

Herbivores and pathogens on *Alnus viridis* subsp. *fruticosa* in Interior Alaska: effects of leaf, tree, and neighbour characteristics on damage levels

Christa P.H. Mulder, Bitty A. Roy, and Sabine Gusewell

Abstract: Parasite damage strongly affects dynamics of boreal forests. Damage levels may be affected by climate change, either directly or indirectly through changes in properties of host trees. We examined how herbivore and pathogen damage in *Alnus viridis* subsp. *fruticosa* (Rupr.) Nym. depend on leaf morphology and chemistry, tree size, and tree neighborhood. Damage and tree properties were measured in 2003 and 2004 on eight trees at each of 20 sites in Interior Alaska. Damage varied significantly among sites and among trees within sites, but Cartesian distances between sites were not correlated with similarity in damage levels. Compared with middle leaves, terminal leaves experienced less damage from phloem-feeding insects and pathogens, whereas leaf-roller damage was largely confined to terminal leaves. Summer drought in 2004 strongly reduced damage from phloem-feeding insects, while damage from chewing insects increased. Overall, herbivore damage was best explained by leaf morphology and chemistry, and pathogen damage by the proximity of other trees; the two damage types were not correlated with each other. Reproduction was negatively correlated with herbivore damage, but positively with pathogen damage. The contrasting relationships found for individual feeding guilds suggest that they must be studied separately in assessing impacts of climate change on parasite damage.

Key words: *Alnus crispa*, chewing insects, climate change, leaf miners, leaf rollers, phloem-sucking insects.

Resume: Les dommages causes par les parasites modifient la dynamique des forets boreales. L'intensite des dommages peut etre affectee par le changement climatique, directement ou indirectement, par des modifications aux proprietes des arbres-hotes. Les auteurs ont examine comment les dommages par les agents pathogenes et herbivores causes à *Alnus viridis* subsp. *fruticosa* (Rupr.) Nym. dependent de la morphologie foliaire, de la dimension de l'arbre et du voisinage de l'arbre. En 2003 et 2004, ils ont mesure les dommages et les proprietes sur 8 arbres sur 20 sites de l'interieur de l'Alaska. Les dommages varient significativement selon les sites et entre les arbres d'un meme site, mais les distances cartesiennes entre les sites ne montrent pas de correlation avec la similitude des degres d'endommagement. Comparativement aux feuilles medianes, les feuilles terminales montrent moins de dommages provenant d'insectes se nourrissant du phloeme et des agents pathogenes, alors que les dommages par les insectes herbivores augmentent. La secheresse de l'ete 2004 a fortement reduit les dommages par les insectes suceurs du phloeme, alors que les dommages par les insectes herbivores ont augmente. Dans l'ensemble, les dommages par les herbivores s'interpretent mieux par la morphologie foliaire et la chimie, et les dommages par les agents pathogenes par la proximite d'autres arbres; les deux types de dommages ne montrent aucune correlation entre eux. On observe une correlation negative entre la reproduction et les dommages par les herbivores, alors qu'elle est positive avec les dommages par les agents pathogenes. Les relations contrastees perçues chez pour les guildes à broutage individuel suggerent qu'ils doivent etre etudie separement lorsqu'on evalue les impacts du changement climatique sur les dommages par les parasites.

Mots-cles : *Alnus crispa*, insectes brouteurs, changement climatique, mineuses des feuilles, rouleuses des feuilles, insectes suceurs du phloeme,

[Traduit par la Redaction]

Introduction

Herbivores and pathogens are known to have strong impacts on their plant hosts, altering survival, growth and reproduction and, consequently, species composition and succession rates of plant communities (e.g., Burdon 1987,

1993; Kranz 1990; Crawley 1993; Dobson and Crawley 1994; Weste et al. 2002). These impacts vary considerably in time and space owing to intrinsic factors and variations in environmental conditions. In particular, climate change is likely to affect herbivores and pathogens (see reviews in Bale et al. 2002; Chakraborty and Datta 2003; Scherm

Received 27 September 2007. Published on the NRC Research Press Web site at botany.nrc.ca on 4 April 2008.

C.P.H. Mulder.¹ Institute of Arctic Biology and Department of Biology and Wildlife, University of Alaska Fairbanks, Fairbanks, AK 99775, USA.

B.A. Roy. Center for Ecology and Evolutionary Biology. University of Oregon, Eugene, OR 97403-5289, USA,

S. Gusewell. Institute of Integrative Biology, ETH Zurich, 8092 Zurich, Switzerland.

¹Corresponding author (e-mail: ffcpm2@uaf.edu).

2004) and to modify their impacts on plant communities (Coakley et al. 1999; Ayres and Lombardero 2000; Malmstrom and Raffa 2000; Niemala et al. 2001; Roy et al. 2004). These effects are likely to be most pronounced in regions with considerable climate warming, and in ecosystems where outbreaks of herbivores or pathogens may radically damage plant communities. For both reasons, it is expected that boreal forests may be particularly affected by effects of climate change on herbivores and pathogens.

Climate change may affect interactions between plants and invertebrate herbivores or pathogens (hereinafter collectively referred to as "parasites") through several direct and indirect pathways, which act at differing spatial and temporal scales. For example, the development of parasite populations may directly depend upon temperature, and their impact on host plants will then change rapidly in response to climate warming. Alternatively (or additionally), the development of parasite populations may depend mainly on the quality of plant tissues (nutrient content, secondary chemistry, toughness), the plant species composition, or the structure of plant communities. Effects of climate will then be mediated by the responses of host plants to climatic conditions, which may occur over years or decades, and the responses of parasites will be delayed accordingly. Large delays are particularly expected if the responses of plants to climate change are themselves mediated by changes in soil conditions, such as nutrient or water availability.

The possibility of indirect long-term effects of climate change on parasite damage introduces a considerable uncertainty in attempts to assess implications of climate change for plant-parasite interactions. Even in regions where there has already been substantial climate warming, long-term changes in parasite damage may be much greater than those observed presently. To evaluate this possibility, it is necessary to know whether long-term, indirect effects of climate change on parasite damage are likely to be more important than short-term, direct effects. A first step consists in determining to what extent plant traits and population structure influence parasite damage, and whether there is evidence that influential factors might be modified as a result of the anticipated climatic change.

Two further questions are relevant in this context. First, how will changes in the amount of parasite damage affect host performance (e.g., growth or reproduction)? The sensitivity of hosts to parasite damage may change under modified climatic conditions. For example, improved plant nutrition on warmer soils may enable plants to compensate for increased losses of resources to parasites. Conversely, increased water stress may reduce the ability of plants to tolerate parasite damage and may exacerbate the effects of parasites on host performance.

The second important question is whether and how the responses of herbivores and pathogens are related to each other. Most plant species harbor multiple herbivores and pathogens, whose effects on hosts may interact (Nakamura et al. 1995; Stinchcombe and Rausher 2001; Siemens and Roy 2005). However, the nature of these interactions has hardly been investigated (Marquis and Alexander 1992; Hatcher 1995; Biere et al. 2002; Siemens and Roy 2005). If the responses of herbivores and pathogens to climate change are positively correlated, implications for plant populations

are likely to be greater than if herbivores and pathogens respond differently. Empirical studies have revealed both positive associations (e.g., Simms and Rausher 1993; Johnson et al. 2003; Biere et al. 2004) and negative associations (e.g., Nakamura et al. 1995; Siemens and Mitchell-Olds 1998; Thaler et al. 2002) between herbivores and pathogens, but most of these studies were concerned with species pairs and did not consider correlations for the entire herbivore and pathogen communities.

The aim of the larger project, of which these results are a part, was to evaluate the role of direct and indirect pathways through which climate change might affect plant-parasite interactions in the boreal forest. To this end, we studied how parasite damage on *Alnus viridis* subsp. *fruticosa* (Rupr.) Nym. (Siberian alder), a common and widespread nitrogen-fixing shrub, varies among and within 20 sites in Interior Alaska. The 20 sites differed considerably in temperature and soil conditions as a result of differences in slope and aspect (south-exposed slopes being much warmer than north-exposed slopes). Thus, variation in parasite damage among these sites might be due to the direct effects of environmental variation on parasites, or due to indirect effects mediated by differences in plant properties and population structure or other biotic factors. The first possibility will be examined in a later paper; in this manuscript we examine the second possibility by studying how plant properties and population structure vary among and within sites, and how they are related to parasite damage at both spatial scales. We further studied how variation in parasite damage affects the performance (reproductive investment) of host plants. Finally, in assessing parasite damage, we distinguished between herbivores and pathogens, and for herbivores, between different feeding guilds, to determine whether and how their impacts on host plants are correlated. We investigated these questions by surveying parasite damage, plant traits, community structure and reproduction of *A. viridis* at the 20 sites in two consecutive years with different environmental conditions, which allowed us to evaluate whether relationships between damage levels and explanatory variables were consistent across years.

Materials and methods

Sites and climatic conditions

All sites were located within the boreal forest in Interior Alaska, an environment characterized by extremely cold winters (Fairbanks mean annual temperature = -3.3°C , January or June means = -25.9°C or 16.4°C) and little precipitation (annual precipitation = 269 mm). The 2003 and 2004 growing seasons differed greatly in temperature and precipitation. The 2003 growing season was characterized by a dry spring season (April + May precipitation = 8 mm or 37% of the 1948-2005 mean) followed by a wet summer (June + July + August precipitation = 213 mm or 167% of long-term mean, with the wettest July on record), but with close to average temperatures (June-August mean = 15.0°C) (climate.gi.alaska.edu/Climate). In contrast, 2004 had a very wet spring (April + May precipitation = 52 mm or 236% of the mean, with the wettest May on record), but the rest of the summer was extremely dry (June + July + August precipitation = 33 mm or 26% of mean) and excep-

tionally warm (June–August mean = 18.1 °C). In 2004, forest fires in Interior Alaska burned a record 2.67 million ha (National Climatic Data Center www.ncdc.noaa.gov/oa/climate/research/2004/fire04.html), including three of our sites, reducing the number of sites used in 2004 to 17.

Twenty sites were selected within 50 km of Fairbanks, ranging in latitude between 64°46'04" - 65°10'04" N and in longitude between 147°27'33" - 148°57'25" W. Each site consisted of a circular area with a 25 m radius (1964 m²). Sites were selected to represent a diversity of elevations (170 to 750 m a.s.l.), slopes (0°–20°) and aspects (due-north to due-south) to maximize the potential for differences in environmental conditions among them. This resulted in wide ranges in variables such as date of snow melt (16 April - 12 May 2003), depth of thaw (July 2004: 30 cm to > 1 m), summer soil temperature (August 2003 range: 1.3–10.2 °C), summer soil moisture (July 2004 range: 4%–13%), and winter below-snow minimum temperature (2003–2004 range: -6.3 to -31.1 °C). Sites on the warm and dry end of the continuum were generally steep south- or east-facing slopes dominated by white spruce (*Picea glauca* (Moench) Voss) and birch (*Betula papyrifera* Marsh.) or aspen (*Populus tremuloides* Michx.), and had low ground cover (<25%) by mosses. Cold sites were generally north-facing, dominated by black spruce (*Picea mariana* (Mill.) BSP), and had a thick layer of moss (near 100% cover). Moderate sites had a mixture of black and white spruce and birch.

Tree properties and population structure

Our focal plant species, *A. viridis* subsp. *fruticosa*, Betulaceae (synonym = *A. crispa*, nomenclature follows Furlow 1987), is a colonizing shrub 1–4 m in height with wind-dispersed seeds and wind-pollination (Viereck and Little 1986). This circumpolar species is ubiquitous throughout the boreal understory and is found across a wide range of environments (Hulten 1968; Viereck and Little 1986).

Within each site, eight trees or shrubs (minimum size: 3 stems > 1 cm in diameter) were randomly selected and marked with metal tags. Almost all individuals at our sites met the minimum size criteria, since very few seedlings or saplings were found. At one site, alder density was very low, and only six trees were sampled. To describe community structure, the distance of each tree to the nearest alder and to the nearest deciduous tree (birch or aspen, up to 10 m away) were measured. These other species of deciduous trees may represent additional potential hosts for herbivores or pathogens, and their density varied greatly among sites. At eight sites, marked alders were mapped by measuring the distance and direction from the central point.

Tree size was estimated by measuring the diameter of all stems > 1 cm in diameter at approx 25 cm from the ground (or, in the case of large dead stems with new sprouts, 25 cm from the base of the sprout). Tree basal area was calculated as the sum of the cross-sectional area of all measured stems. Reproductive investment was estimated in 2003 on a per branch and per leaf basis. This approach was needed to obtain comparable data as trees varied greatly in size and morphology, and a complete catkin count was impractical. We randomly selected two branches on opposite sides of each tree, with a minimum diameter of 1.5 cm, and we counted the number of male and female catkins on the branch, as

well as all leaves distal to the point where the branch diameter was 1.5 cm. We also noted the occurrence of browsing by moose (*Alces alces* L.). Because of the potential for high variability in branch and leaf morphology, we examined reproduction (number of male and female catkins, and percentage of catkins that were female) not only per branch, but also per total leaf mass (number of leaves multiplied by mean leaf mass) on that branch. This allowed us to examine investment in reproduction relative to leaves for branches of similar size.

Parasite species identification

We tried to determine what caused the different kinds of damage (pathogen, herbivore, environmental damage such as drought) through careful field and microscopic observation. For a particular kind of damage to be declared as being caused by a fungal pathogen, definitive characteristics such as spores, hyphae, or asci had to be observed on the sample. All leaf pathogens that could be identified were fungal. Insects were often directly observed causing particular kinds of damage. They were divided by feeding guild into "chewers" (all forms of herbivory caused by the chewing mouthparts of caterpillars and beetles, for example, holes, rasping, edge bite, etc.), "suckers" (caused by phloem-feeding insects such as aphids and psyllids), "miners" (larvae that leave mine traces), and "leaf rollers" (Table 1). Leaf rollers were analyzed separately from other chewing insects because the area damaged is much greater than that consumed. Some types of damage, such as chlorosis (yellowing associated with reduced photosynthesis and loss of chlorophyll) or premature senescence, may be the result of multiple causes. Since they could not be assigned to herbivore or pathogen category they were included in total damage calculations, but not in other categories.

Parasite damage

Trees were surveyed for parasite damage at the end of the growth season in 2003 (11–24 August) and 2004 (9–18 August). To determine whether early-season damage by herbivores or pathogens predicted late-season damage by the other group, we also performed one survey earlier in the growth season (5–16 July 2004). Leaves were sampled from four branches (approximately equally distributed around the tree) at a height between 1 and 2.5 m. To examine possible effects of leaf position, the outermost fully expanded leaf ("terminal leaf") and a leaf from the middle of each branch were evaluated. These leaves most probably were of the same age as alder leaves are generally produced during one initial flush in May (personal observation).

For each leaf we visually estimated the percent of leaf area damaged by each type of herbivore or pathogen. If leaf area had been removed, e.g., by herbivores, damage was expressed relative to the leaf area originally present. This leaf-based damage estimate did not account for possible leaf loss during the growth season. However, a separate leaf marking experiment in 2004 showed that leaf loss was very low (0.72% between 5–16 July and 9–18 August; C. Mulder and B. Roy, unpublished data, 2004) and, therefore, unlikely to affect the outcome. Since, in most cases, the species responsible for the damage was not seen, we primarily described types of damage (e.g., holes on the edges of the leaf versus

Table 1. Types of damage found on leaves of *Alnus viridis* subsp. *fruticosa*.

Description	Damage type	Causal agent	Percent of trees	
			2003	2004
Leaf herbivores				
Brown or purple spots	Insect (sucking)	Psyllidae, Homoptera	91	81
Bite on leaf edge	Insect (chewing)	Caterpillars and beetles ^a	89	94
Hole in leaf center	Insect (chewing)	Caterpillars and beetles	84	92
Small white dots	Insect (sucking)	Thrips	78	59
Rasping	Insect (chewing)	Caterpillars and beetles	69	36
Punctures	Insect (sucking)	Unknown ^b	66	94
Eriophyid galls	Arachnid (sucking)	<i>Eriophyes</i> sp.	66	57
Rolled leaf	Insect (rolling)	<i>Epinotia solandriana</i>	60	92
Linear holes	Insect (mining)	Unknown	49	43
Mining	Insect (mining)	Unknown	20	63
Shredding	Insect (chewing)	Caterpillars and beetles	13	79
Midvein rasping	Insect (chewing)	Beetles	0	9
Small brown spots	Insect (sucking)	Unknown (non-Psyllid) ^b	N/A	28
Leaf pathogens				
Orange flecks	Ascomycete	<i>Phyllactinia guttata</i> f.s. <i>alni</i>	53	41
Mildew	Ascomycete	<i>Microspora penicillata</i>	17	27
Tar spots	Ascomycete	<i>Rhytisma salicina</i>	11	26
Rosy patch	Unknown	Bacteria?	10	21
Tan patch	Unknown	—	9	5
Blister	Unknown	—	6	0
Irregular purple spots	Unknown	—	1	0
Rusty blotch	Unknown	—	1	0
Yellow vein	Unknown	Virus?	0	5
Leaf unclassified				
Mottling	Chlorophyll loss	Physical?	87	82
Orange edge blotch	Unknown	Physical–drought?	9	12
Brown edge	Unknown	Physical–drought?	1	3

Note: Species are listed within each group in order of declining abundance by frequency (percent of trees in which found) in 2003.

^aChewing insects seen on leaves include *Lophocampa maculata*, *Orygia antiqua*, and *Phlogophora* sp. (all in Lepidoptera), and a sawfly (Hymenoptera).

^bSucking insects seen include pentatomid bugs (Pentatomidae), Psyllids, thrips, aphids, and leaf hoppers. We did not separate psyllid from non-psyllid damage in 2003 because we did not yet recognize the difference.

near the midrib, shredding, rasping); the same organism may have been responsible for multiple types of damage, or multiple species may have caused damage of similar appearance.

Leaf morphology and chemistry

All of the leaves on which we measured damage were collected in 2003 for further analysis; in 2004, four of the surveyed leaves per tree (two middle and two terminal) were collected. All leaves were dried at 55°C for 48 h and weighed to the nearest 0.0001 g. Leaf area was measured using a CI-202 area meter (CID Inc., Camas, Wash.). From these data we calculated leaf mass per area (LMA; grams per square metre). A preherbivory leaf area was calculated by adding the area of the leaf removed entirely by herbivores (e.g., holes) to that provided by the leaf area meter. Similarly, we estimated a preherbivory leaf mass by adding in the mass of areas removed entirely by herbivores (based on LMA values). For all damage analyses we report results using the preherbivory leaf areas; analyses for uncorrected estimates of area damaged were almost identical and are not reported.

Leaf chemistry was determined on differing sets of leaves to assess leaf-level variation and the effect of leaf position

while keeping the number of analyses manageable. In 2003 we selected nine sites from which leaves were analyzed individually (three randomly selected trees per site; $n = 194$) or combined by leaf position (the remaining trees). For the remaining sites in 2003 and for all trees in 2004, leaves were bulked per tree. All samples were ground on a roller mill (U.S. Stoneware, East Palestine, Ore.) and, %N, %C, and $\delta^{13}\text{C}$ were obtained using a ThermoFinnigan Delta^{plus}XL (Thermo Fisher Scientific, Inc., Waltham, Mass.) with a ECS4010 elemental analyzer (Costech, Valencia, Calif.). We used %N as an indicator of leaf nutrient content, %C as an indicator of tissue structure (higher lignification leading to greater %C), and $\delta^{13}\text{C}$ as a combined indicator of high photosynthetic activity and water stress (both lead to reduced ^{13}C discrimination and result in less negative $\delta^{13}\text{C}$; Ehleringer and Cooper 1988; Toft et al. 1989; Caldeira et al. 2001). Results for ^{13}C abundance are expressed in the standard notation ($\delta^{13}\text{C}$) in parts per thousand (‰) relative to the international standard (Vienna Pee Dee Belemnite)

Statistical analyses

Analyses were performed using SAS (version. 8.02, SAS

Institute Inc., Cary, N.C.) except where noted. Where appropriate, data were transformed to meet model assumptions. Means in text are presented $\pm$ SE.

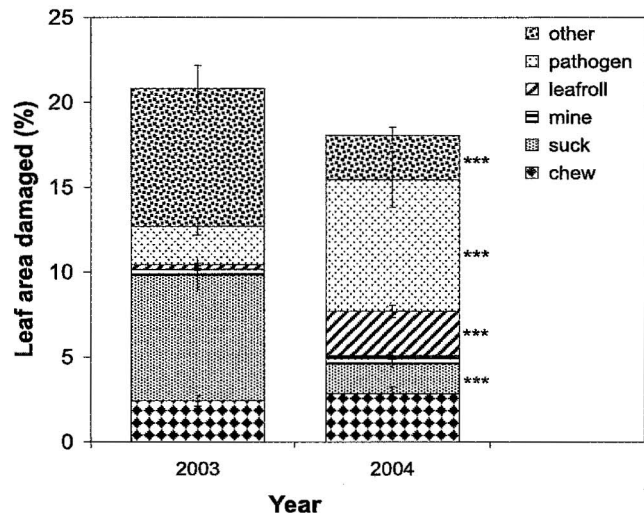
To evaluate the relative importance of between-site and within-site variation in parasite damage, we ran a hierarchical ANOVA including the random factors site, tree within site, branch within tree, and leaf within branch, and reported the variance components for each level of variation as a proportion of the total. As there was large variation in damage among leaves within branches, differences between middle and terminal leaves were tested using a mixed-model ANOVA, with trees as random factor and leaf position as fixed factor. Variation in leaf properties was analyzed with the same models as leaf damage. For tree morphology, the hierarchical ANOVA was simplified to include only the factors site and tree within site.

The dependence of parasite damage on plant properties and population structure was analyzed with stepwise multiple regression (P -to-enter = 0.1, P -to-stay = 0.05). Explanatory variables were those related to leaf morphology and chemistry (area, mass, LMA, %N, %C, $\delta^{15}\text{C}$), tree morphology (basal area and number of stems) and tree neighbourhood (distance to nearest alder, and distance to nearest birch or aspen). These analyses were run both on raw means per tree ("across site relationships") and on data adjusted for site effects (using residuals after removing the site mean for all variables; "within-site relationships"). This distinction was made because across-site relationships were possibly driven by environmental differences between sites, whereas within-site relationships were most probably not, since environmental conditions varied little within sites. Comparing the two types of relationships would therefore provide some information about the importance of environmental variation for plant-parasite relationships.

The effect of parasite damage on reproductive investment was analyzed with simple regression, either across sites (based on raw means per tree) or within sites (using residuals after removing the site mean). Finally, relationships between herbivore and pathogen damage were tested with Pearson correlations. All analyses were carried out separately for 2003 and 2004 to see whether the results were consistent across the two years.

We evaluated the effects of Cartesian distance within and between sites for herbivore and pathogen abundance. For the eight sites at which we had mapped individual trees, we calculated the absolute differences in damage values in 2003 for all pairs of trees. These matrices were correlated to the distances between trees (range = 1.4 m to 48.1 m) using a Mantel test (Mantel 1967). This procedure was repeated for distances between sites (which ranged from 0.13 km to 63.8 km) using differences in means per site. We tested for a "tree effect" (consistency for trees in herbivore and pathogen abundance and leaf characteristics across years) by combining the 2003 and 2004 datasets and running an ANOVA including year, site, and tree in the model. Since trees are consistent across years in genotype and local community structure, a lack of consistency in damage levels would indicate that these factors are relatively unimportant. Furthermore, if leaf characteristics are consistent across years but damage levels are not, this suggests that leaf characteristics are also relatively unimportant in determining damage levels.

Fig. 1. Mean values ($\pm$ SE) for percent leaf area damaged by damage types for *Alnus viridis* subsp. *fruticosa* in 2003 and 2004. Asterisks indicate a significant difference (at $P < 0.001$) between years based on tree means.



Results

Variation in parasite damage

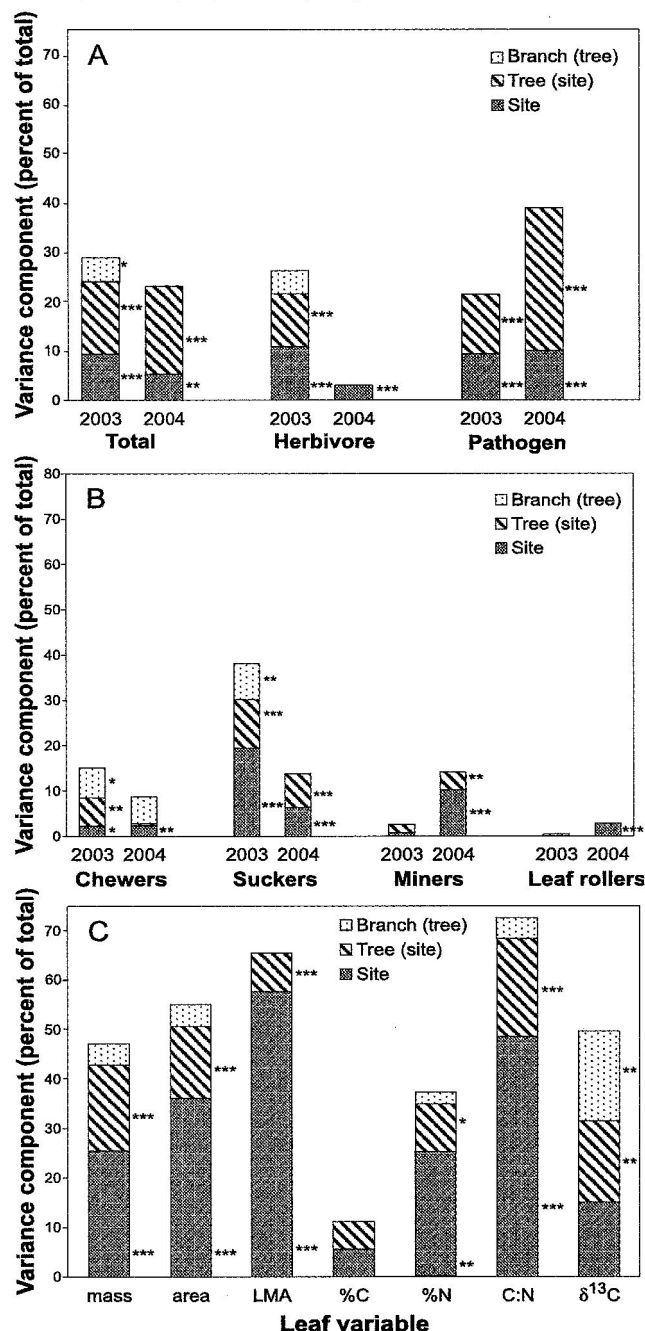
Total mean damage per leaf was slightly greater in 2003 (20.8%) than in 2004 (18.1%; Fig. 1; using tree means $F_{(1,288)} = 6.00$, $P = 0.015$), but whereas herbivore damage comprised the vast majority of the biological damage in 2003 (82%), in 2004 herbivores and pathogens contributed similarly to biological damage (46% and 54% respectively; Fig. 1). For feeding guilds, damage by sucking insects was much greater in 2003 than 2004, while the reverse was true for leaf rollers (Fig. 1).

Variation in total leaf damage, herbivore damage, and pathogen damage (on a per-area basis) were explained by site and (in most cases) by tree within site in both years (Fig. 2A). There was little variation among branches within trees (Fig. 2A). For individual herbivore feeding guilds, results varied between the two years (Fig. 2B); in 2003, trees within sites explained variation for all four groups, while in 2004 tree-level variation was significant only for sucking insects. Branch-level variation was only important for chewing insects. Unlike the other damage types, leaf rolling damage did not vary significantly among trees, and differed among sites only in 2004 (Fig. 2B).

Leaf characteristics showed similar patterns of variation, although generally the total amount of variation explained was greater than for damage variables, and a greater proportion could be attributed to site differences (Fig. 2C). Both sites and trees within sites showed significant variation for all leaf characteristics except $\delta^{15}\text{C}$. In contrast, branches within trees only differed in their $\delta^{15}\text{C}$. For variables measured at the tree level only, the amount of variation explained by site was 44% for distance to nearest alder, 70% for distance to nearest birch or aspen, 34% for number of stems, and 26% for basal area ($P < 0.001$ for all).

Leaf position (middle vs. terminal) affected levels of damage (Table 2): sucking damage and pathogen damage

Fig. 2. Variation in damage by plant parasites and leaf characteristics as explained by site, tree within site, and branch within tree for *A. viridis* subsp. *fruticosa*. Values are variance components based on EMS (expected mean square) expressed as percentage of the sum of the variance components. (A) Variation in total, herbivore, and pathogen damage (percent of leaf area affected). (B) Variation in damage (percent of leaf area affected) for the four guilds of herbivores (chewers, suckers, miners and leaf rollers). (C) Variation in leaf physical and chemical characteristics. Significance levels (for ability of each variable to explain variation) are indicated as follows: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.



were greater on middle leaves, whereas leaf rolling damage and chewing damage were greater on terminal leaves. The effect of leaf position was most pronounced for leaf rolling. Leaf position also affected leaf morphological and chemical characteristics. Terminal leaves had a slightly smaller area, a greater LMA, lower C:N ratio, and higher $\delta^{13}\text{C}$ values than middle leaves (Table 2).

Temporal and spatial correlations

Trees within sites were not consistent in either levels of herbivore damage or pathogen damage across years ($P > 0.8$ for both). In contrast, trees within sites were consistent between years in most leaf characteristics ($P < 0.005$), including mass (pearson's $r = 0.34$), area ($r = 0.44$), %N ($r = 0.35$), $\delta^{13}\text{C}$ values ($r = 0.64$), and C:N ratio ($r = 0.35$), but not LMA or %C ($P > 0.5$ for both).

There was no evidence for spatial correlations in damage levels over the scales at which we measured them. At the across-site scale there was no significant correlation between geographic distance and differences in herbivore or pathogen damage (Mantel tests, $P > 0.1$ for both). At the within-site scale, there was no relationship between herbivore damage and distance between trees for any site for ($P > 0.05$ for all), while for pathogen damage, six sites showed no relationship, one had a weakly positive correlation ($r = 0.35$; $P = 0.039$), and one a weakly negative correlation ($r = -0.39$; $P = 0.042$), again providing no consistent evidence for consistent spatial autocorrelation.

Dependence of damage on tree and leaf properties

Most types of damage were significantly related to one or several tree and (or) leaf properties across sites (Table 3); only leaf-rolling damage was not significantly related to any of these properties. Total herbivore damage was mainly explained by leaf properties (leaf mass and $\delta^{13}\text{C}$ in 2003; LMA in 2004), reflecting the responses of chewing and sucking insects to these properties. In contrast, mining damage and pathogen damage were mainly explained by tree neighborhood (distance to the nearest alder or to the nearest other deciduous tree), but the direction and type of relationships differed between the two years. Since we found that distance to potential additional hosts explained variation in pathogen damage, we examined whether this also affected leaf qualities. In both years, leaves on trees that were closer to birches or aspen were larger in area, thinner (lower LMA values), and had lower $\delta^{13}\text{C}$ values; ($P < 0.0001$ for all); in 2003 they also had higher %N ($P = 0.008$).

Even where relationships with tree or leaf properties were highly significant, they only partly explained between-site variation in parasite damage; if site was entered as additional factor into hierarchical regression models after the variables retained by the stepwise model selection, the site effect was significant in most cases, and it generally explained more variation than all other variables together (Table 3). Within sites, only two weakly significant relationships with leaf mass were found, which hardly exceeds the number of significant relationships expected by chance alone when running twelve models at $\alpha = 0.05$. This suggests that variation in damage among trees within sites was unrelated to tree and leaf properties.

We reran the stepwise regressions using the mass of the

Table 2. Comparison of leaf characteristics and damage levels (mean $\pm$ SE for percent of leaf area) on middle versus terminal leaves of *A. viridis* subsp. *fruticosa*.

Variable	2003			2004		
	Middle	Terminal	<i>P</i>	Middle	Terminal	<i>P</i>
Herbivore	10.9 $\pm$ 0.6	10.2 $\pm$ 0.6	0.26	5.70 $\pm$ 0.5	9.23 $\pm$ 0.8	<0.001
Sucking	8.4 $\pm$ 0.6	6.5 $\pm$ 0.5	<0.001	1.82 $\pm$ 0.2	1.30 $\pm$ 0.2	0.016
Chewing	2.1 $\pm$ 0.2	2.8 $\pm$ 0.3	0.052	2.18 $\pm$ 0.3	3.12 $\pm$ 0.4	0.10
Mining	0.3 $\pm$ 0.06	0.4 $\pm$ 0.07	0.63	0.62 $\pm$ 0.1	0.42 $\pm$ 0.1	0.48
Rolling	0.04 $\pm$ 0.03	0.51 $\pm$ 0.19	0.015	1.67 $\pm$ 0.4	5.61 $\pm$ 0.8	<0.001
Pathogen	2.9 $\pm$ 0.4	1.6 $\pm$ 0.2	<0.001	9.04 $\pm$ 1.3	4.61 $\pm$ 0.8	<0.001
Area (m ²)	0.0026 $\pm$ 6 $\times$ 10 ⁻⁵	0.0025 $\pm$ 6 $\times$ 10 ⁻⁵	0.011			
Mass (g)	0.16 $\pm$ 0.004	0.16 $\pm$ 0.003	0.81			
LMA (g·m ⁻²)	69 $\pm$ 2	73 $\pm$ 0.2	<0.001			
%C	49.4 $\pm$ 0.6	49.2 $\pm$ 0.4	0.39			
%N	2.3 $\pm$ 0.06	2.4 $\pm$ 0.05	0.066			
C:N	21.9 $\pm$ 0.5	21.2 $\pm$ 0.6	0.021			
$\delta^{13}\text{C}$	-28.6 $\pm$ 0.1	-28.3 $\pm$ 0.05	0.003			

Note: No data were available on middle vs. terminal leaf characteristics in 2004. *P* values are based on ANOVA using means per leaf position per tree after including tree in the model. Areas and mass are estimates of pre-damage values (i.e., corrected for loss due to herbivores). Sample sizes for leaf position comparisons: *N* = 314 for 2003 damage variables; *N* = 316 for 2004 damage levels; *N* = 270 for 2003 chemical variables; *N* = 276 for 2003 morphological variables.

leaf affected by herbivores or pathogens as the response variables to determine to what extent relationships between damage (as measured by area) and leaf size (mass or area, which were highly correlated, Pearson's $r = 0.79$) reflected changes in leaf size rather than biomass affected. As expected this altered the direction of the relationship between damage by herbivores and leaf mass from negative to positive and resulted in the addition of leaf mass to the model for pathogen damage, but otherwise it had no effect.

Correlations between pathogens and herbivores

Herbivore and pathogen damage (on a per-area basis) were not correlated, whether evaluated as means of trees across sites or for leaves within trees ($P > 0.1$ for both). For 2004 data, we had estimates from two time periods (early July and mid-August), allowing us to examine the impact of the damage of one group early in the season on damage by the other group toward the end of the season. There was no correlation between early season herbivore damage and late-season pathogen damage ($r = 0.11$, $P > 0.1$) or between early season pathogen damage and late-season herbivore damage ($r = -0.04$, $P > 0.1$).

Damage levels and reproduction

Sites differed greatly in percent of trees reproducing (range = 0%-80%), mean number of catkins per branch (0-45), mean number of catkins per leaf (0-0.1), and the proportion of catkins that are female (0%-86%) (differences among sites: $P < 0.01$ for all). There was no relationship between tree size (basal area or number of stems) and number of catkins per branch or per leaf mass on that branch, whether across or within sites ($P > 0.1$ for all).

Across sites, mean number of catkins per branch was lower in trees with greater herbivore damage ($F_{[1,152]} = 6.86$, $P = 0.0096$; Fig. 3A). This relationship was slightly stronger for female catkins (parameter estimate = -0.033) than for male catkins (parameter estimate = -0.027). As a result, the proportion of female catkins was lower in trees

with greater herbivore damage ($F_{[1,84]} = 4.88$, $P = 0.030$). When calculated on a the basis of catkins per leaf mass, the relationship was also negative for the total catkins ($F_{[1,144]} = 5.03$, $P = 0.026$), although only marginally significant for female catkins ($P = 0.052$) and not significant for male catkins ($P = 0.11$). Within sites, the number of catkins per branch was similarly negatively related to herbivore damage ($F_{[1,133]} = 7.15$, $P = 0.008$), but this relationship was similar for female catkins (parameter estimate = -0.027) and male catkins (parameter estimate = -0.032) (Fig. 3B), and the number of catkins per leaf mass on the branch was unrelated to herbivore damage (total, male and female catkins: $P > 0.1$ for all, data not shown).

Catkin production per branch showed a significant positive relationship with pathogen damage, both across sites ($F_{[1,152]} = 13.69$, $P = 0.0003$; Fig. 3C) and within sites (Fig. 3D). This was driven primarily by changes in the number of female catkins (parameter estimate = 0.13, compared with 0.05 for male catkins), so that the proportion of female catkins was also positively related to pathogen damage ($F_{[1,131]} = 15.09$, $P = 0.0002$). Results were similar for the number of catkins per leaf mass on the branch across sites (for total catkins: $F_{[1,144]} = 5.66$, $P = 0.019$) and in the same direction, but not significant within sites ($F_{[1,124]} = 2.49$, $P = 0.12$).

Male catkin reproduction was greater on plants that had been browsed by moose than on unbrowsed plants (17 ± 4 vs. 5 ± 1 ; $F_{[1,153]} = 10.76$, $P = 0.0013$), but there were no differences in number of female catkins or number of leaves per branch ($P > 0.1$). However, the differences in number of male catkins could be attributed entirely to site differences; once site was included in the model, there was no significant effect of browsing ($P > 0.2$).

Discussion

The dominant paradigm in the ecology of plant-enemy interactions is that intrinsic resistance, either constitutive or induced, controls the amount of damage received by plants

Table 3. Results of stepwise regressions to identify variables best explaining damage levels to leaves of *A. viridis* subsp. *fruticosa*.

Across sites				
Response variable	Year	Model R^2	Variables retained in the model	Site additional R^2
Herbivores	2003	0.14	$-3.0(\text{mass}) - 0.15(\delta^{13}\text{C})$	0.38***
	2004	0.12	0.010(LMA)***	0.14
Chewing	2003	0.03	0.48(LMA)*	0.23**
	2004	0.17	0.011(LMA)***+0.79(%N)*-12.2(basal)*	0.23**
Sucking	2003	0.27	$-3.3(\text{mass})^{***}-0.21(\delta^{13}\text{C})^{***} + 8.78(\text{basal})^{*}-0.07(\text{distA})^{**}$	0.25***
	2004	0.12	0.0063(LMA)***+8.66(basal)*	0.46***
Mining	2003	0.07	0.04 (distA)**	0.18
	2004	0.16	0.08($\delta^{13}\text{C}$)*+0.03(distB)*	0.20*
Leaf rollers	2003	0	[none]	0.11
	2004	0	[none]	0.11
Pathogens	2003	0.07	0.064(distB)**	0.35***
	2004	0.14	0.06(C:N)*+ 21.4(basal)* -0.1156(distB)***	0.35***
Within sites				
Response variable	Year	Model R^2	Variables retained in the model	
Herbivores	2003	0.03	$-16.8(\text{mass})^{*}$	
	2004	0	[none]	
Chewing	2003	0	[none]	
	2004	0	[none]	
Sucking	2003	0	[none]	
	2004	0	[none]	
Mining	2003	0.04	1.29(mass)*	
	2004	0	[none]	
Leafrollers	2003	0	[none]	
	2004	0	[none]	
Pathogens	2003	0	[none]	
	2004	0	[none]	

Note: P -to-enter = 0.01; P -to-stay = 0.05. Dependent variables are $\log_{10}$ (% leaf area +1) for each damage type; distA, distance to nearest alder; distB, distance to nearest birch or aspen; basal, basal area. Units: mass, g; distA, m; distB, m; leaf area, m^2 ; LMA, $\text{g}\cdot\text{m}^{-2}$; basal, m^2 . "Model R^2 " is the total R^2 for the stepwise regression. "Site additional R^2 " refers to additional variation explained by site after variables retained in the model are included. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. $N = 147$ for 2003; $N = 127$ for 2004.

(Ehrlich and Raven 1964; Coley 1983; Agrawal 1998; Parker and Gilbert 2004; Taylor et al. 2004; Wimp et al. 2005; Jones and Dangel 2006). Our results instead suggest that while alder leaf characteristics (for herbivores) or neighbourhood characteristics (for pathogens) explain some of the variation in damage, these variables cannot account for much of the variation in damage among sites. In the boreal forest, the environment may be as or more important than individual tree or leaf characteristics in driving damage by parasites.

Mechanisms for differences in damage within and among sites

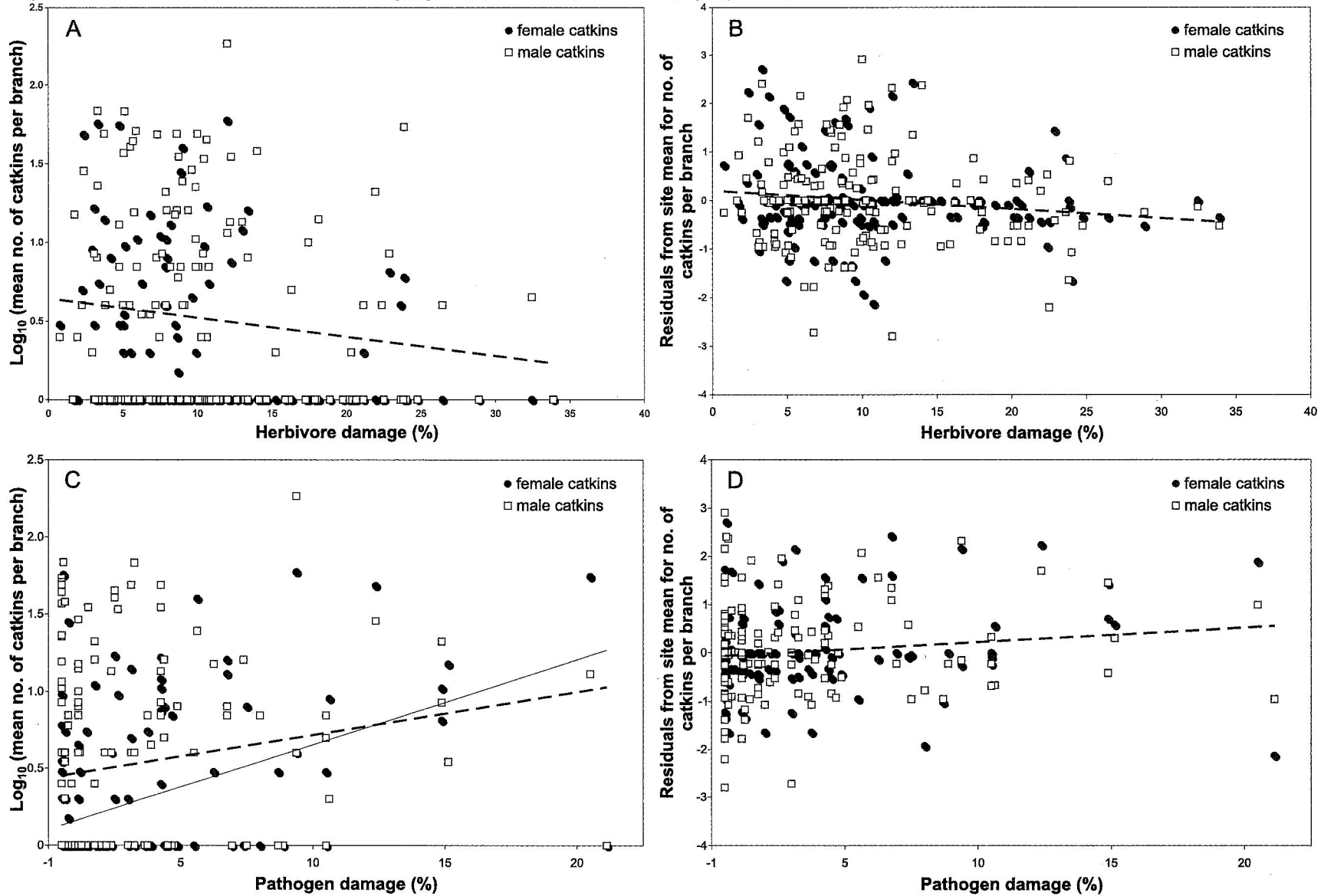
Damage by sucking and chewing insects

Both sites and trees within sites explained a significant proportion of the variation in sucking and chewing damage (Fig. 2). Stepwise regressions on tree-level data indicated that at the across-site level, damage by chewers and suckers was related to leaf characteristics, with some additional variation explained by tree basal area and host density (Table 3). However, at the within-site level, damage was unrelated to leaf characteristics. The most likely explanation for this is that there was insufficient variation in these characteristics within sites to drive variation in herbivore damage: variation in leaf characteristics was much greater

between sites than between trees within sites for almost every variable measured, and almost half of the variation in distance to nearest alder was explained by site. Furthermore, site still explained additional variation in chewing and sucking damage even after all other variables were accounted for. This suggests that either we did not measure some of the characteristics that explain variation (e.g., secondary compound concentrations), or that direct impacts of environmental differences contributed to variation among sites.

For sucking damage, the relationships with leaf characteristics are consistent with the environment having a significant effect. Damage was greater on trees with low LMA values (thinner leaves) and low $\delta^{13}\text{C}$ values in 2003 (when damage by sucking insects represented two-thirds of all damage; Table 3) and, within trees in both years, on the middle leaves, which had lower LMA and $\delta^{13}\text{C}$ values than outer leaves in 2003 (not measured in 2004; Table 2). The negative relationship with $\delta^{13}\text{C}$ values indicates that the sucking insects were sensitive to light or, more likely, drought stress. In low light environments, such as the interior of trees or trees in dense stands, leaves tend to be large and thin (low LMA; (e.g., Loach 1970; Bazzaz 1979; Popma and Bongers 1988; Niinemets and Kull 1994; Beaudet and Messier 1998) and have more negative $\delta^{13}\text{C}$ values (e.g., Ehleringer and Cooper 1988; Toft et al. 1989; Caldeira et al. 2001); they may also have higher water potential (Van-

Fig. 3. Relationship between mean leaf damage and catkin production per branch across and within sites for *A. viridis* subsp. *fruticosa*. (A) Herbivore damage vs. male and female catkin numbers using mean percentage of leaf area per tree. For female catkins: $F_{[1,152]} = 6.55$, $P = 0.01$; for male catkins: $F_{[1,152]} = 2.79$, $P = 0.096$. (B) Herbivore damage vs. male and female catkins numbers using residuals from means per site. For female catkins: $F_{[1,133]} = 3.91$, $P = 0.050$; for male catkins: $F_{[1,133]} = 3.74$, $P = 0.055$. (C) Pathogen damage vs. male and female catkins numbers using raw data. Female catkins: $F_{[1,152]} = 39.18$, $P < 0.0001$; male catkins: $F_{[1,152]} = 3.53$, $P = 0.061$. (D) Pathogen damage vs. male and female catkins numbers using residuals from means per site. Female catkins: $F_{[1,133]} = 8.88$, $P = 0.0034$; male catkins: $F_{[1,133]} = 2.97$, $P = 0.087$.



derklein et al. 2004). Our data suggest a preference for or greater growth, under such conditions, for sucking insects. Leaf water content is often positively correlated with insect growth rates (Scriber and Slansky Jr. 1981), and sucking insects may be particularly sensitive to leaf turgor and source-sink relationships (Jones and Coleman 1991): a recent meta-analysis showed that the majority of sucking insects performed significantly less well on water-stressed plants (Huberty and Denno 2004). The low precipitation in interior Alaska may result in low phloem moisture content: moisture content was 20% lower in phloem of spruce (*Picea glauca*) trees in interior Alaska than in south-central Alaska (Werner and Holsten 1985; Werner et al. 2006). Low light availability may also result in reduced fiber content (Larcher 1980) and it may be easier for sucking insects to reach the veins of these thinner leaves (peeters 2002). Consistent with the hypothesis that drought stress limits the growth or reproduction of sucking insects, in 2004, a year with a record hot and dry summer, damage levels were reduced to 23% of those in 2003 (Fig. 1). In that year $\delta^{13}\text{C}$ values were not retained in the regression model, but this may simply reflect low variation in moisture availability (i.e., that all trees were extremely dry). In general, the hypothesis that damage levels by sucking insects may be limited by moisture levels is supported by comparisons at three different scales: leaves within trees, trees between sites, and differences between years.

Damage by chewing insects was greater on trees with high LMA values (Table 3), and on terminal leaves (with higher LMA values) than on middle leaves (Table 2). Chewing damage was also greater on trees with a higher %N content (in 2004; Table 3), consistent with the results of many studies that have found positive relationships between host leaf %N and insect survivorship, growth and reproduction (e.g., Mattson 1980; Scriber and Slansky Jr. 1981; Mattson and Haack 1987; White 1993). Unlike the results for damage by sucking insects, there was no evidence that drought stress had a negative impact on levels of damage by chewing insects. Other studies have similarly shown few responses of chewing insects to plant stress (Larsson 1989), possibly because they do not discriminate very well between different tissues, and positive effects of stress (e.g., increases in soluble N, White 1984) are counteracted by increased secondary compounds (Gershenson 1984; Mattson and Haack 1987).

Damage by leaf-mining insects

There were significant differences between sites in damage due to leaf-mining insects in both 2003 and 2004, although in 2003 this explained little of the total variation (Fig. 2B). The stepwise regressions (Table 3) did identify some variables that explained variation, including neighbourhood variables and a leaf chemistry variable ($\delta^{13}\text{C}$ in 2004 only). Thus, mining damage differences between sites that do exist can be explained primarily by community characteristics.

Damage by leaf-rolling insects

For leaf-rolling insects, differences between sites were significant only in 2004 (Fig. 2B). No variables were identified in the stepwise regressions for leaf rollers (Table 3); combined with the poor ability of sites or trees to explain

damage levels we have to conclude that leaf rollers appear to be fairly insensitive to host or community characteristics. Leaf rollers were almost entirely limited to terminal leaves (Table 2); since leaf characteristics for inner and terminal leaves overlap substantially (and failed to explain variation at the between-tree scale), this suggests that this pattern may be more related to spatial location. For example, leaf-rolling insects might develop better on leaves at the edge of the canopy, or the moths might have a preference for terminal leaves as has been found for other species (Wearing 1998; Miyashita and Kawanishi 2003). Of all feeding guilds, leaf-rolling insects showed the largest proportional change in damage levels between years, increasing almost 10-fold between 2003 and 2004. This is consistent with the status of *Epinotia solandriana* L., the most abundant and likely the only leaf-rolling insect at our sites, as an outbreak species (Holsten et al. 2001), as well as with other studies demonstrating strong interannual variation in the Alaskan boreal forest (Werner et al. 2006). These results suggest that changes in summer conditions of the kind represented by our range of sites are unlikely to greatly affect damage to alders by leaf rollers.

Damage by pathogens

Both sites and trees within sites differed in levels of pathogen damage, with trees within sites explaining much more of the variation (Fig. 2A). The stepwise regressions identified distance to the nearest birch or aspen as the best predictor for damage levels in both years, but in opposite directions (Table 3). We do not have a good explanation for this, beyond noting that the species composition of the pathogen community changed substantially between years, and our leaf characteristic analysis revealed that leaves on trees closer to birches differed significantly in several characteristics from those that were not close to birches. Sites still explained differences in pathogen damage after all other characteristics were accounted for. Thus, as for damage by chewing and sucking insects, we have either failed to measure important leaf characteristics, or the environmental differences among sites are driving differences in pathogen damage.

Like damage by sucking insects, pathogen damage was greater on middle leaves than on terminal leaves in both years (Table 2). However, unlike for sucking insects, there is little evidence that this is due to drought stress per se. First, damage levels were three times greater in the drier year, whereas sucking damage was low in the dry year. Second, the model selection exercise did not result in the retention of leaf characteristics associated with light or water stress in either year (Table 3). Instead, we suspect that drought stress reduced resistance, enabling increased infection, and that the higher infection on internal leaves is the result of higher humidity inside the canopy [many pathogens require free water on the leaf surface for germination (Bradley et al. 2003)].

Spatial and temporal patterns in damage

Spatial patterns

Differences between sites could be due to geographic iso-

lation of different sites, but there was no relationship between distance between sites and difference for any of the response variables, despite the fact that some sites were very close (e.g., 130 m and 190 m) and others quite far apart (up to 68 km). Furthermore, trees close to each other within a site were not more similar in damage than those farther apart within the same site. This does not necessarily mean that there is no spatial autocorrelation for herbivore and pathogen communities: such patterns may be restricted to intermediate scales (100+ m to a few kilometres) and we may simply have lacked the power to detect them, or larger-scale patterns may have been broken up by our deliberate spatial interspersal of sites with different environmental conditions. However, these results do suggest that at the scales we examined, spatial patterns are overwhelmed by effects of site-specific conditions. Furthermore, any existing spatial autocorrelation patterns are likely driven by pest characteristics (e.g., dispersal limitation) rather than tree characteristics: there is little genetic spatial structure for neutral genes (i.e., trees within a site are not more similar genetically than between sites; B.A. Roy and C. Mulder, unpublished data, 2003), and this is not surprising given that *A. viridis* is wind-pollinated and produces copious seeds that are widely dispersed by wind and water (Viereck and Little 1986).

Temporal patterns

Trees did not show a consistent pattern over time for herbivore or pathogen damage; that is, knowing the damage level for a given tree in one year provided no information about relative damage level in the following year. Thus, even when tree location, size, and neighbour characteristics are constant, damage levels are not. This matches results from the stepwise regressions: herbivore damage was poorly explained by tree morphology or neighbourhood characteristics, while for pathogen damage, results were inconsistent between years (Table 3). In addition, our results suggest that herbivores are relatively insensitive to tree genotype, or that there are environment $\times$ genotype interactions such that the preferred genotype is dependent on environmental conditions. This conclusion is supported by results from a reciprocal transplant experiment which found no maternal family effects for herbivore damage (B.A. Roy and C. Mulder, unpublished data, 2005).

Relationships between herbivores and pathogens

All lines of evidence suggest that herbivores and pathogens are not directly affecting each other, at least not as groups. First, there was no significant correlation between herbivore damage and pathogen damage at any spatial scale. Second, there was no correlation between damage by one group early in the season and damage by the other group late in the season. Third, the variables that best explained variation in herbivore abundance (leaf characteristics) were different from those that best explained variation in pathogen abundance (neighbourhood characteristics) (Table 3). This does not mean that no one individual species from one group affects the other, but rather that at the community level there do not appear to be consistent interactions. Thus there is no reason to believe either that effects of changes in the environment will be similar across groups, or that changes in one will compensate for or exacerbate changes in the other.

Relationships between herbivore and pathogen damage and reproduction

As expected, greater damage by herbivores was correlated with lower investment in both male and female catkins: trees with > 15% of their leaf area damaged produced few (<10) female catkins per branch (Fig. 3A). Sites differed greatly in reproduction levels, and at least for male catkins this may be driven in part by differences in levels of herbivory by moose, who consume alder primarily at sites where willow (*Salix* spp.) are absent (personal observation), and whose browsing results in a release from apical dominance and a subsequent increase in the number of meristems (personal observation). Despite the very large differences between sites in reproduction, this pattern of lower reproduction with higher damage was not solely driven by site differences; the same pattern held within sites as well (Fig. 3B). We cannot determine whether this is because trees that are in poor general health are both more susceptible to herbivores and less able to invest in defense, or whether herbivore damage directly affects reproductive output. Calculating catkin number on a per-leaf-mass basis did not really clarify this: there was a negative relationship between this variable and herbivore damage when examined across sites but not within site (although the direction was still negative). Our measurement of fitness is necessarily an estimate, given that it is not possible to measure lifetime reproduction of a long-lived perennial species. Furthermore, we did not measure additional fitness components such as seed number or viability. However, our data do suggest that high levels of herbivore damage may reduce reproductive fitness.

Pathogen damage was also correlated with catkin production but, unexpectedly, the relationship was positive both across and within sites (Figs. 3C and 3D). This is particularly surprising given the lower levels of pathogen damage compared with herbivore damage, although we were only able to estimate damage on the leaf surface, not within the leaf. Furthermore, even when examined on a per-leaf-mass basis, the relationship held, at least across sites. There are several possible explanations for this relationship. First, if the primary impact of pathogens is to reduce resource availability, then the impacts on reproduction may not be detectable until later years, and there may be a negative relationship between catkin production in 2003 and pathogen damage during the previous year(s). This would explain the lack of a relationship (since trees show little consistency between years in pathogen levels), but it does not explain a positive relationship in 2003. Alternatively, the causal relationship may be in the opposite direction: high catkin production may represent greater general health, and such trees may also provide better conditions for pathogens. If pathogens thrive on more vigorous trees, then one might expect that within sites, larger trees would also have more catkins and higher pathogen damage (within sites, larger size is likely to represent more vigorous growth, since alder recruitment opportunities appear to be rare and trees are likely to be of very similar age). There is little support for this as there was a positive relationship between basal area and pathogen damage levels, but only for one year (2004) and only at the between-site level (Table 3), and there was no relationship between plant size and catkin production. Third, alders may be able to tolerate or compensate for pathogen

damage. Tolerance to damage is not uncommon for herbivory (Strauss and Agrawal 1999) and it has also been reported for some pathogens (Roy and Kirchner 2000; Roy et al. 2001). The increase in the female to male sex ratio associated with pathogen damage is intriguing, and points to a potential role of fungal hormones. Fungal pathogens are known to cause many hormonally regulated changes in plants (Agrios 1988). For example, *Puccinia monoica* and *Puccinia thlaspeos*, which cause flower-like structures to form on host plants (Roy and Widmer 1999), produce both auxin-like and cytokinin-like compounds (Stirk et al. 2006). Fruitful areas for future research include determination of mechanisms that may be altering sex ratios and enabling compensation for pathogen damage.

Implications for damage levels changes under climate change and directions for future research

There are two ways in which the data can give some insight into mechanisms through which climate change might affect the levels of herbivore and pathogen damage experienced by plants. First, plants were selected from sites that differed greatly in environmental characteristics in the hope that this would also result in large differences in plant and community characteristics; this is supported by the very large site differences in leaf variables (Fig. 2C) and by the fact that trees were consistent across years in leaf morphology (a reflection of consistent environmental conditions). This allowed us to examine the extent to which these variables affect herbivore and pathogen damage; if fast-responding variables such as leaf morphology and chemistry are good predictors of damage levels, then we might expect to see rapid indirect effects of climate change that are mediated by plant responses. Second, the extremely hot, dry, and smoky conditions experienced during the summer of 2004 represent an extreme event of the type that is predicted to occur with greater frequency in Interior Alaska in the future (Soja et al. 2007). Thus, the comparison between 2003 and 2004 is more interesting than between most sets of years: if we detect no differences between those two years in either leaf characteristics or herbivore and pathogen damage, then those variables are relatively insensitive to environmental conditions (and possibly climate change in general).

We found evidence for relationships between leaf morphology or chemistry and levels of damage by the most common herbivore guilds (sucking and chewing insects) (Table 3), but very little evidence that tree size, host density, or density of potential alternative hosts could explain damage levels. Thus, there is the potential for any impacts of climate change on host chemistry or morphology to also affect herbivory levels. However, leaf characteristics are expected to respond rapidly to changes in this deciduous plant, so it is unlikely that this will introduce long lagtimes between impacts of climate change and herbivore responses. Furthermore, the two guilds are likely to respond very differently to changes in leaf characteristics. Sucking insects appear to be most sensitive to variables associated with water stress; although the large drop in damage levels in 2004 compared with 2003 could simply be the result of interannual variation unrelated to environmental conditions, it is entirely consistent with the within-year relationships and

evidence from the literature. Any reduction in damage levels by sucking insects under greater drought stress may be countered by increased damage by other herbivore guilds, particularly chewing insects, which do not appear to be sensitive to drought stress. We found that high herbivore damage levels are correlated with reduced catkin production; further research on changes in fitness and mechanisms underlying these changes would help elucidate the potential for indirect effects of climate change on plant fitness via herbivore damage.

We found little evidence for the potential for climate change to alter herbivore damage via variables that change at much slower rates, such as tree morphology or neighbourhood characteristics. There was evidence that pathogen damage was affected by the proximity of other deciduous trees, but it was not consistent between years. However, the question of how plant community structure affects levels of pathogen damage merits further attention.

For all guilds except leaf-rolling insects, there were large differences in damage levels between sites that could not be explained by any of the variables measured, nor by spatial distribution of trees or sites. The most likely explanation is that there are direct effects of different environmental conditions on parasite populations. Future studies should distinguish between herbivore and pathogen damage. There was no evidence for either a positive or a negative relationship between herbivore and pathogen damage, and impacts of each on catkin production were opposite. Thus total impacts on reproduction may depend, in part, in changes to the relative importance of herbivore vs. pathogen damage.

Acknowledgements

We thank M. Burrell, E. Glenn, J. Keay, J. Rohrs-Richey, and J. Stewart for help in the field and lab. Stable isotope analyses were performed by T. Howe at the Alaska Stable Isotope Facility. Research sites were established at the Bonanza Creek and Poker-Caribou creeks LTER sites and the on the University of Alaska Fairbanks North Campus; we thank them for their support. We thank the following landowners and residents for allowing us to establish additional sites on their property: W.S. Armbruster and M. Edwards; S. Grocott and J. Ross; T. Luick; H. McIntyre; R. Ott and S. Pence; A. Prichard and A. Larson; R. and D. Ruess; D. Uliassi. The comments of two anonymous reviewers improved the manuscript. This research was supported by the Office of Science, Biological, and Environmental Research Program (BER), US Department of Energy, through the Western Regional Center of the National Institute for Global Environmental Change (NIGEC) under Cooperative Agreement No. DE-FC02-03ER63613. Financial support does not constitute an endorsement by DOE of the views expressed in this article.

References

- Agrawal, A.A. 1998. Induced responses to herbivory and increased plant performance. *Science* (Wash.), 279: 1201-1202. doi:10.1126/science.279.5354.1201. PMID:9469809.
- Agrios, G.N. 1988. *Plant pathology*. Academic Press, San Diego, Calif.
- Ayres, M.P., and Lombardero, M.J. 2000. Assessing the consequences of global change for forest disturbance from herbivores

- and pathogens. *Sci. Total Environ.* 262: 263-286. doi:10.1016/S0048-9697(00)00528-3. PMID: 11087032.
- Bale, J.S., Masters, G.J., Hodkinson, I.D., Awmack, C., Bezemer, T.M., Brown, V.K., Butterfield, J., Buse, A., Coulson, J.C., Farrar, I., Good, J.E.G., Harrington, R., Hartley, S., Jones, T.H., Lindroth, R.L., Press, M.C., Symmioudis, I., Watt, A.D., and Whittaker, I.B. 2002. Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. *Glob. Change Biol.* 8: 1-16. doi: 10.1046/j.1365-2486.2002.00451.x.
- Bazzaz, F.A. 1979. The physiological ecology of plant succession. *Annu. Rev. Ecol. Syst.* 10: 351-371. doi:10.1146/annurev.es.10.110179.002031.
- Beaudet, M., and Messier, e. 1998. Growth and morphological responses of yellow birch, sugar maple, and beech seedlings growing under a natural light gradient. *Can. J. For. Res.* 28: 1007-1015. doi:10.1139/cjfr-28-7-1007.
- Biere, A., Elzinga, J.A., Honders, S.C., and Harvey, J.A. 2002. A plant pathogen reduces the enemy-free space of an insect herbivore on a shared host plant. *Proc. R. Soc. Lond. B. Biol. Sci.* 269: 2197-2204. doi:10.1098/rspb.2002.2147.
- Biere, A., Marak, H.B., and van Damme, J.M.M. 2004. Plant chemical defense against herbivores and pathogens: generalized defense or trade-offs? *Oecologia (Berl.)*, 140: 430-441. doi:10.1007/s00442-004-1603-6.
- Bradley, D.J., Gilbert, G.S., and Parker, I.M. 2003. Susceptibility of clover species to fungal infection: The interaction of leaf surface traits and environment. *Am. J. Bot.* 90: 857-864.
- Burdon, J.J. 1987. *Diseases and Plant Population Biology*. Cambridge University Press, Cambridge, UK.
- Burdon, J.I. 1993. The structure of pathogen populations in natural plant communities. *Annu. Rev. Phytopathol.* 31: 305-323. doi: 10.1146/annurev.py.31.1090193.001513.
- Caldeira, M.e., Ryel, R.J., Lawton, J.H., and Pereira, J.S. 2001. Mechanisms of positive biodiversity-production relationships: insights provided by $\delta^{13}\text{C}$ analysis in experimental Mediterranean grassland plots. *Ecol. Lett.* 4: 439-443. doi:10.1046/j.1461-0248.2001.00238.x.
- Chakraborty, S., and Datta, S. 2003. How will plant pathogens adapt to host plant resistance at elevated CO_2 under a changing climate? *New Phytol.* 159: 733-742. doi:10.1046/j.1469-8137.2003.00842.x.
- Coakley, S.M., Scherm, H., and Chakraborty, S. 1999. Climate change and plant disease management. *Annu. Rev. Phytopathol.* 37: 399-426. doi: 10.1146/annurev.phyto.37.1.399. PMID: 11701829.
- Coley, P.D. 1983. Herbivory and defensive characteristics of tree species in a lowland tropical forest. *Ecol. Monogr.* 53: 209-233. doi: 10.2307/1942495.
- Crawley, M.J. 1993. *Herbivory, the dynamics of animal-plant interactions*. University of California Press, Berkeley, Calif.
- Dobson, A., and Crawley, M. 1994. Pathogens and the structure of plant communities. *Trends Ecol. Evol.* 9: 393-398. doi:10.1016/0169-5347(94)90062-0.
- Ehrlinger, J.E., and Cooper, T.A. 1988. Correlations between carbon isotope ratio and microhabitat in desert plants. *Oecologia (Berl.)*, 76: 562-566.
- Ehrlich, P.R., and Raven, P.H. 1964. Butterflies and plants: a study in coevolution. *Evolution*, 18: 586-608. doi:10.2307/2406212.
- Furrow, J.J. 1987. *Betulaceae*. In *Flora of North America North of Mexico*, Vol. 3. Edited by F.O.N.A.E. Committee. Oxford University Press, New York, N.Y. pp. 507-508.
- Gershenson, J. 1984. Changes in the levels of plant secondary metabolites under water and nutrient stress. *Recent Adv. Phytochem.* 18: 273-320.
- Hatcher, P.E. 1995. Three-way interactions between plant pathogenic fungi, herbivorous insects and their host plants. *Biol. Rev. (Camb)*, 70: 639-694. doi:10.1111/j.1469-185X.1995.tb01655.x.
- Holsten, E.H., Hennon, P., Trummer, L., and Schultz, M. 2001. *Insects and Diseases of Alaska Forests*. United States Department of Agriculture, Forest Service, Alaska Region, Anchorage, Alaska.
- Huberty, A.F., and Denno, R.F. 2004. Plant water stress and its consequences for herbivorous insects: a new synthesis. *Ecology*, 85: 1383-1398. doi:10.1890/103-0352.
- Hulten, E. 1968. *Flora of Alaska and neighboring territories: a manual of the flowering plants*. Stanford University Press, Stanford, Calif.
- Jones, C.G., and Coleman, J.S. 1991. Plant stress and insect herbivory: toward an integrated perspective. In *Response of plants to multiple stresses*. Edited by H.A. Mooney, W.E. Winner, and E.J. Pell. Academic Press, San Diego, Calif. pp. 249-280.
- Jones, J.D.G., and Dang, J.L. 2006. The plant immune system. *Nature (London)*, 444: 323-329. doi:10.1038/nature05286. PMID: 17108957.
- Johnson, S.N., Douglas, A.E., Woodward, S., and Hartley, S.E. 2003. Microbial impacts on plant-herbivore interactions: the indirect effects of a birch pathogen on a birch aphid. *Oecologia (Berl.)*, 134: 388-396.
- Kranz, I. 1990. Fungal diseases in multispecies plant communities. *New Phytol.* 116: 383-405. doi:10.1111/j.1469-8137.1990.tb00525.x.
- Larcher, W. 1980. *Plant physiological ecology*. Springer-Verlag, Berlin, Germany.
- Larsson, S. 1989. Stressful times for the plant stress hypothesis. *Oikos*, 56: 277-283. doi:10.2307/3565348.
- Loach, K. 1970. Shade tolerance in tree seedlings II. Growth analysis of plants raised under artificial shade. *New Phytol.* 69: 273-286. doi: 10.1111/j.1469-8137.1970.tb02426.x.
- Mantel, N. 1967. The detection of disease clustering and a generalized regression approach. *Cancer Res.* 27: 209-220. PMID:6018555.
- Marquis, R.J., and Alexander, H.M. 1992. Evolution of resistance and virulence in plant-herbivore and plant-pathogen interactions. *Trends Ecol. Evol.* 7: 126-129. doi:10.1016/0169-5347(92)90147-4.
- Malmstrom, C.M., and Raffa, K.F. 2000. Biotic disturbance agents in the boreal forest: considerations for vegetation change models. *Glob. Change Biol.* 6(Suppl.1): 35-48. doi:10.1046/j.1365-2486.2000.06012.x.
- Mattson, W.J. 1980. Herbivory in relation to plant nitrogen content. *Annu. Rev. Ecol. Syst.* 11: 119-161. doi:10.1146/annurev.es.11.110180.001003.
- Mattson, W.J. and Haack, R.A. 1987. The role of drought in the outbreak of plant-eating insects. *Bioscience*, 37: 110-118. doi: 10.2307/1310365.
- Miyashita, T., and Kawanishi, K. 2003. Effects of rice leaf position and growth stage on larval development and survivorship of the rice leaf roller *Cnaphalocrocis medinalis* (Guenee) (Lepidoptera: Pyralidae). *Jpn. J. Appl. Entomol. Zool.* 47: 85-90. doi: 10.13031/jjaez.2003.85.
- Nakamura, R.R., Mitchell-Olds, T., Manasse, R.R., and Lello, D. 1995. Seed predation, pathogen infection and life-history traits in *Brassica rapa*. *Oecologia (Berl.)*, 102: 324-328. doi:10.1007/BF00329799.
- Niernala, P., Chapin, F.S., III, Danell, K., and Bryant, J.P. 2001. Herbivory-mediated responses of selected boreal forests to climatic change. *Clim. Change*, 48: 427-440. doi:10.1023/A:1010787714349.

- Niinemets, U., and Kull, K. 1994. Leaf weight per area and leaf size of 85 Estonian woody species in relation to shade tolerance and light availability. *For. Eco! Manage.* 70: 1-10. doi:10.1016/0378-1127(94)90070-1.
- Parker, I.M., and Gilbert, G.S. 2004. The evolutionary ecology of novel plant-pathogen interactions. *Annu. Rev. Eco! Syst.* 35: 675-700. doi:10.1146/annurev.ecolsys.34.011802.132339.
- Peeters, P.J. 2002. Correlations between leaf structural traits and the densities of herbivorous insect guilds. *Bio! J. Linn. Soc.* 77: 43-65. doi:10.1046/j.1095-8312.2002.00091.x.
- Popma, J., and Bongers, F. 1988. The effect of canopy gaps on growth and morphology of seedlings of rain forest species. *Oecologia (Berl.)*, 75: 625-632. doi:10.1007/BF00776429.
- Roy, B.A., and Widmer, A. 1999. Floral mimicry: a fascinating yet poorly understood phenomenon. *Trends Plant Sci.* 4: 325-330. doi:10.1016/S1360-1385(99)01445-4. PMID:10431223.
- Roy, B.A., and Kirchner, I.W. 2000. Evolutionary dynamics of pathogen resistance and tolerance. *Evolution*, 54: 51-63. PMID:10937183.
- Roy, B.A., Kirchner, I.W., Christian, C., and Rose, L. 2001. High disease incidence and apparent tolerance in a North American Great Basin plant community. *Evo! Eco!.* 14: 421-438. doi:10.1023/A:1010997429365.
- Roy, B.A., Giisewell, S., and Harte, J. 2004. Response of plant pathogens and herbivores to a warming experiment. *Ecology*, 85: 2570-2581. doi:10.1890/103-0182.
- Scherm, H. 2004. Climate change: can we predict the impacts on plant pathology and pest management? *Can. J. Plant Pathol.* 26: 267-273.
- Scriber, J.M., and Slansky, F., Jr. 1981. The nutritional ecology of immature insects. *Annu. Rev. Entomol.* 26: 183-211. doi:10.1146/annurev.en.26.010181.001151.
- Siemens, D.H., and Mitchell-Olds, T. 1998. Evolution of pest-induced differences in *Brassica* plants: tests of theory. *Ecol.* 79: 632-646.
- Siemens, D.H., and Roy, B.A. 2005. Tests for parasite-mediated frequency-dependent selection in natural populations of an asexual plant species. *Evol. Ecol.* 19: 321-338. doi:10.1007/s10682-005-6639-5.
- Simms, E.L., and Rausher, M.D. 1993. Patterns of selection on phytophage resistance in *Ipomoea purpurea*. *Evolution*, 47: 970-976. doi:10.2307/12410202.
- Soja, A.J., Tchepakova, N.M., French, N.H.F., Flannigan, M.D., Shugart, H.H., Stocks, B.L., Sukhinin, A.I., Varfenova, E.I., Chapin, F.S., and Stackhouse, P.W. 2007. climate-induced boreal forest change: predictions versus current observations. *Global Planet. Change*, 56: 274-296. doi:10.1016/j.gloplacha.2006.07.028.
- Stirk, W.A., Thomson, S.V., and van Staden, J. 2006. Effect of rust-causing pathogen (*Puccinia thlaspeos*) on auxin-like and cytokinin-like activity in dyer's woad (*Isatis tinctoria*). *Weed Sci.* 54: 815-820. doi:10.1614/IWS-06-034R.1.
- Stinchcombe, J.R., and Rausher, M.D. 2001. Diffuse selection on resistance to deer herbivory in the ivyleaf morning glory, *Ipomoea hederacea*. *Am. Nat.* 158: 376-388. doi:10.1086/321990.
- Strauss, S.Y., and Agrawal, A.A. 1999. The ecology and evolution of plant tolerance to herbivory. *Trends Ecol. Evol.* 14: 179-185. doi:10.1016/S0169-5347(98)01576-6. PMID:10322530.
- Taylor, J.E., Hatcher, P.E., and Paul, N.D. 2004. Crosstalk between plant responses to pathogens and herbivores: a view from the outside in. *J. Exp. Bot.* 55: 159-168. doi:10.1093/jxb/erh053. PMID:14673022.
- Thaler, I.S., Karban, R., Ullman, D.E., Boege, K., and Bostock, R.M. 2002. Cross-talk between jasmonate and salicylate plant defense pathways: effects on several plant parasites. *Oecologia (Berl.)*, 131: 227-235. doi:10.1007/s00442-002-0885-9.
- Toft, N.L., Anderson, J.E., and Nowak, R.S. 1989. Water use efficiency and carbon isotope composition of plants in a cold desert environment. *Oecologia (Berl.)*, 80: 11-18. doi:10.1007/BF00789925.
- Vanderklein, D.W., Wilkens, R.T., Cartier, A., and Lemke, R.W. 2004. Plant architecture and leaf damage in Bear Oak. I: Physiological responses. *Northeast Nat.*, 11: 343-356.
- Viereck, L.A., and Little, E.L. 1986. Alaska trees and shrubs. University of Alaska Press, Fairbanks, Alaska.
- Wearing, C.H. 1998. Bioassays for measuring ovipositional and larval preferences of leafrollers (Lepidoptera: Tortricidae) for different cultivars of apple. *N Z J. Crop Hortic Sci.* 26: 269-278.
- Werner, R.A., and Holsten, E.H. 1985. Factors influencing generation times of spruce beetles in Alaska. *Can. J. For. Res.* 15: 438-443.
- Werner, R.A., Raffa, K.F., and Tilman, B.L. (Editors). 2006. Dynamics of phytophagous insects and their pathogens in Alaskan boreal forests. Oxford University Press, New York, N.Y.
- Weste, G., Brown, K., Kennedy, J., and Walshe, T. 2002. *Phytophthora cinnamomi* infestation - a 24-year study of vegetation change in forests and woodlands of the Grampians Western Victoria. *Aust. J. Bot.* 50: 247-274.
- White, T.C.R. 1984. The abundance of invertebrate herbivores in relation to the availability of nitrogen in stressed food plants. *Oecologia (Berl.)*, 63: 90-105. doi:10.1007/BF00379790.
- White, T.C.R. 1993. The inadequate environment: nitrogen and the abundance of animals. Springer-Verlag, New York, N.Y.
- Wimp, G.M., Martinsen, G.D., Floate, K.D., Bangert, R.K., and Whitham, T.G. 2005. Plant genetic determinants of arthropod community structure and diversity. *Evolution*, 59: 61-69. PMID:15792227.