GALLING ADELGIDS: GALL FORMATION, DEVELOPMENTAL MORPHOLOGY, CHARACTERIZATION, AND THE GENETIC SUSCEPTIBILITY OF SPRUCE

by

Babita Bains

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ABSTRACT

Adelgids (Hemiptera: Adelgidae) are a small group of insects with complex life cycles and can threaten seed production in British Columbia (BC). Adelgids induce galls on the reproductive and vegetative shoots of spruce trees, reducing the number of future cone sites. Additionally, feeding on the cones and needles of alternate conifer hosts can reduce seed extraction efficiency, and cause discoloration and twisting of needles. Seed orchards are intensively managed for frequent and abundant seed production; hence losses incurred by adelgid pests are of high importance**.** Considering the complexity of insect-galler systems, there are many unanswered questions regarding the basic biology and habits of adelgids.

My research elucidates the influence of two adelgid life stages, the fundatrix and her offspring the gallicolae, through the use of manipulative laboratory experiments and botanical histological processes. It is evident that fundatrices induce the gall formation process and the gallicolae activity completes the gall formation process. Considering the fundatrix is required to stimulate galling it would be sufficient to include only fundatrices in a monitoring system.

Additionally, I tested the theory that susceptibility to adelgid induced galling is under genetic control in spruce. Strong evidence of genetic control of susceptibility was observed with modest individual and high half- and full-sib family narrow-sense heritabilities of 0.17±0.09, 0.87±0.04, and 0.61±0.16, respectively. Breeding values for parental susceptibility to adelgid attack ranged between 0.25 and 0.76, indicating that selection for reduced susceptibility would be possible. These results suggest that adelgid

galling susceptibility could be used as a screening criterion for parental inclusion in future seed orchard establishment.

Furthermore, I attempted to associate structurally unique galls with the inducing adelgid species. Detailed associations of adelgid species and their galls have not been rigorously determined. Rearing of gallicolae alates from various galls suggests that there can be a wide range of variation in the structure that a single species induces. Characterization of galls may allow orchard managers to understand what adelgid species are present and possibly avoid the lengthy laboratory procedure required for species identification.

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Thanks to everyone who has helped me attain this goal.

DEDICATION

To my family

(Satnam, Kashmir, Sharan and Rajeeta)

CO-AUTHORSHIP STATEMENT

The tables below indicate percentages of coauthors' contributions at each stage of research for each chapter.

Chapter 2 – Genetic susceptibility of spruce to gall forming adelgids.

	Bains	**Isik**	**Strong**	**Jaquish**	**McLean**	**El-Kassaby**	**Total**
Problem identification & research design	55	0	25	20	0	0	100
Performing the research	100	0	0	0	0	0	100
Data analyses	10	35	0	0	0	55	100
Manuscript preparation	50	0	15	0	5	30	100

Chapter 3 – The role of *Adelges cooleyi* fundatrices and gallicolae in the gall formation process on interior spruce shoots.

	Bains	**Strong**	**McLean**	**Total**
Problem identification & research design	60	20	20	100
Performing the research	100	0	0	100
Data interpretation	100	0	0	100
Manuscript preparation	65	30	5	100

Chapter 4 – Developmental morphology of the galls of *Adelges cooleyi* on interior spruce shoots.

	Bains	**Shorthouse**	**Strong**	**McLean**	**Total**
Problem identification & research design	50	25	20	5	100
Performing the research	80	20	0	0	100
Data interpretation	70	25	0	5	100
Manuscript preparation	60	30	5	5	100

1 INTRODUCTION

1.1 Taxonomy

The insects of the Adelgidae family belong to the superfamily Aphidoidea in the order Hemiptera. Adelgids are closely related to aphids (Aphididae) and phylloxerans (Phylloxeridae), and are distinguished from their closest relatives by the absence of siphunculi. The Adelgidae are composed of two genera, *Adelges* and *Pineus*. *Adelges* and *Pineus* are distinguished by the number of abdominal spiracles and species are typically identified by morphological characteristics such as wax glands, antenna structure, wing venation and wax plates. There are approximately 36 described *Adelges* species and 29 *Pineus* species. Only 50 of these species have well described life cycles and roughly 15 species can be found in western Canada. There are a lot of uncertainties in the taxonomy and it is believed that several described species may actually be different morphological forms of the same species (Havill and Foottit 2007).

1.2 Life history

Adelgids have a complex life history and can be holocyclic or anholocyclic. Holocyclic species have a sexual generation and four non-sexual generations. Additionally, holocyclic species alternate between a primary host (*Picea*) and a secondary host of another conifer genus. Three of the five generations occur on spruce, whereas two generations occur on the secondary host. The sexuparae generation migrates from the secondary host and lays eggs on the primary host. The sexuparae offspring develop into the sexual generation referred to as the sexuales. The sexuales mate and lay a single egg which becomes the fundatrix. The fundatrix is parthenogenetic, as are the

other asexual generations however she is the most prolific of all the adelgid generations. Her offspring develop into the gallicolae and this summer generation utilizes galls for food and shelter. The gallicolae emerge from the galls, molt into alates and migrate to their secondary host. Upon arrival, the gallicolae lay eggs and die shortly thereafter. The new generation, referred to as the exules, can cycle on the secondary host or their offspring can develop into the migrating sexuparae. The secondary host for adelgids is species specific. Anholocyclic species do not include a sexual generation, do not alternate between hosts and can cycle on either the primary or the secondary host (Havill and Foottit 2007).

1.3 Adelgids as pests

Most research involving adelgids has focused on species that have proven to be serious threats to North American forests. *Adelges picea* (balsam woolly adelgid) and *Adelges tsugae* (hemlock woolly adelgid) are two of the more well-known species and much of the recent research has focused on these introduced forest pests (Havill and Foottit 2007). However, adelgids have also proven to be pests in western Canadian seed orchards. Seed orchards play a key role in breeding and reforestation. Seed orchards capture and package genetic gain and diversity that is attained from breeding programs (El-Kassaby pers. comm.). A healthy seed supply is crucial in maintaining silviculture in British Columbia (B.C.); hence the production and availability of these seeds is of paramount importance. Adelgid feeding on spruce induces galling and can limit branch development. Galling of reproductive buds directly reduces seed yields and galling of vegetative buds decreases the potential number of future cone sites (Strong 2002). On secondary hosts adelgids feed on the needles and cones. Feeding on the needles of a

secondary host can cause twisting and yellowing, hence reducing tree vigour, while feeding on the cones reduces seed yields and impairs seed extraction efficiency. Seed loss is important considering reforestation depends on genetically-improved seed supplied by seed orchards. By 2013, B.C. would like to have 75% of forestry seed supplied by seed orchards (BC Ministry of Forests and Range 2007).

1.4 Objectives

Roughly 60% of the spruce seedlings used for reforestation in B.C. are the product of seed harvested from seed orchards (BC Ministry of Forests and Range 2007). Orchards are the link between breeding and silviculture and they are the conduit for packaging the genetic gain (El-Kassaby pers. comm.). Seed orchards, such as the Kalamalka Seed Orchard, located near Vernon, B.C., are intensively managed for frequent and abundant seed production and losses incurred by adelgid pests are of high importance. The goals of my research were as follow:

1. to determine if susceptibility to adelgid induced galling has a genetic basis (Chapter 2)

2. to clarify the role of *Adelges cooleyi* fundatrices and gallicolae in the gall formation process (Chapter 3)

3. to describe the different morphological phases of *Adelges cooleyi* gall development with botanical histological techniques (Chapter 4)

4. to attempt to associate the different adelgid induced gall structures with the inducing species and to clarify if the described species are distinct species or different morphological forms (Chapter 5)

Prior to commencing this research I was trained by Dr. Robert Foottit and Eric Maw in Ottawa, Ontario on how to accurately identify adelgids to genus and species, using an alate collection made in four-way, up and down Malaise traps (Figs. 1-1, 1-2) in 1996 by Dr. Rory McIntosh. The alates were collected by McIntosh in a spruce plantation at the Kalamalka Research Station. This training has ensured accurate identification of the adelgid species noted in my work. Voucher specimens have been deposited at the Beatty Biodiversity Museum and include specimens from a similar trapping program that we ran in the summer of 2007, and specimens from each stage of research. The mounting procedure for preparing adelgid slides is presented in Appendix A and the key used to identify adelgid alates to genus and species is presented in Appendix B.

1.5 Figures

Figure 1-1. Tamara and Anna helping with the construction of the Malaise traps for our summer 2007 trapping at Kalamalka.



Figure 1-2. Four-way, up and down Malaise trap.



1.6 References

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Strong, W.B. 2002. Pest status and control of Larch Adelgids. Seed and Seedling Extension Topics, British Columbia Ministry of Forests Tree Improvement Branch, **14**: 7-9.

2 GENETIC SUSCEPTIBILITY OF SPRUCE TO GALL FORMING ADELGIDS (HEMIPTERA: ADELGIDAE)[1]

2.1 Introduction

Adelgids (Hemiptera: Adelgidae) are a small clade of insects that feed on conifers and induce galls on most species of spruce (*Picea* spp.). Adelgids have a multi-generation life history and many species alternate between a primary and secondary host (Annand 1928; Cumming 1959; Havill and Foottit 2007). Spruce is considered the primary host because it includes the only sexual generation whereas the secondary host is of another conifer genus (*Pseudotsuga, Larix, Pinus, Tsuga,* or *Abies*) that differs among adelgid species (Annand 1928; Cumming 1959; Furniss and Carolin 1977; Havill and Foottit 2007). On the secondary host, all adelgid generations are parthenogenetic (Havill and Foottit 2007). Adelgid feeding on spruce typically causes galling of vegetative and reproductive shoots, and can limit branch or cone development (Figs. 2-1, 2-2). It is believed that adelgids insert their stylets intra-cellularly and feed on solutes from the phloem of spruce and on the cortical parenchyma or ray parenchyma cells of a shoot (Raman *et al*. 2005). Galling of the reproductive shoots directly reduces seed yield (WBS, unpublished data), whereas galling of the vegetative buds decreases the potential number of future cone bearing sites. Vegetative shoot galling can significantly reduce the aesthetic value of Christmas trees and ornamentals (British Columbia Ministry of Agriculture and Lands 2008; Cranshaw 1989). Foliar infestation of the secondary host can also reduce aesthetic value (Saunders and Barstow 1977). In addition, tree health in

[1] A version of this chapter has been submitted for publication. Bains, B., Isik, F., Strong, W., Jaquish, B., McLean, J.A., and El-Kassaby, Y.A. Genetic susceptibility of spruce to gall forming adelgids.

the forest can be impacted by heavy infestations of non-native galling adelgids (Wilford 1937).

Furthermore, adelgid induced galling threatens the productive output of seed orchards in coastal and interior British Columbia (BC). Presently in BC, roughly 60% of the 80 million spruce seedlings used for reforestation annually are grown from seed harvested from seed orchards (BC Ministry of Forests and Range 2007a). Seed orchards are the link between tree breeding and silviculture, delivering genetic gain to forest plantations (BC Ministry of Forests and Range 2007b). These orchards are intensively managed for frequent and abundant seed production; hence potential seed loss caused by biotic or abiotic agents is of high importance (Faulkner 1975). Chemical and biological controls have been explored in an attempt to manage adelgids, however only insecticidal soaps and dormant oil are currently used in BC (Strong and Bennett 1997; Strong 2002).

Resistance of individual trees to adelgids was first noted by Wilford (1937), who found that 39% of spruce trees in a Michigan forest escaped attack over a 10-year period, despite having their branches intertwined with infested trees. Bjorkman (2000) found further evidence that susceptibility to galling adelgids is under genetic control in spruce, with the identification of two resistant and two susceptible families. This work was further supported by the reported heritability estimates from multiple progeny test trials by Mattson *et al*. (1997), who used 110 half-sib families of Engelmann spruce (*Picea engelmanii*) at 9 study sites in British Columbia.

This study determined the heritability of susceptibility to adelgid galling on interior spruce trees in BC. The adelgids observed in this plantation are native to western North America and comprised a complex of approximately seven species in two adelgid

genera (*Adelges cooleyi, A. lariciatus, A. abietis,* and *A. strobilobius,* and *Pineus floccus, P. similis,* and *P. pinifoliae*). *A. cooleyi* and *A. lariciatus* were the dominant species present, with 57% and 35% of the total respectively. The genetic structure of the trial (partial diallel) permitted the determination of the genetic control of susceptibility, the estimation of individual and family heritability, and parents' breeding values. Exploring the response of different spruce genotypes to adelgid damage could be beneficial to breeding programs and seed orchards. A genetic basis for susceptibility would open the possibility of breeding for resistance as a means of reducing adelgid impact on spruce and secondary hosts.

2.2 Materials and Methods

2.2.1 Trial establishment

The genetic trial (progeny test) consisted of 42 full-sib families of interior spruce (*Picea engelmanni* Parry ex Engelm x *glauca* (Moench) Voss) originating from a 13-parent, incomplete partial diallel mating design (Table 2-1) that was originally established to study genetic resistance to the white pine weevil (*Pissodes strobi* (Peck)) (Alfaro *et al.* 2004). The progeny test was planted at the Kalamalka Research Station, Vernon, BC (50°14'N, 119°16'W, elev. 480m) in 1995. The plantation's design is a complete randomized block with three replications, with crosses randomized within each block. Each cross was planted as a 25-tree square plot with 1.25 x 1.25m spacing among trees (see Alfaro *et al*. 2004, for a detailed description). In spring 2007, the progeny test was thinned to 1492 trees by systematically removing every second tree.

2.2.2 Data collection

Adelgid infestations had been noted in the progeny trial for several years. Current year's adelgid induced galling was assessed on each tree in July-August of 2007. Damage was quantified by counting the number of current year galls on every tree from two randomly selected branches, and a different cardinal direction at breast height (1.3m). Sampling was restricted to new shoots arising from the terminal two years' growth on each branch.

2.2.3 Statistical analysis

For each tree, adelgid attack was considered a binary attribute (0 = no galls, 1 = at least one gall present) following a Bernoulli distribution. A generalized additive linear mixed model [1] was fit to the response variable using the following logit link function:

$$Y_{ijk}l = \log [\pi/(1-\pi)] = \mu + B_i + GCA_j + GCA_k + SCA_{kj} + e_{ijkl} \quad [1]$$

where Y_{ijkl} is the observation of l^{th} tree, π is the mean event (galling average), μ is overall mean, B_i is the i^{th} block effect (fixed), GCA_j is the general combining ability effect of the j^{th} parent (random), SCA_{kj} is the specific combining ability effect of the kj^{th} cross (full-sib family) of parents j^{th} and k^{th} (random), and e_{ijkl} is the random residual error associated with the l^{th} tree of the kj^{th} cross. The covariance matrix of Y vector is $Var(Y) = \mathbf{ZGZ}^T+\mathbf{R}$ where $\mathbf{G}=\sigma^2_i\mathbf{I}$ is the diagonal matrix of random effects**,** and $\mathbf{R}=\sigma^2_e\mathbf{I}$ is the diagonal random errors variance (Schall 1991). In the diallel mating designs, the same parents are used as females and males. Thus, only one GCA variance is estimated for the parents (Griffin 1956), which is equivalent to a quarter of the additive genetic variance (Lynch and Walsh 1998). The SCA variance is equal to a quarter of the dominance genetic effects.

The model was run with the ASReml software to estimate variance components (Gilmour *et al.* 2006), featuring the restricted maximum likelihood to partitioning the phenotypic variance into causal components. The best linear unbiased predicted (BLUP) breeding values were obtained by solving mixed model equations for the random effects (Henderson 1975). The solutions (breeding values) from the generalized model were on logit scale as the logit link function was used in the model. Logit predictions were back transformed to obtain interpretable breeding values (probabilities) according to Isik *et al*. (2005). ASReml provides standard error of predictions along with BLUP values. We calculated correlations between true and predicted breeding values using the standard errors of predictions (Gilmour *et al*. 2006). Using the observed variance components

from the model, narrow-sense heritabilities were calculated for individual trees (h^2_i), half- (h^2_{hs}) and full-sib family means (h^2_{fs}) as follows:

$$h_i^2 = \frac{4\sigma_G^2}{2\sigma_G^2 + \sigma_S^2 + \sigma_E^2} \quad [2]$$

$$h_{hs}^2 = \frac{\sigma_G^2}{\left(p\sigma_G^2 + \sigma_S^2 + \frac{\sigma_E^2}{bn}\right)\frac{1}{(p-1)}} \quad [3]$$

$$h_{fs}^2 = \frac{2\sigma_G^2}{2\sigma_G^2 + \sigma_S^2 + \frac{\sigma_E^2}{bn}} \quad [4]$$

where σ^2_G is the general combining ability variance, σ^2_S is the specific combining ability variance, σ^2_E is the error variance (fixed to 3.29 for the logit model), p is the number of parents, b is the number of blocks, and n is the number of trees per block per cross. Standard errors of heritabilities were obtained by Taylor series approximation as explained by Isik *et al.* (2008).

2.3 Results and Discussion

Adelgid susceptibility/resistance appears to be under strong genetic control as evident by the observed individual and family heritability values (Table 2-2). As expected, the resultant values followed theoretical expectations with lower, but significant, individual (0.17±0.09), followed by full-sib (0.61±0.16) and half-sib (0.87±0.04) heritability estimates (Falconer and Mackay 1996) (Table 2-2). The Best Linear Unbiased Predicted (BLUP) breeding values of parents are the probabilities of adelgid galling incidence. The higher the breeding value, the more likely the tree is prone to galling. For example, parents 165 and 128 are the two most susceptible genotypes while parent 1 and 79 are the least susceptible among all the parents (Table 2-3). For deployment decisions involving seed orchards, Christmas trees and ornamentals, or plantation stock, genotypes with lower breeding values (e.g. Parent 1) should be included. Correlations between true and predicted breeding values ranged from 0.71 to 0.84 suggesting a wide range of reliability of breeding values. Higher correlations mean that the predicted breeding values have smaller variances and are more reliable.

Maximizing growth and yield attributes is the primary goal of the BC's interior spruce tree improvement program. Kiss and Yeh (1988) demonstrated the presence of strong genetic control over interior spruce's height growth and reported family heritability values ranging from 0.67 to 0.82, indicating that selective breeding would be effective. Today, rotation age individual tree volume gain estimates of approximately 20% are expected from spruce seed orchards (Forest Genetics Council of BC 2007). White pine weevil significantly attacks interior spruce thereby reducing tree height growth and tree development. Therefore, resistance to the white pine weevil was

introduced as a secondary attribute for breeding (Kiss and Yanchuk 1991). Selection for white pine weevil resistance proved to be effective due to the observed high family heritability of 0.77, abundant genetic variation for resistance, and the moderate correlated response between height and diameter growth, and resistance (Kiss and Yanchuk 1991; King *et al*. 1997). In the present study, we have demonstrated that adelgid susceptibility/resistance is also under strong genetic control and could be used as a screening criterion for selective breeding. Selected elite genotypes from breeding programs for growth and yield, and white pine weevil resistance, could be screened for adelgid susceptibility in breeding arboreta. An established adelgid population would be needed since augmentation of the natural population is not feasible. Although adelgid damage to spruce and secondary hosts in Western Canada is of modest concern in natural or managed forest stands, Christmas tree plantations and landscape settings of both the primary host and secondary hosts can be greatly affected. In these settings, severe infestations can lead to unacceptable visual damage levels. Adelgids can also lead to economic seed loss in spruce and western larch seed orchards, and require specific pesticide treatments in Douglas-fir (*Pseudotsuga menziesii*) orchards.

We have demonstrated that breeding for adelgid resistance in spruce is a possibility and could be fruitful. Adelgid resistance in spruce could directly reduce galling levels, and indirectly reduce adelgid densities on nearby secondary hosts by interrupting the adelgid life cycle. Moreover, breeding or selection for resistance to adelgids in spruce could be used as an adelgid pest management strategy in landscape settings, and conifer seed orchards.

2.4 Figures

Figure 2-1. Gall induced by *Adelges cooleyi* on an interior spruce shoot.



Figure 2-2. Healthy interior spruce cones (top row) and cones galled by *Adelges* spp. (bottom row).



2.5 Tables

Table 2-1. Illustration of the incomplete partial diallel mating design among the 13 interior spruce parents.

		Males											
		21	**29**	**72**	**79**	**87**	**98**	**117**	**128**	**161**	**165**	**167**	**1645**
Females	**1**	x	x	x	x	x		x	x		x	x	x
	21		x	x	x			x	x	x	x	x	x
	29				x	x				x		x	
	72												
	79			x				x	x				
	87							x		x	x	x	x
	98								x				
	117												
	128												
	161			x	x			x	x		x		
	165			x					x				
	167			x	x					x			

Table 2-2. Variance component estimates (± standard errors) and their percent contribution to the total phenotypic variance; narrow-sense individual tree, half- and full-sib family heritability estimates for adelgid attack on an interior spruce plantation at the Kalamalka Forestry Centre, Vernon, BC.

Source	Estimate (± SE)	%
GCA	0.165±0.019	4.6
SCA	0.114±0.070	3.2
Error	3.29 (fixed)	92.2
h^2_i	0.17±0.09	----
h^2_{hs}	0.87±0.04	----
h^2_{fs}	0.61±0.16	----

Table 2-3. Parent average adelgid attack and their breeding values with the correlations of breeding values.

Parent ID	Average attack	SE of mean	# of trees per parent*	BLUP breeding value (probability of attack)	Accuracy of breeding values
1	0.19	0.021	342	0.25	0.83
21	0.34	0.081	35	0.63	0.84
29	0.24	0.051	71	0.52	0.82
72	0.22	0.028	219	0.36	0.81
79	0.24	0.033	174	0.35	0.83
87	0.12	0.039	68	0.38	0.82
98	0.57	0.083	37	0.64	0.71
117	0.34	0.036	175	0.64	0.81
128	0.45	0.035	208	0.72	0.81
161	0.34	0.040	141	0.48	0.84
165	0.38	0.040	149	0.76	0.82
167	0.21	0.034	147	0.40	0.82
1645	0.20	0.039	105	0.37	0.77

*Since parents can share the same individuals due to diallel mating design, the actual number of trees in the data was 1492, less than the total number of trees in this column.

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3 THE ROLE OF *ADELGES COOLEYI* (HEMIPTERA: ADELGIDAE) FUNDATRICES AND GALLICOLAE IN THE GALL FORMATION PROCESS ON INTERIOR SPRUCE (*PICEA GLAUCA* (MOENCH) VOSS X *PICEA ENGELMANNI* PARRY) SHOOTS.[2]

3.1 Introduction

Adelgids are a small group of insects that are closely related to aphids, and include some of the more destructive introduced pest species threatening North American forest ecosystems (Furniss and Carolin 1977; Havill and Foottit 2007). Additionally, species native to North America, such as *Adelges cooleyi* and *Adelges lariciatus* are seed and cone pests in western Canada. Seed orchards are intensively managed agroecosystems and the seed produced is of high value; hence any losses incurred are important. The seed from orchards is used to produce seedlings for reforestation and seed loss could limit the use of spruce and other conifer hosts in reforestation. Adelgids feed on the buds of spruce trees resulting in galling. Galling of reproductive buds causes swollen scales, swollen bracts and galled cones. This directly reduces seed yields, whereas galling of vegetative buds decreases the potential number of future cone sites (Strong 2002).

Galls are defined as atypical growths that are produced in response to the influence of a foreign organism (Raman *et al.* 2005). Galls represent a complex series of interactions between the tissues of a plant and another living organism (Shorthouse and Rohfritsch 1992). The process by which insects gain control and redirect the growth

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pattern of a plant organ into a gall is believed to represent one of the most complex insect-plant relationships in the natural world (Shorthouse *et al*. 2005). Despite an increasing interest in cecidiology there are many questions regarding adelgids and gall induction on spruce shoots. It is still not clearly understood how gall-inducing adelgids gain control of growing shoots and stimulate them into galls that provide the gall-dwelling stage with food and shelter.

A. cooleyi is a cyclically parthenogenetic, gall forming adelgid that alternates between Douglas-fir (*Pseudotsuga menziesii*) and spruce (*Picea*). Native to Western North America, it was first described in 1907 by Gillette (Annand 1928; Cumming 1959; Havill and Foottit 2007). A complete holocycle takes two years to complete and includes five generations. Four of the generations are asexual and a single generation which occurs on spruce is sexual. This sexual generation is referred to as the sexuales. The sexuales mate in late August, and each female lays a single egg that hatches into the fundatrix by late October. The fundatrices then move to current-year branches of spruce as first instars, insert their mouthparts into the host, and settle permanently. In the spring fundatrices mature into apterous adults that secrete a white, waxy covering and begin to lay eggs in April. The fundatrix is the most prolific of the adelgid generations and she can typically continue to lay eggs for up to two weeks. The eggs hatch approximately five days after being laid, usually synchronous with bud burst, and the gallicolae nymphs move to the breaking buds. The gallicolae become enclosed in the gall that forms on the expanding shoots and by late summer they emerge, molt into alates and migrate to the secondary host, Douglas-fir. The gallicolae settle on the needles of Douglas-fir, lay eggs and die. The offspring of the gallicolae, referred to as the exules, produce progeny that

can either develop into an apterous generation that cycle on Douglas-fir or into the alate sexuparae (Annand 1928; Cumming 1959; Havill and Foottit 2007). Sexuparae migrate to the primary host, spruce; their progeny are the sexuales. A summary of the life cycle for *A. cooleyi* and other holocyclic adelgids is outlined in Figure 3-1.

Conflicting results have been described with respect to the role of the fundatrix and gallicolae in gall formation. Earlier work on adelgid galls were reviewed by Plumb (1959), Rohfritsch (1966), and Meyer and Maresquelle (1983). Plumb's review (1959) suggested that *Adelges abietis* fundatrices and gallicolae could not independently cause the formation of a gall. *A. abietis* is anholocyclic and native to Europe (Havill and Foottit 2007), but has been observed in western Canada. Ozaki (1994) found a similar result for *Adelges japonicus*, which is a Japanese anholocyclic species, though he suggests that the absence of gallicolae results in the formation of a partial gall with open chambers. It has been suggested that all anholocyclic species induce galls similarly. Plumb (1953) and Rohfritsch and Anthony (1992) found similar results with *A. abietis* except they suggest that the gallicolae play a key role in the development of the gall tissues during the gall formation process. Furthermore, Balch and Underwood (1950) suggested that galls induced by *Pineus pinifoliae* are a result of the fundatrices feeding and Speight (1982) suggests that the independent actions of the gallicolae form a gall.

Past works on the role of fundatrices and gallicolae have not included the holocyclic species *A. cooleyi*, and many of the studies have encountered difficulties in successfully isolating the two life stages during experimentation (Ozaki 1994). In an attempt to clarify the influence of the fundatrix and gallicolae we successfully isolated *A.*

cooleyi gallicolae from fundatrices, and tested the role of the two life stages in gall formation through manipulative laboratory experiments.

3.2 Methods

Experiments were carried out at the Kalamalka Research Station, located near Vernon, British Columbia (50°14'N, 119°16'W, elev. 480m) in 2007 and 2008. Branches used for this experiment were collected from a 15-year-old, interior spruce plantation that showed different levels of genetic susceptibility to adelgid-induced galling (Bains *et al*. see Chapter 2). Spruce trees that displayed a moderate to high susceptibility to galling were used for this study.

In May 2007, four one-year-old branches were collected from each of 20 trees and each branch was assigned one of four treatments: an un-manipulated control (fundatrix and gallicolae, Fig. 3-2), a fundatrix without gallicolae (fundatrix-only, Fig. 3-3), gallicolae without a fundatrix (gallicolae-only, Fig. 3-4), and a fundatrix whose eggs had been removed and replaced with the eggs of another fundatrix (switch). For the control, fundatrix-only, and switch treatments, branches with a single settled fundatrix were selected. For the gallicolae-only treatment, branches with no settled fundatrix were used. Each branch was kept in a nutrient-water solution in an individual rose spike (1.7 x 10 cm, 10 mL) mounted in a styrofoam block, all housed in a growth chamber at 21 ºC and 16:8 light:dark (Fig. 3-5). Branches did not touch each other, and rose spikes and the surrounding styrofoam were coated with petroleum jelly to prevent contamination by crawling gallicolae. Branches were observed daily under a stereomicroscope. Eggs found on any fundatrix-only replicate were removed and transferred to an assigned gallicolae-only replicate. Eggs found on a switch replicate were transferred to a different, assigned switch replicate, and the eggs from the second switch replicate transferred back to the assigned replicate. The controls were observed regularly but no egg transfers were

made. Gallicolae transfers and egg hatching were complete by June, and all branches (including any induced galls) were covered with a fine-mesh bag. As galls matured, emerging alates were collected; galls that did not produce alates were dissected to determine if gallicolae were present within the gall chambers.

Because of concerns of gallicolae contamination in the fundatrix-only treatment (see Results), this study was repeated in 2008. In 2008, the switch treatment was not included because it gave results that did not differ from the control treatment (see Results). Furthermore, the fundatrix-only treatment was kept in a separate growth chamber from the other treatments to avoid contamination by roving gallicolae. In all other ways the 2008 study was identical to the 2007 study.

3.3 Results and Discussion

In 2007, the un-manipulated control treatments had galling rates of 69%, and the switch treatment showed an 89% galling incidence. In the control treatment four branches were lost due to early branch death (n = 16) and two branches were lost for the switch treatment (n = 18). The combined galling rate of 27 galls per 34 adelgids likely represents the galling success rate in the field. The gallicolae-only treatments produced no galls (n = 20). Gallicolae were observed moving to the expanding buds but were not able to initiate galling, and did not survive. Fundatrix-only treatments showed complete development of 5 galls, and partial development on 4 branches (n = 20). Upon dissection of the complete galls, evidence of gallicolae were found, thus this treatment was considered to have been contaminated. Results from 2007 are summarized in Table 3-1.

In 2008, the control treatment displayed a galling success rate of 50% with 6% of the branches showing early galling symptoms and 44% of the surviving branches showing no signs of gall induction (n = 18). As was observed in 2007, none of the gallicolae-only replicates showed any symptoms of galling (n = 20), despite gallicolae observed invading the expanding buds. More than half of the fundatrix-only replicates showed gall initiation symptoms such as swelling of the most basal needle bases, with occasional pink colouration (n = 17). Results from 2008 are summarized in Table 3-2.

The switch treatment in 2007 suggested that handling and transferring of the eggs did not influence subsequent galling success. Galling was as successful by a fundatrix and her offspring as was a fundatrix with exogenous offspring. Thus, there is no evidence of kin recognition or specificity in gall initiation. It seems disadvantageous, on an individual basis, for a fundatrix to initiate a gall that can be occupied by the offspring

of other fundatrices. Each fundatrix initiates galling in a bud within a proximal distance (typically less than 5cm) of her location, thus it is likely that her own offspring will be most able to take advantage of her initiation efforts (Sopow *et al.* 2003). The gallicolae however are not limited to invading buds initiated by their mother, but can utilize the resource provided by any fundatrix. This is even more advantageous considering it increases the success of the group.

The partial development of galls in many of the fundatrix-only replicates shows that the fundatrix preconditions a bud, but is not able to induce a complete gall. Sopow *et al*. (2003) found that *A. cooleyi* galls are induced by a chemical stimulus rather than through mechanical stimulation from feeding. This study shows that the chemical induction by fundatrices is incomplete, and must by augmented with either chemical or mechanical stimulation by the gallicolae to form a complete gall.

In five cases, the fundatrix-only treatment of 2007 resulted in a complete gall. In each of these cases, subsequent dissection disclosed gallicolae, which invaded the incipient gall as contaminants. Galls from the switch and control treatments reared up to 195 gallicolae alates, whereas the contaminated galls in the 2007 fundatrix-only treatment had no emerging alates. The complete gall development, even in portions of the incipient gall that are well beyond the reach of the gallicola's mouthparts, suggests that the stimulation to form a complete gall is chemical rather than mechanical. On the other hand, female reproductive buds that are galled usually have only a subset of the total cone scales successfully galled (Strong 2002). Only the scales that are invaded by gallicolae develop successfully; other scales form incomplete gall structures. This localized activity suggests that mechanical stimulation may play a role as well.

It is possible that the fundatrix of *A. cooleyi* has a stimulatory mechanism on the future gall site that may differ from *A. abietis* and *A. japonicus* considering that *A. cooleyi* fundatrices settle proximal to the bud, in line with it along the branch, and *A. abietis* and *A. japonicus* settle directly at the base of the bud (Plumb 1953; Rohfritsch 1966; Ozaki 1994). *A. japonicus* fundatrices were found to induce galls that did not differ in the basic structure of a gall formed by fundatrices and gallicolae together (Ozaki 1994). In this study, the major difference observed was that the fundatrix-only treatment did not induce galls structures like the ones observed in the fundatrix-plus-gallicolae treatment. The fundatrix-only branches only showed swelling of the most basal needles of the expanding shoot. Only 30% of the test shoots from Ozaki's study were observed to induce galls without gallicolae, however the remaining branches were contaminated and gallicolae were observed within the galls. Plumb's cytological analysis of *A. abietis* and observations by Rohfritsch and Anthony (1992) suggest similar findings to our study with *A. cooleyi* and highlight that the greatest increase in the growth of the gall is when gallicolae colonize the expanding shoots.

Adelgid gall formation for *A. cooleyi* requires a fundatrix to induce a gall, and gallicolae to complete the formation of gall chambers and a mature gall. Thus, this holocyclic species differs markedly from the anholocyclic species previously reported on. The ability of *A. cooleyi* fundatrices to initiate galling in developing shoots suggests that pest control efforts targeting the gallicolae may be less successful at preventing seed loss than control efforts targeting the fundatrices. This theory requires further investigation in addition to controlling fundatrices.

3.4 Figures

Figure 3-1. The life cycle of a pentamorphic adelgid. Four of the five generations produce parthenogenetic females. A single sexual generation occurs on spruce. Spruce is the primary host and the secondary host differs among adelgid species.

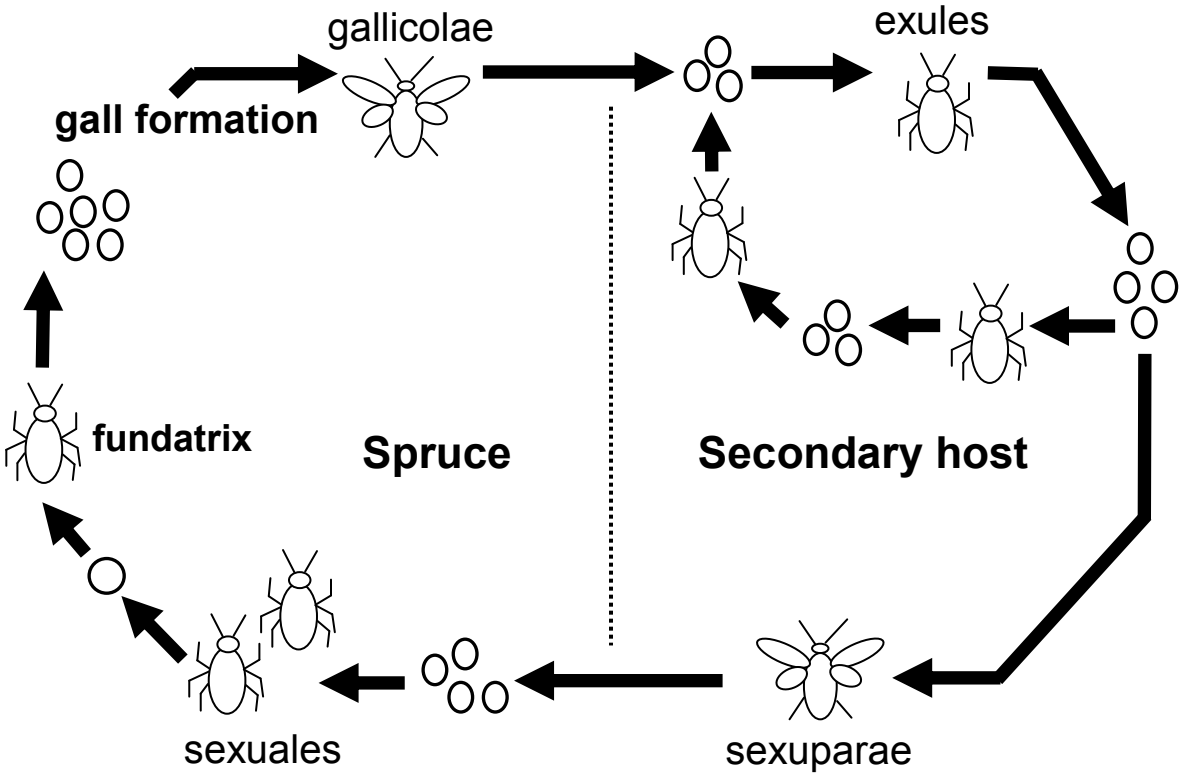


Figure 3-2. Fundatrices with eggs that hatch and develop into the gallicolae (control treatment).



Figure 3-3. Fundatrix with her eggs and white, waxy covering removed (fundatrix-only treatment).

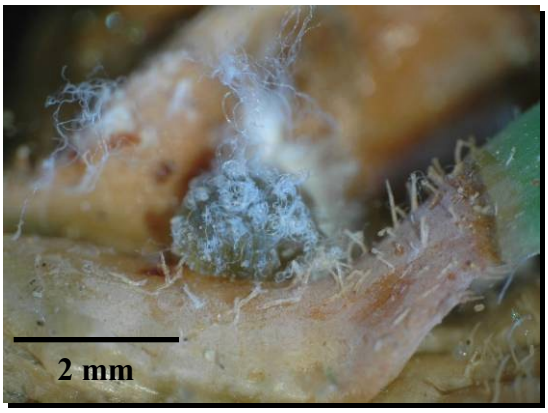


Figure 3-4. Eggs transferred to a branch with no fundatrix (gallicolae-only treatment).

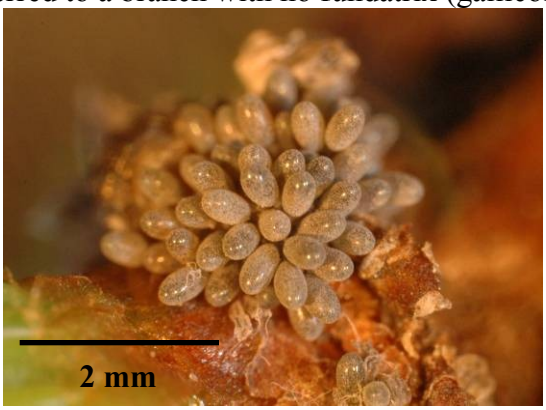


Figure 3-5. Branches kept in a nutrient-water solution in individual rose spikes, mounted in a styrofoam block, and all housed in a growth chamber.



3.5 Tables

Table 3-1. Percentage of branches displaying gall formation or swelling of tissues at the needle bases for control, fundatrix-only, gallicolae-only, and switch treatments in 2007.

	Treatment (N = 80)			
Galling Symptoms	**Control %**	**Fundatrix-Only %**	**Gallicolae-Only %**	**Switch %**
Swelling of tissues	6	20*	0	0
Galled	69	25*	0	89

*evidence of gallicolae were found, thus this treatment was considered to have been contaminated.

Table 3-2. Percentage of branches displaying gall formation or swelling of tissues at the needle bases for control, fundatrix-only and gallicolae-only treatments in 2008.

	Treatment (N = 60)		
Galling Symptoms	**Control %**	**Fundatrix-Only %**	**Gallicolae-Only %**
Swelling of tissues	6	65	0
Galled	50	0	0

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4 DEVELOPMENTAL MORPHOLOGY OF THE GALLS OF *ADELGES COOLEYI* (HEMIPTERA: ADELGIDAE) ON INTERIOR SPRUCE (*PICEA GLAUCA* (MOENCH) VOSS X *PICEA ENGELMANNII* PARRY) SHOOTS.[3]

4.1 Introduction

The life history strategy where select groups of insects control and redirect the growth patterns of plant organs into complex structures known as galls, represents one of the most complex insect-plant relationships in the natural world (Shorthouse *et al*. 2005). By unknown means, gall-inducing insects, gain control of the growth potential of attacked plant organs and stimulate them into developing new structures that provide endophagous insects with high quality food and a shelter. The ability to induce galls has evolved among insects of six orders resulting in the formation of a rich diversity of structures with a wide range of sizes and shapes (Raman *et al.* 2005). Jones *et al*. (1997) described galling organisms as 'physical ecosystem engineers' because they can control the availability of host resources and cause a physical change. More is known about events in the development of galls in some groups of gallers than in others (Meyer and Maresquelle 1983). Aphids and adelgids are two groups of small hemipterous galling insects; however, their galls have received little anatomical attention (Wool 2005), likely because of their complex life cycles and they are difficult to culture under controlled conditions.

[3] A version of this chapter will be submitted for publication. Bains, B., Shorthous, J.D., Strong, W., and McLean, J.A. Developmental morphology of the galls of *Adelges cooleyi* on interior spruce shoots.

Adelgids (Hemiptera: Aphidoidea: Adelgidae) form a small group of about 65 described species and represent some of the more destructive introduced pest species in North American forests (Havill and Foottit 2007). They are a family composed largely of species associated with pine or spruce, also known respectively as "pine aphids" or "spruce aphids". Adelgids include the former family Chermesidae, or "Chermidae" and like their closest relatives, aphids (Aphididae) and phylloxerans (Phylloxeridae), they exhibit cyclical parthenogenesis and complex, multigeneration, polymorphic life cycles that include an association with two species of host plants (Havill and Foottit 2007). Some species of adelgids are anholocyclic; that is they are asexual and do not alternate between conifer hosts (Annand 1928; Cumming 1959; Havill and Foottit 2007) whereas other species are holocyclic and have four asexual generations, one sexual generation, and alternate between a primary and secondary conifer host (Annand 1928; Cumming 1959; Havill and Foottit 2007). Adelgids induce galls on the vegetative and reproductive shoots of their primary hosts. Galls have been known for over 200 years and the earlier work on adelgid galls was reviewed by Plumb (1959), Rohfritsch (1966), and Meyer and Maresquelle (1983).

In many insect groups, galls can serve as diagnostic characters of the species of inducer since some species of gallers induce structurally distinct gall (Stone and Cook 1998; Raman *et al.* 2005). How gall inducers manage this is unknown; however, patterns observed in the growth and structures of galls provide useful information on the biology and evolutionary relationships of the inducers. Such studies require the use of plant histological techniques, knowledge of the insect's life history and the opportunity to harvest large numbers of galls at all stages of development.

The development and anatomy of the galls of three species of European adelgids, *A. abietis*, *A. laricis* (Vallot) and *A. strobilobius* (Kaltenbach) (not listed in Havill and Foottit 2007) have been described (Meyer 1969; Meyer and Maresquelle 1983; Rohfritsch 1966; and Rohfritsch and Anthony 1992). However, none of the galls of North American adelgid species have been examined anatomically. Annand (1928) listed *A. strobilobius* as being nearctic, but suggested that the taxonomy of the species was in doubt.

4.1.1 Objective

In the summer of 2008 a large numbers of *A. cooleyi* galls, at all stages of development were collected in southern British Columbia, and taken to the laboratory for histological examination. We hypothesized that the developmental pathways and anatomy of the galls of *A. cooleyi*, a Nearctic species, would be different from those found in Europe. The purpose of this paper is to report on our findings.

4.1.2 Natural history of *Adelges cooleyi*

The Adelgidae and Phylloxeridae are distinguished from the Aphididae by the absence of siphunculi and the retention of the ancestral trait of oviparity in all generations. Adelgids only lay eggs, and never give birth to live nymphs as do aphids. While phylloxerans and many aphids feed on angiosperms, adelgids feed only on certain genera in the Pinaceae.

A. cooleyi, commonly known as the Cooley spruce gall adelgid**,** is native to western North American and cycles between spruce (*Picea* spp.), the primary host, and Douglas-fir (*Pseudotsuga menziesii*) (Havill and Foottit 2007). *A. cooleyi* has a pentamorphic life cycle which includes four asexual stages and one sexual stage. The

single sexual stage occurs on spruce, is apterous and referred to as the sexuales. In the fall, the sexuales deposit a single egg near the base of a spruce bud which becomes the fundatrix (plural = fundatrices) generation. The apterous fundatrices settle on the stem where they remain for the winter. In the spring the fundatrices produce wax strands that cover their body and protect their eggs as they are laid. Progeny of the fundatrix are known as the gallicolae, and this summer generation colonizes the expanding shoots of spruce. Upon emerging from the gall the gallicolae molt into alates and migrate to Douglas-fir. The offspring of the gallicolae are the exules. Exules produce progeny that can either develop into an apterous generation that cycle on Douglas-fir or into the alate sexuparae that disperse to the primary host and lay eggs that develop into male and female sexuales (Annand 1928; Cumming 1959; Havill and Foottit 2007). The entire cycle takes two years to complete.

Like the galls of most insects, those of adelgids go through four phases of development which can be referred to as initiation, growth, maturation and dehiscence (Meyer and Maresquelle 1983; Rohfritsch and Anthony 1992). Initiation references the phase when the fundatrix preconditions the bud and the gallicolae begin to colonize the bud. During the growth phase the gallicolae continue to feed and become enclosed in the gall chambers, and the cells lining the nymphal chambers differentiate into several layers of specialized cells called nutritive cells (Bronner 1977). The maturation phase is marked by the end of gall growth and the proliferation of gall cells. Most nymphal feeding and growth occurs in the maturation phase. The dehiscence phase occurs only in galls such as those induced by cecidomyiids, aphids and adelgids whereby desiccation of gall tissues causes the gall chambers to open, allowing the gallers to escape. The initiation phase of

adelgids and aphids is different from most gallers in that feeding by the fundatrix initiates the galls and the remainder of gall formation is controlled by the gallicolae (Rohfritsch 1966, Bains *et al.* see Chapter 3).

Galls of all species of the adelgids studied to date are similar in that they are spherical or cylindrical swellings of current the years' shoot. They are composed of a number of chambers where the outer edges of the chambers are bordered by a suture line covered with hairs. All galls are initiated from tissues at the base of immature needles. Feeding by the fundatrix in the European adelgids causes the initiation of the galls near the shoot while the remainder of gall formation is caused by the gallicolae (Rohfritsch 1966; Meyer and Maresquelle 1983).

Mature, fully grown galls of *A. cooleyi*, prior to dehiscence are cylindrical swellings that vary in size, but most mature galls are about 2.5 x 4.0 cm. (Fig. 4-1). Galls cause the termination of shoot growth and normal needles arise from their surface. The surface is usually green or can have a pink coloring, and is typically smooth. Mature galls of *A. laricis*, in contrast, are elongated, pineapple-shaped galls which surround the entire shoot axis and all the needles are incorporated into the gall. Galls of *A. abietis* are elongated and form along one side of the stem axis at its base and contain extensive gall parenchyma which becomes lignified as the gall matures. The unaffected side and the upper part of the shoot, along with the needles, develop normally as the gall grows (Rohfritsch and Anthony 1992). No normal needles protrude from the gall plates, as is seen in *A. cooleyi* galls. Galls of *A. laricis* are whitish green, the epidermis is waxy and hairless, and there are a few white hairs along the distal margins of the flaps. Galls of *A. abietes* are the same dark green of normal needles, but the epidermis is covered with

hairs. Mature galls of *A. abietes* are hard due to a zone of sclerenchyma that develops at the periphery of the vascular elements and throughout the cortical tissues, whereas galls of *A. laricis* remain soft and fleshy and are usually smaller than galls of *A. abietis* (Rohfritsch and Anthony 1992). *A. abietis* is unique from *A. laricis* and *A. cooleyi* in that it does not require a secondary host to complete its life cycle (Havill and Foottit 2007).

Gallicolae pass through four instars in the gall chambers (Fig. 4-2 and 4-3), and as the galls desiccate the chambers open (Fig. 4-4). The gallicolae emerge and molt into adult alates (Fig. 4-5). The alates fly to Douglas-fir and settle on the current year's needles where they lay eggs and then die. The offspring of the gallicolae begin a series of generations consisting of wingless, parthenogenetic exules (Fig. 4-6). Offspring of the exules either develop into apterous generations that cycle on Douglas-fir or migrating sexuparae. Upon arrival on the spruce the sexuparae lay eggs that develop into the single sexual generation known as the sexuales. The sexuales mate in the fall and the female lays a single egg, from which the fundatrix arises. The fundatrix is the most prolific generation and her offspring are the gallicolae (Havill and Foottit 2007).

Previous studies on *A. cooleyi* have explored site selection (Fay *et al.* 1996), fecundity and fitness (Sopow and Quiring 1998; 2001; Ozaki 2000), and gall induction (Sopow *et al.* 2003; Ozaki 1994, 2000; Bains *et al.* see Chapter 3). The role of fundatrices and gallicolae of a related Asian species, *A. japonicus* was studied by Ozaki (1994) who suggested that the fundatrix can form the basic structure of a gall and the gallicolae induce the closing of the gall chambers.

4.2 Materials and methods

Galled and ungalled shoots were collected from a 13-year-old interior spruce orchard at the Kalamalka Research Orchard, located near Vernon, British Columbia (50°14'N, 119°16'E, elev. 480m). Shoots were collected from a variety of interior spruce families (trees from a family share the same mother and father) of known genetic makeup, that demonstrated moderate to high susceptibility to galling, to reduce the genetic variation in galling response among trees (Bains *et al*. see Chapter 2). Shoots and galls were collected between May, 2008 and August, 2008. As samples were collected they were observed under a microscope to determine if adelgids were present, and the bud development phase and adelgid life stage (if present) were recorded before tissues were dissected and fixed for histological processing.

The spruce tissues were cut into 1 cm^3 pieces and vacuum infiltrated with formalin - acetic acid – alcohol (90 parts 70% alcohol, five parts 37% formalin, and five parts acetic acid) at the Kalamalka Research Center (O'Brian and McCully 1981). All further processing of tissues was completed at Laurentian University. Tissues were dehydrated in a series of ethanol and tertiary butyl alcohol, embedded into paraffin, and sectioned into 9 μm specimen samples using a rotary microtome. Paraffin ribbons were fixed to microscope slides using Haupt's adhesive (Jenson 1962), and dried on a 47 ºC hotplate for three days. Slides were stained with safranin-fast green (O'Brian and McCully 1981). Lignified cell walls and nucleoli were stained red, and the non-lignified cell walls of phloem and parenchyma cells were stained blue. The dense nutritive cells stained a deep red. The prepared slides were photographed with a Leica digital camera. Winged adults from structurally similar galls were mounted onto slides and identified to

species. Voucher specimens are deposited at the Beaty Biodiversity Museum (University of British Columbia) and at the Canadian National Collections (Ottawa, Ontario).

4.3 Results

4.3.1 Ungalled shoots

Early in the spring the pre-formed bud from the previous year develops into a dormant bud. By late spring, as days lengthen and the temperatures rise, the bud begins to break and elongate. Shoot growth continues through the early summer. By July, shoot growth for interior spruce is complete and the formation of the next year's bud is underway. Except where otherwise noted, the following detailed descriptions of bud development and tissue structure are a compilation of the works of Plumb (1953) and Esau (1977).

Between the bud and the twig lies the collenchyma plate. This is an area composed of thick-walled cells and it is often referred to as a supporting tissue. In the late growing season, or early dormancy, the medullary cells below the collenchyma plate begin to break-down and leave an open cavity that can be observed as the node in old branches. Above the collenchyma plate are flattened parenchyma cells that pass distally into a cone of large thin-walled medullary cells that are arranged in columns and filled with tannins. The vascular elements are formed from the procambial strands that run laterally to the medullary cone. The needle rudiments then arise from a tissue that is external to the procambial strands (Fig. 4-7). These tissues form the cortex and cortical derivatives, and the needle rudiments will contain provascular elements that are continuous with the procambial strands. Patterns formed by the vascular strands in the stem reflect the close relationship between the stem and the needles. Vascular bundles diverge from the vascular cylinder towards the needles and can vary in length (Fig. 4-7). During normal shoot elongation the provascular elements continue to differentiate and the

distance between the needles expands (Figs. 4-7, 4-8). Dormant buds contain the precursors of all the rudiments found in a mature shoot. From growth initiation to flushing, the growth of the expanding shoot is characterized as linear growth with minimal increases in diameter (Hanover 1980).

In the stem, secondary growth begins early and leads to the internal development of substantial amounts of secondary xylem (Fig. 4-9). The secondary xylem is produced toward the inside of the vascular cambium whereas the phloem is produced towards the outside (Fig. 4-9) (Raven *et al.* 1999). The needles on the shoot are covered by a cuticle and below the cuticle is the epidermis (Fig. 4-10). Beneath the epidermis there are one or more layers of compactly arranged cells, referred to as the hypodermis (Fig. 4-11). The mesophyll is made up of parenchyma cells with conspicuous wall ridges that project into the cells and can be penetrated by two or more resin ducts (Fig. 4-9) (Raven *et al.* 1999). The vascular bundles, most evident in Figures 4-7 and 4-8, are made up of xylem and phloem, and are surrounded by transfusion tissue which is composed of living parenchyma cells and short, non-living tracheids. The transfusion tissue conducts materials between the mesophyll and the vascular bundles. The transfusion tissue is separated from the mesophyll by an endodermis, which is a single layer of cells (Raven *et al.* 1999).

4.3.2 Gall formation

Fundatrices located near the bud base resume feeding in the spring after overwintering as early-instar nymphs, and no modifications to the buds were observed. Upon molting, the fundatrix begins to lay a large clutch of eggs and at this time the first symptoms of galling are evident and no stylet sheaths were observed. Examination of

closed buds suggests that there is a proliferation of the cortical parenchyma cells that arises from the vascular bundle, prior to any influence by the gallicolae (Fig. 4-11). The fundatrix continues to lay eggs for several days, and eggs usually hatch within a week of being laid. As the undifferentiated parenchyma is proliferating in the sterigmata region, the newly hatched gallicolae move to the needle bases of the expanding shoots and begin to feed (Fig. 4-12). In addition to cell proliferation the cells appear enlarged (Fig. 4-12). As the shoot elongates linearly, and the gallicolae continue to feed, the gall parenchyma continues to proliferate at the sterigmata and needle rudiments (Fig. 4-13). The gall parenchyma is concentrated at the sterigmata and needle rudiments, and appears to hinder the formation of an abscission layer in the needles. As the gallicolae continue to feed, and the gall parenchyma continues to proliferate, the gallicolae become enclosed in a chamber within days of colonizing a shoot. Shortly after, the first sign of a cytoplasmically dense tissue appears below the epidermis (Fig. 4-14). The cytoplasmically dense tissues are used as nutrition source by the gallicolae, and hence can be referred to as a nutritive tissue. The proliferation of tissues at the needle rudiments forms an enclosure around the gallicolae, referred to as the gall chamber (Figs. 4-15, 4-16, 4-17, 4-18). The lining of the gall chamber is continuous with the epidermis of the needle rudiments. Unlike ungalled shoots, the growth of a gall is characterized by an increase in the diameter, and often changes in tissue color and stunting of linear growth. *A. cooleyi* galls were observed to stunt shoot growth, occasionally induce a pink colouring of the tissues, but do not hinder the growth of the needles. It was also observed that there is no active abscission layer present in the needles of the galled shoots. Gall formation can be completed within two to three weeks and the basic gall structures are

multi-chambered. Additionally, the gall tissues did not appear to be lignified and there were no visible signs of vascular bundles extending towards the gall chambers.

The gall chambers do not fuse closed, although they appear to be, and an ostiolar opening remains (Figs. 4-18, 4-19). In Figure 4-17 the early signs of narrowing of the ostiolar opening are visible and the development of trichome-like projections also become more evident. As the gall matures the ostiolar opening becomes smaller, and the trichome-like cells become more dense along the opening (Figs. 4-19, 4-20). Gall cells are their maximum thickness at the time of maturation. The enclosed gallicolae feed on the nutritive tissues lining the chamber, causing the lower nutritive tissues to appear as a flattened layer, and the top layer appears chewed (Fig. 4-21). The nutritive tissue layer is only a few layers thick and the depth of feeding does not exceed the length of the gallicola body. By mid–July the tissues begin to dry out and the ostiolar openings increase in size and the gallicolae emerge. Simultaneously, the walls between the gall chambers collapse as a result of the degenerating cells (Figs. 4-22, 4-23, 4-24). The comparison between gall tissues and dehiscing tissues is highlighted in Figures 4-17 and 4-24.

4.4 Discussion

Church (1920) noted that there are developmental and morphological similarities between galls formed by the adelgid species *A. virdis* and primitive seed cones such as cedar (*Thuja* spp.), and suggested that gall formation involves the same chemical factors required for the development of cones. Considering galls are produced in response to a stimulus provided by the activity of adelgids, galls can be regarded as new plant organs (Shorthouse *et al*. 2005). Typically each adelgid species induces a gall that is structurally unique and in some instances more than one species of adelgid can induce galls on the same tree. The differences in the gall structure and appearance can be attributed to differences in stylet-probing behavior, settling site, and phenology (Rohfritsch and Anthony 1992). The induction of galls is one of the more complex insect – host associations (Shorthouse *et al.* 2005).

This is one of the first studies to describe the developmental morphology of a gall induced by *A. cooleyi*. Additionally this study highlights the unique relationship adelgids have developed with their primary host. Past work by Plumb (1953), and Rohfritsch and Anthony (1992) have reviewed the developmental morphology of galls induced by *A. abietis* and *A. laricis,* and Meyer and Maresquelle (1983) examined the galls of *A. abietis* and *A. strobilobius* in detail. Comparisons among the different adelgid galls will highlight the common attributes the galling processes share and any differences that may be species specific. Considering galls supply the gallicolae with shelter and nutrition, at the metabolic expense of the host, it is of interest to better understand the plant – galler interactions.

The fundatrices of *A. cooleyi* settle on spruce branch tips, below newly formed buds. A key event in the life history of gall forming insects is their choice of a site for gall induction (Fay *et al.* 1996) and this choice influences the survival of their offspring. Observations of the shoot tissues, prior to gallicolae emergence, confirm that the fundatrix mother preconditions shoots for galling, considering there is proliferation and enlarging of cortical parenchyma in the sterigmata region (Fig. 4-11). This supports similar interpretations by Plumb (1953), Ozaki (1994), Rohfritsch and Anthony (1992) for various adelgid species, and by Bains *et al*. (Chapter 3) for *A. cooleyi* in a manipulative laboratory study. It has also been suggested that the influence of the fundatrix goes beyond early galling symptoms and includes the formation of gall chambers that are colonized by gallicolae (Rohfritsch and Anthony 1992; Ozaki 1994). Our observations contradict those findings and suggest that the gallicolae are present and active as the gall begins to take its shape, and that gallicolae are required to complete the formation of gall chambers. *A. laricis* and *A. abietis* fundatrices are thought to induce changes to the bud as early as the fall, just after the fundatrix settles and inserts her stylet. Along the pathway of *A. laricis* stylets, the first nutritive tissues are visible and a rich starch layer appears (Rohfritsch and Anthony 1992). In contrast, *A. abietis* settles in October and within a month the fundatrix causes the cortical cells to become modified. The buds manipulated by *A. laricis* were observed to be more modified by the spring than buds attacked by *A. abietis* (Rohfritsch and Anthony 1992). Buds with settled fundatrices for *A. cooleyi* showed no signs of modification in the fall or early in the spring.

Unlike ungalled shoots, the growth of a gall is characterized by an increase in the diameter, and changes in tissue color and stunting of linear growth. It has been observed

that aphids induce characteristic, species-specific galls on their primary hosts (Wool 2005). In typical *A. cooleyi* galls the length of the needles are not stunted. Needle growth is stunted in other adelgid induced galls such as *A. laricis* and *A. abietis*. It is also worthy to note that the absence of an active abscission layer in the needles of the galled shoots may attribute to the presence of needles on dehisced galls. In dehydrated spruces, the needles of ungalled shoots tend to fall off at the point of the abscission layer. Abscission appears to be dependent upon the levels of auxin present on either side of the abscission layer. Auxin is typically in high concentrations in young and rapidly growing organs, and is in low concentrations during senescence. Leading up to senescence the cell walls in the abscission layer weaken and eventually separate, causing the needles to drop on deyhdrated shoots (Hopkins 1999). With needle retention on galls, photosynthate and nutrients are supplied to a gall and benefits the gall inhabitant. Furthermore, the development of the trichome-like structures along the ostiolar opening provides protection to the gallicolae by minimizing exposure to biotic and abiotic stresses.

Tissues of the galls are known to be physiological sinks for nutrients and minerals that are diverted from other parts of the host (Larson and Whitham 1991). It has been proposed that adelgids insert their stylets intra-cellularly and feed on solutes from the phloem of spruce and on the cortical parenchyma or ray parenchyma cells of a shoot (Raman *et al*. 2005; Rohfritsch and Anthony 1992). For *A. abietis* and *A. laricis* the gallicolae are thought to insert their stylets intercellularly and it was noted that the stylets terminate inside the cortical parenchyma cells so they can feed on the plant's stored nutrients (Plumb 1953; Rohfritsch and Anthony 1992). Rohfritsch (1977) also suggested

that adelgids inject a viscous fluid into the plant through their mouthparts that builds a sheath to surround their stylets. The path of the stylets was reported to always be intercellular and the sheaths remained when the stylets were removed. *A. tsugae*, a native of Japan, inserts its stylets intra-cellularly through the epidermal cells and the stylets move either within or among cells until they reach the ray parenchyma cells (Young *et al.* 1995). Our observations of *A. cooleyi* did not show any stylet sheaths in the developing gall beneath the fundatrix or in the proliferating cells lining the developing chambers. Other adelgids are also thought to feed on cortical parenchyma cells and on solutes from the phloem of spruce (Young *et al*. 1995). As mentioned by Wool (2005), adelgid feeding appears to be different from aphids; aphid gallers are phloem feeders and require access to the phloem system of their host plant, or induce the formation of new vascular elements at the site of galling. Aphid stylet sheaths have been observed penetrating cells down to the phloem (Tjallingii and Esch 1993). Our observations of *A. cooleyi* suggest that the enclosed gallicolae feed on the nutritive tissues that line the gall chambers. The mechanism that induces the formation of such tissues is not yet understood, however it is evident that the nutritive tissues are unique to galls. Considering the length of the gallicola stylet, it is not possible for their mouthparts to reach the parenchyma cells; hence feeding is restricted to the nutritive tissue. As the gallicolae mature within the gall chambers it is evident that the integrity of the nutritive tissues changes and they appear to be sucked dry of their contents. Rohfritsch and Anthony (1992) found that the gall chambers of *A. abietis* and *A. laricis* are lined with meristematic epidermal cells that are rich in cytoplasm, starch, and phenols. Our staining procedures could not confirm the presence of starch; however the gall parenchyma cells are definitely hypertrophied with

some cells showing the presence of high levels of phenols. Additionally the nutritive tissues appear to be cytoplasmically dense with enlarged nuclei. Considering only the top few layers of the gall chamber can be used for food, it is likely that with an increase in the number of gallicolae per chamber the competition for food also increases. The number of gallicolae initially colonizing a bud appears to be higher than the number of gallicolae emerging from a gall. Sopow and Quiring (2001) found that the number of gallicolae emerging from a gall was positively related to gall size for *A. cooleyi, Pineus pinifoliae, and P. similis*; hence gall size can be used to estimate the fitness of the fundatrix. It was also noted that gallicolae size decreased with an increase in gallicolae density within galls, suggesting that exploitation competition for food is occurring. It is worthy to note that predation within galls is rare but syrphids have been observed preying within tightly closed galls (Mitchell and Maksymov 1977). Predation within adelgid galls may not be common considering the ostiolar openings are lined with trichomes that possibly keep predators from entering the gall chambers. Additionally, the trichomes are beneficial in that they hold moisture within the gall, yet allow for air exchange to occur.

The synchronization of gallicolae maturity and dehiscence is remarkable and essential for the survival of the gallicolae. Galls provide gallicolae with nourishment, shelter, and protection up to the alate stage (Shorthouse *et al*. 2005). We observed many galls that failed to dehisce and upon dissection we found dead gallicolae within the gall chambers. What triggers dehiscence has not been verified and it is possible that a stimulus is secreted through gallicolae feeding activity. It is well known that many homopteran parenchyma feeders have enzymes in their saliva that assist them in penetrating through the cells of a bud (Shorthouse and Rohfritsch 1992), and it is quite

possible that a stimulus is also released through the saliva to induce dehiscence. For gall induction, Sopow *et al.* (2003) suggested that a chemical stimulus was released through the stylets of fundatrices into the host phloem to induce gall formation. They were able to verify that the mechanical stimulation of the fundatrices mouthparts had no influence on the host tissues. In the case of the gallicolae it is not understood if a chemical stimulus is secreted or mechanical stimulation of their mouthparts induces the hyperplasia and enlargement of the parenchyma. Determining what triggers the formation of gall tissue, and desiccation would further enhance our understanding of the relationships between adelgids and their host.

We have shown that the fundatrix preconditions the bud for successful colonization by the gallicolae nymphs. Further, feeding by the gallicolae is required to complete the gall formation process. The biochemical processes that bring about the phases of initiation, growth, maturation and dehiscence have yet to be demonstrated.

4.5 Figures

Figure 4-1. *Adelges cooleyi* induced gall on the shoot of a spruce tree.



Figure 4-2. Gallicola nymph.



Figure 4-3. Longitudinal dissection of a gall with visible gallicoale nymphs within gall chambers.



Figure 4-4. Dehiscing *Adelges cooleyi* gall.



Figure 4-5. Gallicola alate on a Douglas-fir cone.



Figure 4-6. Exule covered with white, wax secretions and nymphs on a Douglas-fir needle.



Figure 4-7. Longitudinal section of a spruce bud at the time of bud break.



Figure 4-8. Longitudinal section of needles at the point of attachment on a spruce bud.

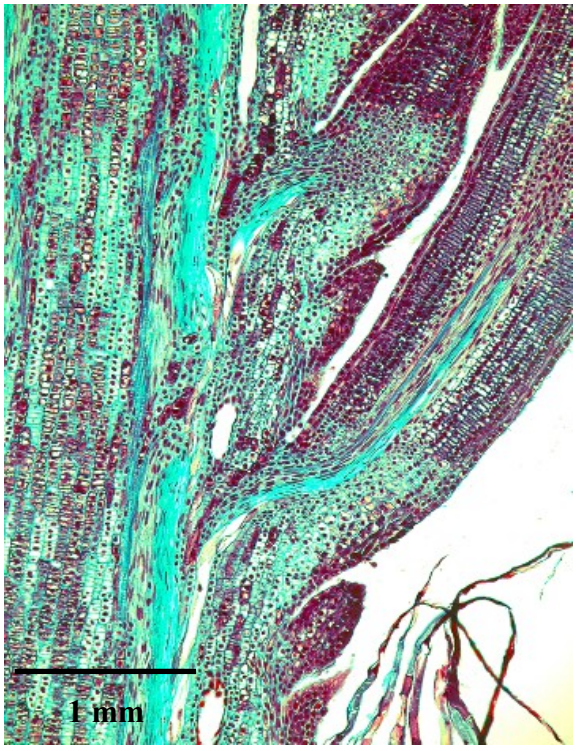


Figure 4-9. Cross-section near the base of a breaking spruce bud.

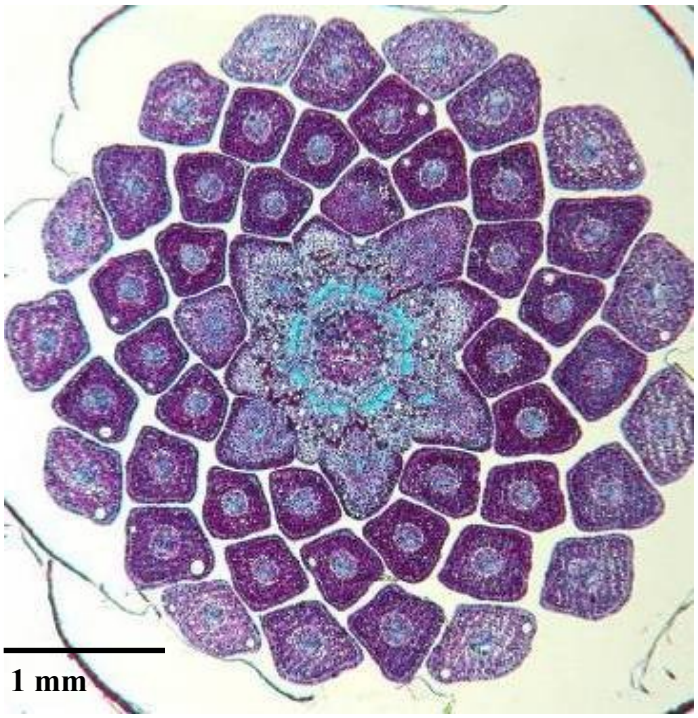


Figure 4-10. Longitudinal section of a spruce needle.

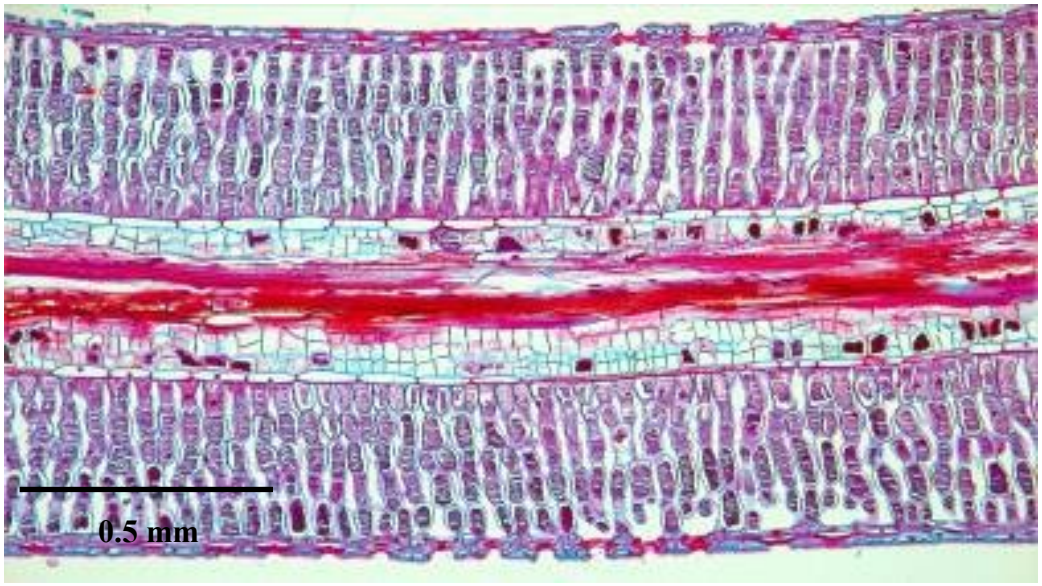


Figure 4-11. Longitudinal section of a spruce bud with early galling symptoms. Undifferentiated, enlarged parenchyma cells proliferating in the sterigmata region.

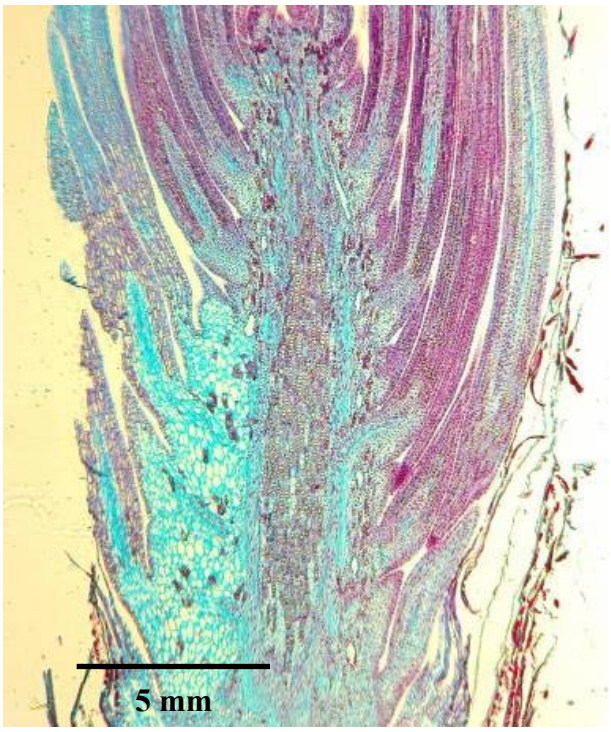


Figure 4-12. Longitudinal section showing two gallicolae nymphs feeding at the base of a needle.



Figure 4-13. Longitudinal section of gall parenchyma proliferating at the needle rudiments.

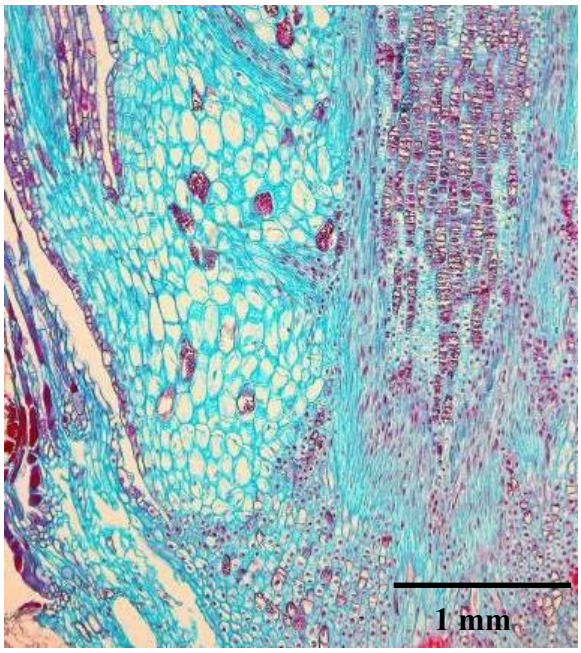


Figure 4-14. Longitudinal section of a gallicolae feeding on the cytoplasmically dense tissue that appears below the epidermis, in the needle rudiment region. The gallicolae use these tissues as their sole nutrition source during development within the chamber.

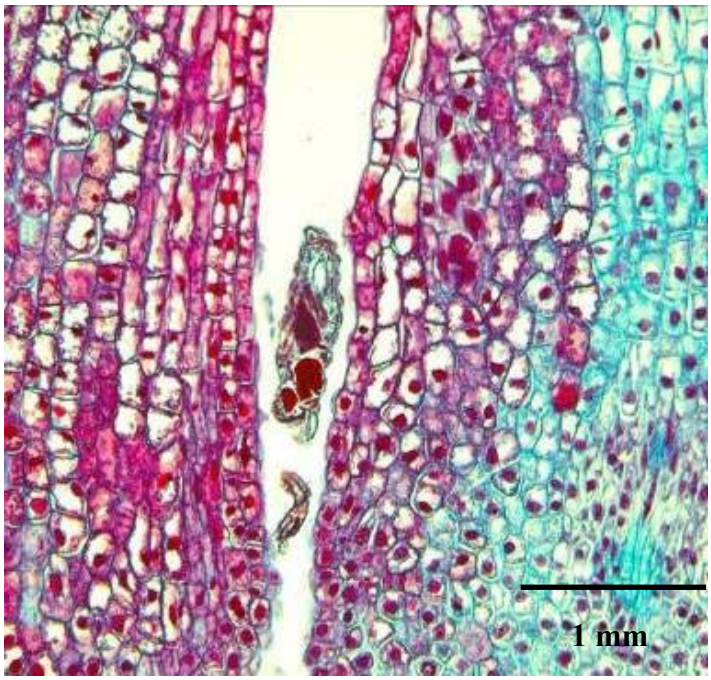


Figure 4-15. Longitudinal dissection of a gall with visible gall chambers.



Figure 4-16. Cross-dissection of a gall with visible gall chambers.



Figure 4-17. Cross-section of a gall with gallicolae nymphs visible within the gall chambers.



Figure 4-18. Longitudinal section of a gall chamber with a visible gallicola nymph and visible trichome-like projections developing at the ostiolar opening.

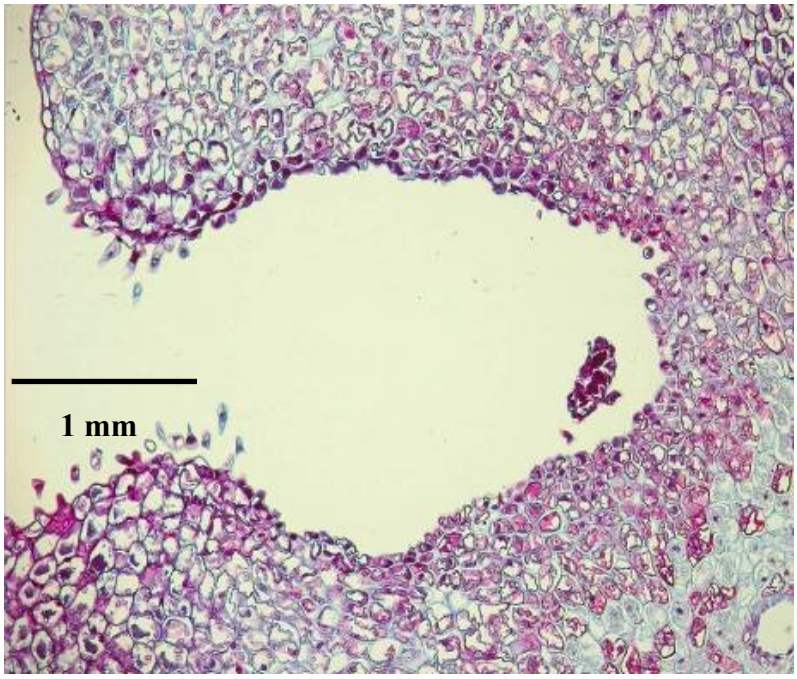


Figure 4-19. Longitudinal section of a gall chamber with a narrowed ostiolar opening lined with trichome-like projections and maturing gallicola.

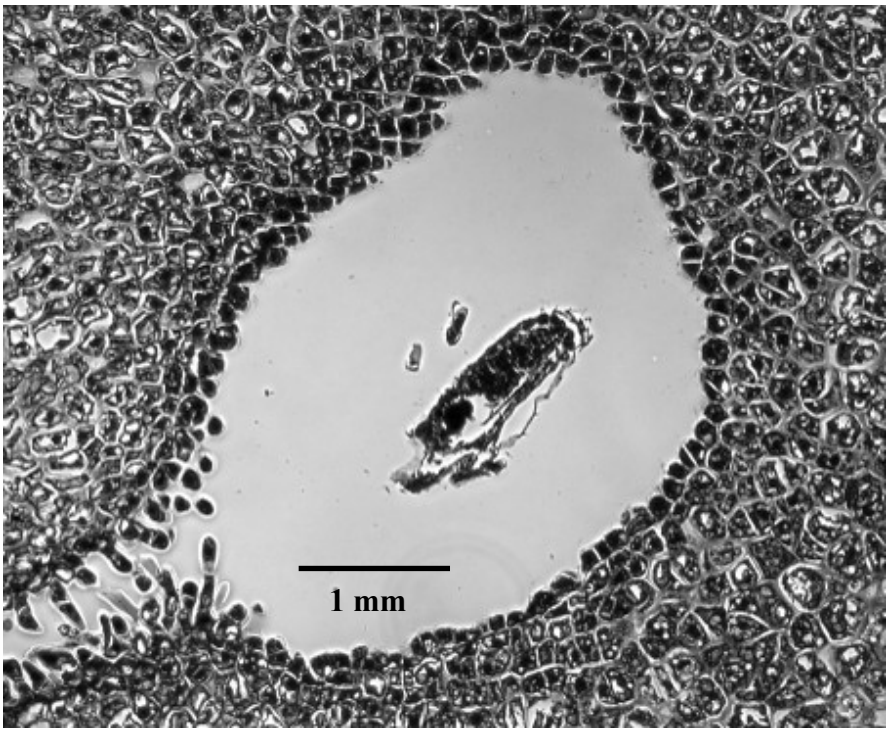


Figure 4-20. Longitudinal section highlighting the narrowed ostiolar opening of the gall chambers.

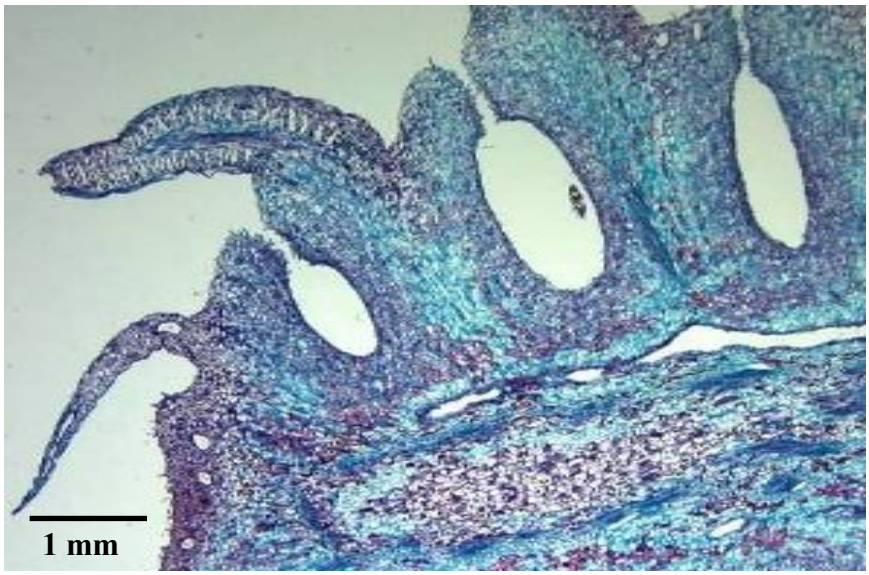


Figure 4-21. Longitudinal section of tissues lining a gall chamber. The top layer of the nutritive tissues have a chewed-like appearance whereas the lower layers appear flattened.

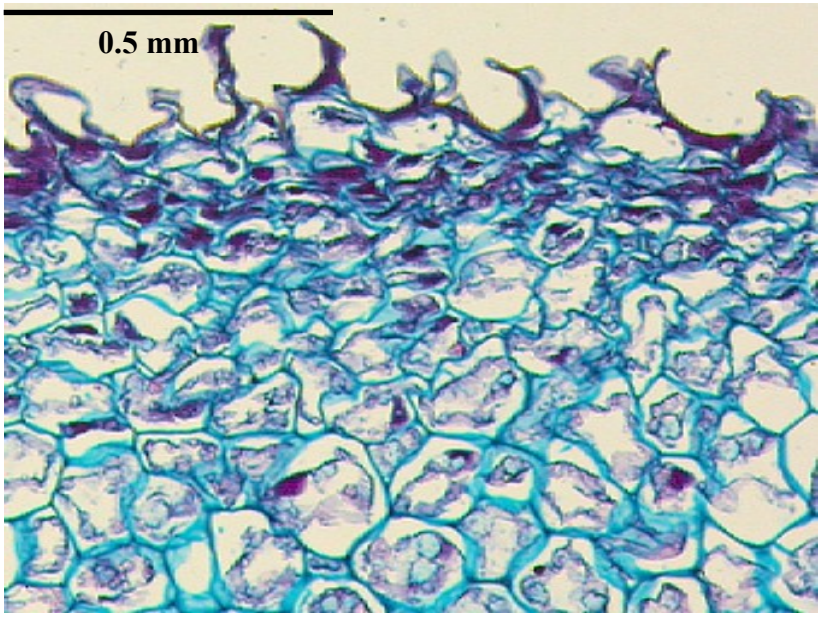


Figure 4-22. Cross-section of a gall as it begins to dehisce. The tissues dry-out and the chamber walls begin to collapse.

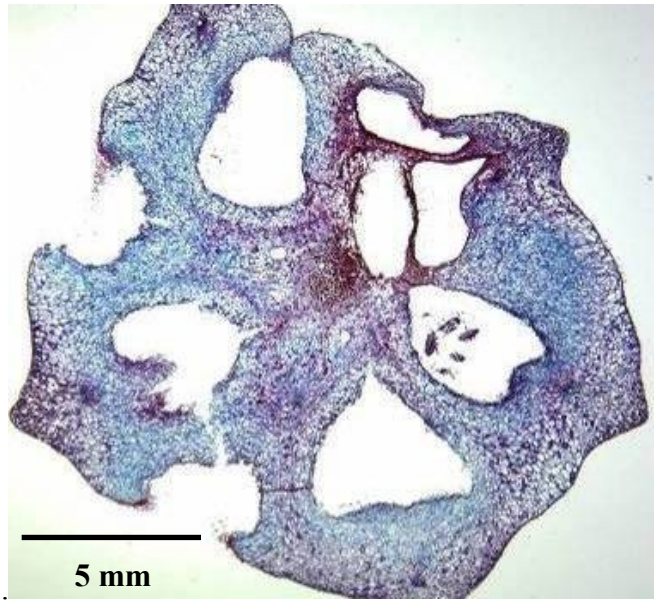


Figure 4-23. Longitudinal section of a gall chamber as the tissues begin to desiccate.

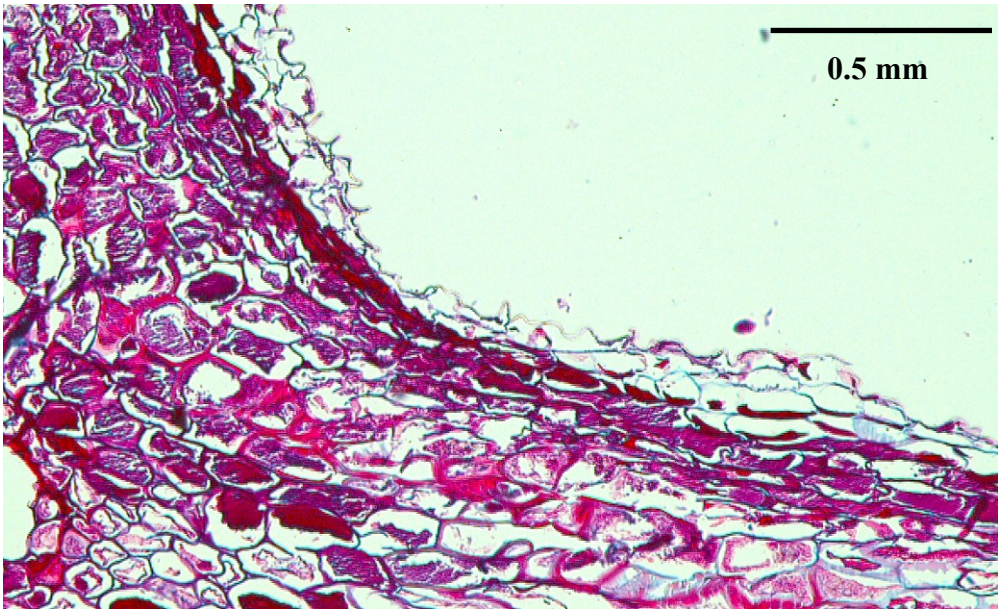
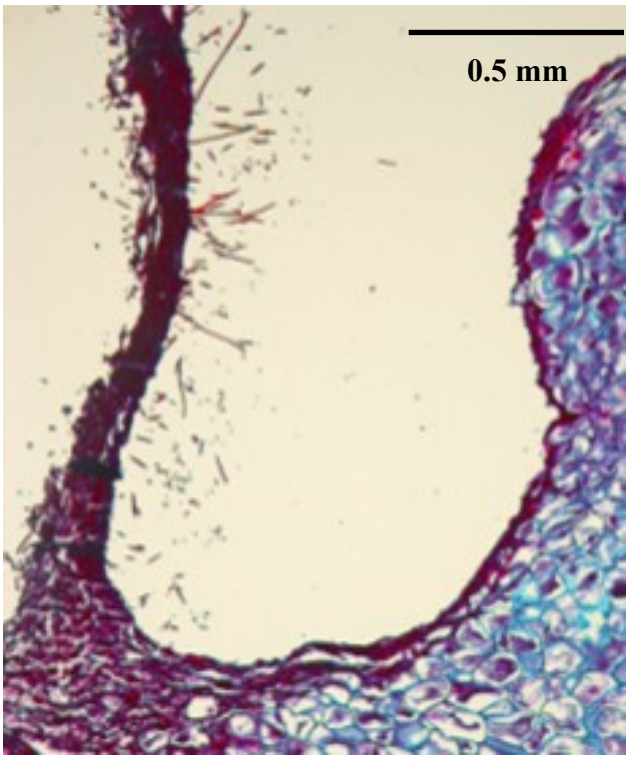


Figure 4-24. Cross-section of the desiccated cells of a gall chamber wall and the expanding ostiolar opening.



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5 ARE ADELGID INDUCED GALLS SPECIES-SPECIFIC?[4]

5.1 Introduction

Galls have been studied for over 200 years and have interested humans because of their use in medicine, industry, and occasionally as food (Raman *et al*. 2005). Some of the earliest notes on galls are thought to be those of Pliny the Elder. Pliny used the term 'galla' to refer to abnormal growths on oaks in the first Century and the systematic study of galls is thought to have started by Malpighi in the 17th Century (Raman *et al.* 2005). More recent research has used galls as experimental systems to understand insect-host interactions and these interactions are viewed as some of the more complex associations between an insect and its host (Shorthouse *et al.* 2005). Galling insects provide a model system for understanding the ecological and evolutionary mechanisms of herbivory (Raman *et al*. 2005). Gall inducing insects are highly specific to their host plants and most galling species form structurally unique galls (Plumb 1953; Meyer and Maresquelle 1983; Rohfritsch 1990; Shorthouse and Rohfritsch 1992; Price 1991; Shorthouse *et al.* 2005). It has been suggested that the differences in gall structure and appearance can be attributed to variations in stylet-probing behavior, settling site, and phenology (Rohfritsch and Anthony 1992). Among aphids, it is thought that many species induce characteristic galls that can be easily identified without taxonomic expertise (Wool 2005).

Adelgid induced galls have been reviewed for very few species and detailed descriptions of species-specific galls are lacking. In an attempt to characterize adelgid galls with adelgid species, we selected a number of galls from the Skimikin Seed Orchard

[4] A version of this chapter will be submitted for publication. Bains, B. Are adelgid (Hemiptera: Adelgidae) induced galls species-specific?.

and Kalamalka Research Station, in southern British Columbia (B.C.) and collected the emerging alates for identification.

5.2 Methods

Galls for this study were collected from the Kalamalka Research Station, located near Vernon, B.C. (50^0 14'N, 119^0 16'W, elev. 480m) in 2007, and Skimikin Seed Orchard, located near Salmon Arm, B.C. (50^0 40'N, 119^0 15'W, elev. 550m) in 2008. Galls of various structures were flagged, labeled, photographed (by Dion Manastyrski), and securely covered with a fine mesh lining to collect the emerging alates upon gall desiccation. The emerged alates were transferred from the mesh bags into 95% ethanol. Alates were slide mounted (Figs. 5-1,5-2) using the procedures outlined in Appendix A and identified to species using keys developed from Annand's (1928) morphological descriptions (Appendix B). Morphological descriptions of slides of the mounted alates were confirmed by Eric Maw (Ottawa, Ontario). Subsets of each sample of alates were sent to Kimberly Wallin (University of Vermont) and Nathan Havill (Yale University) for DNA barcoding, to confirm and compare species identification.

5.3 Results and Discussion

Six different species were found among the two adelgid genera (*Adelges cooleyi, Adelges lariciatus, Pineus pinifoliae, Pineus boycei, Pineus similis,* and *Pineus floccus*). Galls induced by *Adelges* spp. tend to be smaller and tighter than typical *Pineus* spp. galls that are loose in structure and significantly larger. The gall chambers and ostiolar openings of *Pineus* galls tend to be larger. Based on the galls from this study, it is evident that a wide range of variation exists in the structures induced within each genus.

Galls induced by *A. cooleyi* are represented in Figures 5-4a – h. These structures are commonly associated with *A. cooleyi* and share distinctive characteristics, with the exception of Figure 5-4h. The most obvious characteristics are the retention of full needle growth and the termination of shoot growth. Coloring of the galls can range from a green to dark pink, or have a mixture.

The galls induced by *A. lariciatus* are shown in Figures 5-5a – d. These galls have a pineapple-like structure with distinctive rhomboidal plates, reduced needle growth and usually terminate shoot growth. The galls are typically a yellowish color with some variation of green or occasionally a brownish color. Figure 5-5d is not characteristic of *A. lariciatus* and tends to resemble a *Pineus*-like gall. The identification of that alate group was difficult and it will be of interest to review the DNA barcode to confirm the species identity.

A gall that was found to be induced by *P. pinifoliae* is presented in Figure 5-6 and has the large and loose structure characteristic of *Pineus*. The gall is very round and distinct from the other *Pineus* galls described below. A confident characterization of *P. pinifoliae* galls however, would require investigating more galls similar to this description.

Figures 5-7a – j were induced by *P. boycei*. There is wide variation among the gall structures, making a concise characterization difficult. Figures 5-7a – d appear to be most similar and associating those structures with *P. boycei* is most reliable. Figure 5-7e appears to be a hybrid-type gall that was not commonly observed. The bottom portion of the hybrid-gall is similar to the structures in Figures 5-7a –d. Further, the structure of Figure 5-7f is not similar to any *P. boycei* galls in this study and resembles a *P. floccus*

gall listed below. Figures 5-7g and 5-7h resemble each other and so do the pair in Figure 5-7i and 5-7j. Figures 5-7i and 5-7j resemble partial galls. There were many uncertainties with the morphological characteristics of the *P. boycei* alates, with many of their antennae and sensoria ranging from *P. similis* -and *P. floccus* – like structures. Many specimens also showed variations between the left and right sided structures such as abdominal and prothoracic gland patterns, and the circumferential extent of the sensoria. DNA barcode data will confirm these identifications and explain the morphological variations observed among the alate specimens.

The gall represented in Figure 5-8 is questionable and characterization would require DNA barcoding considering the collected alates were difficult to distinguish between *P. boycei* and *P. similis*. Figure 5-9 is a *P. similis* induced gall and this structure is quite different from the gall structure presented in Figure 5-8. Furthermore, the gall structure in Figure 5-8 resembles that of the gall described as *P. floccus* in Figure 5-10. Another gall had alates identified as *P. floccus* (Fig. 5-11), however this gall is similar to the gall in Figure 5-7f, which was identified as a *P. boycei* induced gall.

The gallicolae alates that emerged from the galls investigated were identified based on their morphological characteristics and an analysis of the DNA will clarify the uncertainties of using the current key (Appendix B). A genetic analysis may also show that morphologically distinct species are possibly morphs of a single species. This may also clarify the problems associated with the species that showed inconsistent morphologically characteristics. For example, the abdominal wax gland pattern observed on some western Canadian *P. similis* specimens were very similar to patterns observed for *P. boycei,* however they are also similar to *P. similis* specimens from New Brunswick.

Furthermore, the wax gland patterns on the heads of the Western and Eastern P. similis specimens were different. There were fewer glands in the posterior field, and the middle of the head was not as rugose for the western species. The *P. boycei* alates were also similar to *P. similis* and *P. floccus* with many of their antennae and sensoria ranging from *floccus*-like to *similis*-like, with respect to the circumferential extent, but many specimens also showed differences in their left and right sides – greatly complicating the ability to gather a confident identification of many of the specimens. A comparison of the barcode data will confirm our identifications or may possibly show a divergence; hence it is required to present these results with full confidence.

Preliminary results from this study suggests that variations in gall structures does exist, and some of the adelgids from this work do appear to induce somewhat specific gall shapes, however the *Pineus* group appeared to show great variation, with possible overlap in structures. *A. cooleyi* and *A. lariciatus* do show variation, however some of the galls are still quite distinct and unique. It is possible that individual host responses may account for some of the variation observed among the galls.

Our associations based on morphology did not show the same degree of specificity that is observed in other galling insects. Implications for application include difficulty in deciphering the adelgid species present in a seed orchard and confirmation of species identity would require taxonomic expertise, or the use of DNA barcoding if the morphological keys prove to be inaccurate.

5.4 Figures

Figure 5-1. Flagging adelgid galls on spruce trees at the Skimikin Seed Orchard.



Figure 5-2. Securing galls in fine-mesh bags for gallicolae alate collection.



Figure 5-3a. Preparing gallicolae alates for slide mounts.



Figure 5-3b. Preparing gallicolae alates for slide mounts.



Figure 5-4a-h. Galls induced by *Adelges cooleyi* on spruce.



a



b



c



d



d



f





g h

Figure 5-5a-d. Galls induced by *Adelges lariciatus* on spruce.





a b





c d

Figure 5-6. Gall induced by *Pineus pinifoliae* on spruce.



Figure 5-7a-j. Galls induced by *Pineus boycei* on spruce.



a



b



c



d



e



f



g



h



i



j

Figure 5-8. Gall induced by *Pineus* spp. Identified alates were a morph between *P. boycei* and *P. similis*.



Figure 5-9. Gall induced by *Pineus similis*.



Figure 5-10. Gall induced by *Pineus floccus.*



Figure 5-11. Gall induced by *Pineus floccus.* Identified alates were very similar to *P. boycei.*



5.5 References

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6 CONCLUDING DISCUSSION

Adelgids are fascinating insects that display one of the natural world's more complex life cycles and relationships with their hosts. The objective of my thesis was to increase our understanding of adelgids in an attempt to enhance their current management in BC Seed Orchards. Additionally, I was extremely interested in gaining better insight into the complex association adelgids share with their hosts.

Much of the research involving adelgids has focused on species that have proven to be serious pests of forest ecosystems, such as the balsam woolly adelgid and the hemlock woolly adelgid, and on other anholocyclic species (Havill and Foottit 2007). The work presented in my thesis focuses on western Canadian adelgids that are holocyclic and currently not a threat to natural or managed forest stands. Adelgids have proven, however, to be pests in western Canadian conifer seed orchards. The aim of my research was to provide new insights in adelgid biology, host response, and possibly provide new ideas for monitoring and management.

An important part of this project included understanding the taxonomy of adelgids. Dr. Foottit and Eric Maw at the CNC in Ottawa, Ontario, explained and assisted with familiarizing me with adelgid taxonomy and the laboratory procedures required to prepare adelgid slide mounts for identification.

Susceptibility to adelgid induced galling is under strong genetic control. Understanding the differences in galling susceptibility of a plantation allowed us to better standardize our studies on the life stages involved in gall formation and the developmental anatomy of galls. Based on the best linear unbiased predicted breeding

values of the spruce hosts, we were able to select samples from families that showed a moderate to high susceptibility to galling. This increased the confidence of our results for the manipulative laboratory experiment which was used to decipher the influence of a fundatrix and gallicolae in the gall formation process. Although there are other possible factors such as individual tree health, knowing samples were selected from trees that displayed a similar level of susceptibility better standardized the affects the adelgids had on different samples. For the work on the developmental anatomy, selecting samples from hosts that showed a moderate to high susceptibility also minimized the probability of observing any differences in the development of galls from more or less susceptible trees.

In further attempts to characterize gall structures with the inducing adelgid species it would be of interest to investigate the various gall structures of individuals within the same genetic family or to compare the gall structures among less susceptible families and more susceptible families. Understanding the susceptibility of a tree could possibly explain some of the variation in gall structures observed to be induced by a single species. Host response may also explain some of the variation observed among gall structures induced by a single adelgid species. Additionally, a more detailed review of adelgid galls and the inclusion of DNA barcode data will clarify the uncertainties in the structural variations observed. Increasing the sample size of the galls reviewed in my thesis would have possibly provided a better representation of the gall structures induced by different adelgid species. It would also be necessary to use DNA barcoding to identify the emerging alates; moreover it would be of interest to compare the alate morphology with DNA results. Samples submitted for DNA barcoding may clarify the keys based on

alate morphology. Investigating previous year gall structures would also be of interest to determine if there are enough retained characteristics to associate those structures with an inducing species. Commencing such work would require a strong understanding of the current year gall structures and the associated adelgid species. Characterizing current and previous year galls would be extremely useful in adelgid management and research, and not require the taxonomic expertise which is currently required.

Elucidating the role of fundatrices and gallicolae in gall formation suggested that pest management efforts should target the fundatrices. This supports the current management approaches used by the Kalamalka Seed Orchard, however further investigation of control strategies for fundatrices could provide more effective or alternative management solutions. Biological and chemical controls have been explored for managing adelgid populations, however only insecticidal soaps and dormant oils are currently used in BC (Strong and Bennett 1997; Strong 2002). Furthermore, understanding the stages of gall formation (initiation, growth, maturation and dehiscence) could provide a basis for investigating the biochemical processes involved in each stage.

The increased depth of knowledge of adelgids and their galls could lead to new insights in adelgid biology. More importantly, it would be of great benefit if the findings in this thesis could provide new insights and ideas for future research programs that would explore alternate management tools for treating adelgid populations in conifer seed orchards.

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APPENDICES

Appendix A

Mounting adelgids in Canada Balsam

The following method has been adapted from Dr. Robert Foottit and Eric Maw and can be used for aphids.

1. Pierce the abdomen of the adelgid with a fine point to allow body contents to move out and for the reagents to move in.
2. Heat the pierced specimens at approximately 95 °C in 40% potassium hydroxide (KOH) for 5 to 10 minutes, depending on the size of the adelgid and how easily the body contents are clearing.
3. Allow the specimens to cool in the KOH for a couple of minutes and then transfer them into distilled water for an hour. Specimens can be left over night if necessary.
4. Transfer to chlorophenol (1:1 mix by weight of chloral hydrate and liquefied phenol) for 15 to 30 minutes. Again the time depends on the size of the adelgids.
5. Transfer to a mixture of 1:1 acetic acid and alpha-terpineol (oil of lilac) for 5 to 15 minutes.
6. Transfer specimens into pure alpha-terpineol for approximately five minutes.
7. Place a drop of Canada balsam on the center of a slide and carefully place the adelgid in the center of the balsam, arrange the appendages and gently lower a circular cover slip over the specimen. When arranging the appendages, manipulate the balsam rather than directly maneuvering the appendages. This

will avoid tearing or damage. It also helps to place a small drop of alpha-terpineol on the balsam before placing a cover slide on top.

8. Dry for two weeks at 50^0C for identification.

Appendix B

Key used to identify alate adelgids to species

The following is taken from a bulletin written by C.I. Carter in 1971.

1.	Abdomen with five pairs of spiracles (*Adelges*)	2.
	Abdomen with four pairs of spiracles (*Pineus*)	7.
2.	Head, prothorax, and mesothorax bearing distinc gland areas with definite facets	3.
	Head, prothorax, mesothorax without or with faintly discernible gland areas with indistinct facets	4.
3.	Antennae distinctly five-jointed, sensorial narrow, restricted to the distal one-third of each segment	*Adelges cooelyi*
	Antenna with third, fourth, and fifth segments apparently fused to form a club; sensorial wider, though restricted to distal one-half of each segment	*Adelges strobilobius*
4.	Posterior cephalic, prothoracic, and mesial mesothoracic glands represented by lighter, somewhat granular areas without distinct facets	5.
	These areas entirely lacking	6.
5.	Antenna with sensorium of third segment reaching slightly less than halfway around the segment and distinctly oval; all sensoria rounded. Wrinkles on the dorsal side of last antennal segment strongly arched	*Adelges piceae*
	Antenna with sensorium of third segment reaching slightly more than halfway around the segment and more angular; all sensoria somewhat angluar. Wrinkles on the dorsal side of last antennal segment strongly arched	*Adelges nussilini*
6.	Hindwing with media almost at right angles to the radial sector; first anal of forewing arched toward the distal tip. Margins of sensorial frequently sinuate. Basal portion of fourth antennal segment moderately narrow	*Adelges abietis*
	Hindwing with media leaving radial sector at an acute angle; first anal of forewing nearly straight. Margins of sensorial	

	nearly smooth. Basal portion of fourth antennal segment broad.	*Adelges lariciatus*
7.	Hindwings with media present	8.
	Hindwings with media lacking	11.
8.	Head without gland areas	*Pineus pinifoliae*
	Head with distinct gland areas	9.
9.	Abdominal gland areas of very small facets borne on small distinct plates and posterior cephalic area oval, borne on a distinct convexity; wax canals large and distinct, about equal in diameter to half that of the largest facets. Marginal abdominal areas narrow and crescent-shaped	*Pineus boycei*
	Abdominal gland areas without plates or with plates that are very indistinct; areas not greatly subdivided. Posterior cephalic area only slightly or not all convex, somewhat oval or elongate narrow. Wax canals when area is oval are considerably less than half the diameter of the largest facets. Marginal abdominal areas broadly crescent-shaped or oval	10.
10.	Head with posterior gland area narrow. Antenna with sensorium of last segment reaching more than halfway around end covering nearly two thirds of the length of the segment. Opposite margins of the sensorial nearly parallel. Segments tapering abruptly to the base.	*Pineus floccus*
	Head with posterior gland area broad, nearly oval. Antenna with sensorium of last semgment extending not over halfway around the segment and covering not more than half of the length of the segment. Segments of the antennae tapering gradually to their bases.	*Pineus similis*
11.	Head with at least the posterior areas with facets indistinct.	*Pineus engelmanni*
	Head with facets of both anterior and posterior areas distinct.	12.
12.	Gland facets of head and prothorax angular and contiguous. Posterior cephalic area broad. Posterior mesial and pleural and anterior mesial areas joined to form an L-shaped area on each side.	*Pineus coloradensis*
	Gland facets of head and prothorax tend to be rounded, not	

closely fitted together. Posterior cephalic area narrow, occasionally divided into two parts. Prothorax with anterior mesial area frequently lacking; when present not fused with posterior pleural and mesial areas, which usually are elongate and narrow, close to the posterior margin of the notal plate.	*Pineus borneri** or *Pineus sylvestris**

* these species are difficult to separate using a key. Suggested that *P. borneri* has smaller posterior cephalic and smaller pleural and mesial abdominal areas than *P. sylvestris*.

Appendix C

Gall	Tag	Location
A. cooleyi (Fig. 5-4a)	WKH 4201 1 CC	Skimikin Seed Orchard
A. cooleyi (Fig. 5-4b)	WKH 184 13 I	Skimikin Seed Orchard
A. cooleyi (Fig. 5-4c)	BLK 15 4458 R2	Kalamalka Research Center
A. cooleyi (Fig. 5-4d)	BLK 5 2330 XBT Y008	Kalamalka Research Center
A. cooleyi (Fig. 5-4e)	BLK 13 844 XAG Y033	Kalamalka Research Center
A. cooleyi (Fig. 5-4f)	BLK 3 73 XAX Y010	Kalamalka Research Center
A. cooleyi (Fig. 5-4g)	BLK 5 88 XPB Y010	Kalamalka Research Center
A. cooleyi (Fig. 5-4h)	BLK 13 3366 AC Y032	Kalamalka Research Center
A. lariciatus (Fig. 5-5a)	WKH 4681 13N	Skimikin Seed Orchard
A. lariciatus (Fig. 5-5b)	BLK 13 876 XAE Y033	Skimikin Seed Orchard
A. lariciatus (Fig. 5-5c)	BLK 15 4458	Kalamalka Research Center
A. lariciatus (Fig. 5-5d)	WKH 690 2 BBC	Skimikin Seed Orchard
P. pinifoliae (Fig. 5-6)	WKH 4681 13N	Skimikin Seed Orchard
P. boycei (Fig. 5-7a)	WKH 1339 4V	Skimikin Seed Orchard
P. boycei (Fig. 5-7b)	WKH 1262 25Z	Skimikin Seed Orchard
P. boycei (Fig. 5-7c)	WKH 1273 14H	Skimikin Seed Orchard
P. boycei (Fig. 5-7d)	WKH 4518 13K	Skimikin Seed Orchard
P. boycei (Fig. 5-7e)	WKH 1271 8 S	Skimikin Seed Orchard
P. boycei (Fig. 5-7f)	WKH 1349 16E	Skimikin Seed Orchard
P. boycei (Fig. 5-7g)	WKH 1273 14H	Skimikin Seed Orchard
P. boycei (Fig. 5-7h)	WKH 1262 25Z	Skimikin Seed Orchard
P. boycei (Fig. 5-7i)	WKH 2757 10 CCD	Skimikin Seed Orchard
P. boycei (Fig. 5-7j)	WKH 1343 23 EF	Skimikin Seed Orchard
P. spp (Fig. 5-8)	WKH 4681 13N	Skimikin Seed Orchard
P. similis (Fig. 5-9)	WKH 2707 23CC	Skimikin Seed Orchard
P. floccus (Fig. 5-10)	BLK 15 4307 R1	Kalamalka Research Center
P. floccus (Fig. 5-11)	WKH 690 17BC	Skimikin Seed Orchard

Appendix D

THE UNIVERSITY OF BRITISH COLUMBIA

ANIMAL CARE CERTIFICATE

Application Number: A06-0333

Investigator or Course Director: John A. McLean

Department: Forest Sciences

Animals: Invertebrates Adelges spp. 10000

Start Date: May 1, 2006 **Approval Date:** June 22, 2006

Funding Sources:

Funding Agency: BC Ministry of Forests

Funding Title: Survey of adelgid populations, their biology and impact in the Kalamalka larch and spruce seed orchards

Unfunded title: Adelgid Studies: taxonomy and life history

The Animal Care Committee has examined and approved the use of animals for the above experimental project.

This certificate is valid for one year from the above start or approval date (whichever is later) provided there is no change in the experimental procedures. Annual review is required by the CCAC and some granting agencies.

A copy of this certificate must be displayed in your animal facility.

Office of Research Services and Administration
102, 6190 Agronomy Road, Vancouver, BC V6T 1Z3
Phone: 604-827-5111 Fax: 604-822-5093