

Phenotypic Evidence Suggests a Possible Major-Gene Element to Weevil Resistance in Sitka Spruce

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Abstract

The weevil resistance breeding program against the white pine weevil, *Pissodes strobi* Peck (Coleoptera: Curculionidae), particularly for Sitka spruce (*Picea sitchensis* (Bong.) Carr), is arguably one of the most successful pest resistance breeding programs for plantation forest species, and it has done a lot to rehabilitate this important western conifer. Nearly all planting stock currently comes from this breeding program and so far the resistance seems effective, durable, and stable. We have used this program to also study causes behind this resistance, including: various hindrance mechanisms including induced and constitutive resin cells, sclereid or stone cells, and terpene defenses. All of them appear factors in resistance, but none singly is strongly predictive to resistance - the strongest are sclereid cells. All of these factors are in their nature complex, multifaceted, and appear to offer some partial solution that is likely controlled by complex multigenic systems. We do note, however, that we have very strongly expressed and complete resistance in some individuals. This and some preliminary data investigation indicates that there may also be a major gene element in our observed resistance. Such elements are well described against rusts and other pathogens in forestry, but are also well described for insects and nematodes in crop breeding. Particularly interesting is the Hessian fly in wheat which has a similar life strategy to the weevil. We describe here the elements that suggest this conjecture and how we might go about proving this. Understanding the genetic elements behind this observed resistance has implications for the overall strength and durability to resistance against the white pine weevil.

Keywords: *Pissodes strobi*, white pine weevil, Sitka spruce, resistance breeding program

Introduction and Background

White pine weevil (*Pissodes strobi* Peck) is one of the most devastating pests of young spruce (*Picea* spp.) and pines (*Pinus* spp.) in North America. The weevil is a native insect that occurs across Canada and the northern United States. In eastern North America, it is a major pest of eastern white pine (*Pinus strobus* L.) and introduced Norway spruce (*Picea abies* (L.) Karst.), but in the west it mainly attacks spruce species; Sitka spruce (*Picea sitchensis* (Bong.) Carr.) is particularly susceptible. Adult weevils lay eggs in the bark on tree leaders, emerging larvae mine down under the bark, consuming the phloem, severing the cambial layer, girdling and eventually killing the leader. Damage is so severe that young plantation trees often become stunted and bushy as terminal leaders are repeatedly killed and young trees fail to achieve apical dominance. Because of the weevil, Sitka spruce has been avoided as a re-forestation species in many parts of its former range.

A resistance breeding program initiated in the early 1990s is relatively unique for insect pests in forest trees, but it has been successful and has done a lot to rehabilitate this important western conifer (Alfaro and King these proceedings, King and Alfaro 2009). Nearly all planting stock currently

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comes from this breeding program and, to date, the resistance seems effective, durable, and stable. We have also used this program as a case study for mechanisms of resistance in conifers against shoot infesting insects (Alfaro et al. 2002). To date, various hindrance mechanisms including: induced and constitutive resin cells, sclereid or stone cells; and terpene defenses have been studied (Alfaro 1995, Alfaro et al. 1997, King et al. 2011, McKay-Byun et al. 2006, Robert et al. 2010). All of these appear factors in resistance, but none singly is strongly predictive to resistance - the strongest to date appears to be sclereid or lignified stone cell (King et al. 2011). All of these factors are in their nature complex, multifaceted, and appear to offer some partial solution that is most likely controlled by complex multigenic systems. We do note, however, that we have very strongly expressed, complete, or total resistance in some individuals.

Major gene, or R-gene, resistance is characterized as “total resistance” and is well documented for pathogens in forest trees (Kinloch et al. 2007, Kubisiak et al. 2005), but is also well characterized for insects in many crop plants, especially phloem feeding insects such as is the white pine weevil (examples include aphids, leaf hoppers, and fly larvae). Particularly interesting are the midges, such as the Hessian fly in wheat, which, as a phloem feeder (Harris et al. 2003), has a similar life strategy to the weevil.

In this paper, we review the evidence to date for the hypothesis of “total resistance” in the spruce - insect pest system. We present features associated with this type of resistance and describe what we know about these highly resistant individuals. We suggest methods of confirming this type of resistance and discuss the implications of deploying major gene resistance.

Marked Distribution of Resistance in Native Populations

Early provenance trials indicated strong geographic regions of resistance: the hybrid area of northwest British Columbia and two provenance sources within the high hazard region of southwest British Columbia (Haney and Big Qualicum) (Ying 1991). Screening included clonal selections of individuals from these two provenances as well as a series of open-pollinated (OP) collections from this high-hazard region (King and Alfaro 2009). Results from the first of these OP series indicated weevil resistance was not widely distributed through this region, but had a marked area of resistance close to the original provenances (King et al. 2004). Figure 1 incorporates results from all four of the OP family series trials and graphically demonstrates the distribution of naturally occurring resistance found in the high-hazard zone of southwest British Columbia. The dots indicate provenance sources where trees were sampled in this region. Three to 30 trees per source were included (with over 500 families screened overall) – the relative resistance of these sources is shown by color coding with red expressing higher resistance and blue susceptible. The strongly defined resistance boundaries for the Fraser Valley (Haney) and East Vancouver Island (Big Qualicum) regions are evident. They transition over relatively short distances to susceptible regions to the north (Salmon River and Amor de Cosmos, North Vancouver Island), and south (Duncan and Saanich, South Vancouver Island, and Puget Sound, Washington).

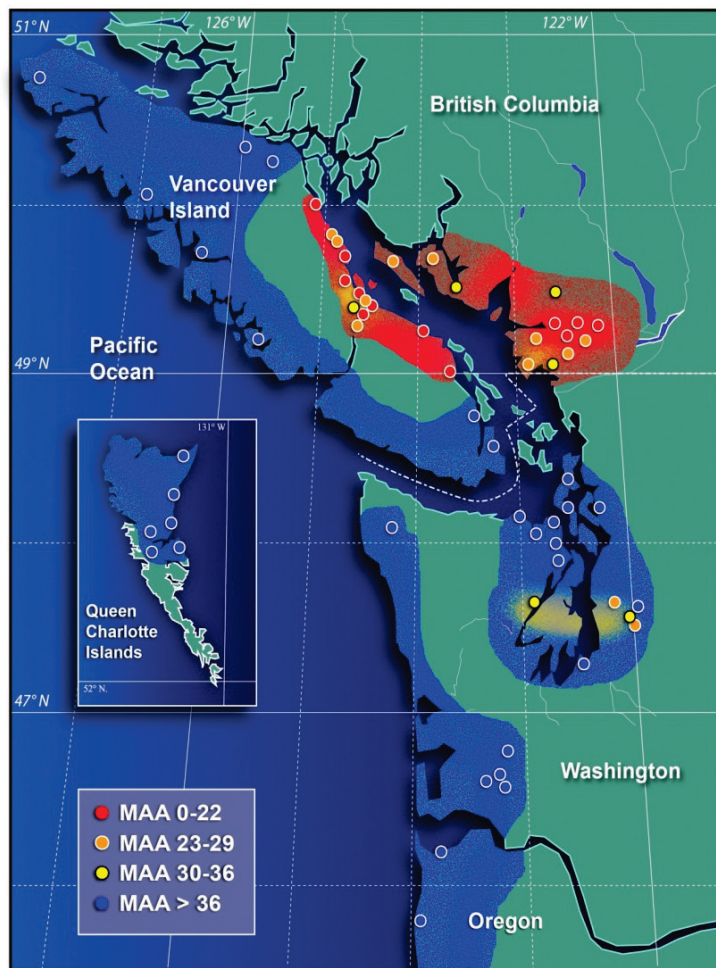


Figure 1—Resistant provenances by mean annual attack (MAA) level and zones of high and low natural resistance; MAA is expressed as a percentage. The resistant region is shown in red, the susceptible region in blue shades (intermediate in yellow).

The strong geographic demarcation noted is also demonstrated clearly by the plots of the frequency distributions based on families from the first OP series families that most widely sampled this high-hazard zone (King et al. 2004). In fig. 2, the distribution f_0 represents the hypothesis that there is one underlying distribution within the high-hazard zone (broad-based resistance). The trendline, f_R , represents the families from the resistant Big Qualicum sources, and f_S represents families from other sources within the high-hazard zone, but outside the resistant (red) region described in fig. 1. The trendline, f_C , represents the combined distribution of f_R and f_S , and fits significantly better than the single distribution f_0 , with a likelihood ratio statistic of 42.5 ($P < 0.0001$). Mean annual attack rates of the Haida Gwaii (Queen Charlotte Islands) families used also in this first OP series range from 0.28 to 0.40 (the archipelago is free of the weevil), the resistant Big Qualicum families (range 0.05 to 0.23) did not overlap at all.

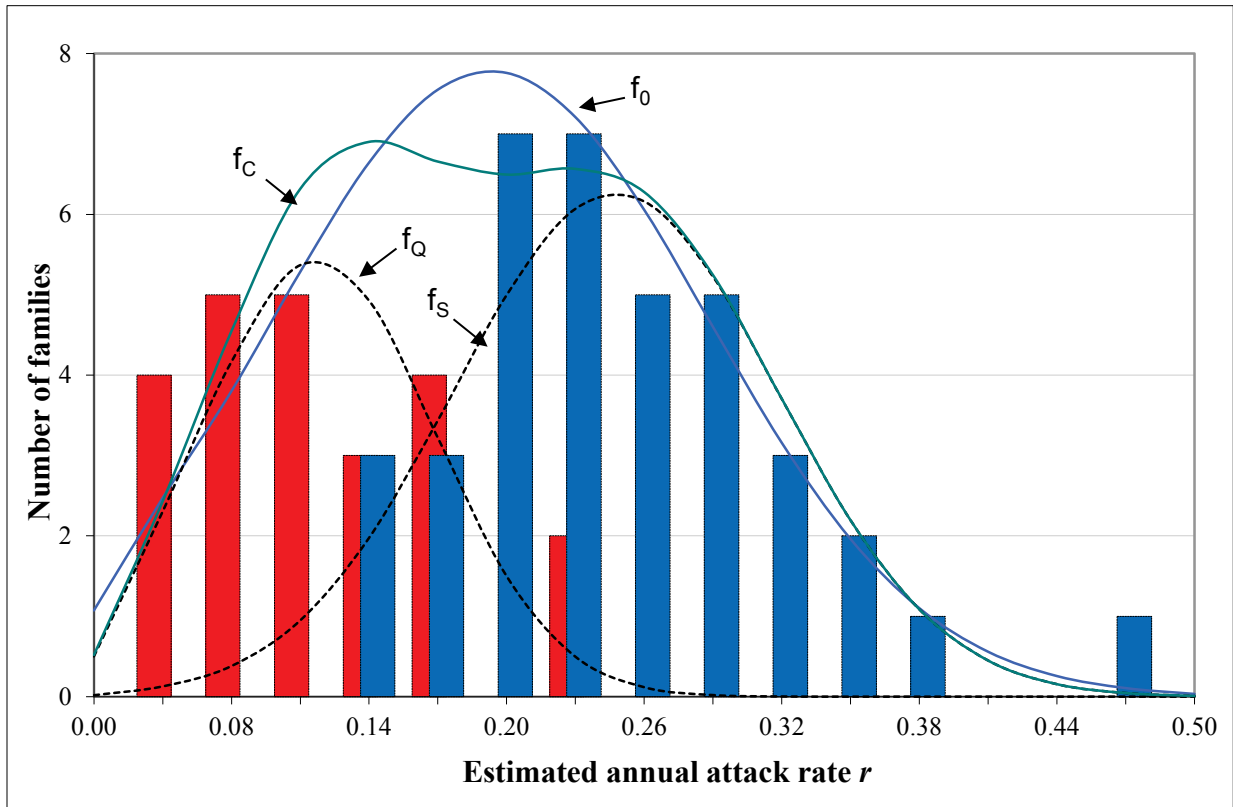


Figure 2—Frequency distributions of weevil attack. f_0 represents the null hypothesis that high-hazard mean families belong to one distribution. f_q depicts families from the high resistant Big Qualicum source (fig. 1). f_s depicts families from the other sources in the high hazard zone. f_c shows the combined frequency distribution ($f_q + f_s$). Bars show frequency of actual data points – the red shaded are those for f_q and blue for f_s .

Observation of Segregation within Families

Besides analyzing the distribution of family means within the high-hazard zone, within family segregation can also offer insights. Simple statistical evaluation techniques to detect segregation within families have been widely used especially in human and animal genetics (Fain 1978, Uimari et al. 1996). Such techniques have recently also been used in forest trees (Zeng and Li 2003). These tests assume that the presence of major genes with large effects will inflate the variance within the families in which the genes are segregating. This segregation may also cause non-normality of the phenotypic data, producing significant skewness and/or kurtosis. One method to explore this within-family phenotypic effect is to regress the offspring variance on the sib or family means (Fain 1978). With no inbreeding there should be no relationship between the family variance and its phenotypic value in traits influenced by many genes with small effects. When a character is controlled by genes with large effects, individuals with the most extreme phenotypes are likely to be homozygotes (extreme means and low variance), whereas intermediate phenotypes are likely to be heterozygotes (intermediate means and high variance). This quadratic (concave down) relationship can be described by a polynomial regression of offspring variance on phenotypic value:

$$\text{var}(z_i) = \beta_0 + \beta_1 z_i + \beta_2 z_i^2 \quad (1)$$

Where $\text{var}(z_i)$ is the phenotypic variance within the i^{th} offspring family, and z_i is the family mean (Fain 1978).

This analysis is designed for continuous normally distributed data, and although major gene segregation will distort the normal distribution, this test is considered robust to non-normality of the data. Our data (the number of attacked leaders divided by number of years of observation) follows more correctly a Poisson distribution, characterized by a single parameter where the variance equals the mean. Specific study of this variance requires a generalization, so an overdispersed Poisson distribution (McCullagh and Nelder 1989) was fitted to the 20 observations within each family. Under this distribution, the expected number of attacked leaders per observation is $E(X_i) = rt_i$ where x_i denotes the number of attacked leaders over t_i years for the i^{th} plot, and r denotes the annual attack rate for the family. The variance is $Var(X_i) = \phi \cdot rt_i$, where ϕ represents an overdispersion parameter – the degree to which the nominal Poisson variance should be inflated or deflated as dictated by the data (i.e., $\phi = 1$ indicates no overdispersion). The scaled residual deviance was used to estimate ϕ .

Results from fitting the Fain model to the estimated Poisson parameters $\hat{r}$ and $\hat{\phi}$ are shown in fig. 3 for the resistant Big Qualicum families. Figure 3 shows a good, significant curvilinear fit ($R^2 = 0.63$, $n = 23$) to the quadratic term in the model ($P < 0.0001$). This same model fit poorly to the susceptible families ($R^2 = 0.37$, $n = 44$), and the quadratic term was not significant ($P = 0.5389$). Using simply the average attack rate and (log) variance as in Equation (1) instead of the Poisson distribution, a curvilinear regression was also highly significant for the Big Qualicum families ($R^2 = 0.86$, $P < 0.0001$ for the quadratic term), and the susceptible families again fit poorly ($R^2 = 0.02$ and $P = 0.5377$ for the quadratic term).

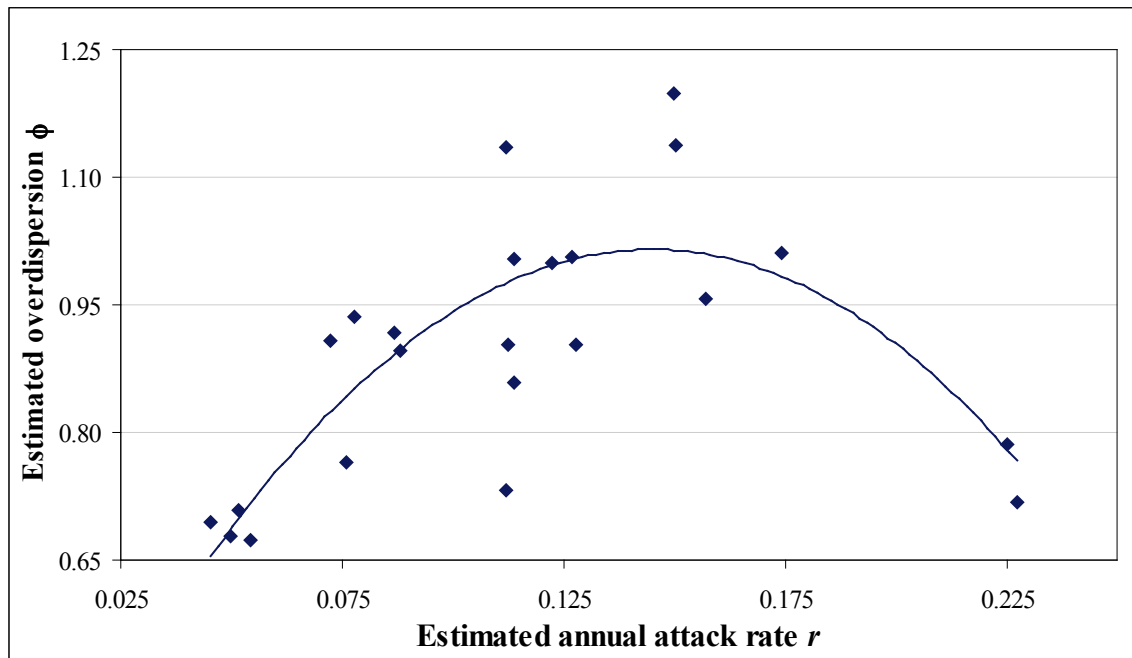


Figure 3—Plot of Big Qualicum families and fitted quadratic (Fain 1978) model ($R^2 = 0.63$, $n = 23$). Overdispersion and annual attack rate are estimated from an overdispersed Poisson distribution.

Studies of Strongly Expressed Phenotypic Resistant Individuals

A series of studies focused on gaining insight into weevil resistance has been conducted on one particular tree, parent clone 898, since this highly resistant individual was first noticed at the Sayward

(Bigtree Creek) trial (Alfaro and Ying 1990, King and Alfaro 2009). This individual has been clonally replicated in many field trials, archives, and nursery experiments. Over a decade of observations have been made in screening trials containing close to 100 898 clonal ramets, and in this time only two trees of this clone have been noted to have leader kill – this compared to observations of leader kill in 100 percent of individuals, many times repeatedly, in many other clones (King and Alfaro 2009). In addition to being weevil resistant, the 898 clone is easily identified in a plantation by its exceptional growth form. Its vigorous growth produces long, straight internodes that typically cause the tree to stand out among its neighbors and make it highly desirable for forestry (fig. 4).

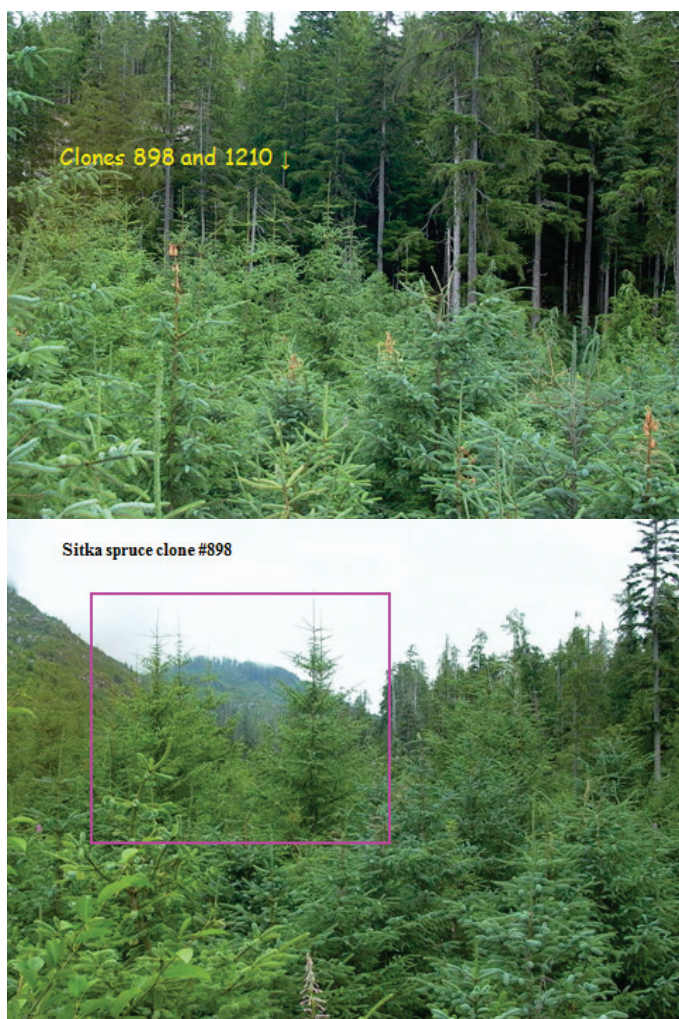


Figure 4—Clonal blocks of 898 (and other immune clone 1210) with susceptible seedlots in foreground. At this site (Hisnit – King and Alfaro 2009) under very heavy attack, not a single 898 individual was successfully attacked.

In efforts to gain insight into the resistance strategy of the 898 clone, feeding and oviposition behavior have been observed through caging experiments over the years (fig. 5). Where weevils had a choice, they preferred not to colonize 898. However, when caged on ramets of clone 898, weevils preferred not to feed on the leader, but rather on the one-year-old internode. Again, in caged (no choice) experiments, gravid females would lay eggs that hatched, but the larvae died at first instar and females that were not gravid did not produce eggs (Tony Ibaraki, personal communication).

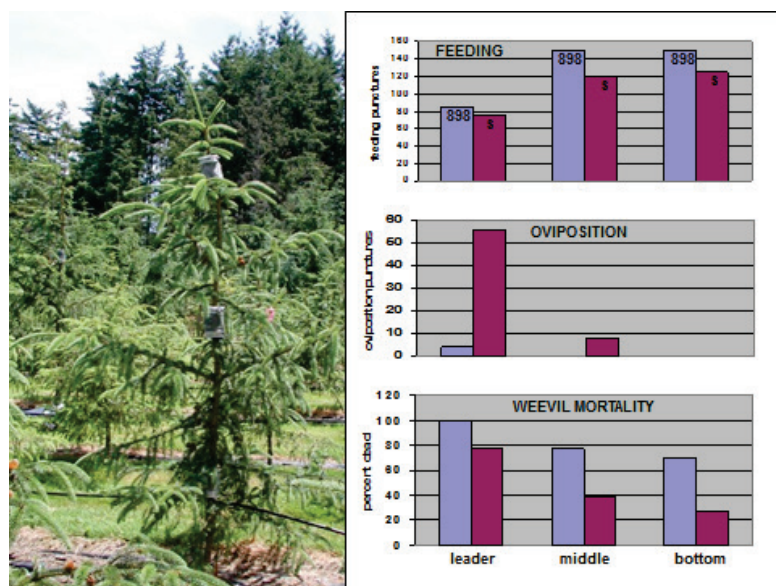


Figure 5—Weevil caging studies showing some of the results of forced caging in different positions on the crown.

Studies of the resin canal systems of Sitka spruce revealed 898 has high bark constitutive resin cell density as well as high sclereid cell density, but most of the trees from the resistant region (East Vancouver Island – Big Qualicum or Fraser Valley – Haney) show this compared to individuals from the more susceptible regions. Interestingly, although 898 is high for both of these traits it is not ‘off the chart’ (King et al. 2011) which suggests other mechanisms may also be present. The traumatic resin response in Sitka spruce has not been thoroughly explored. However, observations to date indicate that this trait does not seem to be as well expressed in Sitka spruce as it is in white spruce (O’Neill et al. 2002). This perhaps accounts for the particular susceptibility of Sitka spruce and why it acts in such an unstable manner to this endemic pest. Our work to date on traumatic resin with 898 was inconclusive.

Various biochemical studies have been made of 898, but none so far have shown anything unique for this clone (McKay-Byun et al. 2006, Robert et al. 2010). One interesting hypothesis is that the resistant 898 clone inhibits the expression of the vitellogenin gene in weevils after they feed. Vitellogenin is an egg-yolk protein precursor necessary for the maturation of eggs in reproductively active female weevils (Leal et al. 1997). Weevils that fed on resistant ortets or cuttings of the 898 clone had fewer eggs form in their ovaries, and ovaries of *P. strobi* females that fed on this clone were less likely to mature. Levels of ovarian growth and expression of the vitellogenin gene were also reduced in weevils that fed on severed leaders of the resistant 898 clone relative to those that fed on severed leaders of a susceptible family (Sahota et al. 2001). However, as we noted earlier, gravid females can successfully lay eggs in 898, although they would not prefer it.

It needs to be noted that 898 is not the only ‘highly resistant’ or immune individual, but it has been the most extensively studied. An F_1 generation breeding program was instituted (King and Alfaro 2009) and some preliminary investigation has begun. Seven of the ten most resistant crosses in the F_1 generation had 898 as either parent (Moreira et al. 2012). A full segregation analysis has yet to be conducted on the F_1 generation, but it should be noted that these trials, primarily made with resistant x resistant crosses, have far fewer attacks, thus the difficulty with getting robust screening compared to the 1st generation OP trials. It is also possible at this time to get to a backcross generation – this would really help us to confirm the segregation – which should be unambiguous compared to either the F_1 or OP generation.

Major Gene Resistance in Other Plant – Insect Systems

Some of the best documented insect resistance systems involving *R* genes are gall midges (Cecidomyiidae) in both wheat and rice. Harris et al. (2003) detailed the genetic and biochemical interactions between gall midge *avr* genes and plant *R* genes. In the wheat-Hessian fly system, close to 30 major genes in the host interact with various biotypes of the pest that display virulence to specific *R* genes in typical gene-for-gene fashion (Ratcliffe and Hatchett 1997). Although antibiosis appears to be the primary mechanism, often leading to the death of the first-instar larvae within a few days of establishment, the nature of this antibiosis is only beginning to be understood. Although hypersensitive responses (HR) have been reported in this system (Grover 1995) this may not be the only or even key defense response to these piercing/sucking type of insects (Kaloshian 2004). Harris et al. (2006) investigated with detailed microscopy the compatibility interactions between larvae and plant. In the compatible interaction in these gall-forming insects, a virulent larva promotes gall formation in the host. This gall does not have any noticeable macroscopic structure, but is defined as aberrant tissue structure resulting from stimulation from a foreign organism, in this case the fly larva (Harris et al. 2003). It had been proposed that injection of salivary substances by the larvae during the first instar might be part of the attack process (Hatchett et al. 1990, Ollerston et al. 1990) and piercing marks by larva mandibles were indicated in the microscopy investigation (Harris et al. 2006). It is hypothesized that *avr* gene products from the larva are secreted into epidermal cells and activate a galling process (Harris et al. 2003). Part of the ensuing aberrant tissue structure (in this compatible interaction) is enhanced protein synthesis combined with breakdown of cell walls – the cells rupturing with ‘nutritive tissue’ providing the larva with a diet rich in soluble amino acids and sugars (Harris et al. 2006). The virulence genes that aid the larva in successfully attacking a susceptible plant would act as avirulence genes in a plant defended by *R* genes (incompatible reaction) (Harris et al. 2003). In fact, it appears the interaction of *R* gene product and *avr* effectors can involve complex recognition and response interactions (Kaloshian 2004). In a defended plant this gall-induced nutritive tissue is not formed and the larva starves. It has been noted that when virulent and non-virulent biotypes colonize the same plant the nutritive tissue can be enough to also sustain the non-virulent larvae (Grover et al. 1989) which has implications for fitness and survival in the evolution of fly virulence (Harris et al. 2003).

This type of galling response, associated with nutritive tissue has been noted in many plant defense responses to insects (Rohfritsch 1992). There is also a phenotypic difference in the expression of these incompatible reactions between resistant genotypes (Harris et al. 2003). Classic HR may also be observed as part of a gall midge attack (e.g., *Salix viminalis* to the gall midge *Dasineura marginemtorquens* (Ollerston et al. 2002). But there appears to be substantial variability in the manifestation of *R*-gene mediated plant defenses in host-insect interactions. HR may be a feature but not always and different forms of limitation to phloem feeding have been observed (Kaloshian 2004).

Summary and Conclusions

There are important hallmarks of large genetic effects for resistance to the weevil: marked geographic distribution, and some evidence of within family segregation. This does not necessarily indicate classic major gene or *R*- gene resistance and it could be explained by relatively few genes controlling the hindrance traits, resin canals, and sclereid cells already investigated. Also, the induced or traumatic resin cell investigation that appears so effective in interior spruce has so far not been managed in Sitka spruce. Genotypes showing ‘total resistance’ in Sitka spruce with respect to the white pine weevil and indication of life-history parallels with similar phloem feeding insects – e.g. the starvation of first instar larvae manifested in *R*-gene defense in the Hessian fly, clearly though point to the need for follow-up investigations. Although investigative work to date has concentrated on one particular cloned individual – other individuals have been identified but as yet have not been followed through in such detail. *F*₁ breeding has been carried out with these individuals (resistant x resistant,

resistant x susceptible, susceptible x susceptible) (Moreira et al. 2012) and it would be very feasible to make backcross segregating lines (with clonal emblings). Developing such segregating lines would certainly help in any further investigation and would set up a good experimental population in further understanding the phenotypic and biochemical natures of this resistance. Molecular genetic surveys based on linked molecular markers could also help in identifying the genetic architecture underlying resistant phenotypes. The molecular and proteomic signatures for R-gene resistance can be also be identified (Liu et al 2004) and structured experimental populations and structured experimental pedigrees would help in this investigation.

Although the genetic variation governing plant-insect interactions is usually observed as continuously variable, and most likely controlled by the segregation of multigenic quantitative trait loci, this does not preclude major genes as important mechanisms of resistance. Certainly for phloem feeding insects that attack crop plants, R genes are widely used as key plant defense mechanisms. We do know that long term stable and manageable resistance is possible by the nature of the resistance we see in white spruce in Eastern North America, where it is not a major problem and acts more like a classic endemic system. An understanding of the genetics behind this resistance is necessary to assess the durability and effectiveness of the resistance found so far in Sitka spruce.

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