Resistance in hybrid aspen to pathogens

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Abstract

Wide-scale plantations of aspen *(Populus tremula)* and hybrid aspen *(P. tremula x Populus tremuloides*) have recently been established in Nordic and Baltic countries after the forest industry has become interested in aspen fibre. As the number of aspen stands increases, the fungal diseases will become economically and ecologically important. *Neofabraea populi* was recorded for the first time in Fennoscandia early in 1960's and subsequent observations of the disease were made later in 1970's. In 2000's, serious damage was observed in second generation of hybrid aspen in Finland. Since conditions in dense coppice stands are probably favourable for the spread of *N. populi*, the fungus could pose a potential threat for short-rotation coppices of hybrid aspen. To study the variation in the resistance of hybrid clones, artificial inoculations were made. The bark of a total of 100 trees (10 clones) was wounded and inocula were placed under the bark. The reactions of the trees and the advance of the cankers were recorded; resistance was considered to be expressed as healing of the cankers. In conclusion, hybrid aspen clones, despite of the fact that the original selection was based on only yield and fibre characteristics, show variability in resistance. A promising observation was made by combining the results from separate trials; the best-growing clone is one of the most resistant ones. Thus it seems likely that there are possibilities to select for both growth and resistance traits in breeding.

Introduction

European aspen (*Populus tremula* L.) is the most widespread poplar species and one of the most widely distributed tree species in the world. Aspen has been found in many diverse habitats throughout its distribution area. In Finland, aspen grows mostly in mixed stands dominated by conifers, and as such makes up only about 1.5 % of the total volume in Finnish forests (Finnish Statistical Yearbook of Forestry, 2001). Wide-scale plantations of aspen *(Populus tremula)* and hybrid aspen *(P. tremula x Populus tremuloides*) have recently been established in Nordic and Baltic countries after the forest industry has become interested in aspen fibre. As the number of aspen stands increases, the fungal diseases will get more important both economically and ecologically. Based on experience from agriculture and clonal forestry with poplars and willows, it is known that damages caused by the fungal diseases may increase as a result of the use of clonal monocultures. To ensure a sufficiently wide range of genetic variation, breeding populations with aspen and hybrid aspen are presently being established at Finnish Forest Research Institute (Metla).

Fig 1. Stem cankers two years after inoculation with N. populi*: A) suscpetible clone, B) resistant clone, C) control, which was inoculated with agar.*

Neofabraea populi Thompson (Thompson 1939) was observed in Norway in early 1960's only a decade after hybrid aspen was imported to Norway and the plantations were established (Semb & Hirvonen-Semb 1968, Roll-Hansen & Roll-Hansen 1969). In late 1960's family trials were surveyed and variation in disease incident was observed between hybrid aspen families (Langhammer 1971). Later in 1970's (Kurkela 1997) and in early in 2000's (Kasanen *et al*. 2002) observations on the same type of symptoms in several stands were recorded also in Finland. After molecular and morphological analyses, Kasanen *et al.* (2002) concluded that *N. populi* was the causal agent of canker disease. In this disease, 2nd generations of trees (root suckers) are seriously damaged; they bear cankers and dead bark. Infections appear as depressed areas in the bark. Later the bark in lesions splits longitudinally. Older cankers can be from 50 to 100 cm long, elliptical and girdling the stem for one-half or more of its circumference. The bark in the center of canker is slightly sunken and split vertically. Cankers can also appear as slightly sunken areas that completely encircle the stems without any callous formation. Since conditions in dense coppice stands are probably favourable for the spread of the cortical pathogen *N. populi*, the fungus could be a potential threat for hybrid aspen cultivation (Kasanen *et al.* 2002).

The breeding system used for aspen and hybrid aspen is time-consuming and expensive (large-scale field tests over the whole rotation period). Such large scale field trials are needed to fulfil the requirements of the EU regulations for marketing forest regeneration material that came into force from 1.1.2002. A method for pre-screening the material in the nursery for e.g. pathogen resistance, in order to exclude unsuitable clones before the field trials are established, would save a lot of costs. In an ongoing project at the Punkaharju Research Station (Metla) such a nursery testing for both family and clonal material of aspen and hybrid aspen is being developed. Both natural and artificial infection may be used to test for resistance in the nursery.

This paper describes the experimental set-up for testing the resistance in hybrid aspen to *N. populi*, briefly reports the preliminary results and finally combines the data from separate trials for growth measurements and resistance testing. The applicability of the results is discussed in relation to the possibility to select for both superior growth and resistance.

Materials and methods

Field trials

The field performance (height increment and viability) of numerous clones planted in late 1990's had been surveyed in 13 field trials, which in total include over 21000 seedlings. Ten hybrid aspen clones, which were in 1999 the most commonly used in forest regeneration, were subject to resistance testing.

The field trial for resistance testing was established in summer 2000 at Suonenjoki Research Station (Metla). A total of 1000 seedlings (10 clones) were planted in rows. Each row consisted of 10 repeats with 10 seedlings per repeat. The clones were placed in rows so that each row was started with a different clone, followed by others in numerical order. The experimental field located in poor sandy soil was fertilized prior to the experiment and occasional drought damages were excluded by watering.

Inoculations

A total of 110 inoculations were made in August 2003. In addition to ten fungal inoculations per clone, one control inoculation was made. Prior to inoculation, an L-shaped wounding (1 cm*2 cm) was cut with knife to the bark. The edge of the wounding was gently lifted and a 1cm*1 cm block of fungal culture (malt agar) was placed under the bark. The bark was closed and the wounding was sealed with parafilm. Control inoculations were made with sterile agar blocks. Prior to the experiment a pilot test was made in 2002 with similar methods. Only one fungal strain was used in the inoculation experiments.

Measurements

The dimensions of the canker (length, width) were measured one year after inoculation, and also diameter of the stem above the canker, breast-height diameter and height of each tree were measured.

Results and discussion

Five out of ten control seedlings, which were inoculated with agar only, were totally healed already one year after inoculation. Regarding seedlings inoculated with the pathogen, four out of ten trees of the most susceptible clone were girdled by the cankers (Fig 1). Although no statistical analysis was made in this preliminary analysis two conclusions can be made; i) the canker height was probably the best variable for describing variation in resistance (Fig 2) and ii) the differences in canker height are most likely statistically significant. As shown in Fig 3 the height increment was also highly variable between hybrid aspen and aspen clones and families.

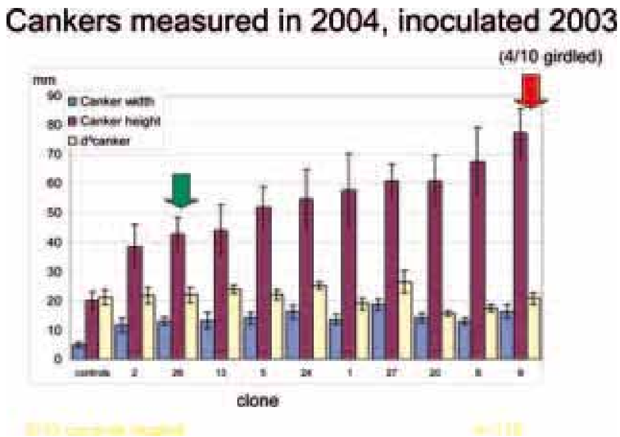

Fig. 2. Canker dimensions measured one year after inoculation.

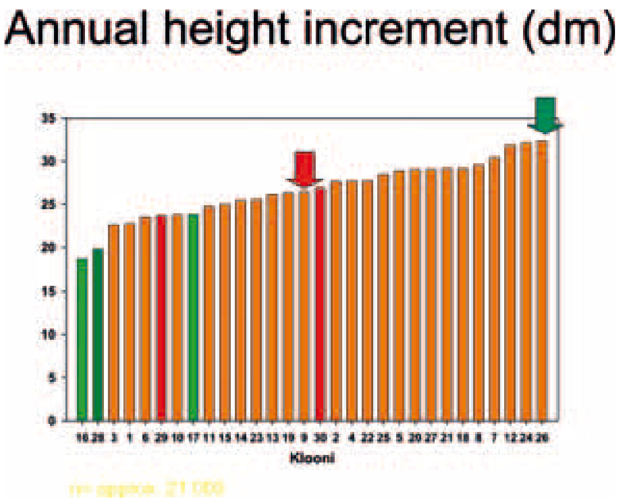

Fig. 3. The annual height increment of hybrid aspen clones (orange), aspen clones (green), hybrid aspen seed families (red) and aspen seed families (dark green). Red arrow points out the most susceptible clone (Fig 2), the clone with the highest disease resistance is shown with green arrow.

It is widely known that fungal strains have variance in virulence. In this study, only one fungal strain was used in inoculations. In our previous study (Kasanen *et al* 2002) we observed that all the isolates of *N. populi* were very similar according to the used markers since practically no variation was observed within ascospore isolates, canker isolates or reference isolates. Although it is known that no marker system can give ultimate resolution of genotypes, and the traits related to virulence are most likely not linked with the RAMS markers used, the absence of marker polymorphism suggests that the fungal isolates studied are very closely related. Thus we conclude that the use of only one fungal strain was justified by the absence of any detectable variation.

It can be concluded that the hybrid aspen clones, despite of the fact that the original selection was based on only yield and fibre characteristics, show variability in resistance. A promising observation was made by combining the results from separate trials; the best-growing clone is one of the most resistant ones. Thus it seems likely that there are possibilities to select for both growth and resistance traits in breeding. Since the occurrence and damages caused by shoot blight *Venturia tremulae* Aderh. were also surveyed on this field trial it will be interesting to see whether the resistance of the clones to several pathogens correlate.

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