Evaluating possible intraspecific variation in resistance of western hemlock (Pinaceae) to *Adelges tsugae* (Hemiptera: Adelgidae)

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Abstract—Western hemlock, *Tsuga heterophylla* (Rafinesque) Sargent (Pinaceae), trees growing in a clonal, commercial seed orchard on Whidbey Island, Washington, United States of America were assessed for *Adelges tsugae* (Annand) (Hemiptera: Adelgidae) infestation levels to determine whether there was any evidence of resistance to this herbivore. Join-count statistics revealed that *A. tsugae* infested trees were not clumped, but instead were randomly distributed throughout the seed orchard. Chi-square analyses suggested that there is a genetic basis for different levels of *A. tsugae* infestation among the genets represented in the orchard. However, since none of the genets were completely free of *A. tsugae* infestation, differences among genets seem to be related more to susceptibility or tolerance than complete resistance. Among the 17 genets that had five or more ramets in the seed orchard, only one had more than one ramet free of *A. tsugae*. That genet had four of five ramets that were free of *A. tsugae*. However, it is not possible to say whether that was due to chance or inherent tree characteristics that make it less susceptible to *A. tsugae* infestation.

Introduction

Adelges tsugae (Annand) (Hemiptera: Adelgidae) was introduced to the eastern United States of America from southern Japan sometime prior to its discovery in Virginia in the 1950s (Stoetzel 2002; Havill *et al*. 2006, 2016a). It is now found from Georgia, United States of America to Nova Scotia, Canada and is causing high levels of mortality to eastern and Carolina hemlocks (*Tsuga canadensis* (Linnaeus) Carrière and *Tsuga caroliniana* Engelmann; Pinaceae) throughout this region (Havill *et al*. 2016b). In the Pacific Northwest, there is a cluster of native *A. tsugae* clones, closely related to the Japanese clone that was introduced to the eastern United States of America (Havill *et al*. 2006, 2016a). Where *A. tsugae* is native in the Pacific Northwest of North America, it does not cause any noticeable damage or mortality to western hemlock, *Tsuga heterophylla* (Rafinesque) Sargent (Pinaceae), even though it occasionally reaches very high densities (Kohler *et al*. 2008). It has been suggested that coevolved host tree resistance and/or native natural enemies keep *A. tsugae* from causing any significant damage to western hemlock.

Three adelgid-specific predators have been found to be frequent and abundant associates of *A. tsugae* in the Pacific Northwest (Kohler *et al*. 2008). *Laricobius nigrinus* Fender (Coleoptera: Deridontidae) was the first of these predators to be studied and released in the eastern United States of America. Despite releasing several hundred thousand *Laricobius nigrinus* adults and eggs, and its establishment at over 100 locations, there is currently no evidence that the beetle is reducing *A. tsugae* populations or hemlock mortality in eastern North America (Mausel *et al*. 2011; Mayfield *et al*. 2015). The other two predators are

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species of silver flies, *Leucopis argenticollis* (Zetterstedt) and *Leucopis piniperda* (Malloch) (Diptera: Chamaemyiidae). Populations of both species appear to be restricted to *A. tsugae* in the Pacific Northwest, although populations in other parts of the world have been collected in association with other adelgid species (Ross *et al*. 2011, 2017; Havill *et al*. 2018). Both *Leucopis argenticollis* and *Leucopis piniperda* from the Pacific Northwest are able to feed and complete their development on the Japanese clone of *A. tsugae* that occurs in eastern North America (Motley *et al*. 2017), and small-scale operational releases have occurred in the last few years. Although several studies have compared species level resistance of western hemlock and several other *Tsuga* (Endlicher) Carrière to *A. tsugae* (Lagalante and Montgomery 2003; Evans 2008; Jetton *et al*. 2008; Weston and Harper 2009; Oten *et al*. 2012), no studies have considered potential intraspecific variation in western hemlock resistance. In this study, western hemlock clones growing in an operational seed orchard were evaluated for *A. tsugae* infestation to identify possible genetic differences in susceptibility.

Materials and methods

This study was conducted in a commercial seed orchard owned by Sierra Pacific Industries on Whidbey Island, Washington, United States of America. Trees were approximately 8–12 m in height with live branches extending to the ground. Trees used in the study were present in two blocks, a northern and a southern block, separated by an open, grassy area about 50 m wide. The northern block was approximately 1.1 ha and the southern block was approximately 1.2 ha.

The trees were rooted cuttings from 133 unrelated parent trees (hereafter, genets). Since the orchard is for production of reforestation seed for Sierra Pacific Industries, it was adapted to their ownership. The parent trees came from elevations of 105–860 m in Whatcom, Skagit, and Snohomish counties, Washington, that is, the west slope of the Washington Cascades. Proprietary genet numbers have been changed for publication. All cuttings were rooted between 1984 and 1988. Past management included irrigation, fertilisation, pesticide applications, and stress treatments to induce flowering. All of these treatments occurred prior to 2010 and all trees were subjected to the same treatments. Numbers of replicates (*i.e*., clones or, hereafter, ramets) of different genets ranged from one to seven individual trees. Ramets were randomly located within each block. The trees selected to be represented in the seed orchard were included due to their desirable silvicultural characteristics, that is, rapid growth rate and good form.

On 5 July 2016, 452 trees were assessed for their *A. tsugae* abundance by categorising infestation levels as absent (no *A. tsugae* flocculence observed), low, or high. Trees categorised with a low infestation had visible *A. tsugae* flocculence on less than 50 % of the branches in the lower 3 m of the crown and those categorised with a high infestation had visible *A. tsugae* flocculence on greater than 50 % of the branches. The subjectivity of this assessment was reduced by verbal consensus between two experienced observers looking at each tree from opposite sides.

There are no published data documenting the population dynamics of *A. tsugae* in western North America. However, two of the authors (D.W.R. and K.F.W.) have followed populations at a number of different locations in western Oregon and Washington for 10–15 years as part of various research projects. Our observations indicate that *A. tsugae* populations remain at high levels (similar to those found in eastern North America on eastern hemlock) on trees at a given location for no more than one to a few years before declining to undetectable or nearly undetectable levels, that is, no ovisacs or only a few scattered ovisacs. Populations remain at low levels for one to a few years before again reaching high densities. We have watched this change in population density through as many as 3–4 cycles at some locations. Consequently, the best time to assess possible resistance or tolerance to *A. tsugae* on individual trees at a given site to document differences in infestation levels is during a year when populations are at high levels in that area. At the time that we evaluated the trees in this study, *A. tsugae* infestation in the seed orchard was obvious on well over 25% of the trees in the orchard and many of those supported high populations. In contrast, by spring 2019, there were only a few scattered ovisacs among all the trees in the seed orchard. We did not make any effort to identify the adelgid species through morphological or genetic methods, because there is only one species (including six geographically distinct lineages) of woolly adelgid associated with hemlocks throughout the world (Havill *et al.* 2011). For this same reason, we did not deposit voucher specimens into a collection.

Data analyses

Join-count statistics were used to test for spatial autocorrelation among the nominal *A. tsugae* data. The *A. tsugae* abundance level data were consolidated into presence/absence. These tests were run separately for the two blocks because the blocks were composed of exclusively different hemlock genets. The *Z*-distribution was used for reference and this theoretical distribution was considered dependent instead of independent. That is, a distribution assuming nonfree sampling without replacement was used instead of one assuming free sampling with replacement; the probability of occurrence for this distribution was determined from the sample area, which was not assumed to represent a larger area; and this probability was not known *a priori* independent of sampling. In the calculations for the join-count statistics, the numbers of neighbours with different categorical attributes were counted for each sampling unit quadrat in space. In this case, sampling quadrats were trees and the categorical attribute was presence/absence (occurrence) of *A. tsugae*. The "queen" neighbouring scheme was used, meaning that neighbours of a focal tree were quadrats containing trees potentially in eight directions from the quadrat representing the focal tree. These eight directions were those diagonally neighbouring the quadrat to the northwest, northeast, southeast, and southwest, as well as those neighbouring straight-on to the west, north, east, and south. A bilateral *Z*-test was used with an alpha value of $P = 0.05$ for the number of joins of presence and absence quadrats.

Chi-square analysis was used to assess the significance of differences in frequency of *A. tsugae* levels on different genets of hemlock. For the χ^2 analysis, hemlock genets with four or more ramets in the orchard were used. The two blocks were not separated in this analysis because the hypothesis being addressed was not spatial. To reduce the risk of type I error stemming from multiple comparisons, the Bonferroni correction was used for adjusting the alpha value for evaluating the *P*-values from these χ^2 tests. Since five tests were conducted, a standard alpha value of $P = 0.05$ was adjusted to 0.01, which was considered significant. All statistical analyses were performed using the R-3.3.2 for Windows (32/64 bit) statistical package (Hornik 2018).

Results

A clumped spatial distribution of *A. tsugae* on the hemlock clones was not supported. *Z*-tests of the two join-count statistics for both the northern and southern blocks were nonsignificant ($Z_{observed} < Z_{critical}$). Thus, the observed distribution was not significantly different from random.

The proportions of ramets with *A. tsugae* levels of absent, low, and high for genets represented by four or more ramets were determined (Table 1). Most of the genets with four or more ramets had no ramets free of *A. tsugae* infestation (21 out of 34 in total; Table 1). None of the remaining 13 genets with four or more ramets, some of which were free of *A. tsugae* infestation, were exclusively *A. tsugae* free. In summary, all genets with four or more ramets had some ramets infested by *A. tsugae*.

The first Pearson's χ^2 test used genets with four or more ramets each and *A. tsugae* levels of absent, low, and high. The frequencies of these levels varied significantly by genet ($\chi^2 = 107.3$; df = 66; $P < 0.001$). The second test again used genets with four or more ramets each, but *A. tsugae* levels were absent and present (combined low and high). The frequencies of *A. tsugae* presence/absence did not vary significantly by genet ($\chi^2 = 48.7$; df = 33; $P = 0.04$). The third test used genets with five or more ramets only and *A. tsugae* levels of absent, low, and high. The frequencies of these levels varied significantly by genet ($\chi^2 = 61.7$; df = 32; $P < 0.01$). The fourth test also used genets with five or more ramets, but *A. tsugae* levels of absent and present. The frequencies of *A. tsugae* presence/absence varied significantly by genet ($\chi^2 = 35.8$; df = 16; $P < 0.01$) (Table 2). Finally, the fifth test used only genets with six or more ramets and *A. tsugae* levels of absent and present. The frequencies of *A. tsugae* presence/absence did not vary significantly by genet ($\chi^2 = 2.1$; df = 4; $P = 0.71$).

Table 1. The proportions of ramets with *Adelges tsugae* absent, low, and high are displayed for genets represented by four or more ramets. Those genets that had no ramets free of *A. tsugae* are bolded.

Generic genet number	Number of ramets (*i.e.*, clones or replicates)	Proportion *A. tsugae* absent	Proportion *A. tsugae* low	Proportion *A. tsugae* high
Northern block				
37	5	0.8	0.2	0
39	7	0.14	0.71	0.14
40	6	0.17	0.17	0.17
42	**4**	**0**	**0.75**	**0.25**
43	**4**	**0**	**1**	**0**
44	4	0.25	0.75	0
45	**6**	**0**	**0.67**	**0.33**
46	4	0.25	0.5	0.25
47	4	0.25	0.75	0
48	4	0.25	0.75	0
51	**5**	**0**	**0.6**	**0.4**
52	**4**	**0**	**0.75**	**0.25**
53	**4**	**0**	**1**	**0**
68	**4**	**0**	**1**	**0**
Southern block				
75	**5**	**0**	**0.4**	**0.6**
76	**4**	**0**	**0.25**	**0.75**
78	**5**	**0**	**0.8**	**0.8**
79	**6**	**0**	**0.17**	**0.83**
80	**5**	**0**	**0.4**	**0.8**
81	6	0.17	0.17	0.67
82	**5**	**0**	**0.2**	**0.8**
83	**5**	**0**	**0.4**	**0.6**
84	**5**	**0**	**0.2**	**0.8**
87	6	0.2	0.2	0.6
88	4	0.25	0.5	0.25
114	**4**	**0**	**1**	**0**
115	**5**	**0**	**0.8**	**0.2**
116	5	0.2	0.8	0
117	**4**	**0**	**0.5**	**0.5**
118	**4**	**0**	**0.5**	**0.5**
119	4	0.25	0.75	0
120	**4**	**0**	**1**	**0**
121	**4**	**0**	**1**	**0**
125	5	0.2	0.8	0

Discussion

There was no evidence that *Adelges tsugae* distribution among the hemlock trees in the seed orchard was significantly different from random. The hypothesis of spatial clumping of *A. tsugae* in the seed orchard could be rejected based on the nonsignificant *Z*-tests of the join-count statistics. This helped to justify pooling all of the genets across the two blocks for the χ^2 analysis because spatial configuration could be eliminated as an explanatory variable for *A. tsugae* infestation levels on the different trees. There was no apparent local uniqueness in space experienced by the hemlock trees that would affect the spatial distribution of *A. tsugae*.

The χ^2 analysis suggests that there is a genetic basis for different levels of *A. tsugae* on western

Table 2. The numbers of ramets with *Adelges tsugae* absent and present for genets represented by five or more ramets. These are the data that were used for the fourth χ^2 test ($\chi^2 = 35.8$; df = 16; $P \leq 0.01$).

Generic genet number	Number of ramets with *A. tsugae* absent	Number of ramets with *A. tsugae* present
37	4	1
39	1	6
40	1	5
45	0	6
51	0	5
75	0	5
78	0	5
79	0	6
80	0	5
81	1	5
82	0	5
83	0	5
84	0	5
87	0	5
115	0	5
116	0	5
125	0	5

hemlock trees in the seed orchard, but the results are equivocal. All of the tests using *A. tsugae* levels of absent, low, and high were significant, meaning that frequencies of ramets with different *A. tsugae* levels varied by genet. The fact that the vast majority of genets with four or more ramets had no trees lacking *A. tsugae* (21 out of 34 in total; Table 1) and none of these genets was exclusively *A. tsugae*-free (Table 1) indicates there is no complete resistance to *A. tsugae* among the clones represented in the seed orchard. This is supported by the nonsignificant result of the χ^2 test of *A. tsugae* presence/absence frequencies among genets with four or more ramets. However, there may be genetic variation in *A. tsugae* susceptibility or tolerance because some genets tended to host more *A. tsugae* than others.

The small numbers of ramets in the different genets reduced the degrees of freedom for the χ^2 tests and thus the ability to infer genetic differences in frequencies of *A. tsugae* levels. If more of the genets had been represented by a larger number of ramets, then more of the data could have been used. However, there was significant variation by genet in presence/absence of *A. tsugae* for genets with five or more ramets. The genets used for this test are worth examining more closely for possible resistance to *A. tsugae* (Table 2). The genet with the generic number 37 uniquely had *A. tsugae* absent on four out of five ramets, whereas all of the other genets had *A. tsugae* present on the majority of ramets and most of these genets had no ramets lacking *A. tsugae*.

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