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NOTE

Biodiversity and sweep sampling of selected leafhopper and beetle species in wild blueberries

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The Canadian blueberry industry is a multi-million dollar commodity and represents the largest fruit crop by area under production in the country (Robichaud 2006). Pest management in lowbush blueberry crops is important to sustain this provincial commodity as the presence of pests through the various stages of production reduces fruit yield and both producers and the government incur economic loss.

Froghoppers (Hemiptera: Cercopidae), and leafhoppers (Hemiptera: Cicadellidae) are pests that use sucking mouthparts to pierce and feed on plant tissues (Hamilton 1982). Numerous species are considered plant pests that either actively feed on plant tissue or vector disease, inflicting economic loss in agricultural systems. From an economic standpoint, puncturing by piercing mouthparts may deform leaves, produce galls, prevent proper fruit and seed formation, and, vector plant diseases (Hamilton 1982; Deitz 2008). Few studies have documented the direct effect of froghoppers and leafhoppers on blueberry plants (de Leeuw 1975; Tozzi et al. 1993).

Blueberry integrated crop management takes into account the standard practices used to manage wild blueberry crops. It aims to minimize pesticide and fertilizer application, yielding the best environmental outcome, while also maximizing the harvest (Yarborough et al. 2001). Early detection of elevated pest populations leads to treating infestations with fewer resources. To determine the damage potential of an infestation, one must take into account the level of infestation required to reach a threshold where the potential economic loss is substantial, i.e., the action threshold (Yarborough et al. 2001). One problem that arises in monitoring is how many sweep samples accurately reflect populations within a given area. A sweep sample comprises only a small portion of the insects present in the vegetation under study. In estimating how many sweep samples represent the population of a unit area, it is required to make an estimate on the average number of sampled species present (Beall 1935).

This study documents the biodiversity of three target groups of insects in selected wild blueberry fields in Nova Scotia: froghoppers (Cercopidae), leafhoppers (Cicadellidae), and flea beetles (Chrysomelidae), the latter of which can be important pests in lowbush blueberry (Yarborough et al. 2001; Yarborough and Collins 1995). Sweep sample densities of the target insects at all field sites were quantified to evaluate whether increased sampling gives a more accurate estimate of the population numbers.

Four blueberry fields in Nova Scotia were monitored using sweep-nets from 15 June 2012 to 25 July 2012. Two sites were located in Earltown (45.5778°N, 63.1379°W), 1 site in Murray Siding (45.3639°N, 63.1633°W), and 1 site in Debert (45.4333°N, 63.4667°W). Fields were swept twice a week in June and July, i.e., 12 June, 15 June, 18 June, 22 June, 29 June, 3 July, 10 July, 12 July, 17 July, and 20 July. Samples were collected using a 30-cm diameter sweep net. Sweeping was synchronized with a walking pace where one step, estimated to be one meter, corresponded to one sweeping motion. At the end of each arc and at the beginning of the next step, the net was quickly twisted at a 180° angle to prevent the escape of captured insects. The net was firmly swept through the vegetation but an attempt was made to minimize the damage to the blossoms or berries on the blueberry plants. A sweep sample constituted 25 step-sweeps. Five sweep samples were collected between 11:00 am and 3:00 pm at each site for each sample day of the trial. The experimenter chose a random path through each field and the sweep represented a distance of approximately 25 m. Samples were

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stored at -20°C at Acadia University until processing.

Frozen samples were emptied into a large plastic container and insects were separated from vegetation, soil, and debris gathered along with insects using a pair of forceps. Insects in each sample were then placed into a labeled vial, outlining site and the date of collection. Samples were returned to the freezer until later pinning and identification of froghoppers, leafhoppers, and flea beetles.

From the five available samples for each site on a day of collection, one was randomly selected and examined independently. The other four remaining samples were pooled together and subjected to the same process. All flea beetle, froghopper, and leafhopper species present in each sample were sorted, counted and pinned. Flea beetles and froghopper were identified to species, and leafhoppers required identification at the Canadian National Collection (CNC) of Insects, Arachnids and Nematodes (Ottawa, ON). Voucher specimens of all species were also sent to CNC for identification, confirmation, and a permanent record of collection data.

To analyze whether one sweep of 25 m could accurately reflect the biodiversity in a given site on a sampling day, the abundance of different leafhopper species, froghopper species, and flea beetle species captured with only one sweep were compared with the number of species captured using four sweeps for each field site. For the flea beetle comparisons, several samples of five sweeps were pooled for another study before the analysis occurred. Shannon-Weiner diversity (S-W diversity) indices were calculated for the leafhopper and froghopper groups for both the single sample and the pooled sample of four sweeps. For flea beetles, S-W diversity could not be calculated due to the pooling of all five sweeps on some test days before analysis.

Overall, two flea beetle species, 15 leafhopper species, and three froghopper species were identified. Leafhopper, froghopper, and flea beetle biodiversity were compared among the two Earltown sites, the Murray Siding field, and Debert field (Table 1). The results correspond to the five pooled sweep samples of 25 m each.

In general, leafhoppers were the most diverse group with 15 different species with variation in the number of species represented at each field. Many leafhopper species had low abundances. Nine species had only one recorded specimen captured (Table 1) and two other species were also not very abundant (*Neokolla hieroglyphica* Say, N=2, *Balclutha impicta* Van Duzee, N=3).

Only three species had a pooled sample of five sweeps with a count of over 100 individuals on an individual day. One species of flea beetle, *Mantura chrysanthemi* Koch (Coleoptera: Chrysomelidae) and two species of froghoppers, *Clastoptera saintcyri* Provanche, (Hemiptera: Clastopteridae) and *Philaenus spumarius* L. (Hemiptera: Cercopidae) were present in the samples in high numbers. Each species count was plotted over time to determine relative population levels throughout the sampling period (Figure 1A). Murray Siding had the highest population of *Mantura chrysanthemi*, although it was present at all four sites, with a lone specimen captured in Debert. All populations reached peak density between 18 June 2012 and 22 June 2012.

All field counts were plotted against one another to determine the peak density (Figure 1B). The population of *Clastoptera saintcyri* in Earltown was higher later in the sampling period. Peak density was reached between 17 July 2012 and 20 July 2012 with a major decrease in number between the dates observed in all locations. At the conclusion of the study population density was still above 200 insects per five sweeps in both Earltown sites. The species was not present in any field before 10 July 2012. The population was high in Debert but not as prevalent in Murray Siding.

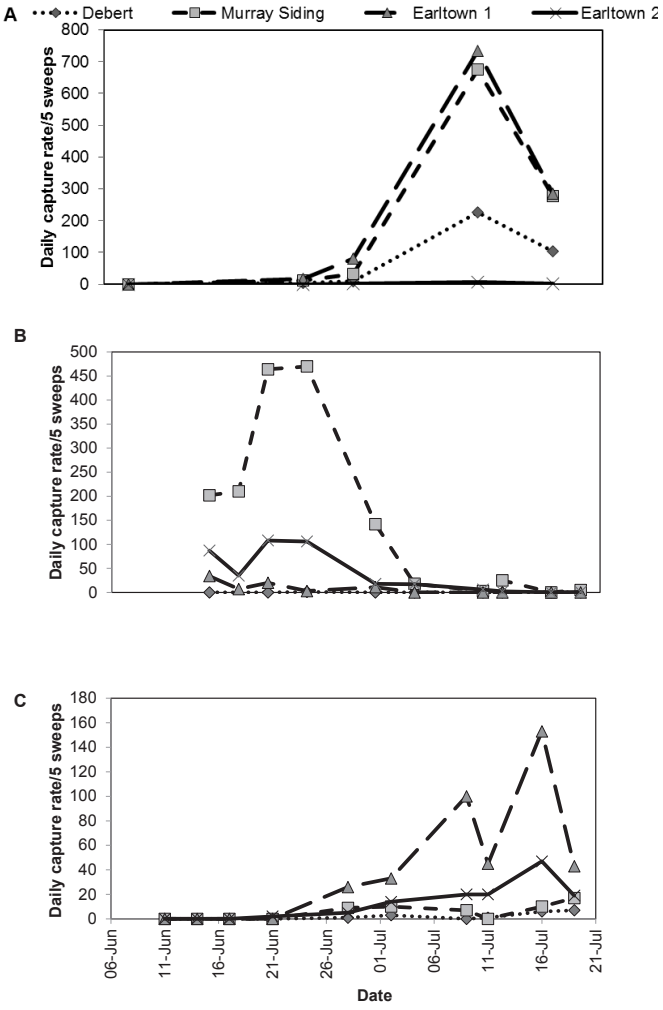
Philaenus spumarius was also abundant, and numerous morphs of coloration and shape were apparent. Population density was highest in Earltown 1, slightly lower in Earltown 2, with much lower abundances in Murray Siding and Debert. Peak density occurred

Table 1. Leafhopper, froghopper and flea beetle species sampled from lowbush blueberry fields in Nova Scotia in 2012. Numbers represent total capture from five pooled samples.

	Species	Site				
		Earltown 1	Earltown 2	Debert	Murray Siding	Total
Cicadellidae	*Ponana scarlatina* DeLong	0	0	0	1	1
	Latalus ocellaris Fallen	2	0	0	0	2
	Helochara communis Fitch	1	1	0	0	2
	Neokolla hieroglyphica Say	2	1	0	0	3
	Athysanus argentarius Metcalf	47	3	0	0	50
	Gyponana spp.	0	0	1	0	1
	Agallia quadripunctata Provancher	6	3	1	0	10
	Elymana sulphurella Zetterstedt	16	24	1	0	41
	Balclutha impicta Van Duzee	0	3	0	0	3
	Cuerna spp.	1	0	0	0	1
	Scaphytopius spp.	15	3	8	20	46
	Graphocephala picta Walker	1	0	0	0	1
	Gyponana serpent DeLong	1	0	0	0	1
	Limotettix spp.	1	0	0	0	1
Cercopidae	*Lepyronia quadrangularis*	31	7	1	1	40
	Philaenus spumarius	401	127	18	59	599
	Clastoptera saintcyri	998	1117	341	8	2464
Chrysomelidae	*Mantura chrysanthemi*	75	372	1	1539	1987
	Altica sylvia	2	7	10	6	25
	Species Present	**16**	**12**	**9**	**7**	**19**

around 17 July 2012 although individual sites were more sporadic in density fluctuation (Figure 1C).

Figure 1. Comparison of populations of (A) *Mantura chrysanthemi*, (B) *Clastoptera saintcyri*, and (C) *Philaenus spumarius* in four lowbush blueberry sites in Nova Scotia in 2012.



Leafhopper species, froghopper species, and flea beetle species (Table 2) were compared to quantify the number of species captured with only one sweep to the number of species captured using four sweeps for each field site. In terms of species diversity, pooled sweeps corresponded to a higher diversity count. For leafhoppers, estimated biodiversity was approximately two times greater for every field when the pooled sweep was compared to the single sweep.

In terms of biodiversity, there was no difference in the number of species of froghoppers or flea beetles between the sites; all fields had the same number of species, although in varied densities. Leafhoppers were the only group with variable species diversity among locations. Earltown 1 had the highest species diversity at 10, followed by counts of 8, 4, and 2 for Earltown 2, Debert, and Murray Siding, respectively. Earltown field sites proved to be the most speciose when leafhopper and froghopper diversity was compared. Leafhopper Shannon-Weiner (SW) diversities were higher in Earltown sites than in Debert and Murray Siding (Table 2). SW diversities for froghoppers were also highest at Earltown 1, and lowest at Debert (Table 2). This could be due to the fact that the blueberry fields are organically grown in a pesticide free-environment. Both sites in Debert and Murray Siding were sprayed for blueberry fruit fly (*Rhagoletis mendax* Curran (Diptera: Tephritidae) in early June. Also, the Earltown sites shared five common species of leafhoppers. This is thought to be due to their relative proximity (50 m) to each other. Nine species of leafhoppers were only caught once, therefore, diversity could not be compared between fields. With increasing distance between field sites, there was observable differences in the diversity of leafhoppers among the four sampled sites.

One species of flea beetle, *Mantura chrysanthemi* and two species of froghoppers, *Clastoptera saintcyri,* and *Philaenus spumarius*, were present in pooled samples at densities greater than 100 counts in a pooled sample of 5 sweeps. In Nova Scotia, a monitoring program exists for blueberry growers to manage pest species. Most thresholds are set per 10 sweeps. For example, blueberry flea beetle, *Altica sylvia* Molloch (Coleoptera: Chrysomelidae), which was present at each site, has a threshold set at 50 adults or larvae per ten sweeps.

Murray Siding had the highest population of *Mantura chrysanthemi*. Peak density of the population was reached between 18 June 2012 and 22 June 2012. When comparing field sites to one another, *Altica sylvia* and *Mantura chrysanthemi* were highly abundant at all fields except Debert.

Peak density of *Clastoptera saintcyri* was between 17 July 2012 and 20 July 2012 at all locations. However, the study concluded with population number remaining high at over 200 insects per five sweeps at both Earltown sites. *Clastoptera saintcyri,* commonly known as the heath spittlebug, uses ericaceous plants (including lowbush blueberry) as hosts for nymphs and adults. They are abundant in Eastern Canada in heath environments in mixed pine-maple woods. Froghoppers have the potential to induce economic loss for growers. Puncturing by piercing mouthparts may deform leaves, produce galls, or prevent proper fruit and seed formation. Froghoppers also may vector plant diseases

Table 2. Diversity of leafhopper, froghopper, and flea beetle species sampled from lowbush blueberry fields in Nova Scotia in 2012 in a single sampling effort and four pooled samples (in parentheses). Numbers represent total capture per site.

	Species	Site				
Family		Earltown 1	Earltown 2	Debert	Murray Siding	Total
Cicadellidae	*Ponana scarlatina*	0 (0)	0 (0)	0 (0)	0 (1)	0 (1)
	Latalus ocellaris	0 (1)	0 (0)	0 (0)	0 (0)	0 (1)
	Helochara communis	0 (1)	0 (0)	0 (0)	0 (0)	0 (1)
	Neokolla hieroglyphica	0 (2)	1 (0)	0 (0)	0 (0)	1 (2)
	Athysanus argentarius	8 (39)	0 (3)	0 (0)	0 (0)	8 (42)
	Gyponana spp.	0 (0)	0 (0)	0 (1)	0 (0)	0 (1)
	Agallia quadripunctata	0 (0)	0 (0)	0 (1)	0 (0)	0 (1)
	Elymana sulphurella	4 (4)	2 (2)	1 (0)	0 (0)	7 (6)
	Balclutha impicta	15 (5)	11 (9)	0 (1)	0 (0)	26 (15)
	Cuerna spp.	0 (0)	0 (1)	0 (0)	0 (0)	0 (1)
	Scaphytopius spp.	1 (0)	0 (0)	0 (0)	0 (0)	1 (0)
	Graphocephala picta	0 (15)	2 (1)	0 (8)	3 (17)	5 (41)
	Gymnonana serpent	0 (1)	0 (0)	0 (0)	0 (0)	0 (1)
	Limotettix spp.	0 (1)	0 (0)	0 (0)	0 (0)	0 (1)
	Number of species	4 (9)	4 (7)	1 (3)	1 (2)	-
	Shannon-Weiner Diversity	1.16 (1.27)	0.83 (1.56)	0 (0.23)	0 (0.64)	-
Cercopidae	*Lepyronia quadrangularis*	11 (20)	1 (6)	0 (1)	0 (1)	12 (28)
	Philaenus spumarius	49 (352)	24 (103)	3 (15)	14 (39)	90 (509)
	Clastoptera saintcyri	36 (962)	255 (862)	86 (255)	1 (7)	378 (2086)
	Number of species	3 (3)	3 (3)	2 (3)	2 (3)	-
	Shannon-Weiner Diversity	0.96 (0.65)	0.32 (0.38)	0.25 (0.52)	0.15 (0.24)	-
Chrysomelidae	*Mantura chrysanthemi*	22 (53)	36 (336)	0 (1)	335 (1204)	393 (1594)
	Altica sylvia	0 (2)	1 (4)	0 (2)	1 (0)	2 (8)
	Number of species	1	2	0	2	-

(Hamilton 1982); therefore, highly abundant populations in lowbush blueberry fields may prove to be damaging.

Other froghopper species present at the fields included *Philaenus spumarius* and *Lepyronia quadrangularis* Say, (Hemiptera: Cercopidae). *Philaenus spumarius*, the common meadow spittlebug, is common throughout eastern and western temperate North America (Hamilton 1982). Adults and nymphs feed on a broad range of host plants of over 380 documented species (Hamilton 1982). *Philaenus spumarius* was present in high numbers in the sweeps. Population density was highest in Earltown 1, slightly lower in Earltown 2, and the species was present in Murray Siding and Debert, but in much lower numbers. Peak density occurred around 17 July 2012. *Lepyronia quadrangularis* is abundant in weedy areas near woodlands throughout North America east of the Rocky Mountains (Hamilton 1982). Nymphs and adults feed on aerial parts of many different plants (Hamilton 1982).

In the Atlantic region of Canada, there are 217 documented leafhopper species, of which 24 are introduced (Hamilton and Langor 1987). Hamilton and Langor (1987) found 65 leafhopper species to be native and common to Cape Breton and Newfoundland. Our study found an introduced European species, *Athysanus argentarius* Metcalf (Hemiptera: Cicadellidae), most abundant at Earltown 1. It was first recorded in North America in Massachusetts around 1920 and was widespread in Cape Breton, NS by 1973 (Hamilton and Langor 1987). It is unclear why it might be more abundant at this specific site, but it is possible that insecticidal treatments at Murray Siding and Debert impacted populations at these sites.

The potential for many of these species to vector diseases within *Vaccinium* species and between crops and wild plants presents a significant concern. A number of phytoplasma diseases affect *Vaccinium* crops, including cranberry false blossom (Xu and Chen 1996), witches' broom of blueberry (de Leeuw 1975), and blueberry stunt disease (Tozzi et al. 1993). It remains unclear which, if any of the species documented in the current study, pose a threat to vector these diseases.

Perhaps of greater concern to the blueberry industry, blueberry stunt phytoplasma is exclusively vectored by the sharpnosed leafhopper, *Scaphytopius magdalensis* Prov. (Hemiptera: Cicadellidae) (Neilson 1968), and *Limotettix vaccini* (Van Duzee) (Hemiptera: Cicadellidae) is a key vector of phytoplasma, including cranberry false-blossom disease (Lee et al. 2014). The presence of these vectors in study fields raises concerns for the potential for these diseases to impact crop health. Finally, despite the lack of evidence for disease transmission in many of these species, there may be a vector potential in many of these species which has simply not been tested.

The presence or absence of a species in a single sample of a 25-m sweep or the pooled sample of four 25-m sweeps was used to estimate biodiversity. Overall, increased sampling did lead to an observable difference in biodiversity. For leafhoppers, sampled biodiversity was equal to, or over two times greater for every field when the pooled sample was compared to the single sweep. For flea beetles and froghoppers a trend was still apparent; however, since both groups had fewer sampled species, it was less clear.

In summary, this study documents species diversity and population levels of potentially damaging pest species of wild blueberries. Thresholds for many blueberry pests are outlined in scouting guides and represent the average number of insects captured for 20 sets of 10 sweeps (Yarborough et al. 2001). However, lowering sample size leaves the grower open to outliers that do not accurately reflect population numbers. Regardless of

the number of conducted sweeps, if a threshold is met in one sweep, the grower should choose to take more samples to gain a better understanding of the pest level and then choose how to properly manage early infestations.

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