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Susceptibility of Norway Spruce Clones (*Picea abies* (L.) Karst.) to Insects and Roe Deer in Relation to Genotype and Foliar Phytochemistry

By

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Summary

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In a 20-year-old plantation of Norway spruce, there was substantial variation among clones in the amount of herbivory from several insect species and from roe deer. Genotypes of all 46 clones were characterized by isozyme markers at 23 polymorphic loci, and 12 clones were also described by chemical composition of foliage (e.g. amino acids, carbohydrates, and phenols).

Among the genotypes there were strong relationships between single isozyme loci and foliar chemical composition and degree of insect or game attack. The results emphasize the need to maintain high genetic variability in forest tree populations. Genetic variation in predisposition to insect and mammal attack is one precondition for long-term stability of forest ecosystems.

Introduction

The susceptibility of trees to insect attack and browsing by mammals is influenced by numerous genotypic and environmental factors (MORAN 1981, MADDOX & CAPUCCINO 1986). On the one hand, many studies have addressed the influence of environmental factors on the predisposition of trees to herbivore attack (review by KAETZEL & MOELLER 1995). On the other hand, little is known on the interaction between genotype, needle compounds, predisposition and intensity of herbivory. More than 40 years of resistance research in forest ecosystems had

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shown that the susceptibility of trees to herbivores is determined in large part by their composition of compounds with nutritional value (e.g. carbohydrates, amino acids) and defense function (e.g. phenols, terpenes, phytoalexins). Because each herbivore species differs in its nutrient requirements and tolerance of defensive compounds, predisposition of each genotype to pests has to be analyzed individually (SCHOPF 1986, MARQUIS 1992). We report the results of studies of genetic differences in susceptibility of Norway spruce clones to some insect species and roe deer.

Material and Methods

Plant material

A Norway spruce (*Picea abies* (L.) Karst.) plantation was established near Waldsieversdorf (Brandenburg) between 1979 and 1981 (WEISER & SCHACHLER 1988) using 59 selected clones with a maximum number of 40 ramets per clone. They originated from a region strongly influenced by SO₂ in the upper part of the Saxon Ore Mountains. The clones were selected in the early seventies by TZSCHACKSCH 1983 and were propagated by cuttings.

All individuals of a clone are growing in one row or in two neighbouring rows. The influence of site on the phenotypic expression of single trees is lower than 10 per cent and can be ignored (tested by multiple correlation). This investigation was conducted on approximately 20-year-old trees, and included 46 clones with 11 to 37 ramets per clone (mean = 25 ramets).

Assessment of infestation level

Degree of infestation by the following insect species were evaluated on a total of 1150 trees belonging to 46 clones from June 21 to June 27 in 1995: spruce gall aphids *Sacchiphantes spec.* and *Adelges laricis* Vall., red and gray forms of the bark louse *Cinaropsis pilicornis* Htg., mealy-bug *Gilleteella cooleyi* Gill., leaf-wasp *Pristiphora abietina* Christ., and *Epinotia tedella* Cl.. Additionally, the browsing by roe deer (*Capreolus capreolus* L.) was scored. Compared with other years, the insect infestation was very high in 1995. Percent of infested trees per clone was estimated for every trait.

Genetic characterization

The genetic characterization of spruce clones by isozyme markers was carried out on bud and megagametophyte tissue. The electrophoretic separation of enzyme proteins in starch and polyacrylamide gels, staining solutions and the interpretation of banding patterns are described by KONNERT & MAURER 1995 and HERTEL & EWALD 1995. Altogether, 23 polymorphic gene loci were evaluated. Isozyme markers can be used to identify classes of clones with identical genotypes at single marker loci. In the case of two alleles at one gene locus, three different genotypes are possible (two homozygotes and one heterozygote), which form three classes.

Phytochemistry

Branches from 8 ramets of each of 12 clones with were collected on July 3, 1995 and stored at -80°C. A detailed description of the analytical methods is given in KAETZEL & MOELLER 1995. The following biochemical parameters were determined in current year but full expanded needles: chlorophyll a and b, carotinoide, ratio of chlorophyll a/b, ratio of chlorophyll/carotinoide, total soluble carbohydrates, total soluble amino acids, proline, ratio of carbohydrates/amino acids, total phenols, procyanidine, ortho-dihydroxy-phenols, vanillin positive compounds, absorbance at 280 nm (extraction in 50 % v/v methanol), and condensation degree of phenols (= total phenols / vanillin positive compounds * absorbance 280 nm, SCHOPF 1986).

Statistical Analysis

Multiple comparisons of medians between clones or between genotype classes were carried out with the non-parametric KRUSKAL-WALLIS Test (Statistical Analysis System Institute Inc. USA, SAS procedure NPAR1WAY).

Results

Infestation level in relation to clone and genotype at single gene loci

Long-term observations have revealed remarkable differences between the clones in their degree of infestation by several insect species. Even single ramets (identified by time of bud burst, morphological flower traits, and isozyme markers) of a resistant clone were avoided by roe deer or insects when growing among a completely attacked clone. Conversely, ramets of susceptible clones were attacked when growing among an avoided clone. Levels of infestation per clone for every trait in 1995 are presented in table 1.

Table 1. Percent of trees within clones infested by the following herbivores: 1 — *Sacchiphantes spec.*, 2 — *Adelges laricis*, 3 — *Cinaropsis pilicornis (red)*, 4 — *Cinaropsis pilicornis (gray)*, 5 — *Gilleteella cooleyi*, 6 — *Pristiphora abietina*, 7 — *Epinotia tedella*, 8 — *Capreolus capreolus*

Clone	1	2	3	4	5	6	7	8
147	0	.	37	.	7	100	4	0
192	3	0	19	.	100	35	3	0
1176	95	0	18	.	9	100	0	27
220	61	33	6	0	0	100	6	50
222	52	0	4	4	52	88	0	68
278	81	0	29	19	19	76	0	29
292	49	37	46	0	100	11	6	3
335	42	12	58	23	0	46	8	46
336	71	10	14	0	5	24	5	24
346	45	0	0	0	0	64	0	55
349	14	8	89	36	17	50	8	22
354	6	3	71	11	100	49	3	100

For the locus NADH-dehydrogenase-B, clones with the homozygote genotype BIB1 were consistently infested with the spruce gall adelgid *Adelges laricis* (Fig. 1). In comparison, clones with the genotype B2B2 were nearly resistant, and clones with the heterozygote genotype B1B2 had an intermediate

level of infestation. Clones with the allele A4 at the locus leucine aminopeptidase-A (Fig. 2), independent of whether it was homozygous or heterozygous condition, were more attractive to roe deer than were clones without this allele. In both cases, a significant correspondence (KRUSKAL-WALLIS Test, $\alpha < 0.05$) between a single gene marker and the phenotypic trait was detected. This does not mean, however, that the respective enzyme necessarily influenced resistance. Other genes in the same or other linkage groups might be involved.

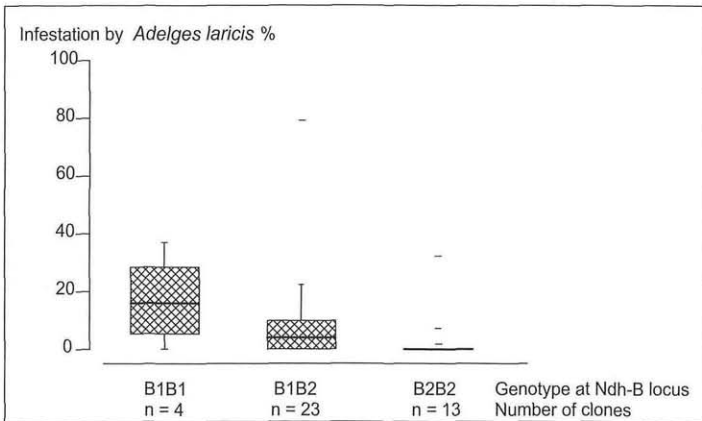


Fig. 1. Box and whisker plot of percent of trees within Norway spruce clones infested with the spruce gall adelgid *Adelges laricis* in relation to genotype at the locus Ndh-B.

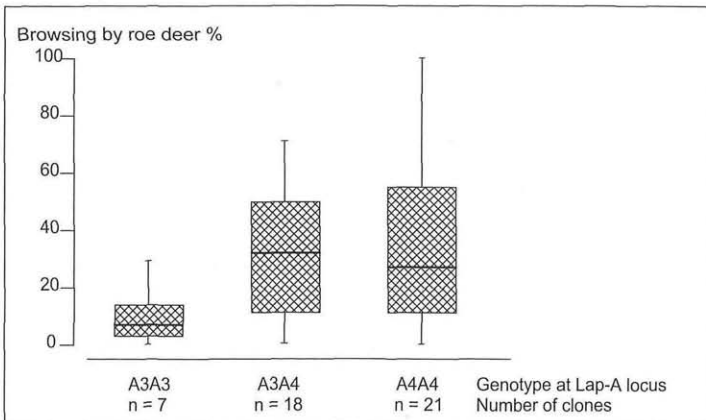


Fig. 2. Box and whisker plot of the percent of trees within Norway spruce clones that were browsed by roe deer in relation to genotype at the locus Lap-A.

Foliar phytochemistry in relation to genotype at single isozyme loci

Highly significant differences between clones were detected for all analyzed needle compounds and their ratios (multiple KRUSKAL-WALLIS Test, $\alpha < 0.001$). Distinguishable patterns of phenolic compounds were observed between clones (Fig. 3). Concentrations of amino acids, including proline, indicate that the trees are growing under sufficient nutrient conditions and without stress.

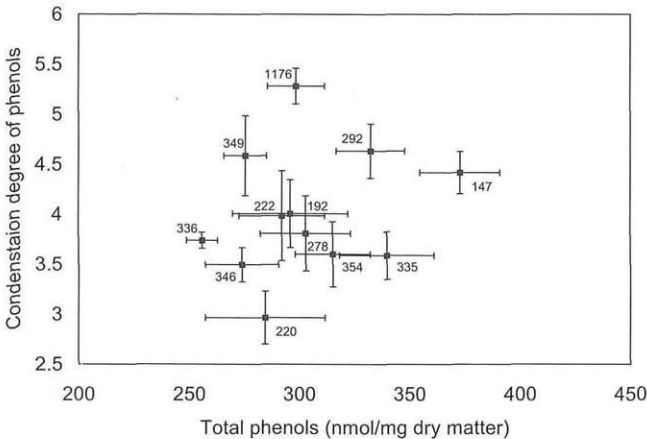


Fig. 3. Total phenolic concentration and condensation degree of phenols in young needles of 12 Norway spruce clones (average and standard deviation of 8 ramets for each clone).

The most conspicuous result was the strong correspondence between carbohydrate concentration and the phosphoglucose isomerase (Pgi-B) locus. Clones with the homozygous genotype B3B3 contain remarkably higher concentrations of carbohydrates than clones with the heterozygous genotype B2B3 (Fig. 4). Since enzyme phosphoglucose isomerase is involved in primary carbohydrate metabolism, this marker enzyme could directly influence this biochemical trait. Unfortunately, clones with the homozygote genotype B2B2 have not yet been analyzed.

All clones highly infested with the leaf-wasp *Pristiphora abietina* (clones 147, 1176, 220, and 222) possess high levels of total chlorophyll (>2.0 mg/g dry matter) and low contents of soluble carbohydrates (maximum 110 mg/g dry matter). In contrast, clones with low chlorophyll content (<1.8 mg/g) and high levels of carbohydrates (>120 mg/g) had lower levels of leaf-wasp infestation (clones 292, 335, 346, 349, and 354).

Clones 147, 192 and 292 were avoided by roe deer, while clones 354, 222, 220, 346 and 335 showed substantial browsing damage. These two groups of spruce clones can be distinguished by their phenolic composition of needles (no browse: high concentration of procyanidine and high absorbance at 280 nm, high browse: lower concentration of procyanidine and low absorbance at 280 nm).

There was no relationship between amino acid and carbohydrate composition and browsing intensity.

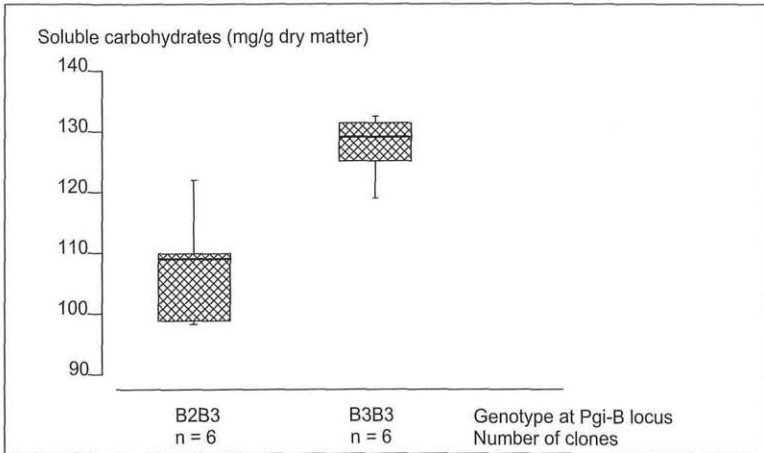


Fig. 4. Box and whisker plot of soluble carbohydrate concentration in relation to genotype at the Pgi-B locus (significant difference between the genotypes at the 0.01 level).

Discussion

Norway spruce clones growing under nearly identical environmental conditions could be differentiated by genotype, foliar phytochemistry, and degree of insect and roe deer herbivory. The decisive influence of genotype on infestation level was shown for two cases. There was a significant relationship between specific isozyme gene markers and attack by spruce gall adelgid *Adelges laricis* and by roe deer. For Scots pine and beech, MOPPER & al. 1991 and GORA & al. 1994 described the dependence of insect attack on genotypes at single isozyme gene loci in individual trees but not clones. The use of clonal material makes it easier to correlate patterns of biochemical compounds with susceptibility to particular herbivores.

The detection of clone-specific patterns of primary and secondary metabolites is very important for biochemical and physiological investigations, which often do not adequately consider genetic variation.

However, clone-specific patterns in qualitative terpene analyses of needles and bark did not correlate with infestation level by any herbivores in this study (LANG unpublished). It is possible that compounds that were not analyzed also have an effect on predisposition of spruce clones to herbivores.

More detailed biochemical characterization of the complete set of 46 clones is underway to test for further correspondence between genotypes at single isozyme loci, organic needle compounds and susceptibility to herbivores.

Regarding the frequency and intensity of excessive insect multiplication and high game populations in the forests, however, our preliminary results underline the necessity of maintaining high genetic variability in forest tree populations in the face of intense pressure from numerous pests. A sufficient level of genetic variation is considered as prerequisite for long-term stability of host-parasite interactions and complex forest ecosystems.

A c k n o w l e d g m e n t s

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