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Intraspecific Variation in Aphid Resistance and Constitutive Phenolics Exhibited by the Wild Blueberry *Vaccinium darrowi*

C. M. Ranger · A. P. Singh · J. Johnson-Cicalese · S. Polavarapu · N. Vorsa

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Abstract *Illinoia pepperi* (MacGillivray) infests cultivated highbush blueberries, *Vaccinium corymbosum* L., in the Northeastern United States. Allopatric resistance to *I. pepperi* was examined in *Vaccinium darrowi* Camp, which evolved in the absence of *I. pepperi* in the Southeastern U.S. *V. corymbosum* cv. "Elliott", was used as a susceptible control. Between population variability in *I. pepperi* resistance was assessed by measuring length of the prereproductive period, fecundity, and survivorship on 14 *V. darrowi* accessions representing 11 discrete wild populations. Length of *I. pepperi*'s prereproductive period and survivorship were not significantly affected. However, differences were detected in fecundity and the intrinsic rate of increase (r_m). Within population variability in resistance was measured by confining first instars to 24 accessions from a single wild population of *V. darrowi* (NJ88-06). Significant differences in the mean total number of aphids occurring after 20 d were only detected between 2 of the 24 *V. darrowi* accessions. A greater degree of diversity in *I. pepperi* resistance exists between populations of *V. darrowi* compared to within a population. Constitutive leaf and stem polyphenolics were identified by HPLC-MS and quantified from 14 of the *V. darrowi* accessions. The accessions varied in concentrations of five phenolic acids and seven flavonol glycosides, but a correlation was not found between individual or total phenolics and aphid performance. Overall, screening within and between populations of *V. darrowi* identified promising sources of aphid resistance, but

C. M. Ranger · A. P. Singh · J. Johnson-Cicalese · S. Polavarapu · N. Vorsa (✉)
Philip E. Marucci Center for Blueberry and Cranberry Research and Extension,
Rutgers-The State University of New Jersey, 125A Lake Oswego Rd., Chatsworth, NJ 08019, USA
e-mail: vorsa@aesop.rutgers.edu

C. M. Ranger
e-mail: ranger.1@osu.edu

A. P. Singh
Department of Plant Biology and Pathology, Rutgers-The State University of New Jersey,
New Brunswick, NJ 08901, USA

Present address:
C. M. Ranger
Horticultural Insects Research Lab, USDA-ARS, 1680 Madison Ave, Wooster, OH 44691, USA

phenolic acid and flavonol glycoside profiles did not predict resistance levels. The mechanism of resistance remains to be identified.

Keywords Allopatric resistance · Blueberry aphid · *Illinoia pepperi* · Metabolic profiling · *Vaccinium darrowi*

Introduction

The aphid, *Illinoia pepperi* (MacGillivary), is the dominant aphid infesting cultivated highbush blueberry, *Vaccinium corymbosum* L., in New Jersey and Michigan (Morimoto and Ramsdell, 1985; Elsner and Kriegel, 1989). The entire life cycle of *I. pepperi* is spent on a *Vaccinium* host, which may include cultivated and wild highbush blueberry, *V. corymbosum*, wild lowbush blueberry, *Vaccinium angustifolium* (Aiton), and wild velvetleaf blueberry, *Vaccinium myrtilloides* Michaux (Lockhart and Hall, 1962; Elsner and Kriegel, 1989). However, *I. pepperi* populations are highest on cultivated blueberry, and movement outside of a field to wild *Vaccinium* plants in the surrounding landscape is considered rare (Morimoto and Ramsdell, 1985).

The holocyclic life cycle of *I. pepperi* consists of overwintering eggs that hatch into wingless, parthenogenic fundatrices during the spring months (Elsner and Kriegel, 1989). Subsequent summer generations consist of alate or apterous virginoparae giving birth parthenogenetically to live young, with the highest populations occurring in June (Morimoto and Ramsdell, 1985). During the fall, sexual morphs of apterous females and alate males are produced, but Morimoto and Ramsdell (1985) collected a few alatae by using water-pan traps after mid-July.

Aphid-transmitted viruses, such as blueberry scorch virus (BlScV) and blueberry shoestring virus (BBSSV), pose economic threats to the commercial production of highbush blueberry, particularly in New Jersey, Michigan, and the Pacific Northwest (Morimoto and Ramsdell, 1985; Martin and Bristow, 1995; Hillman et al., 1996). BlScV belongs to the carlavirus group and is transmitted in a nonpersistent fashion (Hillman et al., 1996), while transmission of the sobemovirus BBSSV occurs in a persistent fashion (Ramsdell, 1979; Terhune et al., 1991). Managing the incidence of BlScV and BBSSV mainly occurs through insecticide applications to reduce aphid population densities, by rouging symptomatic plants and by planting clean root stock (Raworth, 2004). However, as BlScV has a latent period of 1–3 years before symptoms appear (Bristow et al., 2000), an infected plant could continue to serve as a source of inoculum. Therefore, simply rouging symptomatic plants does not represent an effective management strategy. Host-plant resistance can provide a multifaceted approach to vector and virus management. For example, Massonie and Maison (1986) documented reduced virus transmission by using vector-resistant peaches, *Prunus* spp., to reduce the population fitness of the green peach aphid, *Myzus persicae* (Sulzer). The use of plant resistance to limit the population growth of *I. pepperi* could aid in reducing the incidence of aphid-transmitted viruses in cultivated blueberries.

Previous studies found that high levels of insect and disease resistance were exhibited by wild *Vaccinium* spp. (Etzel and Meyer, 1986; Meyer and Ballington, 1990; Stretch et al., 2001). To identify promising sources of aphid resistance, performance parameters of *I. pepperi* were compared after being reared on several wild *Vaccinium* spp. that occur sympatrically and allopatrically with *I. pepperi* (Ranger et al., 2006). Delayed development, along with reduced fecundity and survivorship were associated with several of the *Vaccinium* spp., particularly the allopatric spp. *Vaccinium myrsinites* Lamarck, *Vaccinium*

Table 1 Between population variability in resistance of *Vaccinium darrowi* to the aphid *Illinoia pepperi*

Species and Population ID[b]	Collection Locality	Mean (±SEM)[a]		
		Prereproductive Period (TTR)[c]	Total Fecundity[d]	Survivorship (%) 42 d After Birth
V. darrowi				
NJ88-06-20	Santa Rosa Co., FL	14.7±0.9a	22.0±3.3a	66a
NJ88-15-09	Okaloosa Co., FL	14.7±1.2a	22.1±4.1a	88a
NJ88-13-22	Liberty Co., FL	14.3±0.9a	25.3±2.1ab	87.5a
NJ88-14-03	Gulf Co., FL	14.0±0.9a	29.4±3.9ab	88a
NJ88-04-64	Santa Rosa Co., FL	14.0±0.8a	30.4±4.2ab	66a
NJ88-17-13	Stone Co., MS	14.2±1.3a	34.1±5.0ab	90a
NC84-06a-37	Lake Co., FL	13.8±1.0a	35.0±4.7ab	88a
NJ88-06-01	Santa Rosa Co., FL	13.8±0.8a	36.1±4.8abc	80a
Fla 4B	Marion Co., FL	13.6±0.9a	44.9±4.9bc	100a
NJ88-12-41	Liberty Co., FL	13.3±1.1a	30.1±3.7ab	88a
NJ88-05-29	Covington Co., AL	13.3±1.0a	26.3±4.0ab	88a
NC84-06a-20	Lake Co., FL	13.3±1.0a	38.3±4.8abc	90a
NJ88-11-32	Lake Co., FL	12.5±0.7a	45.4±4.8bc	88a
NJ88-17-27	Stone Co., MS	12.2±0.6a	42.9±4.6bc	100a
V. corymbosum				
Elliott	USDA Breeding Program	11.1±0.4a	58.9±4.0c	100a

[a] Means within columns followed by the same letter are not significantly different [α±0.05; Tukey's studentized range (HSD) test]. N±9 for all accessions

[b] The first value in the population ID (e.g., NJ88-15-09) represents the year of collection, the second value represents the population number, and the third value represents the specimen number.

[c] From birth to reproductive maturity

[d] Total offspring produced over a period of 42 d after birth

hirsutum Buckley, *Vaccinium boreale* Hall and Aalders, and *Vaccinium darrowi* Camp. Wild *V. darrowi* accessions have been used to develop and improve numerous highbush cultivars (Chandler et al., 1985; Erb et al., 1990; Hancock et al., 1992), but *V. darrowi* accessions highly resistant to *I. pepperi* have yet to be identified.

As heterogeneity in resistance levels to insect herbivores occurs in wild plant populations (Fritz, 1992), we hypothesized that intraspecific variability in *I. pepperi* resistance would exist within and between populations of *V. darrowi*. Because variability in phenolics occurs within and between plant populations (Nichols-Orians et al., 1993; Hakulinen et al., 1995; Binns et al., 2002; Van Heerden et al., 2003), constitutive phenolics were also quantified to determine if such compounds could be used to predict resistance levels.

Methods and Materials

Plants and Insects A total of 37 different *V. darrowi* (2×) accessions were evaluated for within and between population variability in resistance to the blueberry aphid, *I. pepperi*, along with the standard highbush cultivar *V. corymbosum* cv. Elliott (4×) as a susceptible control. All *V. darrowi* accessions were collected from wild populations (Table 1) and are maintained in the *Vaccinium* germplasm collection at the Philip E. Marucci Center for

Blueberry and Cranberry Research and Extension, Rutgers-The State University of New Jersey, Chatsworth, NJ, USA. Collection details are described by Bruederle and Vorsa (1994). Specimens collected from different counties represent distinct populations, while those from the same county represent a single population (Table 1). The parent plants are potted in a (1:1 *v*:*v*) sand to peat moss mixture and maintained under greenhouse conditions. Multiple clones of each *V. darrowi* accession were established in May 2004 by excising rhizomes from individual parent plants for each accession. Clones of each *V. darrowi* accession are genetically identical. One-yr-old, *V. corymbosum* cv. Elliott plants were purchased from DeGrandchamp's blueberry farm (South Haven, MI, USA). All plants were grown in a sand: peat moss (1:1 *v*:*v*) mixture and maintained in a greenhouse for approximately 6 mo before their use in experiments. Osmocote 14–14–14 (N:P:K) (Scotts-Sierra Horticultural Products, Marysville, OH, USA) was used as a controlled release fertilizer.

A colony of *I. pepperi* was established in June 2004 by collecting several apterous *I. pepperi* virginoparae from a commercial blueberry planting in Atlantic Co., NJ, USA. As the overall objective was to identify allopatric sources of resistance against *I. pepperi*, multiple *I. pepperi* clones were used to establish and maintain the colony. Apterous *I. pepperi* were occasionally added to the colony to ensure the presence of multiple genotypes. Aphids were reared on excised shoots of *V. corymbosum* cv. Elliott and maintained in a laboratory under fluorescent lights with a photoperiod of 16:8 (L:D).

Between Population Variability in Aphid Resistance Heterogeneity in resistance of *V. darrowi* to *I. pepperi* was measured by using 14 *V. darrowi* accessions representing 11 discrete populations (Table 1), along with *V. corymbosum* cv. Elliott as a susceptible control. Three adult apterous *I. pepperi* were collected from the laboratory-reared colony and transferred to dialysis-tube cages (4-cm diameter, 13-cm long; Ward's National Science, Rochester, NY, USA), containing approximately 6–7 cm of an actively growing shoot, including the apical meristem, from a particular *Vaccinium* accession. Foam plugs were used to seal both ends of the cage, with the bottom plug being cut along the radius to allow for a single stem to enter and be sealed within the dialysis tube. Cages contained leaf and stem tissue and were positioned on the plants 24 hr before their use. The experiment was performed twice, and a total of nine replications were used for each accession.

Plants were arranged in a completely randomized design on a single greenhouse bench. Cages were inspected daily until nymphiposition began, at which point all individuals were removed except for two first instars. Nymphs were allowed to develop until one reached reproductive maturity, after which the second nymph was removed. Fecundity was subsequently measured every 2 d until 42 d after birth. This procedure allowed for calculating the length of time from birth to reproductive maturity, total offspring production, and survivorship over a 42-d period.

The intrinsic rate of natural increase (r_m) was estimated using the equation of Wyatt and White (1977): $r_m = 0.74(\ln M_d)/\mathrm{TTR}$, where 0.74 is a correcting constant, M_d is the number of young produced in a reproductive period equal to *TTR*, and *TTR* is the time from birth to reproduction (Wyatt and White, 1977). Doubling time ($T_d = \ln 2/r_m$), net reproductive rate ($R_o = \sum l_x m_x$), and generation time ($T = \ln R_o/r_m$) were also calculated, whereby l_x=age-specific survivorship and m_x=age-specific fecundity (Andrewartha and Birch, 1954; DeLoach, 1974). As all aphids were monitored from birth, the age specificity of each aphid was known.

Within Population Variability in Aphid Resistance Variation in aphid resistance within a population of *V. darrowi* was measured with 24 accessions from a single *V. darrowi*

population (NJ88-06) collected in Santa Rosa Co., FL, USA, namely, NJ88-06-01; -02; -03; -04; -05; -07; -08; -11; -12; -14; -17; -22; -23; -25; -27; -38; -39; -41; -42; -43; -44; -46; -49; and -51. *Vaccinium corymbosum* cv. Elliott was also used as a susceptible highbush control. Plants were arranged in a completely randomized design on a single greenhouse bench.

Three adult apterous *I. pepperi* were collected from the laboratory-reared colony and transferred to the previously described dialysis-tube cages. Inspections were made until nymphiposition occurred in each cage, at which point, the adults and all but two first instars were removed. As the instars neared reproductive maturity, one nymph was removed, leaving behind a single, robust *I. pepperi* nymph in each cage. Twenty days after birth of the first instars, the total numbers of aphids in each cage were recorded on the *V. darrowi* accessions and *V. corymbosum* cv. Elliott. The experiment was performed twice, and a total of eight replications were used for each accession. Due to the large number of NJ88-06 accessions and replicates of each, it was not feasible to document developmental rates and fecundity as in the between population experiment.

Extraction, Quantification, and Identification of Phenolics Actively growing shoots from each *V. darrowi* accession representing 11 discrete populations, along with *V. corymbosum*, were harvested on three separate occasions during May–June 2005. Excised shoots were placed in paper bags and set in a drying oven set to 60±5°C for 24 hr. After drying, leaves were separated from stems, and each was ground into a homogenous powder. Based on Witzell et al. (2003), 30 mg of leaf or stem tissue were placed in an Eppendorf tube with 1,000 μl of chilled methanol (MeOH). Samples were placed on a shaker (Labnet Shaker 20, Labnet International Inc., Edison, NJ, USA) at 1,000 rpm for 2 min, and then centrifuged at 12,000 rpm for 5 min (Eppendorf centrifuge 5417C, Eppendorf, Hamburg, Germany). The supernatant was collected, and the tissues were re-extracted as described above with 500 μl of solvent. Both supernatants were pooled, filtered, and concentrated to dryness with a Speed Vac concentrator (Thermo Model SPD 2010-220; Milford, MA, USA). Residues were redissolved in 1 ml of MeOH.

Selected phenolic acids and flavonols in leaf and stem extracts were identified by high performance liquid chromatography coupled with mass spectrometry (HPLC-MS). The HPLC system (Shimadzu Co., 10VP Series, Columbia, MD, USA) employed a C_{18} MCM column (4.6×150 mm; 5 μm; MC Medical, Inc, Tokyo, Japan). Twenty microliters were injected into the column, and a gradient elution was used for separations. Solvent A consisted of 10% MeOH in H_2O adjusted to pH 3.5 with formic acid. Solvent B consisted of 20% H_2O (pH 3.5), 20% MeOH, and 60% acetonitrile. At a flow rate of 0.3 ml min^{-1}, the following gradient was used: 0 min, 100% A; 10 min 20% A; 20 min, 40% A; 40 min, 0% A; held at 0% A for 15 min. Five minutes of equilibration at 100% A was performed before and after each injection. Effluent from the column was introduced into a single-quadrupole mass spectrometer (API 150 MCA, PE Sciex, Concord, Ontario, CA) equipped with electrospray ionization (ESI). The following parameters were used: capillary voltage at 5,500 V, nebulizer gas (N_2) at 9 (arbitrary units), curtain gas (N_2) at 10 (arbitrary units), collision gas (N_2) at 10 (arbitrary units), focusing potential at 300 V, entrance potential at 10 V, declustering potential at 75, drying gas (N_2) heated to 320°C and introduced at a flow rate of 8,000 cm^{-3} min/l. Data were acquired in both positive and negative ionization mode, with full scan acquisition being performed from *m/z* 50–1,200. Data were processed by Analyst software 1.4.1. (Applied Biosystems, Applera Co., Foster City, CA, USA).

Retention times, spectral patterns, and Electrospray ionization mass spectrometry (ESI-MS) fragmentations patterns of 5-caffeoylquinic acid and the flavonol glycosides were compared with

Table 2 Population fitness of *Illinoia pepperi* confined to wild accessions of *Vaccinium darrowi* representing distinct populations, along with *V. corymbosum* cv. Elliott

Species and Population ID	Intrinsic Rate of Increase (r_m)[a,b]	95% CI[b]	Net Reproductive Rate (R_o)[c]	Generation Time (T)[d]	Doubling Time (T_d)[e]
V. darrowi					
NJ88-06-20	0.132a	0.098–0.163	18.3	20.4	3.8
NJ88-15-09	0.140ab	0.103–0.178	21.5	21.9	4.9
NJ88-13-22	0.145a	0.130–0.162	25.1	22.2	4.8
NJ88-05-29	0.150ab	0.129–0.175	20.9	20.3	4.6
NJ88-14-03	0.150ab	0.128–0.173	28.8	22.5	4.6
NJ88-12-41	0.160ab	0.134–0.187	29.9	21.3	4.3
NC84-06a-37	0.160ab	10.130–0.187	32.5	21.8	4.3
NJ88-17-13	0.161ab	0.134–0.191	36.2	22.0	4.2
NJ88-04-64	0.163ab	0.141–0.185	28.7	20.7	4.3
NJ88-06-01	0.173abc	0.146–0.201	37.7	20.0	3.8
NC84-06a-20	0.176abc	0.145–0.207	39.7	20.4	3.8
Fla 4B	0.188bc	0.172–0.204	46.8	20.5	3.7
NJ88-17-27	0.191bc	0.165–0.232	41.6	19.6	3.6
NJ88-11-32	0.192bc	0.165–0.220	41.7	19.4	3.6
V. corymbosum					
Elliott	0.216c	0.198–0.232	59.9	18.9	3.2

[a] r_m=0.74(ln M_d)/TTR, where M_d is the number of young produced in a reproductive period equal to TTR, and *TTR* is the time from birth to reproductive maturity. Bootstrap estimate of r_m. N=9 for all accessions.

[b] Nonoverlapping 95% CI corresponds to the rejection of no treatment effect ($\alpha \pm 0.05$).

[c] $R_o = \sum l_x m_x$

[d] $T = \ln R_o / r_m$

[e] $T_d = \ln(2) / r_m$

authentic standards. Authentic standards of 5-caffeoylquinic acid, and quercetin-3-galactoside, quercetin-3-glucoside, quercetin-3-rhamnoside, and quercetin-3-rutinoside were purchased from Indofine Chemical Company, Inc. (Somerville, NJ, USA). The remaining phenolic acids were identified by interpreting ESI-MS fragmentation patterns and by comparing with published data (Clifford et al., 2003; Cho et al., 2004; Vvedenskaya et al., 2004).

The selected phenolics were quantified with an HPLC (Waters, Milford, MA, USA) equipped with a Waters 996 photodiode array detector (PDA). All phenolics were quantified by using the external standard method, with quantification being based on peak area. Quantification of the chlorogenic acid derivatives was based on a standard curve prepared with 5-caffeoylquinic acid. Quercetin derivatives were quantified by using the corresponding authentic standard for each compound, except quercetin-3-oxalylpentoside and quercetin-3-*O*-(6-*p*-coumaroyl)-β-galactoside were based on a standard curve prepared with quercetin-3-rutinoside. For both the standards and extracts, 20 μl were injected and separated by using the aforementioned column and solvent system, but at a flow rate of 1 ml/min. Scanning by the PDA occurred at 320 nm, while data acquisition and processing were performed by Waters Empower™ Chromatography Software.

Statistical Analyses Aphid performance parameters and phenolic concentrations were analyzed with one-way analysis of variance (ANOVA) (PROC GLM; SAS Institute, 1996).

Table 3 Retention times, λ_{max}, *m/z* values, molecular ions, and fragmentation patterns of polyphenolics isolated from *Vaccinium darrowi* and *V. corymbosum* cv. Elliott

RT (min)	λ_{max} (nm)	$[M-H]^-$ and Fragmentation in Electrospray-MS	$[M+H]^+$ and Fragmentation in Electrospray-MS	Structure
17.38	216, 243, 324	353, 191, 178.9, 127, 111	355, 193, 163, 127	3-Caffeoylquinic acid[a]
19.65	216, 243, 324	353, 191, 178.9, 127, 111	355, 193, 163, 127	5-Caffeoylquinic acid[a,b]
20.49	220, 227, 312	337, 190.7, 161, 119	ND	3-*O*-*p*-Coumaroylquinic acid[a]
23.26	210, 227, 312	337, 191, 161, 127	ND	5-*O*-*p*-Coumaroylquinic acid[a]
24.62	218, 295, 323	433, 338.6, 322.8, 161	435, 340.2, 324.1, 162.1	Unidentified
25.46	203, 255, 354	609, 301	611, 303	Quercetin-3-rutinoside[b,c]
26.09	203, 255, 352	463, 301	465, 303	Quercetin-3-galactoside[b,c,d]
26.29	203, 255, 352	463, 301	465, 303	Quercitin-3-glucoside[b,c,d]
26.80	220, 288, 335	594.8, 433, 301	596, 435, 303	Quercetin-3-glucosylpentoside[b,c]
28.51	223, 312	505, 433, 300	507, 435, 303	Quercetin-3-oxalylpentoside[b,c]
28.88	253, 344	447, 301	449, 303	Quercetin-3-rhamnoside[b,c,d]
31.57	205, 257, 353	609, 463, 301	611, 465, 303	Quercetin-3-*O*-(6-*p*-coumaroyl)-β-galactoside[d]

[a] In conjunction with Clifford et al. (2003)

[b] Based on comparisons with authentic standards

[c] In conjunction with Cho et al. (2004)

[d] In conjunction with Vvedenskaya et al. (2004)

To help stabilize the variance, data were square root transformed before analysis, but untransformed data are presented. Means were separated by using Tukey's studentized range [honestly significant difference (HSD)] test at α=0.05. Pearson's Correlation coefficient was calculated to detect a correlation between aphid performance parameters (i.e., length of prereproductive period or cumulative fecundity) and individual or total phenolics (PROC CORR; α=0.05; SAS Institute, 1996). The percentage of aphids surviving on each *Vaccinium* spp. 42 d after birth was compared by using a R×C

contingency table with PROC FREQ procedure (SAS Institute, 1996), and Fisher's exact test (two-tail) was used to test for significant differences between observed and expected frequencies (α=0.05).

The bootstrap technique was used to estimate 95% confidence intervals (CI) for r_m values associated with each *Vaccinium* spp. (Meyer et al., 1986; Petitt et al., 1994; R Project for Statistical Computing, 2005). Nonoverlapping 95% CI corresponds to the rejection of no treatment effect hypothesis at α=0.05 (Maia et al., 2000). As T_d and T are functions of r_m, and R_0 represents the population's response, statistical analyses were only performed on r_m; however, values for each are provided for comparative purposes.

Results

Between Population Variability in Aphid Resistance Length of the prereproductive period of *I. pepperi* (from birth to reproductive maturity) was not significantly affected when reared on the 14 *V. darrowi* accessions representing 11 populations or *V. corymbosum* cv. Elliott (Table 1; P>0.05). Among the *V. darrowi* accessions, length of the prereproductive period ranged from a high of 14.7 d on NJ88-06-20 and NJ88-15-09 to a low of 12.2 d on NJ88-17-27 (Table 1). The shortest time required until reproductive maturity (11.1 d) occurred on *V. corymbosum* (Table 1).

Cumulative mean fecundity of *I. pepperi* up to 42 d after birth was affected by the *Vaccinium* host (Table 1; F=5.15; df=27, 106; P<0.001). On the *V. darrowi* accessions, cumulative fecundity ranged from 22.0 nymphs on NJ88-06-20 to 45.4 nymphs on NJ88-11-32. The two lowest fecundities were associated with *V. darrowi* accessions NJ88-06-20 and NJ88-15-09, which differed significantly from NJ88-11-32, NJ88-17-27, Fla 4B, and *V. corymbosum*. The highest cumulative nymphal production of 58.9 nymphs was observed with *V. corymbosum*, which was significantly different from all of the *V. darrowi* accessions except NJ88-17-27, NJ88-11-32, NC84-06a-20, Fla 4B, and NJ88-06-01 (Table 1). Percent survivorship, 42 d after birth, ranged from 66% on NJ88-06-20 and NJ88-04-64 to 100% on *V. darrowi* Fla 4B, NJ88-17-27, and *V. corymbosum* (Table 1), but significant differences were not detected.

By using the length of the prereproductive period and the number of offspring produced over an equivalent amount of time, the intrinsic rate of increase (r_m), net reproductive rate (R_0), generation time (T), and doubling time (T_d) were calculated for *I. pepperi* when reared on the *V. darrowi* accessions and *V. corymbosum* (Table 2). Longer prereproductive periods and reduced offspring production resulted in the r_m values of *V. darrowi* NJ88-06-20 (r_m=0.132) and NJ88-13-22 (r_m=0.145) being significantly lower than those associated with *V. darrowi* Fla 4B (r_m=0.188), NJ88-11-32 (r_m=0.192), and *V. corymbosum* (r_m=0.216) (Table 2).

Considerable variability in *I. pepperi* net reproductive rates (R_0) was observed across the *V. darrowi* accessions, which represented populations across the species geographic range (Table 2). Among the *V. darrowi* accessions, the lowest net reproductive rate of R_0=18.3 occurred on *V. darrowi* NJ88-06-20, while the highest of R_0=46.8 occurred on *V. darrowi* Fla 4B (Table 2). Generation times (T) among the *V. darrowi* accessions ranged from a high of 22.5 to a low of 19.4 on *V. darrowi* NJ88-14-03 and *V. darrowi* NJ88-11-32, respectively. Doubling time (T_d) ranged from 4.9 for *V. darrowi* NJ88-15-09 to 3.6 for both *V. darrowi* NJ88-11-32 and NJ88-17-27 (Table 2). Population fitness of *I. pepperi* was best on *V. corymbosum*, which exhibited the highest R_0 (59.9) and the lowest T (18.9) and T_d (3.2) (Table 2).

Within Population Variability in Aphid Resistance As *I. pepperi* exhibited comparatively poor performance and population fitness on *V. darrowi* accession NJ88-06-20 compared with representatives from other *V. darrowi* populations (Tables 1 and 2), variability in aphid resistance was compared among 24 accessions collected from a single *V. darrowi* population (NJ88-06; Santa Rosa Co., FL). The mean total number of aphids (occurring 20 d after the confinement of a newly born first instar) was higher on *V. corymbosum* cv. Elliott (17.6±2.1) compared to all of the *V. darrowi* accessions (F=4.24; df=24, 175; P<0.001; data not shown). The fewest individuals were recorded from *V. darrowi* NJ88-06-20 (1.1±0.3 aphids). However, significant differences were only detected between 2 of the 24 *V. darrowi* accessions, namely, NJ88-06-20 and (1.1±0.3 aphids) and NJ88-06-51 (6.6± 1.5 aphids).

Identification and Quantification of Phenolics ESI-MS performed under both positive $[M+H]^+$ and negative $[M-H]^-$ modes allowed molecular masses and fragmentation patterns to be identified for several chlorogenic acid and quercetin derivatives extracted from *V. darrowi* and *V. corymbosum* cv. Elliott leaves and stems (Table 3). All of the identified compounds exhibited characteristic ESI-MS profiles and matched well with published data (Clifford et al., 2003; Cho et al., 2004; Vvedenskaya et al., 2004).

Four chlorogenic acid derivatives were identified, namely, 3- and 5-caffeoylquinic acid, and 3- and 5-*O*-*p*-coumaroylquinic acid (Table 3). 5-Caffeoylquinic acid was identified by comparing retention times and ESI-MS fragmentation patterns with an authentic standard, while the remaining chlorogenic acid derivatives were identified by interpreting their ESI-MS fragmentation patterns, parent and daughter ions, along with comparisons with published data (Clifford et al., 2003). A fifth chlorogenic acid derivative (RT 24.62 min; Table 3) was not definitively identified, but the presence of $[M-H]^-$ ions at *m/z* 338.6 and *m/z* 161 indicated a *p*-coumaric acid unit. For all of the chlorogenic acid derivatives, an intermediate ion was present that corresponded to the loss of caffeic acid or *p*-coumaric acid units, as indicated by the $[M-H]^-$ and $[M-H]^+$ fragmentation patterns (Table 3).

Seven flavonol glycosides were identified from leaves and stems of *V. darrowi* and *V. corymbosum* (Table 3), namely, quercetin-3-rutinoside, quercetin-3-glucoside, quercetin-3-glucosylpentoside, quercetin-3-oxalylpentoside, quercetin-3-rhamnoside, and quercetin-3-*O*-(6-*p*-coumaroyl)-β-galactoside. For each of the flavonol glycosides, the aglycone component was indicated by the prominent ion produced following the loss of the sugar moieties from the pseudo-molecular ion ($[M-H]^-$; Table 3). By maintaining a low source temperature and a high declustering potential in ESI-MS conditions, peak sizes of the daughter ions were intensified. Consequently, the relative intensities of the molecular ion peaks were lower compared to the daughter ion peak, which allowed for the identification of each of the sugar moieties (Table 3; Cho et al., 2004; Vvedenskaya et al., 2004). Identifications of all the flavonol glycosides were based on comparing ESI-MS fragmentation patterns and retention times with commercial standards (Table 3), except for quercetin-3-*O*-(6-*p*-coumaroyl)-β-galactoside, which was based on interpreting ESI-MS fragmentation and comparisons with published data.

The five chlorogenic acid derivatives and seven quercetin derivatives were quantified from leaf and stem tissues of *V. darrowi* and *V. corymbosum* (Tables 4 and 5). Comparisons of individual chlorogenic acid and quercetin derivatives revealed considerable variability between representatives of the distinct *V. darrowi* populations, except for quercetin-3-rutinoside extracted from stem tissues. For example, in leaves of *V. darrowi* NC84-6a-20 and NJ88-06-01, 5-caffeoylquinic acid ranged from 3,001 to 257 μg/g of leaf tissue, respectively (Table 4). Overall, 5-caffeoylquinic acid was among the more abundant leaf

Table 4 Chlorogenic acid and quercetin derivatives extracted from leaves of wild populations of *Vaccinium darrowi* and *V. corymbosum* cv. Elliott

Species and Population ID	Mean (±SEM) Concentration (μg of 5-Caffeoylquinic Acid Equivalent/g of Leaf Tissue)[a]					Mean (±SEM) Concentration (μg of Quercetin Equivalent/g of Leaf Tissue)[b]						
	3-Caffeoylquinic Acid	5-Caffeoylquinic Acid	3-*O*-*p*-Coumaroyl-quinic Acid	5-*O*-*p*-Coumaroyl-quinic Acid	Unidentified Chlorogenic Acid Derivative	Quercetin-3-rutinoside	Quercetin-3-galactoside	Quercetin-3-glucoside	Quercetin-3-glucosyl pentoside	Quercetin-3-oxalyl-pentoside	Quercetin-3-rhamnoside	Quercetin-3-*O*- (6-*p*-coumaroyl)-β-galactoside
V. darrowi												
NJ88-04-64	1403.9± 0.4a	1019.2± 134.2cd	40.4± 11.3e	150.3± 10.6abc	981.1± 19.0abc	122.4± 10.4abcd	19.1± 3.3abc	79.9± 20.7bc	1123.7± 29.8ab	0.0± 0.0e	447.7± 306.7a	511.0± 30.2bcd
NJ88-05-29	229.9± 33.7bcd	355.8± 107.9ef	213.2± 29.1bc	66.9± 9.7cdef	0.0± 0.0g	49.1± 3.6de	10.9± 2.6bcd	47.8± 3.6cd	1155.0± 164.2ab	44.2± 7.5e	157.4± 14.1ab	574.6± 34.5abc
NJ88-06-01	31.9± 1.4e	257.1± 23.5f	107.3± 9.9cde	203.2± 14.9a	365.5± 26.4de	176.9± 67.4abc	2.3± 0.7e	121.0± 9.6ab	54.0± 13.3de	755.6± 120.0bcd	89.9± 1.8ab	397.2± 76.4bcd
NJ88-06-20	99.2± 20.4cde	644.4± 3.2de	151.4± 26.4bcd	27.2± 5.8f	271.9± 24.0e	155.2± 31.6abcd	19.8± 5.4abc	33.6± 7.4de	45.2± 4.1de	676.5± 62.0d	124.3± 0.3ab	330.0± 14.4cde
NJ88-11-32	59.9± 1.4de	382.5± 35.5ef	157.5± 27.8bcd	43.4± 8.4ef	453.9± 14.3de	90.0± 15.6cde	22.0± 1.4ab	13.6± 2.4ef	1106.3± 139.0ab	782.2± 169.3bcd	120.9± 12.8ab	638.1± 24.1ab
NJ88-12-41	360.0± 87.2b	637.2± 24.3de	48.0± 5.6de	157.3± 17.7ab	382.6± 41.9de	125.0± 17.4abcd	17.5± 0.4abc	0.0± 0.0g	0.0± 0.0f	1303.9± 49.9a	123.4± 16.1ab	655.6± 39.9ab
NJ88-13-22	293.8± 97.1bc	624.2± 125.2de	301.3± 54.8ab	43.0± 10.5ef	1083.9± 102.9ab	97.7± 4.1cde	8.6± 0.4cde	49.4± 20.4cd	42.0± 2.1def	1193.2± 41.6abc	60.6± 2.2b	283.6± 51.9de

NJ88-14-03	363.9± 31.9b	935.7± 57.2d	63.2± 16.3de	64.1± 16.3def	466.9± 29.8d	246.6± 32.3ab	11.1± 1.51bcd	185.6± 20.9a	531.2± 4.8c	22.3± 8.3e	64.6± 3.9b	165.0± 9.2e
NJ88-15-09	68.0± 6.5de	549.2± 23.4def	244.1± 58.0bc	182.6± 46.5ab	370.5± 57.2de	105.6± 21.7bcde	5.7± 0.9de	60.8± 2.7cd	751.9± 119.0bc	744.5± 84.5cd	72.3± 4.8b	424.5± 20.5bcd
NJ88-17-13	18.3± 6.5e	407.7± 0.9ef	94.7± 1.7cde	22.2± 6.6f	785.2± 114.9c	247.5± 15.1ab	14.4± 3.3bcd	44.4± 1.3cd	33.8± 6.6ef	826.4± 113.9abcd	83.9± 9.0ab	580.8± 45.5abc
NJ88-17-27	348.7± 14.7b	959.0± 218.9d	144.2± 35.2bcde	99.5± 8.0bcde	1160.3± 84.2a	132.1± 16.7abcd	9.0± 2.0cde	0.0± 0.0g	1243.7± 38.5a	1230.1± 118.0ab	38.5± 4.1b	532.9± 138.3bcd
NC84-6a-20	348.3± 41.6b	3001.0± 176.8a	459.2± 61.1a	124.9± 22.3abcd	1036.4± 23.8abc	226.7± 42.7abc	12.8± 1.7bcd	0.0± 0.0g	713.1± 49.0c	50.9± 4.9e	138.0± 24.9ab	561.5± 80.4bcd
NC84-6a-37	366.6± 53.6b	1585.7± 62.4bc	132.4± 20.7cde	36.9± 1.9ef	799.7± 6.0bc	278.6± 57.4a	13.2± 1.2bcd	57.1± 9.5cd	160.1± 6.6d	986.6± 84.3abcd	138.2± 6.9ab	857.5± 37.8a
Fla 4B	335.8± 103.6b	399.2± 22.0ef	99.7± 16.9cde	45.0± 11.5ef	75.0± 1.6f	23.6± 3.2e	28.4± 3.0a	2.8± 2.8fg	0.0± 0.0f	955.7± 128.0abcd	47.4± 8.8b	334.5± 20.0cde
V. corymbosum												
Elliott	183.0± 7.5bcd	1852.3± 7.5b	158.7± 12.6bcd	23.7± 8.4f	11.7± 0.8g	115.3± 12.3bcd	2.7± 0.1e	85.1± 3.1bc	10.6± 1.2ef	29.7± 7.2e	38.5± 4.3b	453.8± 1.2bcd
F and *P* value	29.56; <0.001	43.37; <0.001	13.07; <0.001	17.19; <0.001	124.22; <0.001	8.71; <0.001	12.72; <0.001	53.89; <0.001	113.24; <0.001	76.21; <0.001	2.96; =0.0062	11.01; <0.001

[a] Means within columns followed by the same letter are not significantly different [α=0.05; Tukey's studentized range (HSD) test; *df*=14, 30]. *N*=3 for all accessions. Chlorogenic acid derivatives are expressed as µg of 5-caffeoylquinic acid equivalent per g dry weight.

[b] Means within columns followed by the same letter are not significantly different (α=0.05; Tukey's studentized range [HSD] test; *df*=14, 30). *N*=3 for all accessions. Quantity of each quercetin derivative based on corresponding standards, except quercetin-3-oxalylpentoside and quercetin-3-*O*-(6-*p*-coumaroyl)-β-galactoside were based on quercetin-3-rutinoside.

Table 5 Chlorogenic acid and quercetin derivatives extracted from stems of wild populations of *Vaccinium darrowi* and *V. corymbosum* cv. Elliott

Species and Population ID	Mean (±SEM) Concentration (μg of 5-Caffeoylquinic acid Equivalent/g of Stem Tissue)[a]					Mean (±SEM) Concentration (μg of Quercetin Equivalent/g of Stem Tissue)[b]						
	3-Caffeoylquinic Acid	5-Caffeoylquinic Acid	3-*O*-*p*-Coumaroyl-quinic Acid	5-*O*-*p*-Coumaroyl-quinic Acid	Unidentified Chlorogenic Acid Derivative	Quercetin-3-rutinoside	Quercetin-3-galactoside	Quercetin-3-glucoside	Quercetin-3-pentoside	Quercetin-3-oxalyl-pentoside	Quercetin-3-rhamnoside	Quercetin-3-*O*-(6-*p*-coumaroyl)-β-galactoside
V. darrowi												
NJ88-04-64	1.1± 1.1ef	15.3± 15.3b	51.8± 13.1ab	18.9± 2.1b	297.3± 15.0a	26.0± 2.3a	2.1± 0.6ab	4.5± 4.5c	0.0± 0.0c	322.7± 82.6a	65.9± 7.0a	135.1± 35.7ab
NJ88-05-29	0.1± 0.1f	82.9± 8.5ab	28.8± 6.7ab	9.4± 4.3b	108.1± 9.5a	29.2± 5.6a	1.8± 0.0ab	21.5± 5.6bc	143.4± 25.8b	1.3± 1.3d	26.8± 4.2b	63.4± 6.7bcd
NJ88-06-01	28.4± 1.8bc	193.9± 54.7a	61.2± 19.3ab	10.2± 2.7b	98.3± 26.8a	21.0± 4.2a	3.2± 1.1ab	1.3± 1.3c	0.0± 0.0c	169.7± 6.7abcd	18.5± 4.5bc	79.1± 2.2abcd
NJ88-06-20	19.3± 1.6bcd	133.6± 27.3ab	26.5± 4.2ab	9.1± 1.1b	316.2± 25.3a	20.7± 6.2a	3.4± 0.5ab	1.1± 1.1c	118.5± 18.7b	221.5± 22.5abc	12.6± 1.3bc	54.0± 6.5cd
NJ88-11-32	17.4± 5.8bcde	72.1± 4.9ab	31.5± 11.0ab	26.7± 5.0ab	66.5± 1.9a	14.4± 4.2a	3.5± 0.3ab	1.9± 1.2c	267.5± 27.4a	223.8± 4.4abc	4.2± 0.5c	65.4± 6.2bcd
NJ88-12-41	0.2± 0.2f	120.1± 52.2ab	39.3± 10.7ab	13.6± 2.4b	95.7± 11.1a	14.3± 1.8a	3.3± 1.0ab	16.7± 5.9bc	4.5± 4.5c	108.3± 6.8cd	12.2± 2.1bc	83.2± 11.1abcd
NJ88-13-22	5.8± 5.1def	89.8± 36.6ab	51.4± 11.9ab	11.8± 3.2b	81.1± 1.0a	32.5±± 2.1a	3.7± 1.2ab	0.0± 0.0c	185.0± 18.2ab	123.1± 11.0bcd	4.1± 1.1c	113.1± 19.1abc

NJ88-14-03	2.3± 2.3ef	66.2± 5.0ab	28.6± 10.0ab	9.4± 5.6b	99.7± 20.2a	38.7± 5.8a	1.1± 0.2b	2.8±± 2.8c	9.6± 9.6c	169.0± 27.3abcd	18.2± 8.1bc	86.3± 7.7abcd
NJ88-15-09	0.0± 0.0f	71.3± 27.5ab	6.0± 3.1ab	8.4± 0.7b	91.2±± 22.8a	15.9± 8.0a	5.0± 2.3ab	1.3± 1.3c	158.3± 32.0b	90.4± 13.7cd	10.6± 0.5bc	87.7± 11.5abcd
NJ88-17-13	53.7± 13.3ab	89.6± 26.0ab	75.9± 24.0a	45.3± 12.2a	175.8± 5.3a	44.1± 25.3a	7.0± 1.5a	9.1± 4.8bc	3.2± 3.2c	284.9± 6.5ab	12.3± 4.9bc	117.5± 1.3abc
NJ88-17-27	8.1± 4.7cdef	204.8± 44.9a	42.0± 13.9ab	26.5± 9.4ab	164.2± 59.7a	16.6± 8.9a	3.2± 0.9ab	31.7± 10.2b	0.0± 0.0c	218.9± 80.7abc	8.0± 2.4bc	72.5± 15.5abcd
NC84-6a-20	16.8± 5.1bcde	154.4± 63.3ab	22.9± 12.4ab	24.9± 4.6b	96.8± 38.5a	22.3± 9.0a	3.7± 1.2ab	0.5± 0.5c	173.7± 34.8b	2.8± 2.8d	14.4± 4.7bc	143.0± 15.9a
NC84-6a-37	1.8± 1.4def	72.0± 13.5ab	0.0± 0.0b	16.4± 3.0b	147.9± 21.4a	20.4± 16.4a	5.3± 2.3ab	0.0± 0.0c	0.9± 0.9c	2.0± 2.0d	3.0± 0.8c	72.6± 25.4abcd
Fla 4B	81.7± 3.4a	111.0± 27.8ab	38.8± 20.4ab	3.1± 1.0b	12.9± 1.1a	9.0± 3.6a	5.5± 0.3ab	69.7± 7.8a	0.0± 0.0c	162.0± 29.3abcd	1.2± 0.5c	32.3± 5.1d
V. corymbosum												
Elliott	0.9± 0.6ef	34.7± 5.2ab	12.8± 9.5ab	5.2± 3.1b	0.0± 0.0a	25.4± 7.3a	1.3± 0.3ab	15.0± 3.9bc	153.5± 0.8b	6.2± 1.0d	5.5± 0.3c	74.5± 2.7abcd
F and *P* value	17.79; <0.001	3.24; = 0.0034	4.22; = <0.001	4.87; <0.001	5.24; <0.001	1.02; = 0.46	2.25; = 0.03	11.24; <0.001	51.26; <0.001	28.25; <0.001	13.50; <0.001	4.62; <0.001

[a] Means within columns followed by the same letter are not significantly different [α=0.05; Tukey's studentized range (HSD) test; *df*=14, 30]. *N*=3 for all accessions. Chlorogenic acid derivatives are expressed as micrograms of 5-caffeoylquinic acid equivalent per g dry weight.

[b] Means within columns followed by the same letter are not significantly different [α=0.05; Tukey's studentized range (HSD) test; *df*=14, 30]. *N*=3 for all accessions. Quantity of each quercetin derivative based on corresponding standards, except quercetin-3-oxalylpentoside and quercetin-3-*O*-(6-*p*-coumaroyl)-β-galactoside were based on quercetin-3-rutinoside

and stem phenolic acid. For example, 5-caffeoylquinic acid represented 83% of the chlorogenic acid derivatives extracted from leaves of *V. corymbosum*.

In addition to differences between populations of *V. darrowi*, variability was also detected among *V. darrowi* accessions from the same population. For instance, of the 12 quantified leaf phenolics, significant differences were detected for four compounds between NJ88-06-01 and NJ88-06-20, nine compounds between NJ88-17-13 and NJ88-17-27, and eight compounds between NC84-6a-20 and NC84-6a-37 (Table 4). Regarding stem phenolics, significant differences were detected for two compounds between NJ88-06-01 and NJ88-06-20, five compounds between NJ88-17-13 and NJ88-17-27, and four compounds between NC84-6a-20 and NC84-6a-37 (Table 5). In general, phenolic acid and flavonol glycoside concentrations were lower in stems compared to leaves (compare Tables 4–5).

To determine if concentrations of a particular compound predict levels of aphid resistance exhibited by *V. darrowi*, Pearson's correlation coefficients were calculated for each individual leaf and stem phenolic and aphid performance (i.e., length of prereproductive development or cumulative fecundity). A significant correlation was not detected for any of the 48 comparisons ($P>0.05$). Similarly, totals of the 12 phenolics quantified in this study did not correspond with resistance levels of the *V. darrowi* accessions ($P>0.05$).

Discussion

Allopatric resistance is genetically based resistance to insect pests that occurs between plants and insects that did not coevolve with each other (Harris, 1975; Kennedy and Barbour, 1992; Smith, 2005). Such is the case with *I. pepperi* and *V. darrowi*. *Illinoia pepperi* occurs in the Northeastern and upper Midwestern U.S. (Elsner and Kriegel, 1989; Blackman and Eastop, 2000), while *V. darrowi* occurs in the Southeastern U.S., occupying Florida, Georgia, and westward to eastern Texas (Lyrene and Sherman, 1980; Lyrene, 1997). Harris (1975) argued that allopatric resistance has been the most successful source of host plant resistance to insects for agricultural crops. Furthermore, due to the lack of gene-for-gene interaction between the pest and host plant, allopatric resistance is often polygenic and typically more stable (Harris, 1975; Smith, 2005). Allopatric resistance could be the result of genetic drift, immigration, or genes conferring resistance to insect herbivores found in the plant's native habitat (Kennedy and Barbour, 1992).

Resistance to a variety of abiotic and biotic stresses and the ability to be successfully crossed with *V. corymbosum* (Ortiz et al., 1999; Qu and Vorsa, 1999), has made *V. darrowi* of particular interest for improving the resistance properties of cultivated highbush blueberry, *V. corymbosum* (Chandler et al., 1985; Erb et al., 1990; Hancock et al., 1992). Fla 4B, reported as a *V. darrowi*, has been extensively used to improve highbush blueberry (Draper, 1995; Qu and Hancock, 1997). However, Fla 4B is not rhizomatous, which is characteristic of *V. darrowi* (Vander Kloet, 1977). Instead, Fla 4B appears to be the result of introgression between *V. darrowi* and *V. corymbosum*. Fla 4B was not identified as a promising source of aphid resistance traits in this study or others (Ranger et al., 2006).

V. darrowi is not unique compared to other *Vaccinium* spp. in having considerable variation for phenotypic and horticultural traits within and between wild populations (Camp, 1945; Lyrene and Sherman, 1980; Lyrene, 1997). The current study further supports this phenomenon as reflected in *V. darrowi* intra- and interpopulational variability for aphid resistance and phenolics. Such variation is likely attributed to genetic drift in

individual populations, introgression with sympatric *Vaccinium* spp., and/or by environmental differences between plant individuals (Vander Kloet, 1977; Lyrene and Sherman, 1980; Karban, 1992). By characterizing the intraspecific variability in *I. pepperi* resistance exhibited within and between populations of *V. darrowi*, discrete populations and individual accessions with promising levels of aphid resistance were identified. Identifying such individuals is useful for developing and improving resistance levels in future cultivars. While considering the variability in horticultural traits exhibited by wild *Vaccinium* spp., Lyrene and Sherman (1980) noted that selecting the best accession for a particular trait within a species is almost as important as selecting the most appropriate species.

Offspring production was the only biological parameter of *I. pepperi* that varied among populations of *V. darrowi*, which may be attributed to a phloem-based defense (Givovich et al., 1992). Since r_m values were calculated based on length of the prereproductive period and fecundity, significant differences in r_m were only detected between three of the *V. darrowi* accessions. Andrewartha and Birch (1954) argued that r_m is the only statistic that adequately represents the physiological qualities of an organism relative to its capacity of increase. Greater variability in aphid resistance within a population of *V. darrowi* may have been detected by calculating r_m values for *I. pepperi* (as was done for between population variability), rather than by measuring the mean total number of aphids after 20 d.

Due to their antioxidant capacity, considerable attention has been paid to the fruit phenolics of *Vaccinium* spp. (Häkkinen and Törrönen, 2000; Taruscio et al., 2004; Ayaz et al., 2005). *Vaccinium* leaf and stem phenolics have received less attention. To our knowledge, this study is the first quantitative description of chlorogenic acid and quercetin derivatives in leaves and stems of *V. darrowi* and *V. corymbosum* cv. Elliott. Witzell et al. (2003) quantified temporal variation in phenolic acids and flavonoids in leaves and stems of boreal bilberry, *Vaccinium myrtillus* L. Similar to our study, Witzell et al. (2003) detected quantitative differences in phenolic profiles between leaves and stems, with higher concentrations generally occurring in the leaves. Furthermore, Witzell et al. (2003) also found a chlorogenic acid derivative (tentatively identified as 5-*O*-caffeoylquinic acid) to be the most abundant compound in leaf samples, while quercetin derivatives were the most abundant flavonols in leaf and stem tissues.

Secondary metabolites, including phenolics, can serve as host-recognition cues and feeding stimulants for monophagous or oligophagous aphids (Montgomery and Arn, 1974; Joerdens-Roettger, 1979; Takemura et al., 2006). As theorized by Fraenkel (1959), the presence of such secondary metabolites can drive the evolution of arthropods from polyphagy to monophagy, whereby the compounds are no longer detrimental but instead, act as beneficial cues. As previously noted, *I. pepperi* is monophagous on *Vaccinium* spp. (Elsner and Kriegel, 1989). Thus, the phenolic acids and flavonol glycosides extracted and identified from the sympatric species *V. corymbosum* may provide host-recognition cues for *I. pepperi*. As previously noted, 5-caffeoylquinic acid represented 83% of the phenolic acids extracted from leaves of *V. corymbosum*. Due to the lack of coevolutionary history between *I. pepperi* and *V. darrowi*, unique or novel ratios of phenolics present in *V. darrowi* may have contributed to resistance.

The localization of secondary metabolites within plant tissues has greater implications for resistance against phloem-feeding insects than other feeding guilds. Phenolic compounds are typically stored in vacuoles or specific storage tissues (Harborne, 1967; Harborne and Grayer, 1993), but they may diffuse into the phloem in their glycoside form (Macleod and Pridham, 1965; Mullin, 1986; Peng and Miles, 1991). If present mainly in the mesophyll, such compounds may be encountered during probing, resulting in the deterrence of phloem feeders (Calatayud et al., 1994). For example, the phenolics

procyanidin, *p*-hydroxybenzaldehyde, and dhurrin deter *Schizaphis graminum* (Rondani) from feeding on resistant sorghum cultivars (Dreyer and Jones, 1981, Dreyer et al., 1981). If localized in the phloem, nutritional deficiencies may occur, resulting in reduced developmental rates and/or fecundity (Givovich et al., 1992). *Aphis fabae* (Scopoli) exhibits reduced fecundity on cowpea, *Vigna unguiculata* L. Walp., lines with elevated quercetin glycoside levels (Lattanzio et al., 2000). Similarly, Calatayud et al. (1994) found that three flavonoid glycosides (rutin, kaempferol glycoside-1, and kaempferol glycoside-2) and cyanogenic glycosides were translocated in the phloem of cassava, *Manihot esculenta* Crantz, and consumed by the mealybug, *Phenacoccus manihoti* Matt. Ferr.

While a direct cause and effect relationship between phenolics and plant resistance to insects is not well documented (Smith, 2005), phenolic compounds play at least a partial role in resistance to several aphid pests (Hedin and Waage, 1986). Furthermore, while identification of the specific mechanism of resistance may not be crucial for the development of resistant cultivars, such information can allow for the utilization of phenotypic or genotypic characters, thereby, facilitating breeding or selection programs (Smith, 2005). For instance, if resistance is chemically based, concentrations of allelochemicals could potentially provide a diagnostic characterization of resistant germplasm. Metabolic profiling can predict levels of plant resistance to insects (Brennan et al., 1992; Brignolas et al., 1998; see Smith, 2005), thereby aiding plant breeders in improving the resistance levels of selected cultivars (Andersson et al., 1980; Hedin et al., 1991). Concentrations of isochlorogenic acid in roots of lettuce, *Lactuca sativa* L., predict resistance levels to the lettuce root aphid, *Pemphigus bursarius* L. (Cole, 1987). Similarly, Lattanzio et al. (2000) found a positive correlation between the flavonoid glycoside content of cowpea, *V. unguiculata*, and aphid resistance.

Vaccinium darrowi and other wild *Vaccinium* spp. represent useful sources of genes providing resistance to abiotic and biotic stresses (Etzel and Meyer, 1986; Meyer and Ballington, 1990; Stretch et al., 2001). The current study characterized variability in aphid resistance and constitutive phenolics exhibited within and between populations of the wild blueberry *V. darrowi*. Several *V. darrowi* accessions were identified as being particularly disruptive to offspring production by *I. pepperi*, which resulted in poor population fitness. As a correlation was not detected between aphid performance and constitutively expressed chlorogenic acid or quercetin derivatives, additional studies should be conducted to assess the role of induced defenses following herbivory by *I. pepperi*. Phloem constituents should also be considered. Offspring resulting from crosses between *V. darrowi* and *V. corymbosum* are currently being screened for *I. pepperi* resistance in an attempt to identify molecular markers associated with insect resistance. Marker-assisted selection of plants based on genotype rather than chemotype could expedite the screening process of advanced *Vaccinium* selections.

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