TRACKING RESISTANCE GENES AGAINST THE GREEN SPRUCE APHID

Elise Skov[1], Hubert Wellendorf[1], and Mogens Lind Jorgensen[2]

OBJECTIVES

i): Identification of genetic markers in *Picea sitchensis* linked to major genes behind resistance against the greene spruce aphid and ii): Perspectives for implementation of marker aided selection in breeding strategies.

BACKGROUND

The original host for the aphid is believed to be Norway spruce. In Europe co-evolution between the host and the insect has developed into a stable balance, where Norway spruce is not showing serious symptoms of needle loss. For the introduced N-American *Picea sitchensis, glauca,* and *pungens,* the aphid is causing severe needle loss after mild winters in NW-Europe. In the mild climate here, the aphid develops a very effective asexual reproduction which exploits the mild early spring for vigorous reproduction (Bejer-Petersen, 1962). In contrast, sexual reproduction is occurring in continental parts of Europe (Kloft et al. 1964, von Scheller, 1963) where wintering eggs are able to resist severe winter frost prevailing here. Genetic diversity in the aphid populations is detected with RAPD-markers in NW-Europe (Sigurdsson et al. 1998 - personal communication).

The importance of the pest is judged to be increasing due to climatic changes in the direction of milder winters in NW-Europe. The associated needle loss causes in severe cases mortality especially in combination with late spring frost - otherwise the damage is limited to loss of increment.

Keywords: *Picea sitchensis, Elatobium abietinum,* RAPD, resistance, linkage, breeding strategy.

MATERIAL AND METHODS

Plant material and field observations

The investigation is carried out in 2 of 15 open-pollinated families in which phenotypic variation in aphid attack has been recorded in February 1990 (Jensen et al 1997). Population size was 60 in each family. These families originate from the open pollinated mating in 1968 of 15 clones present in a seed orchard, FP611 on the location Vosnæs in Eastern Jutland. Abundant flowering was recorded in the orchard that year and seed were harvested on all clones for the first time.

The ortets behind the parent clones were selected for resistance against the Sitka spruce aphid back in 1958. The realized gain in 1990 of this early selection has been demonstrated in the investigated open-pollinated progeny test, namely trial F154C at the plantation of Kase in North western Jutland (Jensen et al 1997). Here a general genetic gain of field resistance was demonstrated for the whole set of open-pollinated progeny from the 15 clones in the orchard. However a significant variation remained between families.

In the 1990 investigation, the aphid attack was visually judged as percentage of needles lost in the whole tree. Supplementary observations on aphid reproduction on individual trees were carried out in one of the investigated families in 1998. On each tree, two cages each with one 4th instar nymph was left for

[1]Royal Veterinary- and Agricultural University, Hørsholm Arboretum, Copenhagen, Denmark
[2]Royal Veterinary- and Agricultural University, Section of Zoology, Copenhagen, Denmark

reproduction for two months during the spring. Counting of nymphs and adults was then taken as an expression of natural aphid reproduction on individual trees within this particular family.

DNA extraction and the RAPD assay

The procedures of DNA-extraction (CTAB method, Bousquet et al. 1990, Carlson et al. 1991) and RAPD assay (Williams et al. 1990) have been modified by Skov (1998a, 1998b). RAPDs are highly polymorphic dominant markers obtained by PCR.

Two sets of 100 10-mer RAPD-primers each were obtained from John Carlson and John Hobbs, University of British Columbia and used for the extensive primer screening. The primers of these sets were selected from a ranking of primers for their ability to detect polymorphism in conifers. Additional 50 primers from Operon Technologies (Alameda, California) series A, F, G, J, and Y were used based on earlier experience in *Picea abies* (Skov 1998a; Skov and Wellendorf 1998).

PLAN AND RESULTS OF THE INVESTIGATION

Within the two open-pollinated offspring families, which were heavily attacked by the green spruce aphid in 1989, 1000 dominant RAPD-markers distributed to 250 primers have been screened for co-segregation with recorded field resistance. The screening was performed in 3 steps, i) by bulk-segregant analysis (Michelmore et al. 1991), ii) by selective genotyping of candidate markers identified during step 1, and iii) by whole family co-segregation analysis between resistance and candidate markers surviving the two initial steps. Three of these markers, one in the first family and two in the second family, co-segregated. In the first family further checks of the zygosity of the female parent performed on her haploid megagametophytes confirmed her heterozygosity in the marker locus. The allele frequency amongst the remaining orchard clones was unity for the recessive allele, i.e. a near-perfect test-cross situation was revealed for this particular marker. In the second family the two markers co-segregating with resistance segregated independent of each other. A highly significant interaction between these two markers concerning field resistance indicates epistatic gene effects between two non-linked resistance genes. Considering this evidence, it is concluded, that three RAPD-markers have been identified, each of which is linked to segregating resistance gene-loci coding for aphid resistance in the offspring from these two particular parent clones. The total number of detected resistance gene-loci is two, possible three. Average effects of each combination of resistant gene and linked marker covered a range of 0.6 - 1.2 times the within-family phenotypic standard deviation in field resistance.

Within the family in which two resistance genes showed interaction, aphid reproduction was recorded. No significant correlation occurred between needle loss and aphid reproduction. It was further tested if RAPD markers co-segregating with needle loss also co-segregated with aphid reproduction. This was the case for that combination of resistant gene and linked marker which partially covered the effect of the other combination.

PERSPECTIVES

Resistance mechanisms

Considering this evidence, a model is sketched for gene action for two resistant genes each coding for different resistance mechanisms. Resistance gene no 1 is coding for the most important resistance mechanism which has the double effect of retarding Aphid reproduction and limit needle loss. Resistance gene no 2 is coding for a secondary defense mechanism, which especially if resistance gene no 1 is absent, is effectively restricting the needle loss.

Breeding strategy

The obtained evidence of co-segregation between markers and QTL for resistance is used to demonstrate a case of combining family selection and within-family selection in 2. generation breeding. Marker aided selection is in this case used to broaden the genetic diversity.

ACKNOWLEDGEMENTS

Financing of the cited activities is partially provided by the EU - Fair proj ect, "Improving Protection and Resistance of Forests to the Spruce Aphid" - RESFORAPHID.

LITERATURE CITED

Bejer-Petersen, B. 1962. Peak years and regulation of numbers in the aphid *Neomyzaphis abietina* Walker. Oikos 13: 155-159.

Bousquet, J., L.Simon , and M.Lalonde. 1990. DNA amplification from vegetative and sexual tissues of trees using polymerase chain reaction. Canadian Journal of Forest Research. 20: 254-257.

Carlson, J. E., L.K.Tulsieram, J.C.Glaubitz, V.W.K.Luk, C.Kauffeldt, and R.Rutledge. 1991. Segregation of random amplified DNA markers in F_1 progeny of conifers. Theoretical and Applied Genetics. 83: 194-200.

Jensen, J. S., S. Harding, and H. Roulund. 1997. Resistance to the green spruce aphid *(Elatobium abietinum* Walker) in progenies of Sitka spruce *(Picea sitchensis* (Bong) Can.). Forest Ecology and Management. 97: 207-214.

Kloft, W.H., H. Kunkel und P. Erhardt. 1964. Weitere Beitrage zur Kenntnis der Fichtenröhrenlaus *Elatobium abietinum* (Walker) unter besonderer Berücksichtigung ihrer Weltverbreitung. Zeitschrift air angewandte Entomologie. 55: 160-185.

Michelmore, R. W., I.Paran, and R.V.Kesseli. 1991. Identification of markers linked to disease-resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregating populations. Proc.Natl.Acad.Sci.USA. 88: 9828-9832.

Skov, E. 1998a. Mendelian Inheritance and Tissue Expression of RAPD-markers in *Picea abies (L.)* Karst. Silvae Genetica. 47 (5-6): 262-270.

Skov, E. 1998b. Timing of DNA extraction from megagametophytes for PCR during initial steps of seedling developments in *Picea abies (L.)* Karst. Silvae Genetica. 47 (5-6): 270-273.

Skov, E. and H.Wellendorf . 1998). A Partial Linkage Map of *Picea abies (* L.) Karst. based on Recombination of RAPD-markers in Haploid Megagametophytes. Silvae Genetica. 47 (5-6): 273-282.

von Scheller, H.D. 1963. Zur Biologie und Schadwirkung der Nadelholzspinnmilbe *Oligonychus ununguis* Jacobi und der Sitkafichtenlaus *Liosomaphis abietina* Walker (Horn. Aphid.). Teil II: *Liosomaphis abietina* Walker. Zeitschrift für angewandte Entomologie. 51: 258-284.

Williams, J. G. K., A. R. Kubelik, K. J. Livak, J. A. Rafalski, and S. V. Tingey 1990. DNA polymorphisms Amplified by Arbitrary Primers Are Usefull as Genetic Markers. Nucleic Acid Research. 18: 6531-6535.