Japanese beetles on raspberries was significantly higher than the number of Japanese beetles on geranium (Student's t-test,  $\alpha = 0.05$ ;  $F = 6.55$ ;  $df = 1$ ;  $P = 0.0197$ ).



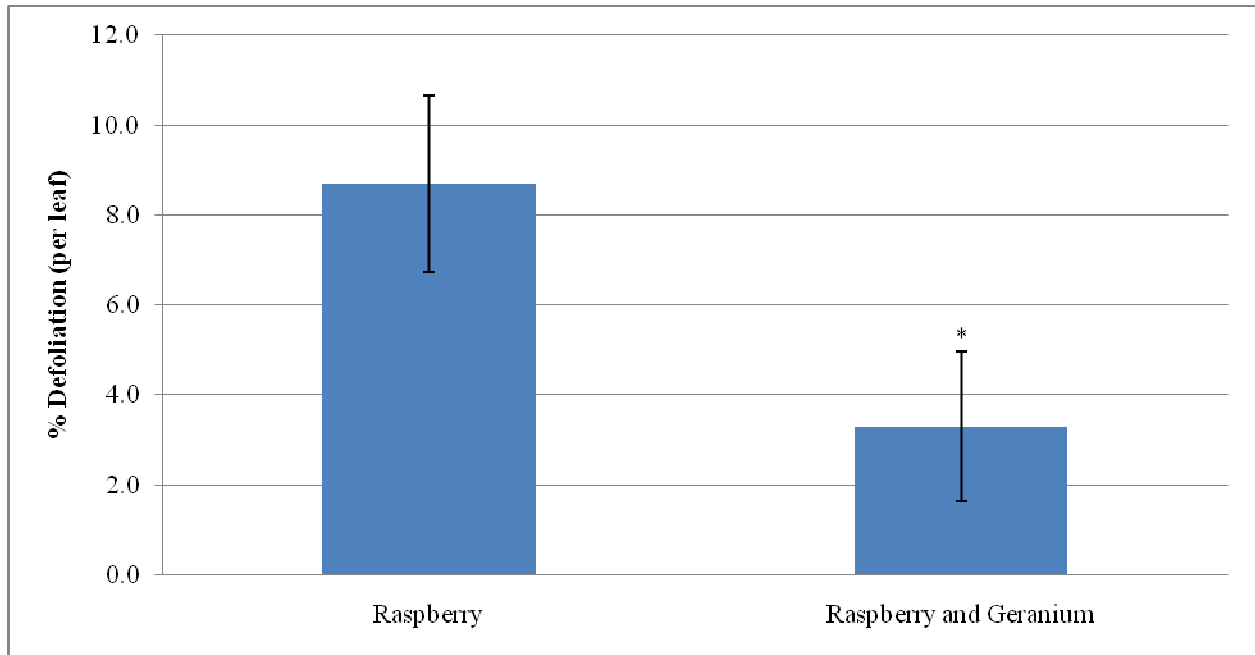


Figure 4.10 Two Japanese beetles were exposed to a geranium flower along with a raspberry leaf or to a raspberry leaf only. After 48 hrs the percent defoliation of the raspberry leaves were compared and the values were significantly different (Student's t-test ( $\alpha = 0.05$ ;  $F = 4.43$ ;  $df = 1$ ;  $P = 0.0444$ ); data was ArcSine ( $\sqrt{x}$ ) transformed).

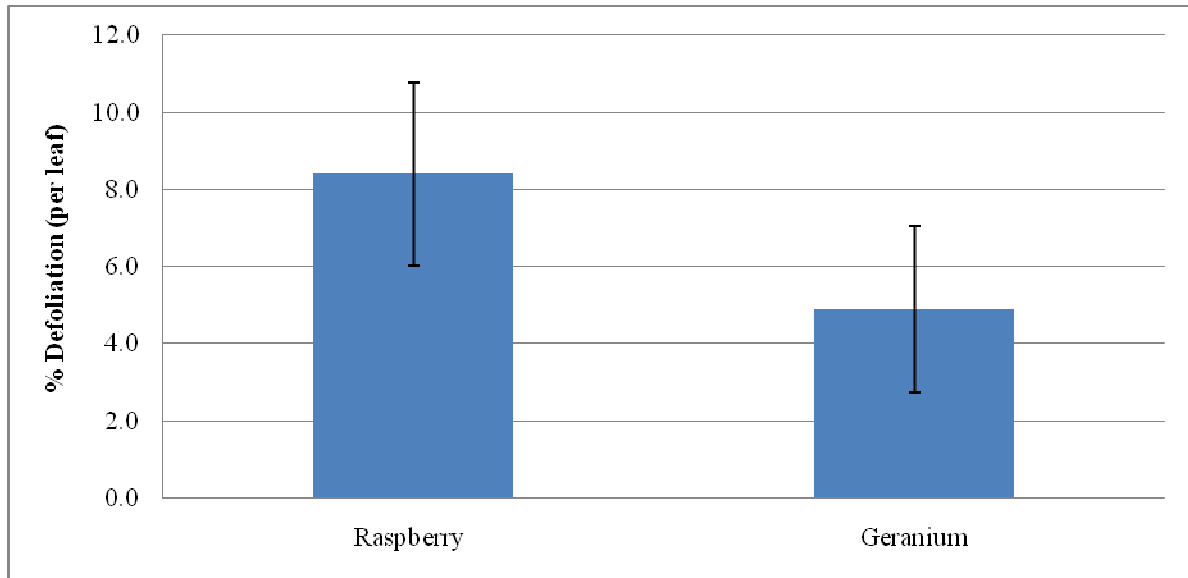


Figure 4.11 Defoliations from Japanese beetle exposed to either a geranium flower or a 'Prelude' raspberry leaf and then exposed to a fresh raspberry leaf. The defoliations of the raspberry leaves were compared and the treatment source of variation was not significant according to ANOVA ( $\alpha = 0.05$ ;  $F = 2.37$ ;  $df = 1$ ;  $P = 0.1353$ ) after an ArcSine ( $\sqrt{x}$ ) transformation.

## CHAPTER 5: A Survey of the Stink Bug (Hemiptera: Pentatomidae) Species Present in a Primocane-bearing Raspberry Planting in Southwest Virginia and Identification of Stink Bug Injury to Raspberries

### Abstract

Knowledge on the species complex and damage caused by stink bugs (Pentatomidae) to raspberries is generally lacking in the mid-Atlantic U.S. Over a two year study, the species of stink bugs present in a Southwest Virginia primocane-bearing raspberry field were identified. Also, stink bug injury to raspberries was identified and the images are presented. During the two-year study *Euschistus servus* (Say) made up 48.1 % of the overall species composition. The other species collected in decreasing order of the percent captured include: *Cosmopepla lintneriana* (Thomas) (20.3 %), *Acrosternum hilare* (Say) (16.3 %), *E. tristigmus* (Say) (9.5 %), *E. variolarius* (Palisot) (2.3 %), *Thyanta accerra* McAtee (1.4 %), *Banasa calva* (Say) (0.2 %), *B. euchlora* (Stål) (0.2%), *Brochymena quadripustulata* (Fabricius) (0.2%), *Coenus delius* (Say), *Dendrocoris humeralis* (Uhler) (0.2%), *Hymenarcys nervosa* (Say) (0.2%), and *T. calceata* (Say) (0.2%). Stink bugs were observed feeding on the veins of the leaves, on the sepal of the fruiting structure, and between the drupelets of the berries. Most feeding injury to ripened or ripening berries was minor in that feeding left holes between the drupelets. Stink bugs also injured the berries through depositing frass rendering the berries distasteful. Identification of the species present in 2008-2009 and documenting the injury they caused to raspberries, has clarified the role of this pest complex on this specialty crop.

## Introduction

Stink bugs (Hemiptera: Pentatomidae) feed on a wide range of fruits, vegetables, nuts, and grains in North America (McPherson and McPherson 2000). All life stages of most stink bug species, except for the first instar (Froeschner 1988), pierce plant tissues and extract fluids from stems, petioles, foliage, flowers, fruits, and seeds of plants (McPherson and McPherson 2000, Panizzi et al. 2000). Stink bug injury to most plants can be identified by discolored spots on the plant along with a stylet sheath at the feeding location (Schuh and Slater 1995, McPherson and McPherson 2000). This injury to mature plant structures is usually not noticeable; however, injury to the preferred younger plant structures can result in severe damage (Spangler et al. 1993, Schuh and Slater 1995, McPherson and McPherson 2000). Stink bug injury on immature plant structures is identified by shriveling, decrease of size, or abortion (McPherson and McPherson 2000). Other resulting injuries to the plant are foliar retention, early plant maturation, delayed maturation, abnormal plant growth, and the reduction of fruit size and weight. The observed injury is the result of the loss of plant fluids, the injection of digestive enzymes, the deformation and abortion of seed and fruiting structures, pathogenic and decay organisms colonizing at the feeding location, and/or the delay of plant maturity. The overall impact of stink bug feeding depends on the crop in question, the time of feeding, and the location of feeding. However, heavy feeding on any plant stage can have a significant impact on the crop's marketable yield (Panizzi et al. 2000).

The pest significance of stink bugs on raspberries is not well understood. In Australia, Coombs and Khan (1998) found that when *Plautia affinis* Dallas fed on raspberries for two weeks the fruit was more distorted, discolored, and had reduced firmness compared to the control. Stink bugs can also cause secondary injury to caneberries through depositing frass with

an obnoxious odor on the berries (Kieffer et al. 1983, Ellis et al. 1991, Alford 2007). Stink bugs are also a pest of raspberries by contaminating caneberries harvested by a mechanical harvester (Kieffer et al. 1983). When these berries were harvested, not only did the actual insect bodies contaminate the product, the stink bug's defensive chemicals also tainted the berries. The tolerable amount of stink bug damage depends on the views of the producer, the processor, or the consumer and on the crop's quality, availability, and yield reductions (McPherson and McPherson 2000). The acceptable amount of feeding, or presence of frass or insects after being mechanically harvested all depends with stakeholder opinion.

In the northern United States, most Pentatomidae species have one generation per year and in the southern portions of North America, up to five generations per year have been documented (McPherson and McPherson 2000). With the multiple species present and their life cycles, an adult stink bug can be found somewhere within North America year round (Froeschner 1988).

The current species complex of stink bugs found on raspberries in Virginia is not known. Kamminga et al. (2009b) lists several common species on agricultural crops in the southeastern U.S. including: the green stink bug, *Acrosternum hilare* (Say), *Euschistus quadrator* (Rolston), the brown stink bug, *E. servus* (Say), the dusky stink bug, *E. tristigma* (Say), the brown marmorated stink bug, *Halyomorpha halys* (Stål), the harlequin bug, *Murgantia histrionica* (Hahn), the southern green stink bug, *Nezara viridula* (L.), the rice stink bug, *Oebalus pugnax* (Fab.), the redbanded stink bug, *Piezodorus guildinii* (Westwood), the redshouldered stink bug, *Thyanta accerra* McAtee, and, *T. custator custator* (Fab.). These and other damaging species are mainly weed feeders that can move into cultivated crops, cause serious damage, and then move to another host (McPherson and McPherson 2000, Panizzi et al. 2000). Recently, the

brown marmorated stink bug, an invasive species, is of great concern in the Mid-Atlantic states, including Virginia, because of the wide range of fruit and vegetable crops it infests (Hoebeke and Carter 2003, Hamilton 2009).

Stink bugs are occasionally mentioned and grouped along with other true bugs (Hemiptera) as pests of commercial caneberries (*Rubus*) (Spangler et al. 1993). Across the United States, McPherson and McPherson (2000) listed the following *Rubus* species as hosts of stink bugs: American dewberry (*Rubus flagellaris* Willdenow), red raspberry (*Rubus idaeus* L.), and cut-leaf blackberry (*Rubus laciniatus* Willdenow). In a New York caneberry planting, a stink bug complex was reported to be occasionally or moderately abundant (Spangler et al. 1993). The adults in that study were present in greater numbers on canes with ripe berries in late August 1989 and also during the primocane-fruiting stage compared to the florican-fruiting stage. Since stink bug populations are greater in the fall, primocane-bearing raspberries are at a greater risk of being injured by these pests.

Both national and international reports of stink bugs on caneberries indicate the need for future studies on this topic. While stink bugs have been reported to injure raspberries (Coombs and Khan 1998, Kaya and Kovanci 2004), photographic documentation of stink bug injury to raspberries has not been published. The objectives of my study are to identify the stink bug species present within a southwest Virginia raspberry planting and to provide photographic documentation of stink bug injury to raspberries.

## Materials and Methods

### Species Survey

Surveys were conducted to determine the species of stink bugs present in a 2005 planting of primocane-bearing raspberries and blackberries. This planting is on an elevated site above the New River in southwestern Virginia (37° 12.417'N, 80° 35.513'W, 615.7 m elev.). The caneberry planting consisted of six raised bed rows in a randomized complete block design containing 11 raspberry cultivars adjacent to seven raised bed rows containing two blackberry cultivars. Each cultivar plot is 6.2 m long and 1.0 m wide; rows are 3.0 m apart. This caneberry planting is bordered by an apple orchard, forest, and pasture. The primocane bearing raspberry cultivars present included, 'Anne', 'Autumn Bliss', 'Autumn Britten', 'Caroline', 'Dinkum', 'Fall Gold', 'Heritage', 'Himbo Top', 'Josephine', 'Nova' and 'Prelude'. In the years surveyed, there were also insecticidal trials conducted in selected raspberry cultivars. Only raspberry plants that were not used in the insecticidal trials were used during the stink bug survey. Plants in all levels of maturity were shaken over a heavy duty sweep net to collect the stink bugs present. Stink bugs were collected on 6, 16, 21, and 31 August, and 14 and 19 September 2008, and 7 and 21 July and 5, 10, and 17 August 2009. Stink bugs were placed in a vial containing alcohol and taken to the laboratory for identification. The taxonomic key, Stink Bugs of Economic Importance in America North of Mexico (McPherson and McPherson 2000) was used to identify the stink bugs to species. Stink bug species composition is reported for each year (Tables 5.1 and 5.2). Also, to obtain an overall stink bug species composition of the two years studied, the counts from 2008 and 2009 were added together and reported (Table 5.3).

## **Identification of Stink Bug Damage to Raspberries**

### ***2008 Field Experiments***

In 2008, stink bugs of unidentified species were bagged onto unsprayed raspberry plants in the field to document stink bug damage on raspberries. All stink bugs applied were living and active. The stink bugs were collected from the raspberry planting either with a sweep net or by hand picking. On the dates that the bags containing stink bugs were applied, the berries were examined and a digital image was taken observing any previous damage. All digital images taken in 2008 were with a Sony Cyber-shot 12 megapixel digital camera with macro setting. Canes containing berries of different maturity levels without injury were used. For every bag that was applied containing stink bugs, another empty control bag was applied to a nearby cane. The bags used were made out of nylon mesh and were 20 × 27 cm. These bags were applied to the top of the raspberry canes exposing this plant area to the stink bugs.

On 6 August 2008, five bags each containing five stink bugs (species undetermined) were bagged onto red raspberry canes. Five control bags were also applied. The bags were applied to ‘Autumn Bliss’, ‘Caroline’, or ‘Prelude’ raspberries on this date. The stink bugs used were also collected on the same date they were bagged onto the canes. Digital images were taken on 11 and 15 August and the images from the control and stink bug bags were compared.

A similar test was then conducted starting on 18 August 2008. Five bags each containing five stink bugs of undetermined species were bagged onto red raspberry canes. Five control bags were also applied. The bags were applied to ‘Autumn Bliss’, ‘Autumn Britten’, or ‘Josephine’ raspberries on this date. The stink bugs used were also collected on the same date they were bagged onto the canes. Digital images were taken on 21 and 26 August and the images from the control and stink bug bags were compared.



On 2 September 2008, stink bugs were collected and only provided a water source until 11 September when they were bagged onto 'Prelude' and 'Nova' red raspberries. Only living, active stink bugs were used. Only two bags containing stink bugs and two control bags were applied on 11 September. Then on 19 September, the bags were removed and digital images were taken so comparisons could be made.

### ***2009 Potted Raspberry Experiments***

'Fall Gold' yellow raspberries and 'Autumn Bliss' red raspberries were taken out of the field and potted to capture video images of stink bugs feeding on raspberries. Stink bugs were captured from the raspberry planting either with a sweep net or by hand picking. These stink bugs were only given a water source for a week and then placed onto the potted plants and allowed to roam freely. Individual stink bug activity was then recorded with a Sony Digital Handycam with macro lenses. The digital videos were then edited to only include footage of stink bug's feeding on different plant parts. Still frames from the video were then used as digital images of stink bugs feeding on raspberry.

## **Results and Discussion**

In 2008, a total of 116 stink bugs were collected from the primocane-bearing raspberries (Table 5.1). Out of the 116 specimens collected, 62.1 % were brown stink bug, *E. servus*. The other species collected in 2008 (in decreasing order of the numbers collected) include: the dusky stink bug, *E. tristigma* (11.2 %), the twicestabbed stink bug, *C. lintneriana* (7.8 %), the onespotted stink bug, *E. variolarius* (6.9 %), the green stink bug, *A. hilare* (5.2 %), the redshouldered stink bug, *T. accerra* (4.3 %), *Banasa euchlora* (0.9 %), *T. calceata* (0.9 %), and *Coenus delius* (0.9 %).

*Euschistus servus*, was again the predominant species in 2009 comprising 41.2 % of the 233 stink bugs collected (Table 5.2). Other species included: *C. lintneriana* (26.6%), *A. hilare* (21.9 %), *E. tristigmus* (8.6 %), *Banasa calva* (0.4%), *Dendrocoris humeralis* (0.4%), *Brochymena quadripustulata* (0.4%), and *Hymenarcys nervosa* (0.4 %).

When the stink bug species counts were added together from 2008 and 2009, *E. servus* made up almost half (48.1 %) of the overall species composition (Table 5.3). Out of the 349 stink bugs collected in the two years, 20.3 % were *C. lintneriana*, 16.3 % were *A. hilare*, 9.5 % were *E. tristigmus*, 2.3% were *E. variolarius*, and 1.4 % were *T. accerra*. Species that were only collected once in the two years were considered as a minor stink bug species (*B. calva*, *B. euchlora*, *B. quadripustulata*, *C. delius*, *D. humeralis*, *H. nervosa*, and *T. calceata*). These minor stink bug species made up 2.0 % of the species present in 2008 and 2009.

The three most abundant stink bug species on the raspberries surveyed were *E. servus*, *A. hilare*, and *C. lintneriana* (Table 5.3). Of these three species, *E. servus* and *A. hilare*, are listed as being economically important to agriculture in North America (Hoffman 1971, McPherson and McPherson 2000, Panizzi et al. 2000, Kamminga et al. 2009b) and are the two most common pentatomid pests of agricultural crops in Virginia (Hoffman 1971, Nault and Speese 2002, Kamminga et al. 2009a, Koppel et al. 2009). *C. lintneriana* is only listed as a less important pest species by Kamminga et al. (2009b) and not discussed in McPherson and McPherson Stink bugs of Economic Importance in America North of Mexico (2000).

According to McPherson and McPherson (2000) and Panizzi et al. (2000) the brown stink bug, *E. servus* has the greatest economic importance of all *Euschistus* species. The brown stink bug was reported to be the most abundant and widespread stink bug in Virginia by Hoffman (1971). There are two subspecies of *E. servus* in Virginia, *E. servus servus* (Say) and *E. servus*

*uschitoides* (Vollenhoven) (McPherson and McPherson 2000). The two subspecies are difficult to separate because of similar descriptions in the taxonomic key that is used to separate the two. Therefore, only the species was reported but both subspecies were present. Raspberries are an ideal host for this polyphagous species because they prefer plants that have fruits or pods (McPherson 1982). They also inhabit areas that were previously disturbed by humans (Hoffman 1971). Raspberries are a reported host of *E. servus* (Stoner 1922, Furth 1974) and this species bivoltine life cycle (Munyaneza and McPherson 1993) allows the nymphs and adults to be present while most raspberries are in season.

*Acrosternum hilare* is also highly polyphagous and an important pest in fruits (McPherson and McPherson 2000). Even though the green stink bug is a general feeder on a wide variety of plants (Hoffman 1971), they prefer woody shrubs and trees (Jones and Sullivan 1982). Since raspberry plants are woody perennials, they are a suitable host for this pest species. Other reports of *A. hilare* using raspberry as a host include Knowlton and Harmston (1940) and Furth (1974) *A. hilare* is one of the most abundant pentatomids in Virginia (Hoffman 1971). Most of the feeding done by *A. hilare* occurs from mid-June through mid-September in Virginia (Underhill 1934). This feeding period exposes both primocane-fruiting and floricanes-fruiting raspberries to this pest.

The twicestabbed stink bug, *C. lintneriana* was the third most abundant stink bug species captured in 2008 (Table 5.1), the second most abundant stink bug in 2009 (Table 5.2), and the second most abundant species captured overall (Table 5.3). This phytophagous species is bivoltine with both adult and nymphal stages present while raspberries are in season (McPherson and Tecic 1997). Lugger (1900), Stoner (1922), Blatchley (1926), Tonks (1953) and Furth (1974) list raspberry as a host of *C. lintneriana*. It was my observation that when present, *C.*

*lintneriana* was found in aggregations of the same species. Since these stink bugs aggregate, there is a greater chance of an infested plant being damaged because of the potential of numerous stink bugs being present and causing injury in a restricted area. This aggregation could also explain why *C. lintneriana* was the second most abundant species collected, through a sampling bias. Spotting one twice-stabbed stink bug usually led to the collection of more than one stink bug. The greater number of this species collected can also be explained by their conspicuous reddish-orange and black markings that made them easier to spot and collect. Therefore, my findings reported here may be a biased representation of the actual species composition present in the raspberries. The impact this species has on raspberries is unclear. Even though many *C. lintneriana* were collected on raspberries, specimens were not observed feeding on the plant. Therefore, neither video nor image proof was obtained and this species' impact on raspberries is not fully understood.

Through digitally video recording stink bug activity on potted raspberries, feeding on multiple raspberry plant parts by stink bugs was documented. Stink bugs fed on the veins of the leaves (Figure 5.1), on the sepal of the fruiting structure (Figure 5.2), and between the drupelets of the berries (Figures 5.3 and 5.4). In the 2008 field experiments, countless thrips were observed on the plants in both the stink bug and control bags. The presence of these thrips made it difficult to distinguish thrips injury from stink bug injury. However, after stink bug feeding on the raspberries was observed in 2009, damage observed in the 2008 field trials was easier to identify as stink bug injury. Stink bug injury can now be identified as the holes between the drupelets of a raspberry (Figure 5.5). When the stink bug feeds on one drupelet continuously and/or on all sides, the drupelet can collapse (Figure 5.5). However, most stink bug feeding observed did not result in a deflated berry, only in punctures between the drupelets. The

punctures between the drupelets are conspicuous but may not necessarily make the berry unmarketable. Yet, great amounts of stink bug feeding on green or ripening fruit often may result in significant yield loss to raspberries.

There is potential for stink bug feeding to cause significant injury to raspberries. The impact that stink bug feeding has on raspberries depends on the maturity of the fruiting structures that are fed upon and the degree of feeding (the time spent feeding and/or the number of stylet insertions). The stink bug injury to raspberries observed by Coombs and Khan (1998) included berry distortion, discoloration, and reduced firmness. These injuries were not observed in my study. The different findings are most likely a result of the different growth stages of the berries used in each experiment, the different durations of time the stink bugs were allowed to feed on the raspberries, and/or the different stink bug species considered. Coombs and Khan applied the stink bugs to flowers and newly set fruit; whereas, stink bugs were bagged onto green and pink berries in my experiments. Since greater injury was observed by Coombs and Khan, it is implied that stink bug injury is more of an issue when flowers and newly set fruit is feed upon versus when green or ripening berries are consumed. This finding is consistent with research on other plants, which demonstrated that stink bug injury to mature plant structures is usually not noticeable and that injury to younger plant structures can be predominant (Spangler et al. 1993, Schuh and Slater 1995, McPherson and McPherson 2000). The different degrees of injury observed in each experiment can also be explained by the different durations of time that the stink bugs were allowed to feed. In Coombs and Khan's trial, stink bugs were allowed to feed for two weeks; whereas, in my experiments stink bugs were on the berries only 8 or 9 days. Moreover, different species of stink bugs were used in the two experiments, and it is possible that *Plautia affinis* Dallas, which was used by Coombs and Kahn (1998), is more damaging to

raspberries than *E. servus*. Overall, the different procedures used in the experiments support the need for a more conclusive study considering all stages of fruit maturation during one experiment. The stink bug complex's overall impact on raspberries can then be documented.

The impact that stink bug feeding has on raspberries can also be further explored through studying stylet sheaths. Stink bugs leave a stylet sheath at the feeding location (Schuh and Slater 1995, McPherson and McPherson 2000). Therefore, further experiments can be conducted to identify from where within the berry the stink bugs are pulling fluids. These experiments would also identify the specific feeding site, i.e., if the stink bugs are feeding on the berry or on the receptacle that holds the berry to the rest of the plant. Studies looking at the stylet sheaths can also be used to better understand how many proboscis insertions one stink bug makes during a given time frame. With knowledge of where and how often a stink bug feeds on a raspberry, there will be a better understanding of this pest complex impact on raspberries and if their injury warrants action.

Throughout the two-year study, stink bug frass was observed on multiple occasions on the berries (Figure 5.6). This presence of frass confirms the reports of Kieffer (1983), Ellis (1991), and Alford (2007) that stink bug excretions taint raspberries. Personal observation also confirms that raspberries with stink bug frass on them are distasteful. The offensive taste is easy to identify as stink bug injury through the taste being similar to the unpleasant smell for which stink bugs are notorious. Since excretions from one stink bug can ruin an entire berry and feeding injury from one stink bug results in a minute hole between the drupelet, stink bug frass may be a more important issue in some situations. A large population of stink bugs therefore would be of concern to raspberry growers.

In summary, *E. servus*, *C. lintneriana*, *A. hilare*, *E. tristigmus*, *E. variolarius*, *T. accerra*, *B. calva*, *B. euchlora*, *B. quadripustulata*, *C. delius*, *D. humeralis*, *H. nervosa*, and *T. calceata* were collected throughout the 2008 and 2009 raspberry field seasons. These stink bugs injured ripened or ripening raspberries by making holes between the drupelets while feeding. The injury to the more mature fruiting structures observed in this study was minor or unnoticeable. Coombs and Khan (1998) however, documented more severe injury to raspberries when the stink bugs fed on flowers or newly set fruit. The most damaging injury observed in this trial was the stink bug excretions on the berries. The holes left in ripened and ripening berries, the injury observed by Coombs and Khan, and the frass present where the stink bugs are located, indicate that the importance of this pest complex would increase with an increasing stink bug population. Therefore, the increasing population of the polyphagous brown marmorated stink bug (*Halyomorpha halys*) to Virginia maybe of concern to raspberry growers. During the survey of stink bug species present in 2008 and 2009, *H. halys* was not collected, but high populations were noted on caneberries in this area in 2010. Documenting stink bug injury to ripening raspberries and identifying the species present in a southwest Virginia planting of primocane raspberries, has clarified the importance of the stink bug complex on raspberries.

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## Tables and Figures

Table 5.1 The number and percentage of each stink bug species collected in 2008 from primocane-bearing raspberries.

Species	Common Name	Number	Percentage
<i>Euschistus servus</i>	Brown stink bug	72	<p style="text-align: center;"> <span style="color: blue;">■</span> <i>Euschistus servus</i>                      <span style="color: red;">■</span> <i>Euschistus tristigmus</i>  <span style="color: green;">■</span> <i>Cosmopepla lintneriana</i>            <span style="color: purple;">■</span> <i>Euschistus variolarius</i>  <span style="color: cyan;">■</span> <i>Acrosternum hilare</i>                    <span style="color: orange;">■</span> <i>Thyanta custator accerra</i>  <span style="color: lightblue;">■</span> <i>Banasa euchlora</i>                        <span style="color: pink;">■</span> <i>Thyanta calceata</i>  <span style="color: lightgreen;">■</span> <i>Coenus delius</i> </p>
<i>Euschistus tristigmus</i>	Dusky stink bug	13	
<i>Cosmopepla lintneriana</i>	Twicestabbed stink bug	9	
<i>Euschistus variolarius</i>	Onespotted stink bug	8	
<i>Acrosternum hilare</i>	Green stink bug	6	
<i>Thyanta accerra</i>	Redshouldered stink bug	5	
<i>Banasa euchlora</i>	Juniper stink bug	1	
<i>Thyanta calceata</i>	N/A	1	
<i>Coenus delius</i>	N/A	1	
	Total	116	

Table 5.2 The number and percentage of each stink bug species collected in 2009 from primocane-bearing raspberries.

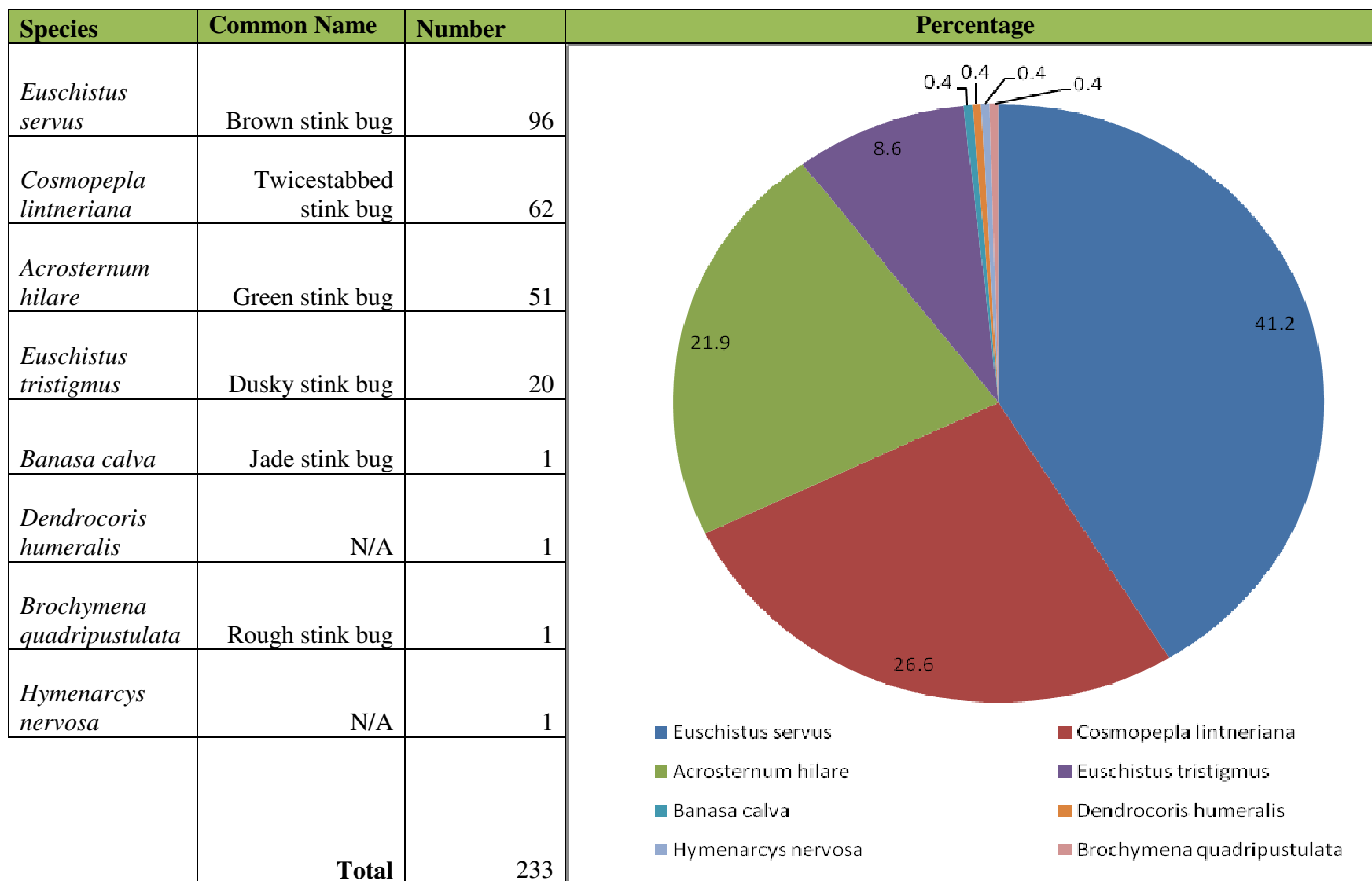


Table 5.3 Stink bug species collected from 2008 and 2009 were added together to get an overall stink bug species composition of the two years. The percentage of the overall species composition is also listed. A species is considered minor if it was only collected once in the two years.

Species	Common Name	Number	Percentage
<i>Euschistus servus</i>	Brown stink bug	168	
<i>Cosmopepla lintneriana</i>	Twicestabbed stink bug	71	
<i>Acrosternum hilare</i>	Green stink bug	57	
<i>Euschistus tristigmus</i>	Dusky stink bug	33	
<i>Euschistus variolarius</i>	Onespotted stink bug	8	
<i>Thyanta custator accerra</i>	Redshouldered stink bug	5	
<i>Banasa calva</i>	Jade stink bug	1	
<i>Brochymena quadripustulata</i>	Rough stink bug	1	
<i>Banasa euchlora</i>	Juniper stink bug	1	
<i>Dendrocoris humeralis</i>	N/A	1	
<i>Hymenarcys nervosa</i>	N/A	1	
<i>Thyanta calceata</i>	N/A	1	
<i>Coenus delius</i>	N/A	1	
	<b>Total</b>	349	



Figure 5.1 A brown stink bug (*Euschistus servus*) feeding on a vein of the underside of a raspberry leaf.



Figure 5.2 A brown stink bug (*Euschistus servus*) feeding on the sepal of a raspberry plant.





Figure 5.3 Brown stink bugs (*Euschistus servus*) feeding in-between the drupelets of unripe red raspberries.



Figure 5.4 Green stink bug (*Acrosternum hilare*) feeding in-between the drupelets of a 'Fall Gold' raspberry.



Figure 5.5 Enlarged image of stink bug damage on a red raspberry. Injury is seen as holes in between the drupelets and this caved-in drupelet.

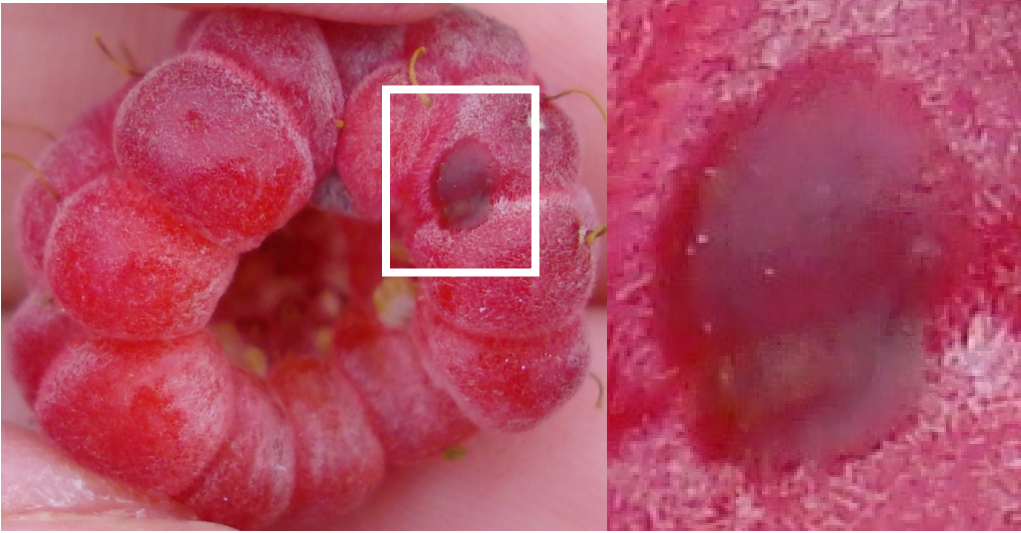


Figure 5.6 Enlarged image of stink bug frass on a red raspberry. Berries with stink bug frass have a bad taste making them unmarketable.

## Summary

### *Japanese beetle:*

The gregarious Japanese beetle (JB), *Popillia japonica* Newman, injures primocane-bearing caneberries (blackberries and raspberries) through skeletonizing the leaves and chewing irregular hollows in the berries. After the berries are injured they are more susceptible to transferable fruit diseases which spread to uninjured fruit making them unmarketable. The injury caused by these pests promoted the testing of different control methods for Japanese beetle (JB) in a primocane-bearing caneberry planting. To find an effective pesticide with a low-preharvest interval, insecticides were applied. Cultivar comparisons were also evaluated to identify the cultivars of raspberries and blackberries that were less susceptible to JB infestation and injury. Lastly, field and laboratory experiments were conducted to evaluate the effectiveness of using geranium's toxicity toward JB as a control method in raspberries.

The chemical control studies tested several insecticides with short pre-harvest intervals for their control of JB in caneberries. In field and laboratory experiments, deltamethrin displayed control through reducing JB infestation, reducing defoliation, and producing greater marketable yields on blackberry plants treated with this treatment. This insecticide is therefore recommended for JB control in caneberries. Chlorantraniliprole significantly reduced defoliation in 2008 and 2009. When lime-alum was used in the field, the number of JB present on the treated plants was reduced. However, the marketable yield was not increased and the defoliation was not decreased by lime-alum. Further studies using fewer applications or less active ingredients should be conducted to better understand the effectiveness of this insecticide. Thyme oil

lessened blackberry defoliation but did not decrease the number of JB present or prevent berry injury. Therefore, further tests should be conducted with this treatment before being recommended for use. Deltamethrin had greater marketable yield than the untreated control in the 2007 blackberries, however, no other treatment applied decreased the percent of unmarketable berries or yielded greater marketable yields. The low populations present during this study make it hard to know which treatments would control JB when a larger population is present.

The chemical bioassays found that deltamethrin, acetamiprid, an azadirachtin/ pyrethrin combination, and the *Chenopodium* extract kept more JB off of treated leaves than the untreated control. Deltamethrin, acetamiprid, chlorantraniliprole, and an azadirachtin/ pyrethrin combination killed more JB than the untreated control. Also, the *Chenopodium* extract, deltamethrin, acetamiprid, chlorantraniliprole, and the azadirachtin/ neem oil extract combination prevented defoliation. Deltamethrin and chlorantraniliprole were the only insecticides that exhibited control in both field and laboratory experiments which again support recommending these insecticides for control of JB. Overall, the field and laboratory insecticide experiments exhibited the importance of a larger JB population when trying to detect control. The instances where control was detected increase our knowledge of the treatments used and how they affect JB in caneberries.

Studies comparing JB on different raspberry and blackberry cultivars were conducted to test if the cultivar affected the JB numbers present, prevented injury to the berries, or prevented defoliation. There were significantly more JB counted on 'Prelude' compared to 'Anne', 'Caroline', 'Heritage', 'Dinkum', or 'Himbo Top'. There were also

more beetles on 'Prime-Jim' than on 'Prime-Jan' blackberries. Reasons for the different cultivar susceptibilities to JB infestations could be due to multiple stimulants that attract JB, including physical characteristics, naturally occurring volatiles, and injury-induced volatiles. 'Prelude' and 'Prime-Jim' possesses a combination of these characteristics that make these cultivars preferred for infestation.

'Heritage' had a higher percent of injured fruit compared to 'Autumn Bliss', 'Fall Gold', 'Himbo Top', or 'Prelude' in 2007. Also in 2007, 'Caroline' had a higher percent of injured fruit than 'Autumn Bliss'. 'Caroline' had a higher percent of injured fruit compared to 'Autumn Bliss', 'Dinkum', 'Fall Gold' or 'Prelude' in 2009. Also in 2009, 'Anne' and 'Himbo Top' had a higher percentage of injured fruit compared with 'Autumn Bliss', 'Prelude', 'Fall Gold', and 'Dinkum'. In the blackberries, 'Prime-Jan' was more susceptible to injury than 'Prime-Jim' in 2007 and 2008. The cultivars that were more susceptible to injury can be explained by differing factors including the plant's physical characteristics, the plant's naturally occurring volatiles, JB injury induced volatiles or the berries' chemical content.

The raspberry cultivars also experienced differing levels of defoliation. In 2008, 'Prelude' experienced more defoliation than 'Anne', 'Heritage', 'Caroline', or 'Dinkum'. Also in 2009, 'Prelude' and 'Fall Gold' experienced more defoliation than 'Caroline' or 'Anne'. The differing susceptibilities of foliar injury can be explained by differing factors including the plant's physical characteristics, the plant's naturally occurring volatiles, JB injury induced volatiles or the foliar chemical content. More studies need to be conducted to determine the exact factor(s) responsible for a cultivars' susceptibility to JB infestation, fruit injury, and defoliation. However, my study increased what is known

about the different primocane-bearing caneberry cultivars and their susceptibilities to JB infestation and ingestion.

Geraniums (*Pelargonium* spp.) are the most commonly documented plants that are toxic to JB. My tests conducted enhance our understandings of utilizing geraniums as JB control in raspberries. Field tests found that previous exposure to geraniums lessened the average raspberry defoliation. Also, defoliation was lessened by continual exposure to geraniums in a laboratory experiment. Though JB did not choose to be on a geranium petal over a 'Prelude' raspberry leaf, other choice tests using less JB susceptible raspberry cultivars are suggested. It is proposed that placement of the geraniums can be used to orient JB towards the geraniums instead of the raspberries. However, my results indicate that geranium's effectiveness as a trap crop need elaboration. These preliminary findings can be used when developing more in-depth studies of geraniums as JB control and support the production of a treatment containing quisqualic acid, the toxic compound in geraniums. Once an effective control method is identified, more natural, consumer-friendly pest management strategies can be implemented using geraniums.

Overall, the experiments conducted strengthen the field of JB control in primocane-bearing raspberries and blackberries. The natural benefits provided by caneberries along with the destructive nature of JB, increase the importance of the research conducted on this pest in these cropping systems. If JB populations increase compared to the populations observed in 2007-2009, I would recommend further tests to be conducted using my research and experiences as a backbone. As long as new control methods are created and new caneberry cultivars are genetically engineered, the



information learned about JB in caneberries will continue. My research only adds to the library of information provided on JB control and caneberries.

***Stink bugs:***

There have been multiple reports of raspberries being a host to the stink bugs complex. There have also been reports of stink bugs injuring raspberries. However, photographic proof has not been provided. My study strengthens what is known about this pest complex in raspberries through 1) identifying the species present within a southwest Virginia raspberry planting and 2) through documenting caneberry injury caused by stink bugs.

The following stink bug species were collected and identified in 2008 and 2009: *Acrosternum hilare*, *Banasa calva*, *B. euchlora*, *B. quadripustulata*, *Coenus delius*, *Cosmopepla lintneriana*, *Dendrocoris humeralis*, *Euschistus servus*, *E. tristigmus*, *E. variolarius*, *Hymenarcys nervosa*, *Thyanta custator accerra*, and *T. calceata*. The three most common species collected were *E. servus*, *C. lintneriana*, and *A. hilare*. Two of these stink bug species, *E. servus* and *A. hilare*, were observed injuring raspberries by making holes in-between the drupelets while feeding. Most feeding injury observed was minor or unnoticeable, but injury did occur from the stink bug excretions on the berries. Because holes are left after feeding and frass is secreted where the stink bugs are located, the importance of this pest would increase with an increasing stink bug population. Therefore, the increasing population of the polyphagous brown marmorated stink bug (*Halyomorpha halys*) to Virginia maybe of concern to raspberry growers. During the survey of stink bug species present in 2008 and 2009, *H. halys* was not collected.

The impact that stink bug feeding has on raspberries can be furthered explored through studying the position and frequency of stylet sheaths. Also, other stink bug species can be observed feeding on raspberries to compare their behavior to the behaviors observed in my experiments. The commonly found *C. lintneriana* or the invasive *H. halys*, would be two stink bugs species of current interest for such experiments. Through identifying stink bug injury to raspberries along with identifying the species present in a Southwest Virginia planting of primocane raspberries, the importance of the stink bug complex on raspberries is better understood.