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Cover photo. Some of the 10% ash dieback resistant *Fraxinus excelsior* trees in a central Lithuanian progeny trial at Kedainiai, Photograph Alfás Pliura.

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# Some diseases and pests affecting forest trees

## Objectives

The objective of this publication is to present:

General information about diseases and pests including some definitions

Harmful pests and diseases in some temperate tree species

Information on genetic variation in attacks by fungi or insects following spontaneous or artificial inoculation or insect exposure

Measures taken to mitigate fungal and insect attacks.

This paper does not give a full coverage of the vast genetic research related to forest tree pests and diseases. Pathogens affecting poplars and eucalypts are not treated. They deserve separate treatments.

## Some important forest tree diseases and pests

### Conifers

Disease resistance is of utmost importance in several conifers. Fusiform rust, *Cronartium quercuum*, causes great losses for forest owners in South Eastern USA owing to attacks on the commercially important pine species, *Pinus elliotii* and *P. taeda*. The symptoms of fusiform rust are spindle-shaped galls on stems or branches (Picture 1). During early spring, bright yellow aecial pustules might be observed (aecial = a cuplike structure of some rust fungi that contains chains of aeciospores, pustule = a small often distinctively colored elevation or spot resembling a blister pimple). Blister rust caused by *Cronartium ribicola* is another important disease affecting white pine species such as *Pinus strobus*, *P. strobiformis*, *P. lambertiana*, and *P. monticola*. Brunchorstia disease on *Pinus sylvestris* and *P. contorta* in Scandinavia caused by *Gremmeniella abietina* leads to severe damage certain years. *Dothistroma septosporum* causes serious damage in *Pinus radiata* in Australia and New Zealand. It is sometimes referred to as red band needle blight or *Dothistroma* blight. The former was attributed to the red color of the needles. Initially following infection, the needles turn yellow and later on the needle tips become reddish brown. Mist and rain spread the disease, as well as direct contact, with most damage in humid climates.

There are numerous insects that are dependent on forest trees for their existence, some of them extremely harmful. Genetic studies are sometimes missing even if some insect species cause serious damage to their host tree



*Picture 1.* A *Pinus taeda* tree severely infected with fusiform rust, which attacks both stems and branches. Photograph Gösta Eriksson.

species. In this chapter we illustrate some examples from genetic studies on important insect herbivores. *Dendroctonus ponderosae* is a small beetle (mountain pine beetle) that causes severe damage in various pine species in western North America from Mexico to British Columbia. In British Columbia alone, 16 million out of 55 million hectares were destroyed by the mountain pine beetle (B.C. Forest service 2012).

*Pissodes strobi*, the white pine weevil, is one of the most harmful pests on *Picea sitchensis*, *P. engelmanni*, and *P. glauca* in western North America. The eggs are deposited in spring in the leader from last year. The larvae feed from the bark phloem, which mostly kills the leader. This in turn results in deformities of the stem. One example of the severity of *Pissodes strobi* attacks on Sitka spruce was given by King et al. (2004), who reported that only five trees in a 10-ha Sitka spruce plantation had survived the white pine weevil attack. Results from two studies of resistance to this insect will illustrate genetic variation in susceptibility to this harmful insect. In periodic outbreaks the spruce budworm, *Christoneura fumiferana*, defoliates *Picea* and *Abies* trees in its distribution areas in eastern North America, and causes mortality and great losses in wood yield during such outbreaks.

### Deciduous trees

Two Dutch scientists described a dieback in elms in 1921, which gave rise to the name Dutch elm disease, DED. This disease was caused by a fungus, *Ophiostoma ulmi*. During the late 1960s a still more virulent fungus,



*Ophiostoma novo-ulmi*, attacked elms. The American elm, *Ulmus americana*, and the European elm species are seriously, and most often, lethally damaged by DED. In Europe the disease is spread by two beetles, *Scolytus scolytus* and *S. multistriatus*. In addition to the latter species, *Hylurgopinus rufipes* is also a transmitter of DED in America. DED was spread to America somewhat later than its first appearance in Europe.

The American chestnut, *Castanea dentata*, was a dominating deciduous tree species in forests of eastern USA until the early 1900 when chestnut blight, caused by the fungus *Cryphonectria parasitica*, was introduced by imports of the Asian *Castanea crenata*, which is almost resistant to this fungus. Since then this serious disease has spread and more or less wiped out *C. dentata* in Eastern USA. In 2020 almost only young seedlings of this species could be found in its distribution area. There is a great public interest to restore this charismatic species. Great efforts were, and still are, devoted to restoration of this keystone species.

In 1992 dieback in *Fraxinus excelsior* was detected in Poland, and a few years later it was found in neighboring Lithuania. This disease is caused by the Asian fungus *Hymenoscyphus fraxineus*; it is still spreading in Europe in 2020 and has killed millions of *F. excelsior* trees. The seriousness of this disease has triggered several forest geneticists to study resistance to it.

The three insect species *Lymantria dispar*, *Popillia japonica*, and *Hyphantria cunea* are herbivores feeding from *Castanea dentata*. *Lymantria* originates from Latin and means destroyer. The moth *Hyphantria cunea* is native to North America, ranging from Canada to Mexico. It is one of the few insect pests introduced from America into other continents. *Popillia japonica* in North America is a noted pest of about 300 species of plants. The leaves of the host plants attacked are skeletonized by this insect species.

*Dryocosmus kuriphilus* is a harmful insect that was observed on *Castanea sativa* in Europe during the present century. In one provenance trial in Italy the affected percentage of trees increased from a few per cent in 2004 to around 90% in 2007.

## Resistance - tolerance

The severity of diseases or pest attacks may vary, and different terms are used to describe the severity, sometimes with different definition of the terms used. The following definitions were given by [Woodcock et al. \(2019\)](#):

*Complete resistance*: no or minimal damage is caused by pathogens or pests

*Partial resistance*: the effects of attacks are limited

*Tolerance*: symptoms are displayed, but growth and performance are maintained to some extent.

In the search for genetic variation in disease resistance it is of importance to analyze if the resistance is caused by active defence in the host tree or if it could be attributed to escape from being exposed to a particular pest or pathogen. A false resistance might exist if the pathogen or pest has not reached the area of observation at the time of assessment of resistance. Thus, such a resistance is attributed to avoidance of exposure to biotically harmful organisms. In particular, with global warming a faster expansion of pathogens and pests might take place than the expansion of host species. This means that the host species will become exposed to harmful organisms that they were not exposed to earlier. Since there was no selection for resistance before, a high susceptibility is expected under these conditions.

Different phases in the response to a pathogen may be identified. An hypothesis with three steps in defence against *Phytophthora* attacks of chestnut tree roots was presented by [Santos et al. \(2017\)](#):

1. Avoidance of attacks by morphological change or antifungal defence around the roots
2. Recognition of the parasite
3. Activation of host resistance genes

This model may apply to many other pathogens and not be limited to *Phytophthora* attacks. An analogous model for insect attacks was presented by [Sniezko and Koch \(2017\)](#):

- Traits that deter or repel insect feeding or oviposition
- Traits that reduce insect fitness and/or survival
- Tolerance, which means that hosts withstand or recover from insect attacks

Examples indicate that there is a low frequency of trees that exert an active defence.

It is worthwhile to consider the question as to whether resistance is polygenically inherited or achieved by one or a few major genes. Since the generation times of forest trees usually vastly exceed those of the pathogens or pests, it is unlikely that the resistance depends on major genes. If resistance depended on one or a few major genes, it could easily be lost by mutations in the pathogens or pests enabling renewed virulence. Therefore, in species with much longer generation times than their pathogen(s), polygenic inheritance is more likely than single-gene resistance. From a breeding perspective durable resistance is aimed at, and this is more easily obtained with polygenic inheritance. The importance of long-time field experiments for identification of durable resistance was stressed by [Sniezko et al. \(2019\)](#): Because trees are long-lived, resistance must be effective for decades to centuries to be useful. Resistance needs to be durable, stable, and present at a useful level. Field plantings provide the best opportunity to assess resistance durability and stability across a range of environments.

**Table 1.** Examples of relationships between diseases and host species. These diseases listed have great impacts on the host species.

Disease and host species	Origin of host species	Origin of disease/pest
Fusiform rust <i>Pinus taeda</i> <i>Pinus elliottii</i>	native	native
Blister rust American white pines	native	introduced
Chestnut blight <i>Castanea dentata</i>	native	introduced
Ink disease <i>Castanea sativa</i>	native	introduced
Dutch elm disease European and American elms	native	introduced
<i>Dothistroma</i> , <i>Pinus radiata</i>	introduced	introduced
<i>Gremminiella</i> , <i>Pinus contorta</i> (Sweden)	introduced	native

### What are the requirements for successful breeding for pest and disease resistance?

Requirement for knowledge of the following factors emerged from a scrutiny of five long-term breeding programs scrutinized by Woodcock et al (2019):

- Long-term commitments and central coordination
- The frequency and heritability of resistance, in order to assess the potential for resistance breeding
- The relationship between the target trait and other pests and diseases in the host species
- Demand for resistant trees by end users
- The urgency for operational deployment; the breeding strategy will most likely differ at extensive mortality caused by pests and diseases
- The risks that the selection pressure in the pest or pathogen may result in regained virulence of the pathogen; these should be communicated to the end users
- Continued monitoring of any signs of susceptibility to pests or diseases in the field.

Long-term commitment and central coordination were found to be key issues for success.

Both native and introduced species may be affected by diseases and pests. Serious attacks by introduced pathogens have caused great losses in many natural species.

In Table 1 some spectacular cases of introduced fungal diseases are listed together with native pathogens. Fusiform rust in *Pinus taeda* and *P. elliottii*, blister rust in *Pinus strobus* and other white pines, white pine weevil (*Pissodes strobi*) in American spruces, mountain pine beetle (*Dendroctonus ponderosae*) in *Pinus contorta* and *P. monticola*, spruce budworm (*Christoneura fumiferana*) in *Picea glauca*, chestnut blight in *Castanea dentata*, Dutch elm disease in American and European elms, and ink disease in *Castanea sativa*, and ash dieback (*Hymenoscyphus fraxineus*) in *Fraxinus excelsior* are examples of serious fungal diseases in tree species. Blister rust, Dutch elm disease, and chestnut blight were introduced to Europe and North America via imports of wood from other continents, notably Asia. The host species were not exposed to these pathogens in their previous history. Therefore, a build-up of tolerance or resistance had not taken place in these species. Various means to overcome the susceptibility were tried:

Selection within wild populations

Crosses with related species having resistance against the disease; followed by back crosses with the domestic species

Transfer of major resistance genes

Even if a species was not exposed to the introduced pathogen or pest, resistance at low frequency may exist in the tree species. Under such conditions screening of huge populations must take place.

Transfer of major resistance genes seems to be a simple solution to obtain resistance. The first prerequisite is to identify the resistance gene and locate it in the proper chromosome. Then it must be transferred in single copy to its proper location on the chromosome. Thus, there are several critical steps that must work for a successful transfer of a major resistance gene.

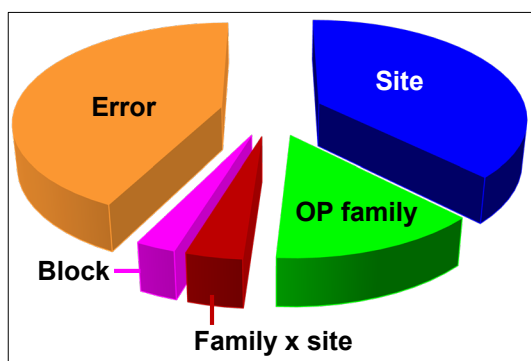
Genetic variation in resistance following spontaneous exposure to the pathogen as well as after inoculations will now be presented.

## Diseases

### Conifers

The low frequency of resistant individuals in natural populations is characteristic for most pathogens.

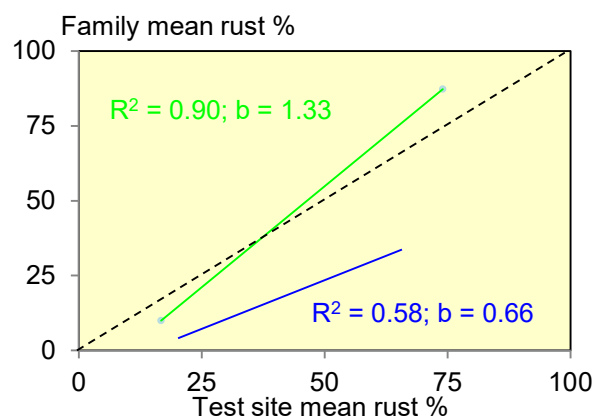
Range-wide studies are useful to get information on the severity of a disease or pest in the distribution area of the species. Much useful information might be gained about the potential for breeding, including delineation of breeding zones, if combined provenance and progeny trials are established.



**Figure 1.** Partitioning of the variance for fusiform rust incidence based on 28 *Pinus taeda* progeny trials with 43 open-pollinated families.

In one such range-wide study including 28 test sites the occurrence of fusiform rust was assessed for 43 open-pollinated families (McKeand et al. 1999). Fig. 1 shows that the test site and family effects contributed much in the partitioning of the variance. This figure reveals that the family effect was much stronger than the family x site interaction. The test site means for fusiform rust attacks varied in the range 10–75%. Based on all data from the 43 OP-families, the expected fusiform rust percentage at a site with a mean value of 50% was calculated. The OP-family with the lowest expectation had a value of 22.4% while the most sensitive family had an expected value of 75.3%. The effects of family as well as the family x test site interaction were both strongly significant. The partitioning of the variance components from this series of trials reveals that the family effect is much more important than the family x test site interaction (Fig. 1). These results mean that delineation of breeding zones for fusiform rust will not have top priority in breeding. One way to elucidate the stability of family performance over a series of test sites is to plot the family mean values against test site means. Such plots are called Finlay-Wilkinson diagrams, after the authors who presented this technique for the first time. The regression lines for two extreme families are visualized in Fig. 2. The green family with its regression coefficient  $b > 1.0$  suffers more from fusiform rust at sites with high mean fusiform rust occurrence. In contrast, the blue family was the least affected family at test sites with high incidence of fusiform rust attacks, with the regression coefficient  $b < 1.0$ . Besides, the blue family would be most attractive to include in a breeding population with fusiform rust resistance as one of the target traits. This family had low percentages of fusiform rust in the majority of test sites.

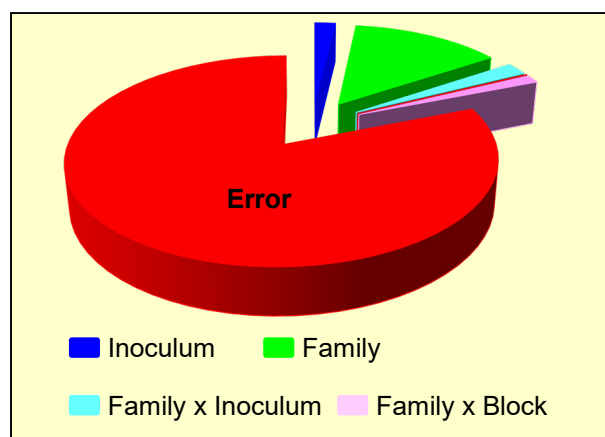
A study with the objective of estimating the relationship between artificial inoculation with *Cronartium quercuum* (fusiform rust) and observed field resistance/susceptibility to this pathogen was carried out with progenies from 25 fast-growing *Pinus taeda* parents (Isik et al. 2008). Ten sources of fusiform rust were used for the green-



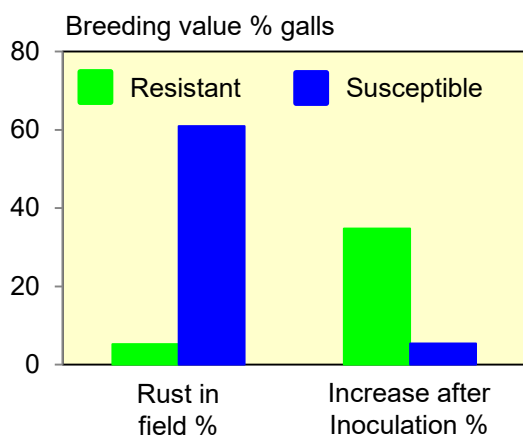
**Figure 2.** Family mean fusiform rust, %, of two *Pinus taeda* families plotted against test site fusiform rust mean %. The dashed line has a slope of 1.0. McKeand et al. 1999

house inoculation. The selection of the parents was based on trials with a range of fusiform rust incidence varying between 33 and 56%. Of the families selected, 17 were recorded as resistant with mean breeding values of 5% and eight parents were recorded as susceptible with mean breeding values of 64% for fusiform rust incidence. The inoculations took place at eight weeks after sowing, and were assessed two months after treatment. The seedlings were classified as diseased or non-diseased.

The partitioning of the variance showed that the family effect was much larger than inoculum, family x inoculum, block, or family x block effects (Fig. 3). The source of the inoculum contributed 1.9% of the variability, which shows that the source of the inoculum has only limited effect on susceptibility. The  $h^2$  for disease resistance was estimated at 0.56, which suggests that considerable progress in breeding for fusiform rust resistance may be obtained. In all but one progeny there was an increase in



**Figure 3.** partitioning of the variability in an experiment with inoculation of 25 open-pollinated families of *Pinus taeda* with ten inocula originating from the whole range of *P. taeda* distribution in southeastern USA. The block effect was too small to illustrate, 0.1%, in this pie chart. Isik et al. 2008.



*Figure 4. The mean percentage of fusiform rust infections in field of 17 resistant and 8 susceptible Pinus taeda parents and the increase in fusiform rust incidence in percentage units after artificial inoculations of seedlings with a mix of inoculum from ten sources across the distribution area of Pinus taeda. Isik et al. 2008.*

fusiform rust incidence in the artificial inoculations. There was a pronounced difference in the increase between the progenies from resistant and susceptible parents. The high percentage of fusiform rust in the susceptible parents with a mean value of 61 % does not allow as large an increase as there is room for in progenies from resistant parents. There was a wide span of the increase in the resistant progenies, 2x – 37x. The poor agreement between field breeding values of the resistant parents (1% – 18%) and breeding values from the artificial inoculation (12% – 70%) was one disturbing result (Fig. 4). In contrast, the agreement for the susceptible parents was good. The difference among the progenies from the resistant parents might be attributed to varying virulence of the spores used for individual inoculations, which consisted of a 30 inoculum gall mix. This means that the inoculum might have some pathogen type that can overcome the resistance in the progeny of a particular “resistant” parent. Even if there was a wide span in increase in rust incidence among the “resistant” parents, culling of susceptible parents in breeding populations can accurately be carried out.

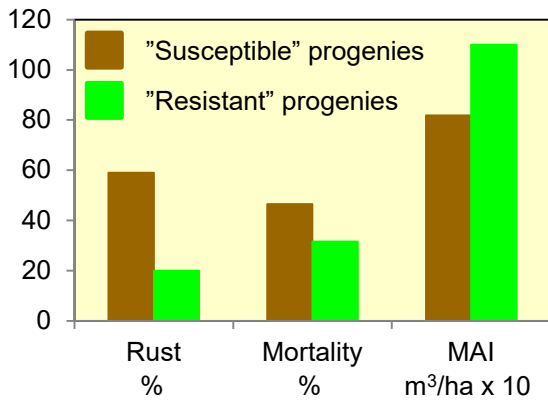
A 32-year old *Pinus elliottii* tree, denoted 10-5, was selected in a naturally regenerated forest for a study of inheritance of fusiform rust resistance, since a certain frac-

tion of its progeny was free of fusiform rust (Myburg et al. 2006). Full-sib families (denoted B, C, and E) from three offspring trees to 10-5 were also included in this investigation. They had a common male. In addition this male tree was also crossed with 10-5, denoted family A. Seedlings at an age of 8–9 weeks old were inoculated with basidiospores from two isolates of *Cronartium quercuum*. Occurrence of rust galls was assessed at nine months. The association of detected fusiform rust loci with RAPDs (random amplified polymorphic DNA) was determined. For family A, 314 segregating polymorphic DNA sequences were identified. The corresponding figure for family B was 232. Even if there was a 1:1 segregation of resistant:susceptible, no significant association between RAPD markers and fusiform rust resistance was noted in a first study of two full-sib families. In a second test several markers from linkage group 9 showed clear differences between resistant and susceptible groups of seedlings. Several significant associations between some of these markers from linkage group 9 and resistance to fusiform rust were disclosed. However, no completely unambiguous disclosure of a resistant gene could be obtained, but a region in chromosome 9 might harbor one or more loci with resistance genes for fusiform rust.

Most genetic studies contain a large number of entries often with single-tree plots. Such a design does not allow for estimations of the yield per area, which is the ultimate goal of breeding. One approach to estimate mean annual increment used 48-tree plots containing a mix of open-pollinated *Pinus elliottii* progenies from six resistant and six susceptible parents (Vergera et al. 2007). Five trials with such seed lots were established in Georgia and Florida, USA, in which the occurrence of fusiform rust infection was assessed. Each 48-tree plot was surrounded by 2-3 border rows. Oak seedlings, which are alternative hosts of fusiform rust, were eradicated as one treatment. Growth, mortality, and rust incidence on stems or branches were assessed at ages 5, 12, and 16. The design was a 2 x 2 x 2 x 3 study:

- Two seed lots: resistant or susceptible
- Two treatments: oak-remaining or oak-free
- Two cultural treatments: fertilization and herbicide treatment or no treatment during the first year in the field
- Three blocks





**Figure 5.** The fusiform rust incidence and mortality in %, and mean annual increment (MAI) x 10 in m<sup>3</sup>/ha for rust resistant progenies and rust susceptible progenies in five *Pinus taeda* field trials at age 16 in Georgia and Florida, USA. Each progeny consisted of a mix of six open-pollinated families of rust resistant or rust susceptible parents. Vergera et al. 2007.

The included parents had 13 and 11% superior growth rate compared to non-bred *P. elliotii*, with the highest figure for the resistant families.

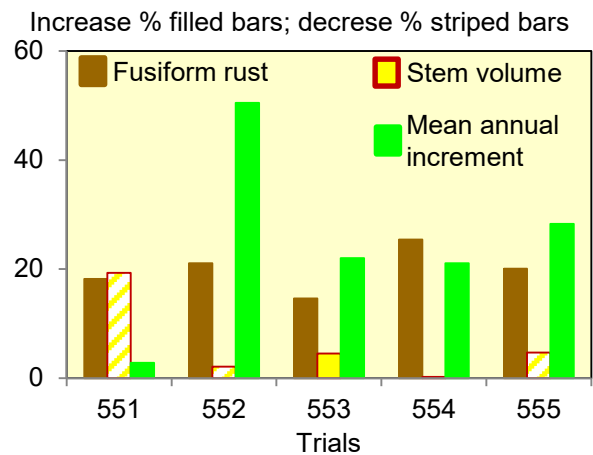
The general performance of the two types of mixes is illustrated in Fig. 5, which reveals that the incidence for fusiform rust was much higher in the plots with progenies from susceptible parents than in the other type of progeny. Similarly the mortality was highest in the “susceptible” progenies.

The evaluation of the effect of the pathogen was estimated in a somewhat complicated way. The genetic resistance was calculated as realized percentage of total rust incidence of the resistant seed lot of progenies when rust hazard in a site is 50%. Thus calculated percentages (gains or losses) are illustrated in Fig. 6. This figure reveals that the superiority of resistant progenies as regards fusiform rust varied in the range 15–25%. Except for trials 553 and 554 the stem volume was lower in the progenies from the resistant parents. This was attributed to an increased mortality in the plots with progenies from susceptible parents – 46% versus 31%. As a consequence of this the competition in “susceptible” plots was lower than in the “resistant” plots, allowing for a better growth of the surviving trees. In trial 551 the much larger stem volume in the “susceptible” plots means that the mean annual increment was only marginally improved in this trial, while there was a pronounced improvement of mean annual increment in trial 552. The other three trials had gains in the range 20–30% (Fig. 7). The seed type x site interaction was limited, meaning that at least the seed lots used had good stability.

The percentage gain was calculated according to the following formula:

$$\frac{[(\text{Resistant seed lot} - \text{susceptible seed lot}) / \text{susceptible seed lot}] \times 100}{}$$

Fig. 7 reveals that the superiority of the “resistant” as re-



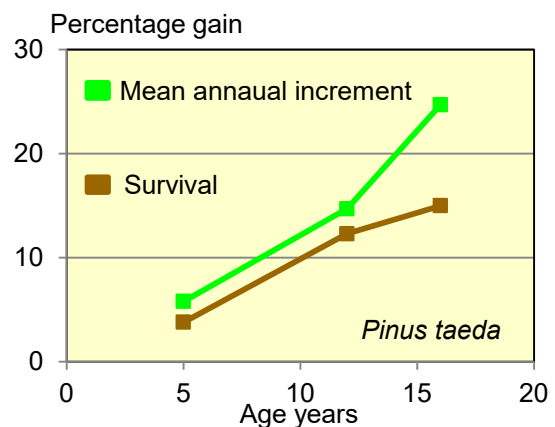
**Figure 6.** The gain in percent of rust resistant progenies over rust susceptible progenies in five *Pinus taeda* field trials at ages 15–17 for fusiform rust incidence, stem volume, and mean annual increment. Filled columns = superiority, striped columns = inferiority of the rust resistant progenies. Each progeny consisted of a mix of six open-pollinated families of rust resistant or rust susceptible parents. Vergera et al. 2007.

gards mean annual increment and mortality was consistent over years and even increased.

The most significant result for breeding was the improved mean annual increment in the “resistant” plots, 11m<sup>3</sup>/ha versus 8.2m<sup>3</sup>/ha for the “susceptible” plots. Part of this gain can be attributed to improved survival of the “resistant” seed lot.

Five single-genotype isolates of *Cronartium quercuum* were inoculated on seven fusiform rust resistant families in the search for identification of fusiform rust resistance genes, denoted *Fr*, in *Pinus taeda* (Amerson et al. 2015).

Based on the results in Table 2 nine *Fr* genes were identified. Only family 152-329 was resistant against three of the five single-gene isolates while family D was resi-



**Figure 7.** *Pinus taeda*. The percentage gain in survival and mean annual increment by use of a seed lot consisting of a mix of 6 progenies from fusiform resistant parents compared to a seed lot of a mix of 6 susceptible parents at ages 5, 12 and 16. Vergera et al. 2007.



**Table 2** The response in seven *Pinus taeda* families to inoculations with five single-genotype isolates, I-1 – I-5 of *Cronartium quercuum*. Red = incompatible, resistant; yellow compatible, susceptible; orange = two Fr (fusiform rust resistance) genes, one compatible and the other incompatible. Families B and C had two Fr genes. Amer-son et al. 2015.

	1-1	1-2	1-3	1-4	1-5
10-5	Red	Yellow	Yellow	Yellow	Red
A	Yellow	Red	Yellow	Yellow	Red
152-329	Yellow	Red	Red	Yellow	Red
29R	Yellow	Yellow	Red	Red	Yellow
B	Orange	Orange	Orange	Orange	Orange
C	Red	Yellow	Orange	Orange	Yellow
D	Red	Yellow	Yellow	Yellow	Yellow

stant against one isolate only. Families B and C each had two resistance genes, *Fr5+Fr9* and *Fr6+Fr7*. Thanks to mapping of the *Pinus taeda* genome with RAPDs it was possible to locate the *Fr* genes to certain chromosomes:

Linkage group 2, *Fr1, Fr3, Fr4, Fr6, Fr7 and Fr9*

Linkage group 3, *Fr2*

Linkage group 10, *Fr5 and Fr8*

Unlike the study presented by Isik et al. (2008) no association of *Fr* genes to linkage group 9 was detected.

This investigation shows that resistance to fusiform rust is complex, and identification of *Fr* genes is an important step towards a deeper understanding of the resistance to fusiform rust.

Variation in fusiform rust incidence of host trees may depend on the genetic structure of the host plants as well as variation in virulence of isolates. In one investigation eight isolates from each of seven populations from North Carolina to Louisiana in USA were collected in natural populations for inoculation of three *Pinus taeda* materials (Powers et al. 1977). Seedlings from two open-pollinated families were included in this experiment: one resistant and one susceptible parent. In addition, seedlings from one population with intermediate resistance to fusiform rust were included. The incidence of rust was recorded nine months after inoculation.

The following effects were significant:

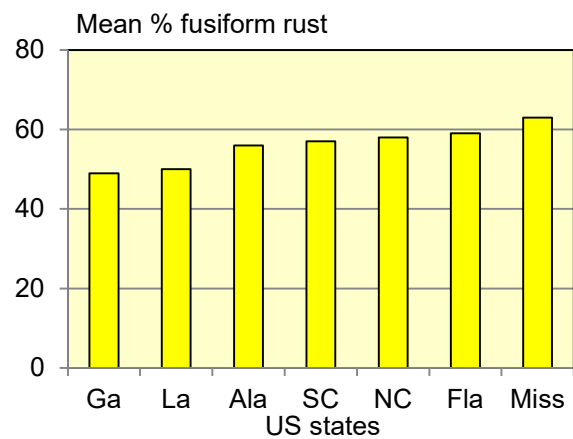
Family

Isolates within states

State origin of the isolates

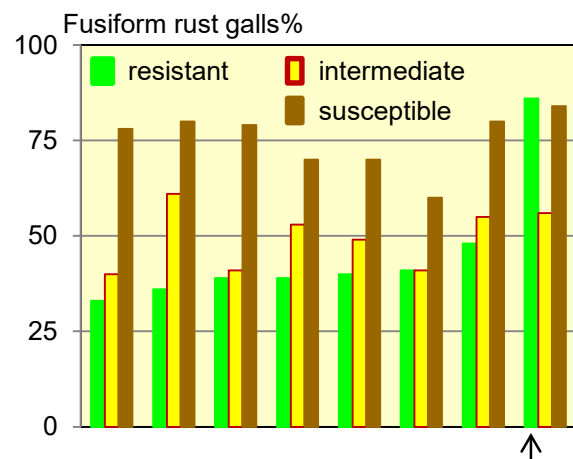
Interaction family x state

The grand means of the eight isolates from each of the seven states were significantly different but no conspicuous differences in virulence were observed (Fig. 8). The variation among isolates in a population from South Carolina is illustrated in Fig. 9. This population was selected since the intermediate population had a mean of 50% that would guarantee detection of genetic variation

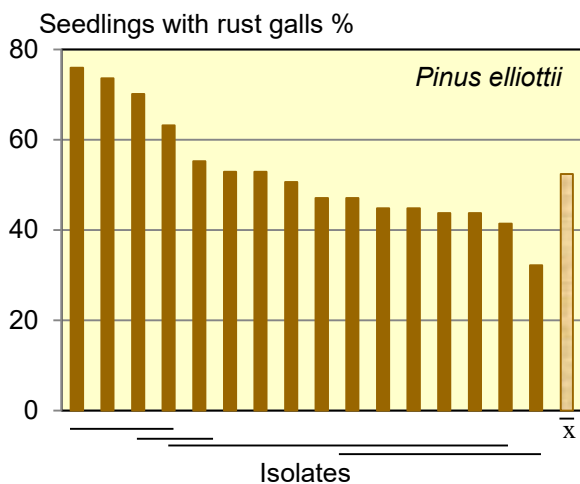


**Figure 8** Mean percentage across three *Pinus taeda* populations of fusiform rust galls in seedlings at 9 months after artificial inoculation with 8 different isolates of *Cronartium quercuum* from each of seven states in USA. One open-pollinated family originated from a resistant parent and another originated from a susceptible parent. The third population was a bulked population with intermediate susceptibility to fusiform rust. Powers et al 1977.

if any exists. As seen from the figure, one isolate (arrow) had an extremely strong and negative effect on the resistant offspring population. It might be speculated that this isolate had been exposed to resistant loblolly pine and as a consequence of this was forced to change its virulence via mutations and subsequent selection. This observation was the most deviating in the entire study. Generally, the resistant family remained the most resistant in all seven groups of isolates, with a mean value of 40%. The corresponding values for the intermediate and susceptible populations were 54 and 74%, respectively. The susceptible OP-family had in 93% of the cases the highest percentage



**Figure 9.** Percentage fusiform incidence in two open-pollinated *Pinus taeda* families; one resistant and one susceptible parent as well as bulked seedlings from a population with intermediate resistance to fusiform rust. Percentage of actively growing galls was recorded nine months after inoculation. Powers et al. 1977.

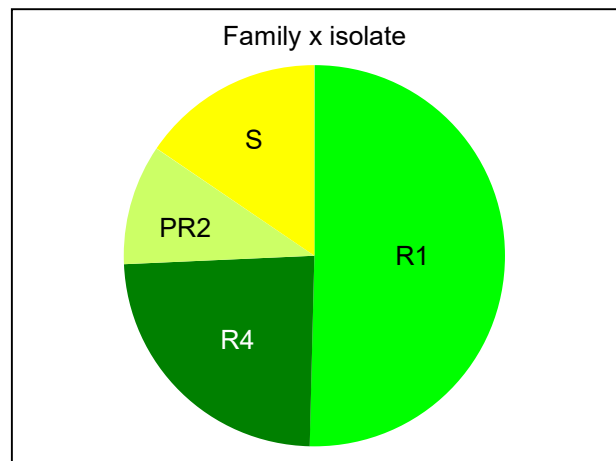


**Figure 10.** The percentage of fusiform rust galls in seedlings in a *Pinus elliottii* open-pollinated family, R1, at 6-9 months after artificial inoculation with 16 different isolates of *Cronartium quercuum*. Lines below the diagram show non-significant differences among 16 isolates. Gramacho et al. 2013.

of rust. With strong significance for isolate, the identification of rust-resistant parents becomes complex. Resistance might be attributed to true resistance but it may equally well be attributed to evasion of exposure to virulent isolates. Mixture of isolates for inoculation tests might be a remedy to avoid false resistance.

A study with 16 isolates will be presented, in which open-pollinated progenies from two *Pinus elliottii* trees classified as fusiform-rust-resistant (R1 and R2) were inoculated with 16 isolates of *Cronartium quercuum* (Gramacho et al. 2013). In addition, progenies from one tree classified as partially resistant (PR) and another tree classified as susceptible (S) were included in this investigation. The formation of rust galls was examined 6-9 months after inoculation.

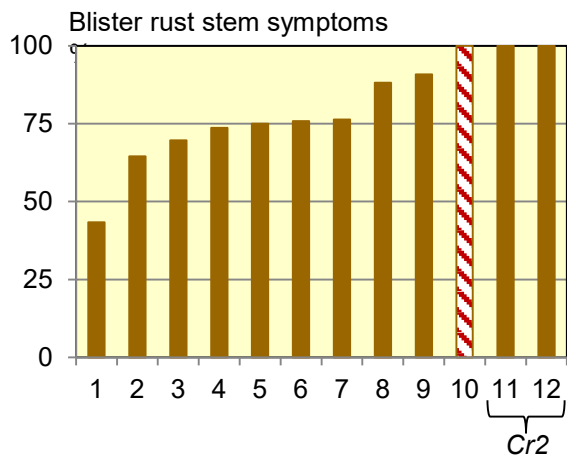
The largest variation in fusiform galls was noted for tree R1, with the extreme percentages being 32 and 76% (Fig. 10). The possibility to detect variation was in this case at its maximum, with the mean percentage around 50%. The variation in the susceptible progenies was limited, with 14 of the isolates having percentages above 90%. The R1 progenies showed the largest family x isolate interaction (Fig. 11), responsible for 50.2% of this interaction. With the large variation in response to the 16 isolates of the pathogen, it is logical that R1 would be a great contributor to the family x isolate interaction. This investigation clearly shows that it is necessary to test fusiform resistance against several isolates to identify true resistance to this disease.



**Figure 11.** The percentage contribution of four progenies to the interaction family x isolate variance after inoculation of a *Pinus elliottii* open-pollinated family with 16 isolates of *Cronartium quercuum*. Evaluation of fusiform rust galls took place 6-9 months after inoculation of the seedlings. R = resistant, PR = partial resistant, and S = susceptible. Gramacho et al. 2013.

Contrary to the hypothesis presented above that major gene resistance is unlikely in long-generation time forest trees, there are results indicating that a major gene may confer resistance against blister rust via hyper-response reaction (Kinloch et al. 1999). This gene was denoted Cr2 and it works with a fast enclosure of the fungus and prevents further development of the pathogen. In an investigation including 28 full-sib families with an expected 3:1 segregation, two of the 28 families deviated strongly from the expected segregation 3:1, with one out of 40 seedlings and 15 out of 31 seedlings showing resistance, instead of the expected 30 and 24 seedlings. The others clearly supported a 3:1 segregation.

Genetic variation in resistance to blister rust in a series of three progeny trials with twelve families of *P. monticola* will be illustrated (Sniezko et al 2012). In these trials, stem symptoms and survival were assessed twelve years after sowing. Significant family differences for stem symptoms of canker (Fig.12) were noted for all three trials, while survival did not vary much in one trial, 90–100%. Survival among the families in the two other trials differed considerably, 19–94% and 43–100%. One family was included as a susceptible control material in this series of progeny trials (striped column in Fig. 12). Two of the families (11 and 12) contained the dominant resistance gene Cr2. It is evident that it did not confer resistance in these families against the *Cronartium ribicola* strain used in this inoculation experiment. Alternatively, the genetic constitution modifies the effect of the Cr2 gene dramatically. These twelve families were previously arti-

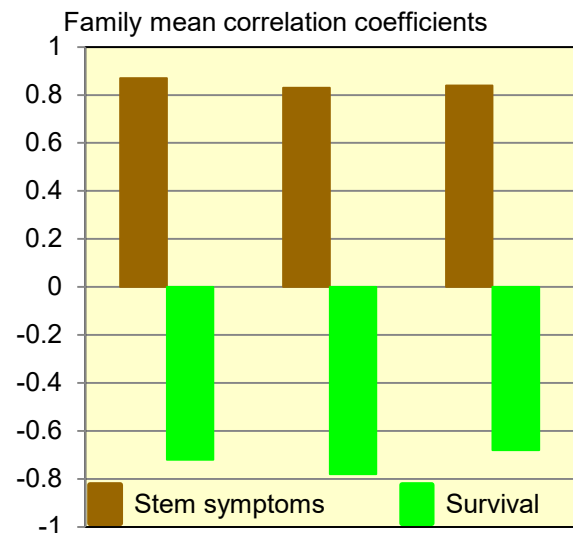


**Figure 12.** Percentage blister rust stem symptoms in 12 *Pinus monticola* families with *Cronartium ribicola*. Ten of them are open-pollinated families from natural stands. One OP-family originated from a tree in a seed orchard and the twelfth is a full-sib family. Families 11 and 12 contained the dominant resistance Cr2 gene. Assessments of blister rust took place twelve years after sowing in a field trial in Oregon, USA. One susceptible family was included as control material (striped column). Sniezko et al. 2012.

ficially inoculated with *Cronartium ribicola* in a nursery, which enabled an estimation of the relationship between artificial inoculations of juvenile material with field performance at later ages. The correlation coefficients from each of the three field trials reveal that mortality in the nursery is fairly strongly correlated with percentage of stem symptoms in the field (Fig. 13). Similarly, there is a fairly strong relationship between juvenile mortality and survival in the field, but in this case the relationships were negative as expected.

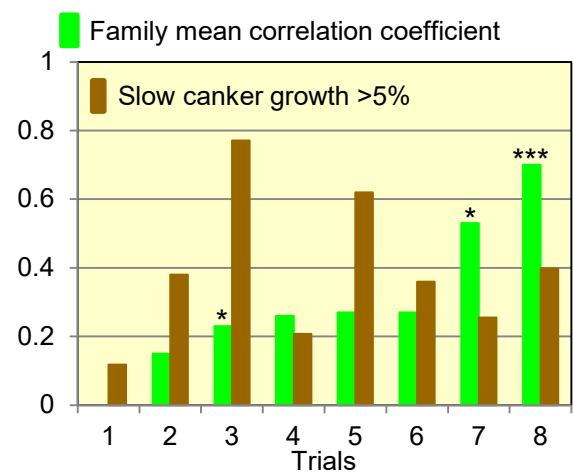
All studies were not as positive as regards the relationship artificial inoculation results – field performance. Another example with *P. monticola* is illustrated in Fig. 14 (Hunt 2002). The proportion of OP-families with slow canker growth is also illustrated in this figure. The reason for this was that a low proportion of affected trees in the field means that the relationships are expected to be weak. However, as seen in this figure, there was no clear relationship between the family mean correlation coefficients and the proportion of trees with more than 5% slow pathogen growth in this study.

There are hopes that pine species exposed to the blister rust in their native habitats may have developed resistance that can be transferred to susceptible white pine spe-

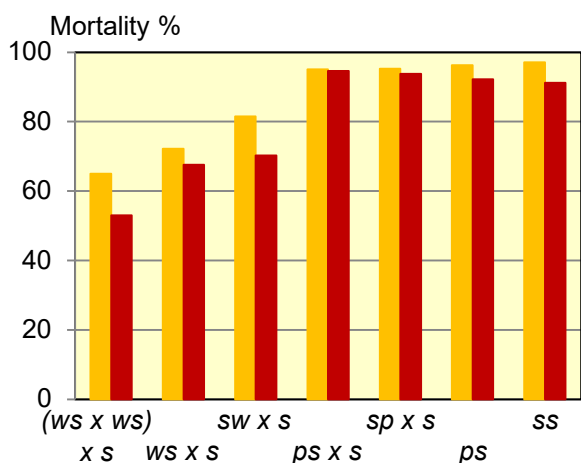


**Figure 13.** *Pinus monticola*. Family mean correlation coefficients between mortality in juvenile material after artificial inoculation with *Cronartium ribicola* and field survival or stem symptoms in field twelve years after sowing. Results from each of three field trials are shown. Sniezko et al. 2012.

cies in North America. Since the American pine species were not exposed to blister rust in their native habitats before the introduction of *Cronartium ribicola*, no selection for blister rust tolerance had taken place. The American white pine species were therefore crossed with related pine species from other continents. Some results from

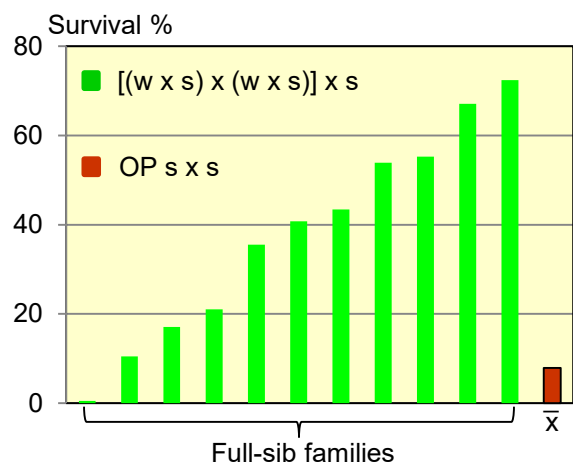


**Figure 14.** *Pinus monticola*. Family mean correlations between slow canker growth after artificial inoculation and the same trait in eight field trials. Significant relationships are indicated. The mean proportion of families with above 5% slow canker growth in the eight field trials is also shown. Hunt 2002.

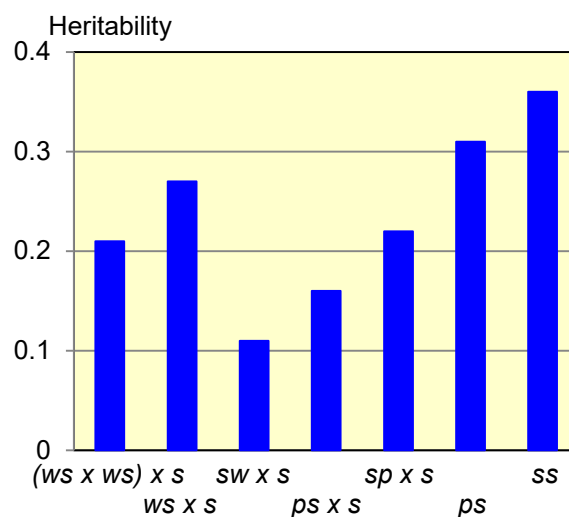


**Figure 15.** Mortality in *P. strobus* and interspecific crosses between *Pinus strobus* (*s*) and *P. peuce* (*p*) or *P. wallichiana* (*w*) following two separate inoculations (orange and brown) with *Cronartium ribicola*; *ws* stands for *P. wallichiana* x *P. strobus* and *sw* is its reciprocal cross etcetera. Six-month-old seedlings were inoculated. Assessment of fusiform rust took place around ten weeks after inoculation. Lu et al. 2005.

four inoculation experiments with interspecific crosses will be presented here. The investigation by (Lu et al. 2005) comprised several species hybrids and backcrosses with *P. strobus*. Fig. 15 shows that the mortality varied in the range 50–95% following strong, artificial inoculation of 6-month-old seedlings. The assessments of blister rust took place around ten weeks after the start of the inoculation. Some resistance is evidently contributed by the Himalayan *P. wallichiana*, while *P. peuce* from the Balkans



**Figure 16.** Mean survival, %, 14 months after inoculation with *Cronartium ribicola* of eleven interspecific families with *Pinus strobus* (*s*) and *P. wallichiana* (*w*) and mean value for 53 open-pollinated *Pinus strobus* families. Lu et al. 2005.

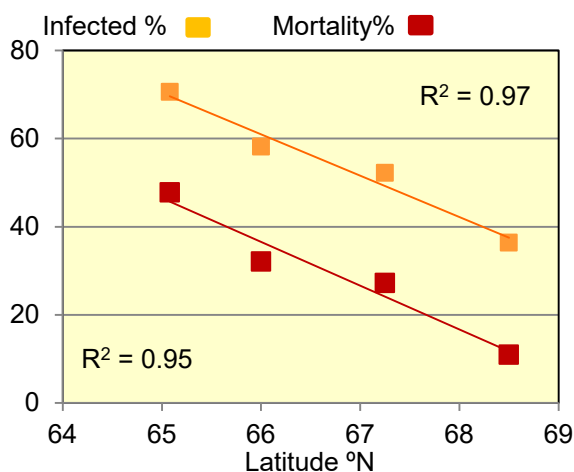


**Figure 17.** Mortality rate heritabilities in different types of families in an experiment with artificial inoculation of *Cronartium ribicola*. The estimates emanate from the point of time when 50% mortality was noted for the *P. strobus* families. *p*, *s*, and *w* stand for *Pinus peuce*, *P. strobus*, and *P. wallichiana*, respectively. Lu et al. 2005.

does not improve resistance against blister rust. Only the types of progeny with *P. wallichiana* as one parent had significantly lower mortality than pure *P. strobus*. There was large variation among families in mortality as is exemplified for the [(w x s) x (w x s)] x s families in Fig. 16. The mortality mean for these eleven families was ≈ 38% with a wide range in survival, 0.5–72.4%. The heritabilities for each type of cross were calculated for the point of time when the mortality in the *P. strobus* families was 50% (Fig. 17). At this point of time there is a differentiation of the families within types of cross. At a later occasion when almost all *P. strobus* families have reached 100% mortality, the differentiation among families will be minimal and any potential genetic differentiation cannot be identified. The heritability estimates for mortality rates in Fig. 17 indicate that there are potentials for improvement in blister rust resistance in pure *P. strobus* as well as in some of the interspecific hybrid families. Even if the final mortality approached 100% in some of the family types following the strong pressure from the inoculated pathogen, the observed heritabilities suggest that there might be possibilities for partial resistance in areas with lower selection pressure from blister rust.

Although the Chinese white pine, *P. armandi*, was represented by only two OP-families in one of the inoculation experiments, it is worth mentioning that these two families had almost 90% survival, when all other family types had less than 50% survival after inoculation with *Cronartium ribicola*. Future studies will reveal whether or not hybrids with *P. armandi* will bring resistance into American white pines.

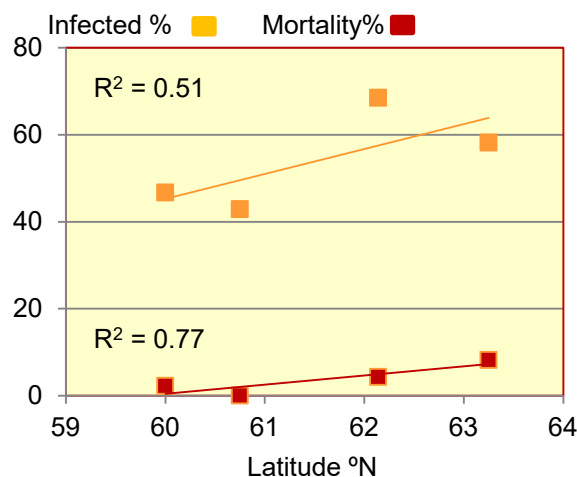




**Figure 18.** Percentage of infected seedlings 13 months after inoculation with *Gremmeniella abietina* in four populations of *Pinus sylvestris* from northern Sweden. The percentages of dead seedlings 26 months after inoculation are also shown. Hansson 1998.

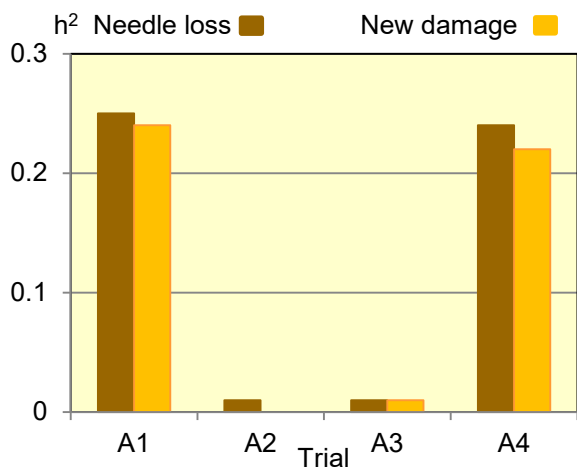
Genetic differences in susceptibility to *Gremmeniella abietina* assessed 13 months after inoculation of seedlings from four *Pinus contorta* and four *P. sylvestris* populations are presented in Figs. 18-19 (Hansson 1998). The low mortality of the *P. contorta* seedlings must be attributed to their substantial recovery. A comparison of Figs. 18-19 reveals that the four *P. sylvestris* populations are more susceptible to *G. abietina* attacks than the *P. contorta* populations. As regards *P. sylvestris* the relationships between population latitudinal origin and the two traits assessing the effect of *G. abietina* inoculation were extremely strong. The northern populations showed the strongest resistance to *G. abietina* attacks. In *P. contorta* the southern populations were most resistant and the relationships with latitudes at population origin are weaker.

Serious attacks of *Gremmeniella abietina* on *Pinus sylvestris* occurred in large parts of Sweden in 2001. Sonesson et al. (2007) used this opportunity for estimates of genetic parameters for resistance to the disease caused by this fungus. The results from four progeny trials are presented in Fig. 20. As seen from this figure, the needle loss and new damage were considerable in two of the trials. They were accompanied by high heritabilities, while

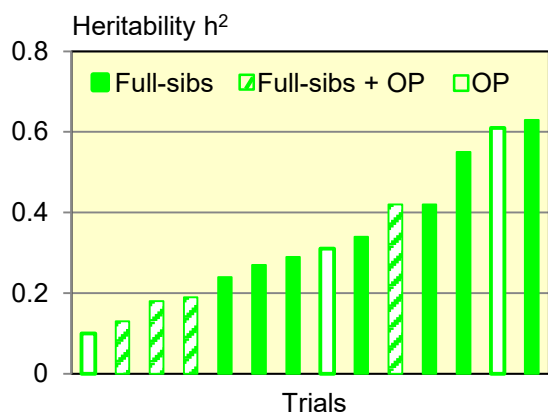


**Figure 19.** Percentage of infected seedlings 13 months after inoculation with *Gremmeniella abietina* in four populations of *Pinus contorta* from northern Sweden. The percentages of dead seedlings 26 months after inoculation are also shown. Hansson 1998.

low estimates were noted in the two trials with the lowest damage. As stated elsewhere, the possibility to identify genetic differences is greatest at a mean level of 50%. The high heritabilities indicate that breeding for resistance against *G. abietina* may be successful.

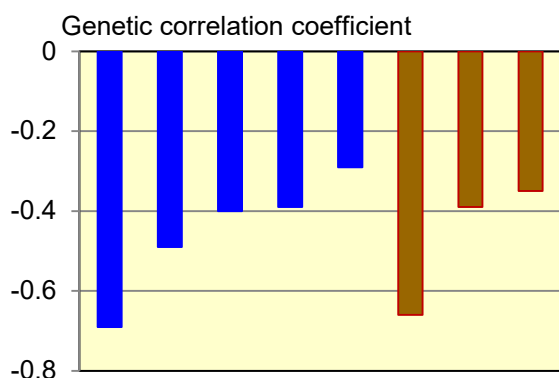


**Figure 20.** Heritabilities for needle loss and new damage in four progeny trials with *Pinus sylvestris*, A1 – A4, following *Gremmeniella abietina* spontaneous attacks in year 2001. Sonesson et al 2007..

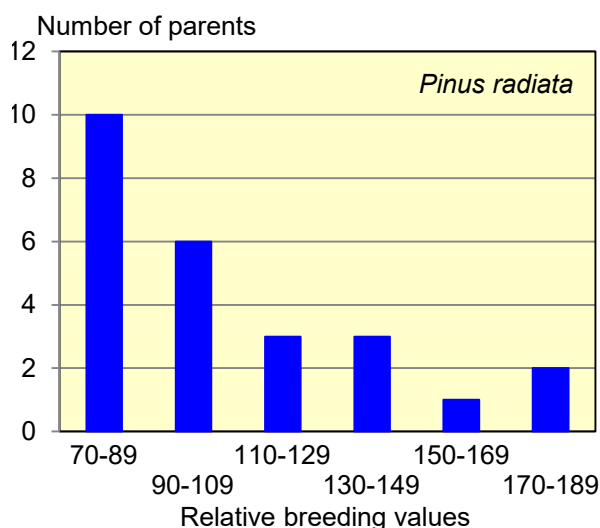


**Figure 21.** Narrow-sense heritability for resistance to *Dothistroma blight* in 14 trials with *Pinus radiata* in Australia. Seven trials contained full-sib families only, while the corresponding figure for OP-family trials was four. Finally three trials contained both types of families. Ivkovic et al. 2010.

The occurrence of *Dothistroma blight* in 16 *Pinus radiata* progeny trials in northeastern Victoria, Australia was assessed at an age of 3-4 years by Ivkovic et al. (2010). The majority of trials had full-sib families exclusively or in combination with open-pollinated families from seed orchards. Three trials had only OP-families. Infections were assessed in eleven classes, 0–10, 0 = <5% defoliation and 10 = >95% defoliation. The intermediate scores comprised ten percentage units each. All trials were located in a geographically limited area, but with a large variation in *Dothistroma* score, <5% – 65% of the crown affected. The narrow-sense heritabilities in the individual trials varied in the range 0.10–0.63 (Fig. 21). The results indicate that there are good possibilities for improvement of resistance to *Dothistroma blight*. It is expected that the highest genetic resolution among families with respect to *Dothistroma blight* susceptibility would occur at 50% affected trees in a trial; so it was logical to test



**Figure 22.** Genetic correlations between *Dothistroma septosporum* score at ages 3 (blue) or 4 (brown) breast height diameter in eight *Pinus radiata* trials in Victoria, Australia. Ivkovic et al. 2010.



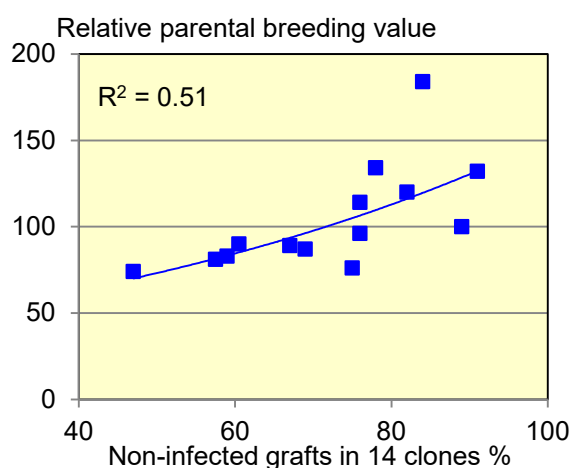
**Figure 23.** Relative breeding values of 25 parents for resistance to *Dothistroma blight* in *Pinus radiata* based on a disconnected diallele mating with 25 parents grouped in five sets with five parents in each group. Assessments took place at an age of five years. Parents were grouped into six classes of breeding values. Wilcox 1982.

any relationship between field trial score and heritability. However, no such relationship was observed. Since the trials did not contain enough numbers of common families, no genotype x environment could be estimated.

The disease influences the growth of the affected trees. To estimate the relationship between *Dothistroma* score at age 3–4 and tree growth at age 10–16 in field trials, genetic correlations between these two traits were calculated. Fig. 22 shows that there are negative relationships but only twice did they explain more than 50% of the variation.

Wilcox (1982) assessed the occurrence of *Dothistroma blight* at age five in a New Zealand progeny trial with *Pinus radiata* full-sibs from a disconnected diallele mating including 25 parents grouped into five sets. A disconnected diallele mating means that only crosses within each of the five groups were carried out. The parents used in this experiment were selected before the arrival of the *Dothistroma blight* to New Zealand and were growing in a clonal seed orchard. This means that no selection for resistance to the blight disease took place at selection of these plus trees.

There was a large variation in blight infection, with five families having 3% infected trees while the most affected had 69% infected trees. Separate analyses of general and specific combining ability were carried out for each group of five parents. Only in one group was there significance for the specific combining ability while the general combining ability was strongly significant in all five groups. The relative breeding values of the parents for resistance to *Dothistroma blight* were estimated (Fig. 23). Three parents showed strong resistance to the disease; relative

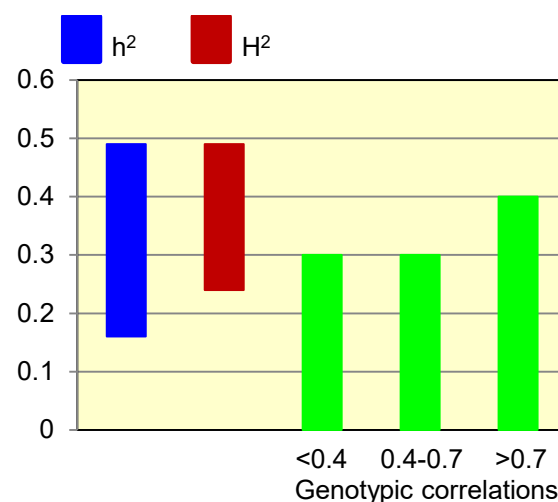


**Figure 24.** The relationship between percentage of non-infected grafts in 14 clones in a *Pinus radiata* seed orchard and relative parental breeding values based on progenies from these clones at age five in a progeny trial. Wilcox 1982.

estimates 153–184, while 13 of the parents had values below 100. The three former are useful for the continued breeding for *Dothistroma* blight resistance. The progenies from the selected trees did not differ much from the three check lots with respect to mean damage score, 1.90 versus 1.72 (scale 0–4). As stated above, the absence of any difference reflects that there was no selection of the plus trees included in this investigation

The relative parental breeding values of 14 clones were related to the percentage of non-infected grafts in 14 clones (Fig. 24). The moderately strong relationship explained approximately 50% of the variation. The scientist who carried out this study stated that evaluation of *Dothistroma* blight in grafted material is less reliable than in trees developed from seeds. This means that a perfect relationship is not expected. Thus, scoring in seed orchards or clonal archives will not lead to optimum selection of blight resistant trees, but culling of the trees with poorest resistance is a valuable possibility for the breeders.

Clone x trial interaction for *Dothistroma* blight resistance was analyzed in five clone trials in New Zealand by Li et al. (2018). The resistance was estimated as the percentage of needles free of blight. The ranges for narrow-sense heritabilities and clonal repeatabilities for resistance in individual trials were 0.16–0.49 and 0.24–0.49, respectively (Fig. 25). 40 % of the genotypic correlations were larger than 0.70 and 30% were below 0.40. High genotypic correlation coefficients mean that the clone x trial interaction is limited. It should be added that all parents and individuals per family were not planted in all trials, which makes an analysis of clone x trial interaction somewhat imprecise, but it was concluded that for *Dothistroma* blight this interaction seems to be of minor importance.



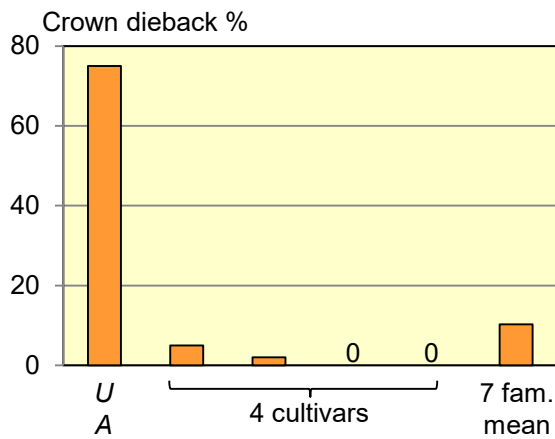
**Figure 25.** The ranges of narrow-sense heritability,  $h^2$ , and clonal repeatability,  $H^2$ , as well as fraction of genotypic correlations in three classes of strength for *Dothistroma septosporum* resistance in five *Pinus radiata* clone trials in New Zealand. Li et al. 2018.

## Deciduous trees

### Elms

Two Dutch scientists described dieback in elms in 1921, which gave rise to the name Dutch elm disease, DED. This disease was caused by a fungus, *Ophiostoma ulmi*. During the late 1960s a still more virulent fungus, *Ophiostoma novo-ulmi*, attacked elms. The American elm, *Ulmus Americana*, and the European elm species are seriously, and most often, lethally damaged by DED. In Europe the disease is spread by two beetles, *Scolytus scolytus* and *S. multistriatus*. In addition to the latter species, *Hylurgopinus rufipes* is also a transmitter of DED in America. DED was spread to America somewhat later than its first appearance in Europe. In 2004 Mittempergher and Santini presented a history of elm breeding. Several breeding programs were initiated in Europe and USA thanks to the ornamental values of elms in urban areas (Mittempergher and Santini 2004). The Dutch breeding program was pioneering but was closed after the retirement of the prominent elm breeder, Hans Heybroek. One of the American programs, led by Eugene Smalley, was terminated after his death in 2002. These are good examples of risks for closure of breeding programs that rely on only one scientist, however prominent. Hans Heybroek (Heybroek 2000) summarized this problem in the following way: *Finally, tree breeding programs, being long term by nature, are susceptible to administrative fatigue*. Heybroek stressed further that DED resistance has a clear entomological component, which is mostly overlooked.

The initial steps in the various elm breeding programs were selection of resistant trees within domestic populations. This was followed by crosses among cultivars with satisfactory DED resistance. Since most existing culti-



**Figure 26.** Crown dieback two years after inoculation with *Ophiostoma ulmi* in *Ulmus Americana* (UA) seedlings, four cultivars, and mean percentage for seven interspecific hybrid families. Townsend and Douglas 1996.

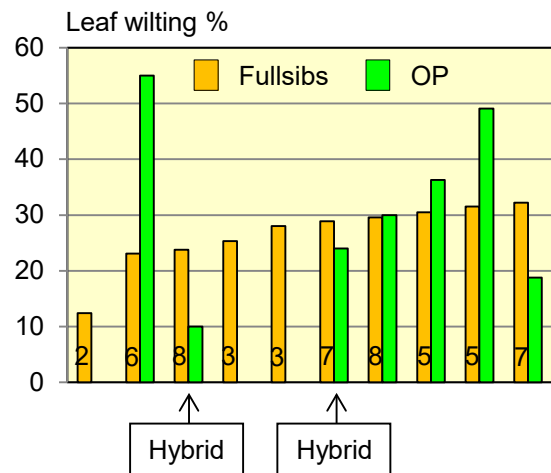
vars at the time of appearance of *Ophiostoma novo-ulmi* turned out to be susceptible, the interest for hybridization with exotic elm species increased. Such hybridization with exotic elms, mainly Asian elms, had already started in the mid-1930s.

The Dutch breeding program showed that:

- Exotic elm species might be useful for hybridization with *U. glabra* to obtain resistance to DED
- Non-harmful organisms for the domestic elm species may be harmful to exotic species and interspecific hybrids

American elm, *Ulmus Americana*, trees are highly susceptible to Dutch elm disease, *Ophiostoma novo-ulmi*, which is a great constraint to within-species improvement of resistance against this disease. Screening of approximately 35,000 American elms resulted in two resistant individuals (Smucker 1944 according to Mitterpergher and Santini 2004), which well illustrates the problem of finding resistant American elms. Even if the frequency of resistant American elm trees is extremely low, a few cultivars were patented and released. Fig. 26 illustrates the large difference in DED resistance between non-selected American elm seedlings and four cultivars (Townsend and Douglas, 1996). One of the seven families between these cultivars had a mean value of 46% for crown dieback and contributed most to the mean value of 10.3% for these seven families. The other six families had low percentages.

There are few studies designed to enable estimates of genetic parameters for DED resistance. One Spanish investigation by Solla et al. (2015) aimed at estimating variance components for DED resistance and growth factors. Ten parents in a partial diallele mating design were studied after inoculation with *Ophiostoma novo-ulmi*. Eight of the parents were *Ulmus pumila* while the other two were interspecific *U. minor x U. pumila* hybrids. The design



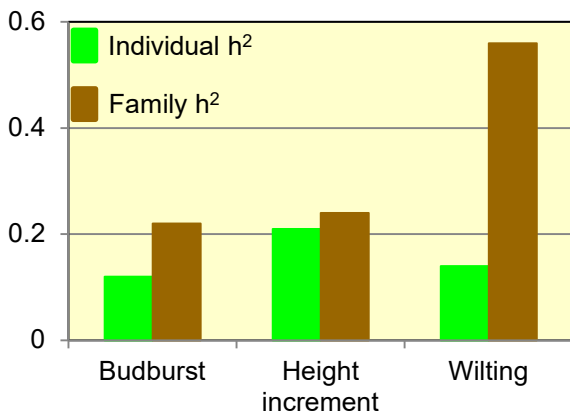
**Figure 27.** Percent leaf wilting of fullsibs (orange) and open-pollinated families of *Ulmus minor* and interspecific *Ulmus minor x U. pumila* females. Wilting was recorded 60 days after inoculation with *Ophiostoma novo-ulmi*. The number of fullsib families for the individual parents is presented. No open-pollinated progenies were obtained from three females. Solla et al. 2015.

was unbalanced owing to absence of seeds from three *U. minor* clones, so that these clones could only be used as males. Leaf wilting was recorded at 60 days after inoculation.

The mean leaf wilting percentages of the progenies from individual parents are illustrated in Fig. 27 together with results from offspring in open-pollinated families. The parent with the lowest mean percentage, 12.4%, was represented in only two full-sib families and is therefore less precise than the other estimates. The other parents had a fairly narrow range of wilting %. It is further seen that the agreement between the percentages following open-pollination and the partial diallel matings is poor. This suggests that open pollination testing is less reliable in this material. The mean values for the two full-sib families with interspecific hybrid parents do not deviate in a significant way from the rest of the mean values. The narrow-sense heritability for leaf wilting was estimated at 0.14 (Fig. 28). It is possible that the heritability would have been somewhat higher if the global mean for the entire material had been closer to 50% than the observed 37%. Since family selection will probably be used in applied breeding, it was useful to estimate the family heritability, which turned out to be high. Height increment had the highest  $h^2$ , 0.21, which does not deviate in a significant way from estimates in other tree species.

The relationship between leaf wilting and height growth is plotted in Fig. 29. As seen from this figure, there is no relationship between these traits. This means that selection for DED resistance will not lead to any strong decrease in height growth. Fig. 29 also shows that the two *U. minor x U. pumila* parents were ranked as second and third parent with respect to height increment.

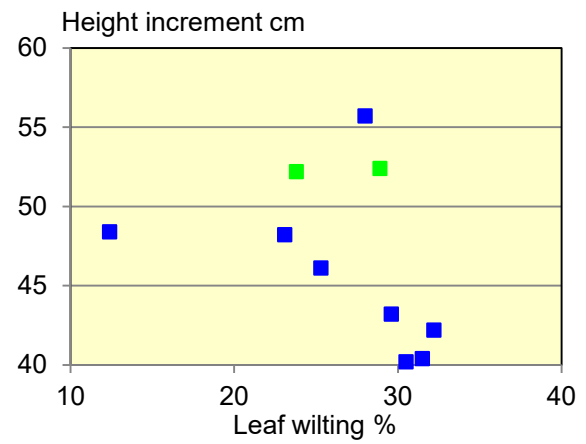




**Figure 28.** Narrow-sense heritabilities and family heritabilities (repeatabilities) for bud flushing, height increment, and leaf wilting percent in an investigation with ten parents crossed according to a partial diallele mating design. Eight *Ulmus minor* clones and two *U. minor* x *U. pumila* clones were included in this mating design. Solla et al 2015.

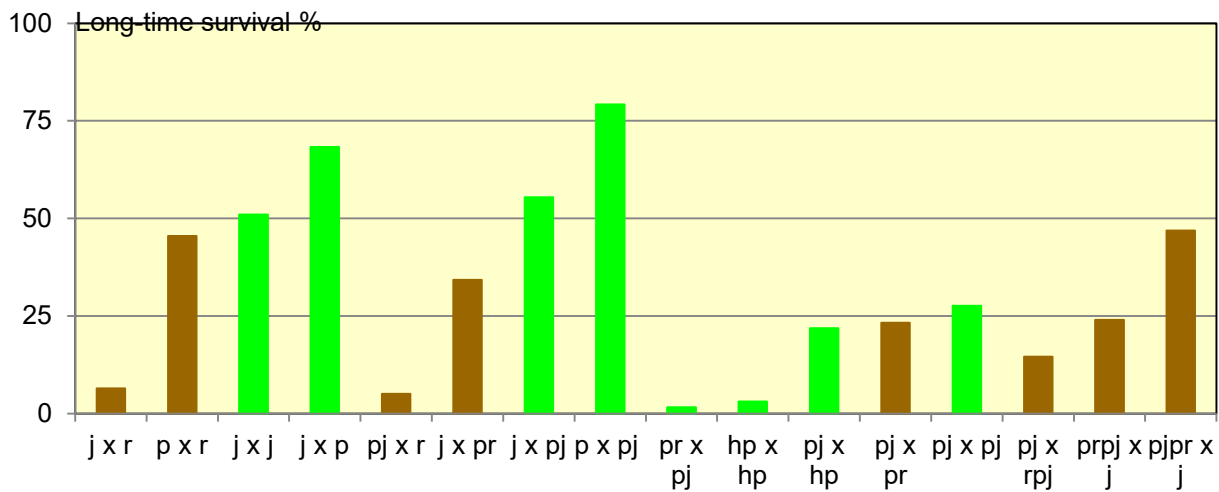
The observation that the non-additive variance for bud flushing and height increment was three times larger than the additive variance is worth mentioning. At least phenology traits usually show strong additive variance. The large presence of non-additive variance also explains the fairly low heritability for budburst.

The American elm is tetraploid, which is a great constraint to hybridization with the diploid elms. In an American hybridization program involving ten elm species 1,600

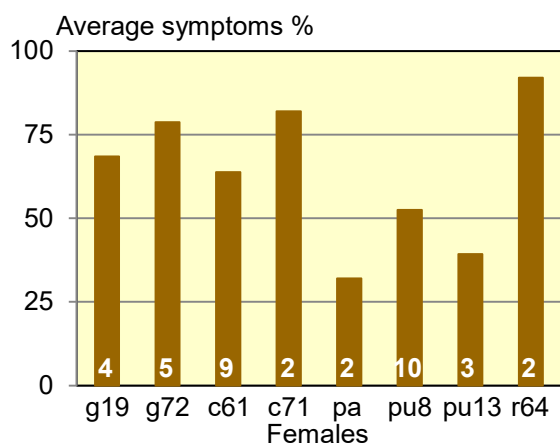


**Figure 29.** The relationship between mean increment and leaf wilting percent for ten clones. The mating design was partial diallele with eight *Ulmus minor* clones (blue) and two *U. minor* x *U. pumila* clones (green). Solla et al. 2015.

seedlings were obtained, of which 12 were hybrids with American elm (Smalley and Guries 1993). If these hybrids are diploid they may be crossed with other diploid elm species. Owing to this constraint in species hybridization with the American elm, American elm breeders focused on hybrids between other elm species. The results of an investigation by Smalley and Guries (1993) are illustrated in Fig. 30, which shows that *U. rubra* (Indian elm) is a susceptible species to DED while *U. japonica* and *U. pumila* (Siberian elm) are more promising for obtaining DED resistance.



**Figure 30.** Percentage of long-time survival of various kinds of elm hybrids following inoculations with *Ophiostoma novo-ulmi* in Wisconsin, USA; h = hollandica (from The Netherlands), j = japonica, p = pumila, r = rubra. Families including *U. rubra* are shown in brown. Smalley and Guies 1993.



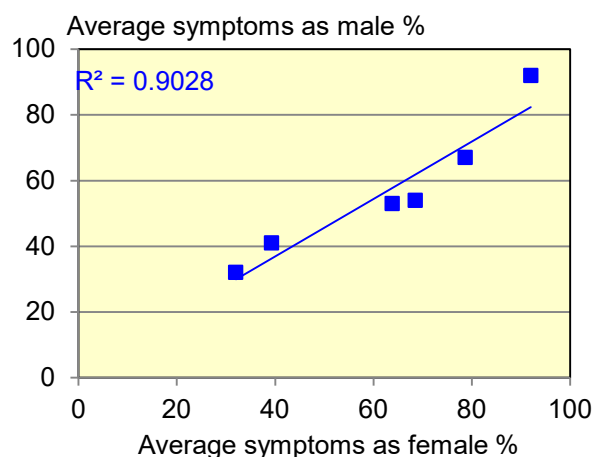
**Figure 31.** Percentage symptoms of Dutch elm disease in offspring from eight females in different interspecific hybrids at 56 weeks after inoculation with *Ophiostoma ulmi*; c = carpinifolia (field elm), g = glabra (wych elm), pa = parvifolia (Chinese elm), pu = pumila (Siberian elm), r = rubra (Indian elm). The number of families with the various parents are indicated. Townsend 1979.

The usefulness of hybridization with *U. pumila* was also noted in another American species hybridization study (Townsend 1979, Fig. 31). The two *U. pumila* parents and the *U. parvifolia* (Chinese elm) had the lowest mean values for disease symptoms. Six of these eight trees were also used as males. There was a strong relationship between the symptoms observed as females and males (Fig.32). The low number of families for certain parents means that too far-reaching conclusions should not be drawn. The Himalayan elm, *U. wallichiana*, was also found to be a resource for resistance to DED.

Even if DED is the major threat to elms in Europe and North America, other threats were identified such as elm yellows, which is caused by phytoplasma. Some herbivore insects may also be harmful for growth of elms. Besides these threats, it is self-evident that the cultivars to be released must be adapted to the ambient conditions in the zone of cultivation.

### Chestnuts

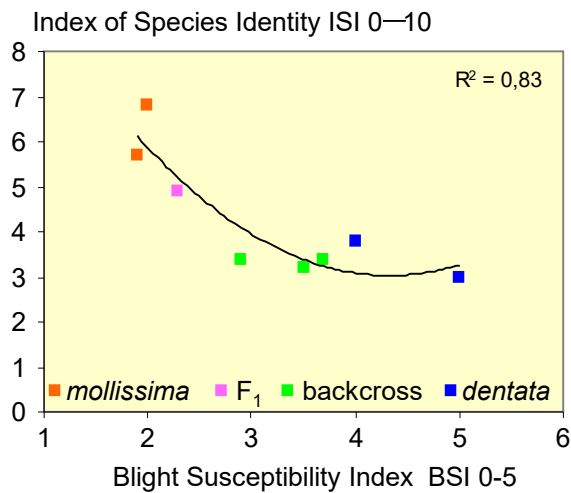
The American chestnut, *Castanea dentata*, was a dominating deciduous tree species in forests of eastern USA until the early 1900 when chestnut blight, caused by *Cryphonectria parasitica*, was introduced by imports of the Asian *Castanea crenata*, which is almost resistant to this fungus. Since then this serious disease has spread and more or less wiped out *C. dentata* in Eastern USA. In 2020 almost only young seedlings of this species could be found in its distribution area. There is a great public interest to restore this charismatic species. Great efforts were, and still are, devoted to restoration of this keystone species. The American chestnut does not seem to have any tolerance towards chestnut blight. Towards the end



**Figure 32.** The relationship between percentage symptoms of Dutch elm disease in offspring from six trees used as females and males in different interspecific hybrids. Symptoms were assessed 56 weeks after inoculation with *Ophiostoma ulmi*. Townsend 1979.

of the 20th century attempts were made to introduce the resistance to chestnut blight from *C. mollissima* (Chinese chestnut) into *C. dentata*. Root rot caused by *Phytophthora* species is another serious disease to chestnuts in Europe and America.

Early studies on inheritance of blight resistance suggested a simple inheritance with genes conveying resistance at 1–3 loci. The results from a backcross study by Cipollini et al (2017) in Georgia, USA, will be used to illustrate some results from backcrosses. This investigation included three full-sib third generation backcross families, one F<sub>1</sub> two *C. dentata*, and two *C. mollissima* (Chinese chestnut) open-pollinated families. The material was artificially inoculated with *Cryphonectria parasitica* at the beginning of the fifth growing season. Pesticide and fungicide treatments were used to avoid attacks by insects and root rot. No symptoms of chestnut blight were noted before the inoculation. On each tree two inoculations were applied with each of two strains of *Cryphonectria*, one of them strongly virulent. Trees with small stem diameters were inoculated once. Blight infection was recorded five months and ten months after inoculation. Three classes of chestnut blight cancer were distinguished following visual inspection at five months after inoculation and a blight susceptibility index, BSI, was calculated. This index was arranged such that a BSI = 1.0 means low susceptibility, while BSI = 5 means high susceptibility. Twenty morphological traits known to differ between the parental species were recorded on undamaged branches growing on the south side of the trees at about two meters above ground. A standardized index of species identity, ISI, was calculated based on this examination. The ISI values were arranged such that *C. dentata* values were low while high values were related to *C. mollissima*. The ISIs are the means of thus standardized values for the different families.

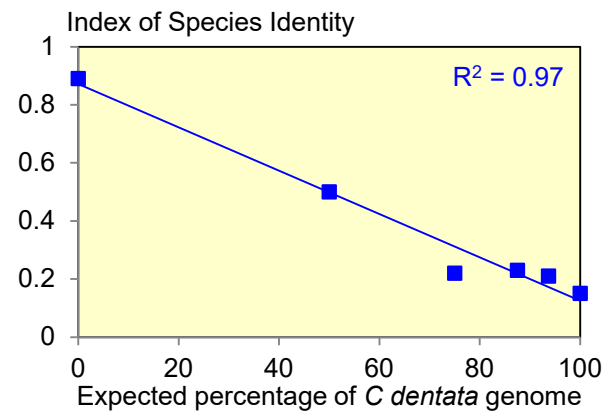


**Figure 33.** The relationship between Blight Susceptibility Index (BSI) and morphological Index of Species Identity (ISI) for *C. dentata*, *C. mollissima*, their  $F_1$ , and third backcross generation. A low ISI value indicates strong *C. dentata* phenotype and a high BSI value means high susceptibility to *Cryphonectria parasitica*. Artificial inoculation took place approximately five months before recording of blight. Cipollini et al. 2017.

In Fig. 33 the main results from this investigation are summarized. The chestnut blight susceptibility is lowest in the *C. mollissima* progenies and highest in the *C. dentata* progenies. The ISI values of the  $B_3$ -families are close to the ISI estimates of pure *C. dentata*. However, the blight resistance is unsatisfactory in the three  $B_3$ -families. The mean percentage of trees with a blight index below 3.0 was 17.1 with a large variation among the three  $B_3$ -families; range 5.1–31.1%. To reach the blight resistance level in *C. mollissima*, it is likely that larger numbers of trees in  $F_1$  and  $F_2$  generations are required to enable a stronger selection for blight resistance. The agreement between the species identity indices of the backcross and the pure *C. dentata* is of greatest interest for restoration of *C. dentata* USA. It was noted that three trees in one of the backcross families were morphologically indistinguishable from pure *C. dentata* and intermediate with respect to blight resistance. These three trees were vigorous 3.5 years after the inoculation.

The assumption the blight resistance would be fixed in  $B_3 \times B_3$  families as is expected if resistance is regulated by genes in two independent loci was challenged. It was speculated that the inheritance is less simple than previously believed. From an evolutionary perspective it is more likely that resistance to blight is polygenically inherited, since the resistance would easily be overcome if it relies on genes at a few loci. The pathogen may pass several generations during one generation cycle of the host species and new mutations overcoming the resistance may occur.

Search is also going on to identify major resistance genes, which might be transferred via modern gene technology

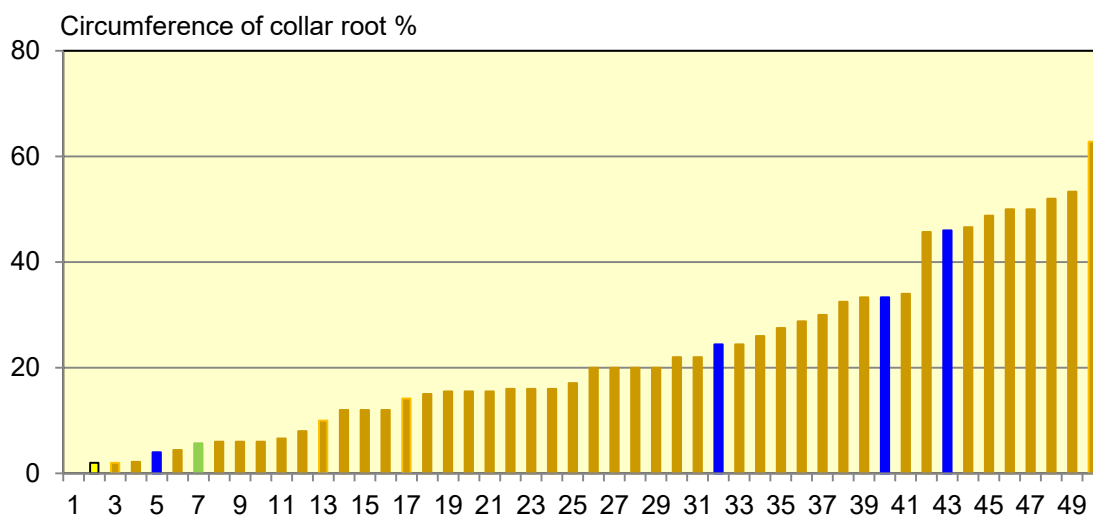


**Figure 34.** The relationships between Index of Species Identity (ISI) and percentage *C. dentata* genome in  $F_1$ ,  $B_1$ ,  $B_2$ ,  $B_3$ , and *C. dentata* following interspecific crosses between *C. dentata* and *C. mollissima*. B stands for back cross with *C. dentata*. Value 1.0 for ISI is *C. mollissima*. Diskin et al. 2006.

to the American chestnut. Understanding of the molecular processes leading to resistance might improve the possibilities to incorporate blight resistance into *C. dentata*.

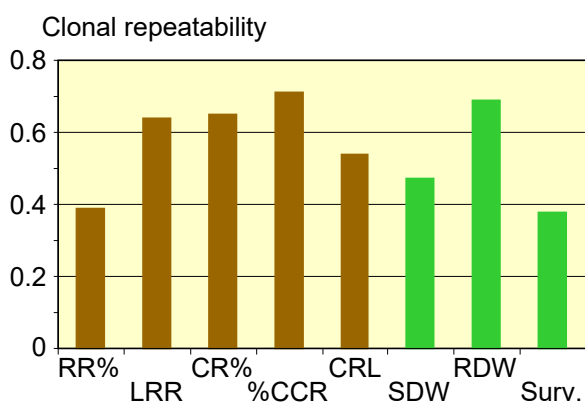
Another example of results from back crosses to *C. dentata* in a species hybrid cross will be presented. The results from three generations of back crosses with *C. dentata* were published by Diskin et al. (2006). Thanks to early flowering the generation turnover of chestnuts can be five years or less. This makes a back-cross breeding program a valuable option. In each generation of this back-cross program, disease-tolerant trees were selected as parents. Twenty-four morphological traits in  $F_1$  and the three generations of back-crosses were recorded. Also the parental species *C. dentata* and *C. mollissima* were studied. The morphological traits were weighed to obtain an index of species identity (ISI) with a scale 0–1, in which 0 stands for *C. mollissima* and 1.0 stands for *C. dentata*. Such an index gives a general picture of the morphological approach against the *C. dentata* phenotype. In the case of simple inheritance a straight line is expected between the percentage of *C. dentata* genome and the different traits studied. Fig. 34 shows that the fit to the straight line for the relationship with ISI is extremely good. The strong fit suggests that most of the traits analyzed showed additive inheritance or alternatively that dominance effects from both parents are cancelled. These results are promising steps for the restoration of the American chestnut with incorporated tolerance against *Cryphonectria parasitica*. It requires that other important traits also are included in the American chestnut.

Root rot caused by *Phytophthora cinnamomi* in *Castanea sativa* is a serious disease. Besides selection in domestic populations, interspecific hybrids with Asian chestnuts were thought to be a remedy to combat this disease.



**Figure 35.** Mean clonal value for the percentage circumference of collar rot of 50 clones tested for resistance against *Phytophthora cinnamomi* based on visual examination of roots 14 weeks after inoculation. 44 *C. crenata* x *C. sativa* clones (orange), 4 *C. sativa* clones (blue), 1 *C. mollissima* x *C. sativa* clone (green), and 1 *C. crenata* clone (yellow). Miranda-Fontaina et al. 2007.

Results from an experiment with *Phytophthora cinnamomi* inoculations of 50 vegetatively propagated clones, mainly *C. crenata* x *C. sativa* hybrids (44), will be used to illustrate the genetic variation for resistance to this pathogen (Miranda et al. 2007). A large and continuous variation for the percentage circumference of collar rot was observed (Fig.35). The continuous variation for this trait suggests polygenic inheritance of resistance to root rot.



**Figure 36.** Clonal repeatabilities of resistance-related traits and growth traits after inoculation with *Phytophthora cinnamomi*. Fifty clones, mainly *C. sativa* x *C. crenata*, were included in this investigation. Trait abbreviations are:

Root rot percentage = RR%

Level of root rot = LRR

Collar root percentage = CR%

Percentage circumference of collar rot = %CCR

Collar rot length = CRL

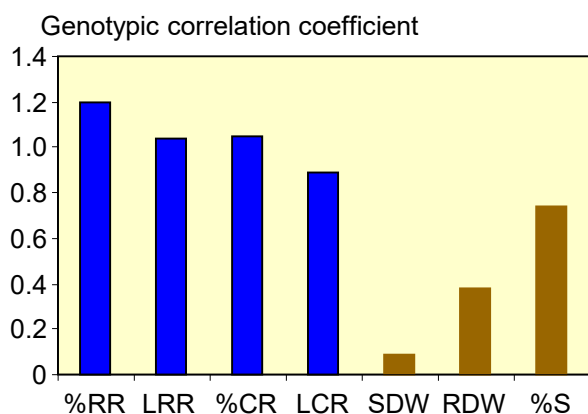
Shoot dry weight = SDW

Root dry weight = RDW

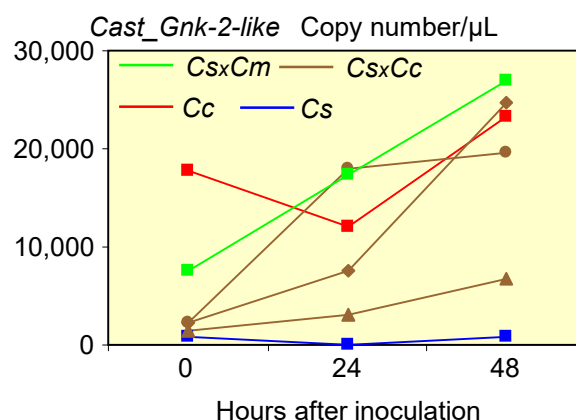
Survival percentage = Surv. Miranda-Fontaina 2007.

Interestingly, one of the *C. sativa* clones was one of the least susceptible clones in this experiment. As expected, the two Asian clones belong to this category as well. Except for the root rot percentage, four other traits showed strongly significant differences among clones. This is reflected in the high clonal repeatabilities of most traits with small standard errors (0.04 – 0.05) of the estimates. The clonal repeatabilities for three growth traits and percentage of circumference collar rot are illustrated in Fig. 36 together with four other traits related to resistance to *Phytophthora cinnamomi*. The repeatabilities were relatively high for the two growth traits as well. There were significant differences between the isolates for the three traits involving collar rot. However, except for collar rot length, the interaction clone x isolate was non-significant. This means that testing just one isolate of the pathogen would be satisfactory to identify resistant genotypes. Of interest is to estimate relationships among different disease-related traits to decide how many traits to include in applied breeding. The relationships between disease-related traits with growth traits will reveal if there is a cost for resistance owing to loss of productivity. The most reliable estimates of such relationships are genotypic, which can be estimated in clone trials. The genotypic correlations between collar rot disease-related traits as well as growth traits are presented in Fig. 37. As seen from this figure the correlations among the rot-related traits are all strong and positive, while there are negative relationships with the dry weight traits and percentage survival. It is evident that the infection had a negative impact on growth and survival. Experimental shortcomings might be responsible for the coefficients exceeding the permitted correlation coefficient of 1.00. Strong genotypic correlations among the disease-related traits mean that all traits need not be assessed in applied breeding.





**Figure 37.** Genotypic correlation coefficients between percentage of circumference collar rot and:  
 Root rot percentage = %RR  
 Level of root rot = LRR  
 Collar rot percentage = %CR  
 Length of collar rot = LCR  
 Shoot dry weight = SDW  
 Root dry weight = RDW  
 Survival percentage = %S  
 Red bars = negative coefficients. Miranda-Fontaina 2007.



**Figure 38.** Cast\_Gnk-2-like gene expression in four types of progeny following inoculation with *Phytophthora cinnamomi*.  
 Cs = *Castanea sativa*  
 Cc = *Castanea crenata*  
 Cm = *Castanea mollissima*  
 CsxCm = interspecific hybrid between *C. sativa* and *C. mollissima*  
 CsxCc = interspecific hybrid between *C. sativa* and *C. crenata*. Santos et al. 2017

Results from another investigation (Santos et al. 2017) will be used to illustrate some results on genes that might be involved in resistance to *Phytophthora*. Expression profiles of eight genes before and after inoculation of four taxa (1 *C. sativa*, 1 *C. crenata*, 3 *C. sativa* x *C. crenata*, 1 *C. sativa* x *C. mollissima*) with varying susceptibility against *Phytophthora cinnamomi* were studied. Among the 283 differently expressed genes, eight were selected for a detailed investigation. These genes should represent the three layers of defence against fungal attacks:

1. Means to avoid attacks in form of morphology or antifungal defence around the roots
2. Recognition of the parasite
3. Activation of host resistance genes

All *C. sativa* plants died one week after treatment, while 83% of the *C. crenata* plants survived. Among the hybrids, those with *C. mollissima* were the most resistant, with 46% survival. The development of expression for one gene is shown in Fig. 38. For *Cast\_Gnk-2-like* gene there is a pronounced increase over time in three of the interspecific hybrids, while the fourth hybrid and *C. sativa* seedlings did not show any increased gene expression. The expression in *C. crenata* did not change much during the test period but it was the only taxon with high expression at the start of the experiment (Fig. 38). This

gene is believed to have an antifungal activity, and its function may be to prevent pathogen growth owing to its antifungal properties or by inducing cell death.

Based on the results a hypothetical response mechanism in *Castanea* for attacks by *Phytophthora cinnamomi* was presented, i.e. a crucial role of the *Cast\_Gnk-2-like* gene. It was suggested that the first layer of defence was decisive for obtaining resistance. The lower expression in *C. sativa* of the eight genes studied, as well as their delayed expressions, may be the reason for the susceptibility of *C. sativa*. Since the two Asian chestnuts have coexisted with *Phytophthora cinnamomi* for generations it is likely that selection for resistance against this pathogen has taken place over many generations. The task for breeders is to identify these genes and transfer them into the susceptible chestnut species.

### Ash

In 1992 dieback in *Fraxinus excelsior* was detected in Poland, and a few years later it was found in neighboring Lithuania. This disease is caused by the Asian fungus *Hymenoscyphus fraxineus*; it is still spreading in Europe in 2020 and has killed millions of *F. excelsior* trees. The seriousness of this disease has triggered several forest geneticists to study resistance to it.

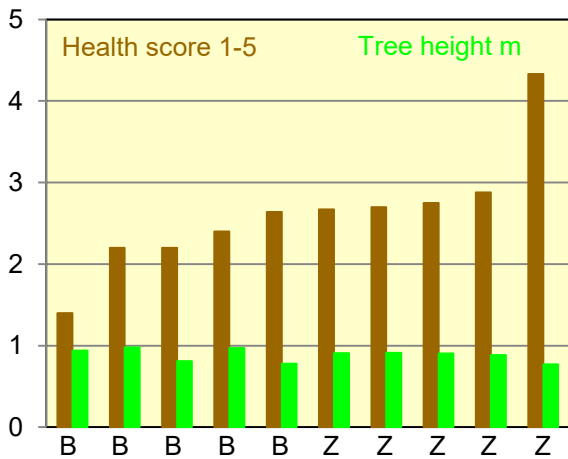


Figure 39. Tree health score and seedling height at age four of ten OP-families of *Fraxinus excelsior* from two Lithuanian populations, Biržal and Zeimelis, in a Lithuanian greenhouse trial. Tree health score 1 = dry stem and branches, 5 = externally healthy tree. The trees were exposed to an artificial freeze testing at  $-5^{\circ}\text{C}$ , May 16 at age four. Pliura et al. 2015.

In a Lithuanian investigation by Pliura et al. (2017) including ten OP-families of *Fraxinus excelsior* from two populations in Lithuania, survival varied over the range 40–100% at age four. Two treatments, besides the control, were included in this trial: an artificial freeze test at May 16 and simulated drought in the last growth period. Several traits related to the ash dieback disease were assessed. The health status of the individual trees was classified as class 1 = brown stem and branches – to class 5 = externally healthy tree. A great majority of the trees were affected by the ash dieback disease. The incidence of disease in the OP-families was high in all three treatments

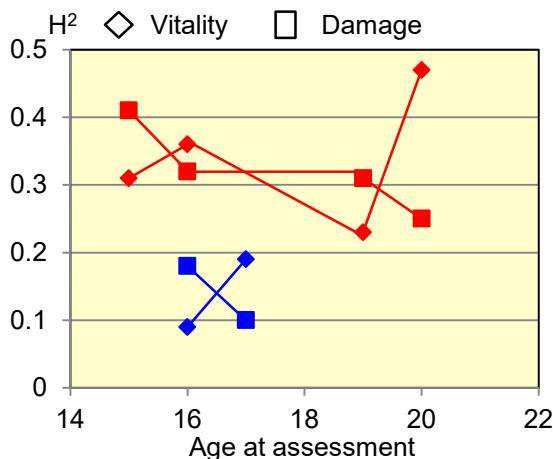


Figure 40. The development over time of clonal repeatability,  $H^2$ , for tree vitality and damage in older shoots and stem caused by ash dieback in two southern Swedish seed orchards (red and blue) with *Fraxinus excelsior*. Both traits were scored in ten classes. The seed orchards had 100 and 104 clones.

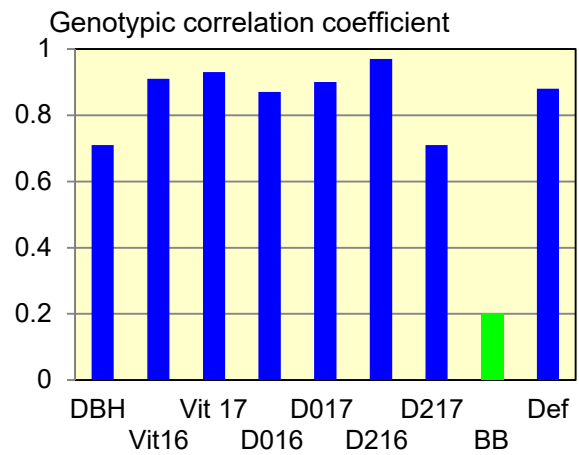
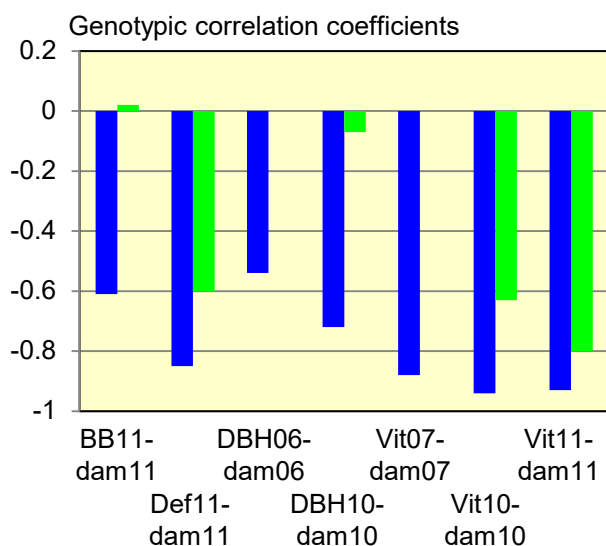


Figure 41. The genotypic correlations between the same trait in the two Swedish seed orchards with *Fraxinus excelsior*. DBH = breast height diameter, Vit = vitality at ages 16 and 17, D0 = damage of leader, D2 = damage of older shoots and stem damage, BB = budburst, Def = defoliation. Green = negative correlation. Stener 2013.

and varied in the range 90–95%. Fig. 39 reveals a considerable OP-family variation in the health condition in the individual OP-families in the spring frost treatment. The variation was strongly significant.

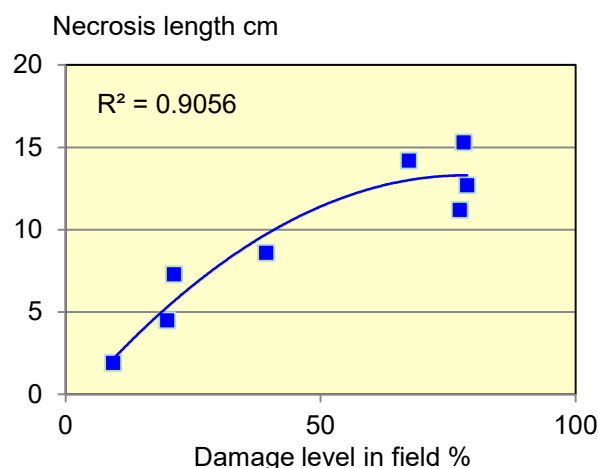
Ash dieback in two southern Swedish *F. excelsior* clonal seed orchards was assessed at ages 15–20 years by Stener (2017). Clonal repeatabilities for two of the traits related to ash dieback are illustrated in Fig. 40. In the orchard with observations over several years there is an indication that clonal repeatability for the damage trait declines with age. The damage caused in individual years might be attributed to different ambient conditions rather than to host tree age. Of great significance was the strong genotypic correlation between the same trait in the two orchards (Fig. 41). This strongly suggests that clone  $\times$  site interaction is of limited importance. Phenology traits were assessed to find whether there is any relationship between them and damage or vitality. The genotypic correlation coefficients for such relationships are illustrated in Fig. 42. In both seed orchards there were fairly strong negative relationships between defoliation and the mean damage; while bud flushing was not correlated with mean damage in one of the seed orchards but this correlation was fairly strong in the other seed orchard. The increased susceptibility with late growth cessation was shared with other studies. The correlations between vitality and mean damage were strong in both seed orchards. This means that one damage trait will more or less give the same information about tree vitality. Scoring of damage could therefore rely on just one of the damage traits to minimize the cost for assessment in applied breeding.



**Figure 42.** The genotypic correlations between various traits a certain year (11 = 2011, 06 = 2006 etc.) in two Swedish seed orchards (blue and green) with *Fraxinus excelsior*. Assessments in the green orchard were carried out only in years 2010 and 2011. BB = budburst, Def = defoliation, DBH = breast height diameter, Vit = vitality, dam = mean for three types of damage D0 = damage of leader, D2 = damage of older shoots and stem damage. Stener 2013.

In a Danish investigation by Kjær et al. (2015) the damage in eight clones in the field was compared with the extension of necrosis in their progeny following artificial inoculation (Fig. 43). This set of eight clones represented the range of susceptibilities obtained among 39 previously tested clones. Fig. 43 reveals a strong relationship between field damage and necrosis in the offspring,  $R^2 = 0.90$ . The estimated narrow-sense heritabilities for resistance to *H. fraxineus* were rather strong and varied in the range 0.37–0.53. The clone with the highest resistance became infected with small spots of necrosis, but it seemed to have an ability to prevent the pathogen from further expansion; an example of an active response in the host species.

The genomic basis was studied in 1,250 European ash trees by Stocks et al. (2019) with varying resistance to ash dieback. No fewer than 3,149 single nucleotide polymorphisms were associated with low or high resistance to ash dieback. Of the more than nine million SNP loci detected, 203 were significantly associated with degree of ash dieback damage. In ten of these 203 SNP loci, missense variants were found for seven genes. Missense means that a nucleotide in DNA is substituted such that a change of amino acid in the produced protein takes place. One conclusion was that resistance to ash dieback disease is polygenically inherited.



**Figure 43.** The relationship between damage level in field and length of necrosis in offspring from eight *Fraxinus excelsior* clones artificially inoculated with *Hymenoscyphus fraxineus* in a Danish investigation.

Common to the studies of ash dieback is:

- Low frequency of resistant genotypes in *F. excelsior* populations and so far no totally resistant tree detected
- Strong genetic variation in susceptibility to this disease
- Polygenic inheritance of disease resistance
- Limited genotype x site interaction

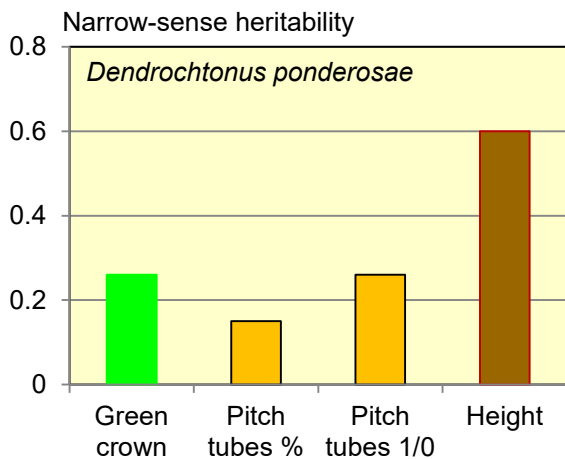
Late growth cessation seems to increase the susceptibility.

## Insects

Numerous insects are dependent on forest trees for their existence, some of them extremely harmful. Genetic studies are sometimes missing even if some insect species cause serious damage to their host tree species. In this section we illustrate some examples from genetic studies on important insect herbivores. *Dendroctonus ponderosae* is a small beetle (mountain pine beetle) that causes severe damage in various pine species in western North America from Mexico to British Columbia. The devastation caused by the mountain pine beetle in *Pinus contorta* forests in British Columbia was mentioned earlier (BC Forest service 2012).

*Pissodes strobi*, the white pine weevil, is one of the most harmful pests on *Picea sitchensis*, *P. engelmanni*, and *P. glauca* in western North America. The eggs are deposited in spring in the leader from last year. The larvae feed from the bark phloem, which mostly kills the leader. This in turn results in deformities of the stem. Results from two studies of resistance to this insect will illustrate genetic variation in susceptibility to this harmful insect.

In periodic outbreaks the spruce budworm, *Christoneura fumiferana*, defoliates *Picea* and *Abies* trees in its distribution areas in eastern North America, and causes mortality and great losses in wood yield during such outbreaks.



**Figure 44.** Narrow-sense heritabilities for tree height and three traits related to spontaneous mountain pine beetle infestation in a Canadian *Pinus contorta* progeny trial including 165 open-pollinated and six full-sib families. Pitch tubes 1/0 stands for absence or presence of this trait. Infestation at age 20 was recorded while height data emanate from age ten. Yanchuk et al. 2007.

#### *Dendroctonus ponderosae*

An attractive way to analyze if resistance to mountain pine beetles is genetically conditioned is to compare the genetic constitution of the few survivors with dead trees (Six et al. 2018). To overcome problems of obtaining DNA from dead trees, breast height diameters of killed trees were recorded and the samples of survivors for this study comprised surviving trees with similar breast height diameters. The beetles do not attack trees of small size. The genetic constitution of the survivors was compared with a second set of living trees for each species with much lower breast height diameters. The trees in the second set were assumed to reflect the genetic constitution of the original stand without any selection caused by the mountain pine beetle. A national forest in Montana, USA was used for this study. This forest had experienced a serious outbreak of mountain pine beetle on *Pinus albicaulis* and *P. contorta* a few years before, with the death of 93% of *P. albicaulis* and 75% of the *P. contorta* trees. Inter-simple sequence repeats (ISSR) were used for this purpose. Three ISSR primers were used for *P. albicaulis* while four were used for *P. contorta*. The studied trees were selected in long but narrow transects to obtain 30 living trees.

The estimates for differentiation between the two sets of population with analysis of molecular variance resulted in 13% and 7% between-population differentiation within *P. albicaulis* and *P. contorta*, respectively. These estimates are really high considering that all trees, survivors and comparison trees grow intermixed in the same forest. Other analysis methods also revealed large variation between survivors and the general population. Fewer than 10% of the trees in the comparison population had “survivor” genotypes.

A significant outbreak of mountain pine beetle attacks in British Columbia in 2005 called for an analysis of any genetic differences in a *Pinus contorta* progeny trial of 165 open-pollinated families, representing three populations (Yanchuk et al. 2008). In addition, six full-sib families and a regional control population were included in this trial. Green crowns, pitch tubes per tree, and presence or absence of pitch tubes were recorded. Green crown = no visual signs of beetle infestation. Pitch tube is a hole that is left by a beetle when it tries to bore into the tree. The relationship between tree height at age ten and infestation traits was estimated to see if the beetle attacks had any impact on growth.

The percentages of attacked trees in 2003 and 2004 were 2% and 11%. This means that 87% of the trees had a green crown in 2005. However, more than 60% of the trees had pitch tubes, i.e. holes in the bark from penetration of the mountain pine beetles, but the number of pitch tubes per tree was low, 1.5. The proportion of trees with pitch tubes present varied in the range 0.39-0.60 among the three populations. The corresponding proportion in the regional control material was slightly higher than the most affected population, 0.69. The estimated heritabilities for the traits investigated are illustrated in Fig. 44. With the low values for percentage of affected crowns, 13%, and the low number of pitch tubes per tree it is not expected that these two traits would have high heritabilities. Therefore the narrow-sense estimates of 0.20 and 0.15 must be regarded as unexpectedly strong. Similarly, the heritability for tree height was extremely high for a forest tree, 0.60. Population differences might have contributed to the high heritability estimate. There was a large variation among the family mean values for tree height, 3.45–5.46 meters, which explains the large heritability estimate for tree height. Besides, the population effect was inflated in the heritability estimates. It was assumed that one third of the estimates might be attributed to the variation among the three populations. The heritability for presence or absence of pitch tubes was fairly high, 0.26, which suggests that selection for resistance to mountain pine beetle would be rewarding. A prerequisite is that the relationship between resistance and growth is weak, which was the case,  $R^2 = 0.23$ .

#### *Pissodes strobi*

In an investigation by Alfaro et al. (2008) 67 open-pollinated families and six bulk populations of *P. sitchensis* were studied in two field trials over five and ten years. In one part of each trial weevils were released while the other part of the trial was left without any treatment. The cumulative attacks by the weevil were assessed and amounted to 54% (Cr) and 81% (Jr) in the areas with released weevils and to 33% (Cnr no release) and 62% (Jnr) in the other areas of the two trials. In the Cnr area 12 OP-families did not show any weevil attacks at the end of the experiment after five seasons. The high percentages of attacks in three of the areas allowed a meaningful ana-



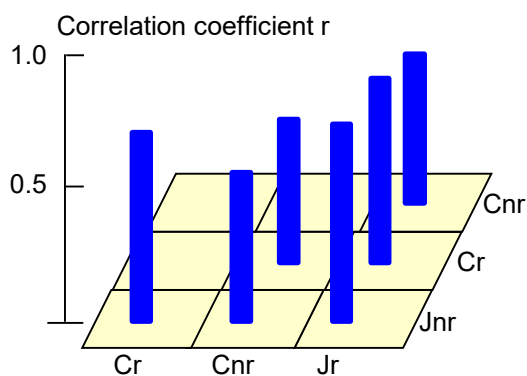


Figure 45. Pearson correlation coefficients for all pairwise relationships of *Pissodes strobi* cumulative attacks in two *Picea sitchensis* trials, C and J, with release of *Pissodes strobi* (*r*) or without release (*nr*) of this insect. Alfaro et al. 2008.

lysis of possible genetic differences. In all, eight families belonged to the tenth percentile with highest resistance to the weevil. None of them were common to all three areas (Cr, Cnr, and Jr). Some of these OP-families belonged to the tenth percentile for several but not all years. Of interest is to estimate the agreement in weevil attacks in the four treatments. Pearson correlations for the pairwise comparisons of insect cumulative attacks were calculated. All of them were significant but the degree of explanation of the relationships varied in the range 28–56% (Fig. 45). In the J trial the correlation coefficient between the two treatments was the highest of all relationships,  $r = 0.75$ , while the corresponding estimate for the C trial was the lowest of all relationships,  $r = 0.53$ . The fairly low degree of explanation in the relationships is distressing for breeding for resistance against weevil attacks, since breeding cannot rely on one test locality or one treatment only.

Forty-two full-sib families from *P. glauca* x *P. engelmanni* were studied in another investigation on genetic variation in *Pissodes strobi* attacks (Alfaro et al. 2004). Eleven males and nine females were used in the crossings. Some parents were used in more than one cross and in a few cases both as female and male. Also in this case weevils were released in the progeny trial, and the death of leaders was assessed over four years after the release of weevils. Fig. 46 shows that the resistant x resistant families suffered least from the weevil attacks and the resistant x susceptible families had slightly lower percentage of dead apical leaders than the susceptible x susceptible families. The latter type of family was represented by six families only, which makes the estimates somewhat less precise than for the other two types of family, with 16 and 20 families, respectively. The mating design used meant that estimates of genetic parameters could not be carried out. Attack mean values for four of the parents included in two types of crosses (A–D in Fig. 47) and represented by at least three full-sub families in each of the crosses are also shown in this figure. Parent A had

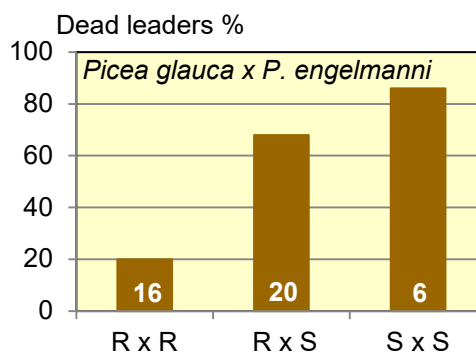


Figure 9-46. Percentage of dead leaders following attacks by *Pissodes strobi* in three types of full-sib families; R x R = resistant x resistant, S x S = susceptible x susceptible, and hybrids between resistant and susceptible parents (R x S) of interspecific *Picea glauca* x *P. engelmanni*. Alfaro et al. 2004.

mean values above the average in both types of crosses, R x R and R x S, while parent C had the highest mean value among the R x R families but had a mean value below the average in the R x S families. Parent D was included in three of the six S x S families, which means that it contributed much to the S x S mean value. It is hard to draw any conclusions as regards the stability of the parents from these data.

The presence of failed attacks, defined as ovipositions carried out but without brood development, was determined with the following result: R x R 17%, R x S 12%, and S x S 6%. It seems as if there is a defense mechanism in the resistant trees preventing further development after oviposition.

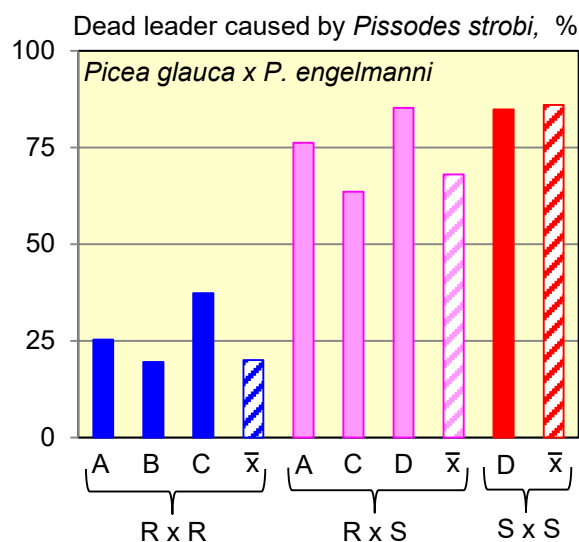
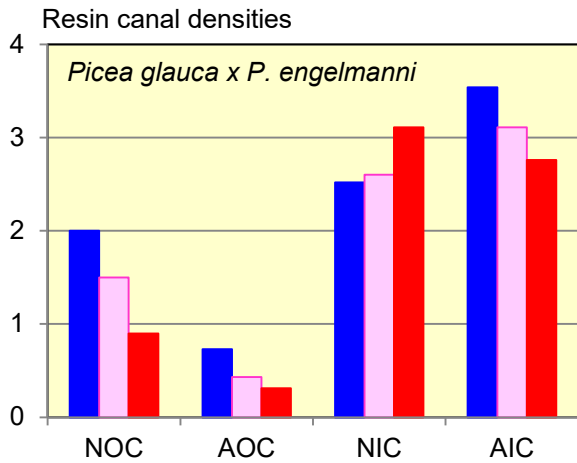


Figure 47. Percentage of dead leaders following attacks by *Pissodes strobi* in three types of full-sib families; R x R = resistant x resistant, S x S = susceptible x susceptible, and hybrids between resistant and susceptible parents (R x S) of interspecific *Picea glauca* x *P. engelmanni*. Alfaro et al. 2004.

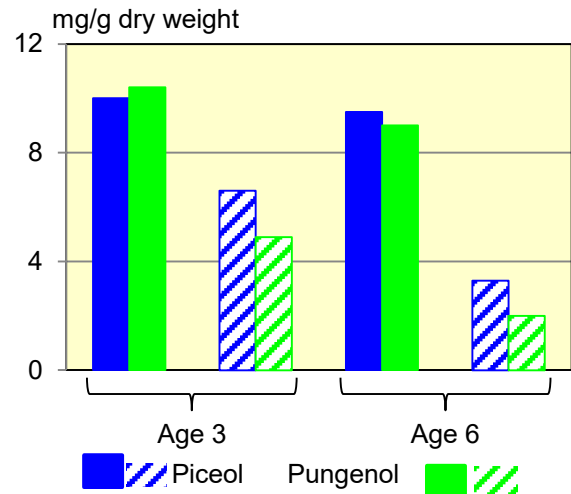


**Figure 48.** Resin canal densities in three types of full-sib families; R x R = resistant x resistant (blue), S x S = susceptible x susceptible (red), and resistant x susceptible (lilac) between resistant and susceptible parents. NOC = number of outer resin canals/mm<sup>2</sup>, NIC = number of inner resin canals/mm<sup>2</sup>, AOC = % of bark area occupied by outer resin canals, AIC = % of bark area occupied by inner resin canals. Alfaro et al. 2004.

To test whether or not resin canal characteristics are of any significance for resistance to white pine weevil attacks, four resin canal traits were assessed: number of outer resin canals/mm<sup>2</sup>, number of inner resin canals/mm<sup>2</sup>, percentage of bark area occupied by outer resin canals, and percentage of bark area occupied by inner resin canals. As seen from Fig. 48 there is a difference between the three types of families for the outer resin canal traits. The resistant x susceptible families take an intermediate position between the two other types of family. Moreover, there were significant and negative relationships with weevil attacks. However, these relationships explained less than 20% of the variation around these relationships. This means that other factors influence the attacks by the weevils more than the resin canal traits.

#### *Christoneura fumiferana*

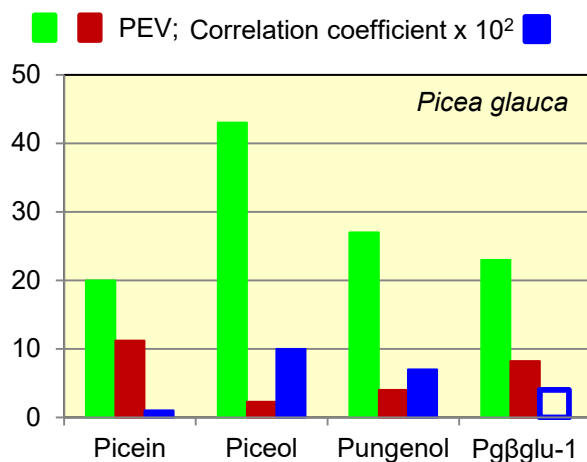
Efforts were devoted to understand the molecular mechanism underlying resistance to *Christoneura fumiferana* (spruce budworm) in *Picea glauca* (Mageroy et al. 2015). The expression of more than 20,000 genes in resistant and susceptible *Picea glauca* trees was screened in one investigation. Transcripts of 236 genes were more abundant in the resistant trees (defined as 0–20% defoliation) than in the susceptible trees (defined as 30–70% defoliation) while transcripts of 250 genes were more abundant in the susceptible trees. Especially transcripts of one gene, *Pgβglu-1*, were 770 times higher in current year needles in resistant trees than in susceptible trees. Progenies from the resistant trees had higher expression of the *Pgβglu-1*-gene. There was a weak correlation between abundance of this transcript of *Pgβglu-1* and the levels of two biologically active acetophenones, piceol and pungenol. The



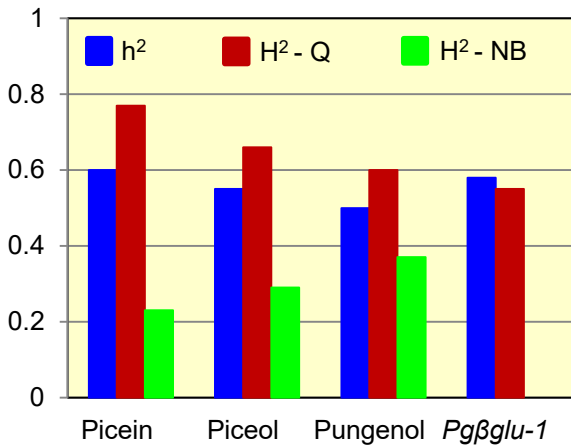
**Figure 48.** Resistant/susceptible ratios of two acetophenones, piceol and pungenol, in *Picea glauca* in an eastern Canadian population. Data from 3 and 6 years after spruce budworm outbreaks. Filled bars refer to assessments in June and striped bars refer to assessments in August. Mageroy et al. 2015.

ratios between their levels in resistant and susceptible trees varied in the range 2.0–10.4 (Fig. 49). The role of these two acetophenones in tree defence was detected in the early 2010s. The detection of a gene contributing strongly to resistance to spruce budworm is important information for breeding of *Picea glauca*.

In further studies of acetophenones by Lamara et al. (2018), single nucleotide polymorphisms (SNPs) were analyzed. In this effort 35 SNPs from newly formed needles were significantly associated with *Pgβglu-1* transcript



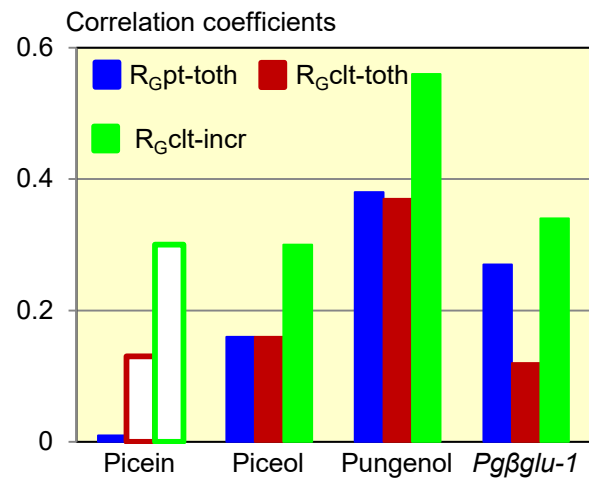
**Figure 50.** Phenotypic variation explained, PEV, by all significant SNPs identified by two different analysis methods (green and brown bars). The phenotypic correlation coefficients x 10<sup>2</sup> between breast height diameter and the four compounds related to resistance to spruce budworm attacks are given. Empty bar = negative relationship. Lamara et al. 2018.



**Figure 51.** Narrow sense heritability,  $h^2$  and clonal repeatability  $H^2$  at two *Picea glauca* trials in Quebec (Q) and New Brunswick (NB) for four spruce budworm resistance biomarkers, picein, piceol, pungenol, and  $Pg\beta glu-1$ . Mendez-Espinosa et al. 2018.

and the three compounds, piceol, picein, and pungenol. All three are involved in the metabolism of phenols. In conifers phenols play an important role in the defence against herbivores. Picein had the highest concentration with 6% of the dry mass. The phenotypic variance explained (PVE) by SNPs was analyzed by two different methods. One way resulted in PEVs in the range 20–43% and the other in the range 2.3–11.2% (Fig. 50). It is evident that resistance to spruce budworm is more complex than single-gene inheritance. The results in this study suggested that resistance was attributed to few genes with a large effect and several other genes, each with a small effect. The relationships between growth traits (annual ring width, tree height, and breast height diameter) and the three acetophenones or  $Pg\beta glu-1$  were all weak (Fig. 50). This means that the production of biomarkers for resistance to spruce budworm does not take place at a cost in biomass production.

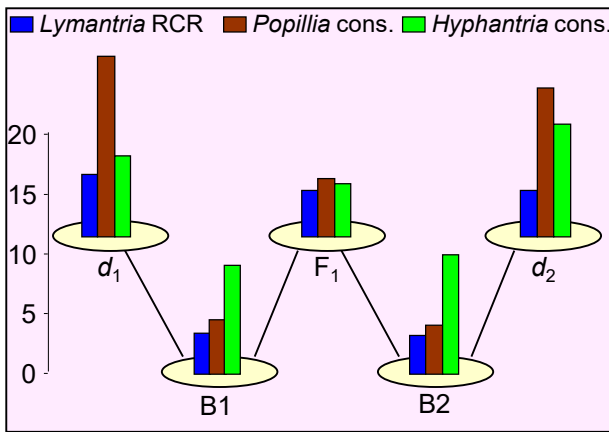
To elucidate the potential for resistance breeding, genetic parameters were also estimated for these biomarkers based on two Canadian progeny trials in Quebec and Ontario as well as five clone trials with *Picea glauca* in these provinces (Mendez-Espinosa et al. 2018). High nar-



**Figure 52.** Genetic correlation coefficients between four spruce budworm resistance biomarkers and total height (toth) at age nine in a *Picea glauca* progeny trial (pt) in Quebec and clonal correlation coefficients for total height at age six and height increment (incr) between ages 3–6 in *Picea glauca* clone trials (clt) in Quebec. Empty columns = negative correlation coefficients. Mendez-Espinosa et al. 2018.

row-sense heritabilities and clonal repeatabilities were noted for the four biomarkers in the Quebec trials (Fig. 50). Especially the narrow-sense heritabilities were higher than most similar studies with other quantitative traits. One reason might be the wide origin of this material, with an accompanying differentiation owing to adaptation to different site conditions. Except for picein, the three other biomarkers showed strong genetic correlations.  $R_G$  varied in the range 0.79–0.88. The weak relationship picein – piceol was unexpected since picein is the precursor of piceol. This might be attributed to high accumulation of picein in the needles.

There were no strong negative relationships between the biomarkers and tree height or height increment (Fig. 52). Only in one case did the correlation coefficient exceed 0.50, the correlation between pungenol and height increment in the clonal trial. It may be concluded that the four biomarkers do not affect tree growth negatively to any great extent.



**Figure 53.** *Lymantria dispar* relative consumption rate mg/(mg x day); *Popillia japonica* consumption leaf area cm<sup>2</sup>; *Hyphantria cunea* consumption leaf area cm<sup>2</sup>. F<sub>1</sub> = *C. dentata* x *C. mollissima*, B1 = back cross with *C. dentata* d1, B2 = back cross with *C. dentata* d2. Kellogg et al. 2005.

The interaction between chestnut blight in *Castanea dentata* and three herbivores, *Lymantria dispar*, *Popillia japonica*, and *Hyphantria cunea*, was investigated by Kellogg et al. (2005). Two backcross families, B1 and B2, between one F<sub>1</sub> tree from a cross between *C. mollissima* and *C. dentata* were included in this study. The families contained 24 and 18 trees respectively and were half-sibs. Chestnut blight was inoculated in June during the fourth growing season of the two progeny families; also the three parental trees were inoculated. The rating of blight resistance was carried out five months after inoculation with the following result.

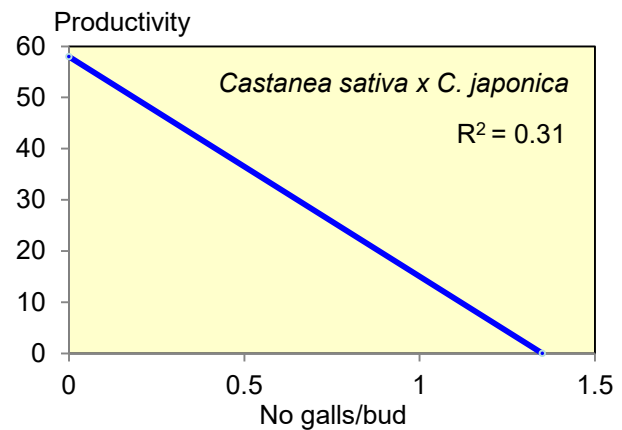
<i>C. dentata</i>	5.0 highest susceptibility
F <sub>1</sub>	2.0
Back cross 1	3.0
Back cross 2	5.0

Two twigs from each tree were sampled for herbivore assay and foliar properties, respectively. The insects were kept in cages under controlled conditions: 23°C and 15:9 h light and darkness. The *Lymantria dispar* larvae were reared in small rearing boxes after 24 hours of starvation. The relative growth rate (RGR) was determined and estimated as:

$$\frac{[\text{final larval weight}] - (\text{initial larval weight})}{(\text{initial larval weight}/\text{day})}$$

The *Popillia japonica* beetles were also starved for 24 hours before groups of three beetles were exposed to a single leaf in rearing boxes for 48 hours or in groups of nine exposed to three leaves, one from each parent and their progeny. The amount of dry matter consumed was determined.

Caterpillars of *Hyphantria cunea* were starved for 24 hours and then placed in groups of nine on chestnut leaves in rearing boxes. Five foliar characteristics were analyzed at three occasions, June 10, July 8, and July 29, during the growth period:



**Figure 54.** The relationship between number of galls per bud and productivity = kg nuts per trunk area in cm<sup>2</sup>. The productivity refers to mean values for the Marsol cultivar (*Castanea sativa* x *C. japonica*) at ages 8-14 in a northern Italian orchard. There was wide scatter around the line, which is reflected in the low R<sup>2</sup> estimate. Sartor et al. 2015.

- Toughness, mg
- Density, mg/cm<sup>2</sup>
- Carbohydrates, %
- Nitrogen, %
- Tannins, tannic acid equivalents

Correlations between foliar content/characteristics and the impacts of insect damage were estimated.

The three classes of blight resistance, 3.0 – 5.0, did not affect any of the traits studied in any of the three insect species included in this study. In the three-choice test the *Popillia* beetles preferred the two *C. dentata* parents, p < 0.001. There were no significant differences between the five genetic entries with respect to any of the traits studied in the *Lymantria* assay (Fig. 53). This figure reveals that there were large differences for the *Popillia* and *Hyphantria* assays. As regards *Popillia* the two back crosses and F<sub>1</sub> were least affected by this insect. This suggests that there is some dominance for resistance against *Popillia* contributed by *C. mollissima* that is conferred from F<sub>1</sub> to the two back cross progenies. However, in the three-choice test, F<sub>1</sub> and the *C. dentata* parents were the most affected entries, and that speaks against such an interpretation. The F<sub>1</sub> parent was least affected by *Hyphantria* insects. It is possible that an increased contribution of the *C. dentata* genome in the back crosses contributed to an increased susceptibility of these towards *Hyphantria*. The results from this investigation are good illustrations that straight-forward interpretations of biological material are not always obtained.

In an investigation by Sartor et al. (2015) 62 cultivars of *C. sativa* and its interspecific hybrids with *C. crenata* were infested with *Dryocosmus kuriphilus*. Infestation was carried out in June-July in screen houses with one insect per five buds. The response to infestation was estimated as galls formed per number of buds at infesta-



tion. Yield loss in one cultivar (Marsol) was estimated in a north-western Italian orchard at lat. 44.49°N and 500 masl during seven years at ages 8-14 years. At the end of the growing season the tree circumference was measured at 20 cm above the graft union and the nut weight was recorded. The relationship between galls/bud and production data was determined.

Seven cultivars did not develop any galls. This total resistance was confirmed after stronger infestation application of these seven clones. Two of the resistant cultivars were *C. sativa* and one was *C. crenata*; the remaining four were interspecific hybrids between these two species. Three of the four hybrids had a common parent, a *C. crenata* clone selected in France. The galls per bud in the remaining 55 clones varied continuously up to approximately 1.2 galls per bud. Especially for breeding, the finding of two resistant *C. sativa* cultivars was welcomed. It was noted that eggs were deposited in all cultivars but with different preference. It was speculated that bud size, bud texture, and volatile substances might be responsible for this difference in preference.

As regards the effect on productivity of number of galls/bud, one significant relationship was detected. However, the degree of explanation for this relationship was only 31% (Fig.54). The contents of crude fats, crude fibers, carbohydrates, starch, and crude proteins were lower in leaves with galls than in unaffected leaves. In contrast, sugar content was higher in the infested leaves.

## Summary

Important pests and diseases affecting conifers and deciduous trees from the temperate and boreal regions are presented. Several of the serious attacks on host trees are attributed to exotic fungi and insects. Various combinations of host and parasite occur; not only native host and exotic parasite but also exotic host and native parasite as well as other combinations. It is pointed out that confusion exists as regards terminology related to resistance. The following definitions have been put forward:

Complete resistance: no or minimal damage is caused by pathogens or pests

Partial resistance: the effects of attacks are limited

Tolerance: symptoms are displayed, but growth and performance are maintained to some extent.

Different phases in the response to a pathogen may be identified:

1. Avoidance of attacks by morphological change or characteristics of the host that deter or repel fungi and insects
2. Recognition of the parasite
3. Activation of host resistance genes

The general approach of the genetic studies to mitigate the attacks of pathogens has been:

Screening of domestic populations for resistance  
Screening of variation in virulence of fungal parasites

Interspecific hybridization to transfer resistance from exotic species

Identification of genes for resistance and transfer of genes via molecular technology

Generally, the frequency of resistance or tolerance in natural populations is mostly extremely low. The majority of studies suggests that resistance is polygenically inherited. Results from artificial inoculation at young age reflected results in field trials in some cases. In these cases inoculation at young age might be an option in breeding for resistance. Major resistance genes were identified but they did not confer resistance in all genetic backgrounds. Interspecific hybridization has led to improved resistance in several cases, especially when related East Asian tree species are used in the hybridization. In the program for restoration of the American chestnut the third backcross generation has been reached with some improvement of the resistance to the chestnut blight disease. An accompanying approach to the American chestnut phenotype has taken place. Variation in virulence among fungal isolates was in some cases pronounced but in most cases much less than host resistance variability.

A scrutiny of five resistance breeding programs showed the vulnerability of programs relying on one or a few persons. It was concluded that long-term commitment and central coordination were key issues for success. Expressed in another way: *tree breeding programs, being long term by nature, are susceptible to administrative fatigue.* This quotation comes from one leading elm breeder and geneticist, Hans Heybroek. He also stated that there ought to be more focus on the interaction between the fungus and the beetles transferring the fungal Dutch elm disease than what has been the case so far.

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