Examensarbeten **2009:3**
Institutionen för skogens ekologi och skötsel

Susceptibility of hybrid aspen *(Populus tremula x tremuloides)* to pine twisting rust *(Melampsora pinitorqua)*

Emma Kassfeldt

Sveriges Lantbruksuniversitet Skogligt magisterprogram/Jägmästarprogrammet
Examensarbete i skogshushållning, 30 hp, avancerad D
Handledare: Andreas Bernhold och Per Hansson, SLU, Inst för skogens ekologi och skötsel
Examinator: Björn Elfving, SLU, Inst för skogens ekologi och skötsel ISSN 1654-1898
Umeå 2009

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Nyckelord/*Keywords:*
Pine twisting rust, *Melampsora pinitorqua* , hybrid aspen, *Populus tremula x tremuloides* , European aspen, rust disease

ISSN 1654-1898 Umeå 2009

Sveriges Lantbruksuniversitet / *Swedish University of Agricultural Sciences*
Fakulteten för skogsvetenskap / *Faculty of Forestry*
Skogligt magisterprogram/Jägmästarprogrammet / *Master of Science in Forestry*
Examensarbete i skogshushållning / *Master of Science thesis*, EX0481, 30 hp, avancerad D

Handledare / *Supervisors*: Andreas Bernhold och Per Hansson
SLU, Inst för skogens ekologi och skötsel / *Dept of Forest Ecology and Management*
Examinator / *Examiner:* Björn Elfving
SLU, Inst för skogens ekologi och skötsel / *Dept of Forest Ecology and Management*

Content

Sammanfattning

Knäckesjuka *(Melampsora pinitorqua)* är en rostsvamp som orsakar skador på plantor och unga träd av tall *(Pinus sylvestris)* i Sverige, vilket lokalt kan innebära stora problem. Svampen har en komplicerad livscykel som innefattar en värdväxling mellan tall och asp *(Populus sp.)*. Syftet med studien var att undersöka om sporer av knäckesjukasvampen är lika vanligt förekommande på hybridasp *(Populus tremula x tremuloides)* som på europeisk asp *(Populus tremula)*. Med tanke på att hybridasp kan komma att bli ett alternativ som biomassaproducent är det viktigt att studera tänkbara risker detta trädslag kan föra med sig. Min hypotes var att hybridaspen är mer resistent än europeisk asp mot knäckesjuka, och att den därmed också sprider mindre mängd sporer som i sin tur kan infektera tall. Genom att inventera 12 hybridaspplanteringar etablerade åren 2005-2007 i Jämtland och Västernorrland och jämföra sporförekomsten på blad av hybridasp med blad på rotskott av europeisk asp på samma lokaler, samlades data in för att kunna testa denna hypotes. Signifikanta skillnader kunde ses mellan hybridaspen och den europeiska aspen på samtliga 12 lokaler. Totalt var endast 1 % av hybridaspens löv infekterade jämfört med 35 % av den europeiska aspens löv. Studien kompletterades med en inventering av tre hybridaspplanteringar etablerade i Västerbotten 1993, för att se om knäckesjuka förekommer även på äldre träd. Här var 3-6 % av hybridasplöven infekterade. Slutsatsen blev att knäckesjuka kan infektera även hybridasp, men att infektionsgraden är mycket låg i jämförelse med europeisk asp. Denna slutsats styrks av liknande resultat i tidigare studier. Eventuellt kan detta resultat öka motivationen för plantering av hybridasp. De skogsbrukare som tvekat inför hybridasp, på grund av smittorisken till närliggande tallföryngringar, kan med stöd av denna studie känna större tilltro till detta snabbväxande trädslag.

Abstract

Pine twisting rust *(Melampsora pinitorqua)* is a rust fungus that causes damage in plantations and young stands of Scots pine *(Pinus sylvestris)* in Sweden, which can cause extensive problem on a local scale. The fungus has a complicated life-cycle which involves two host species, Scots pine and European aspen *(Populus tremula)*. The purpose of this study was to examine whether the pine twisting rust is as common on hybrid aspen *(Populus tremula x tremuloides)* as on European aspen. Considering that hybrid aspen likely will become an alternative for biomass production, it is important to know which risks it might bring. My hypothesis was that the hybrid aspen is more resistant to pine twisting rust than the European aspen, and therefore it will spread a less amount of spores that can infect pine. By measuring infection on 12 hybrid aspen plantations established in 2005-2007 in Jämtland and Västernorrland, and comparing the amount of uredospores on hybrid aspen leaves with leaves on suckers of European aspen at the same sites, data was collected to test the stated hypothesis. Significant differences were found between the hybrid aspen and the European aspen on all 12 sites. Only 1% of the leaves on the hybrid aspens were infected compared to 35% of the leaves on the European aspens. The study was complemented with an inventory of three hybrid aspen plantations established in Västerbotten in 1993, to conclude if spores of the rust were present on older trees as well. On these sites, 3-6% of the leaves were infected. The conclusion was that pine twisting rust can infect hybrid aspen, but the rate of disease incidence is very low compared to European aspen. This conclusion is also supported by similar results in earlier studies. Perhaps these results increase the possibilities for future planting of hybrid aspen. The forest owners that have hesitated to plant hybrid aspen, because of the risk of rust infection spreading to Scots pine regenerations, might with support of the present study have higher faith in this fast growing tree.

Introduction

Hybrid aspen *(Populus tremula x tremuloides)* grows faster than the native European aspen *(Populus tremula)* and reaches a very high productivity on farmland sites (Elfving 1986, Rytter & Stener 2005). With its short rotation period and aesthetic advantages it is an interesting alternative to Norway spruce *(Picea abies)* on fertile soils no longer used for farming purposes. However, to be able to judge whether the hybrid aspen is suited for biomass production we need to learn more about potential problems it can bring. A new introduced species always comes with risks, such as higher susceptibility to diseases, and if we are not careful the result can be a change in the ecosystem balance (Karlman 1981).

A group of disease agents connected to aspens, and that sometime causes severe problems are *Melampsora* rusts (Sinclair & Lyon 2005). In Sweden, pine twisting rust *(Melampsora pinitorqua)* causes damage on young Scots pine *(Pinus sylvestris)* stands and have the European aspen as its alternate host (Klingström 1963). The pathogen reduces the growth and quality of the pines and might even kill small seedlings (Skogsstyrelsen 1995). This is why forest managers for a long time have tried to eliminate aspen from Scots pine stands (Eidmann & Klingström 1976).

There seems to be a shortage of recent studies concerning hybrid aspen and its interactions with pathogens. Little effort has been put into research since the middle of the 20th century, when hybrid aspen was introduced in Swedish forestry and considered a promising alternative for the future (Johnsson 1955). Nowadays, when biomass production is a hot topic (due to their coal sink effect), the fast growing species have regained some attention (Pettersson Ostelius 2008). However, caution needs to be taken and the presumptions made more than 50 years ago needs to be complemented with more research.

Pine twisting rust

Melampsora species cause damage on several different tree species in many parts of the world (Sinclair & Lyon 2005). Fungi that cause rust diseases are biotrophic pathogens meaning that they prefer healthy hosts for growth and development. *Melampsora* rusts require two different plant species as hosts to complete their life cycle (Lagerberg 1945, Manion 1991). For example, the collective species of *Melampsora populina* consists of many different species that all infect poplar species but have different alternate hosts. *Melampsora larici-tremulae* alternate between aspen and *Larix* and *M. rostrupii* alternate with *Mercurials perennis* (Lagerberg 1945, Sinclair & Lyon 2005). In Sweden, it is usually only the pine twisting rust that is accounted for from the *Melampsora* genus. This species is found all across Europe from the northernmost parts of Norway all the way down to Cyprus and into the Asian parts of Russia. It alternates between many different pine and poplar species. However, in Sweden the pine twisting rust mainly alternates between the native European aspen and Scots pine (Klingström 1963, Eidmann & Klingström 1976).

Life cycle

The life cycle of pine twisting rust takes one year and starts with the forming of basidia and basidiospores on leaves of aspen in the spring (Klingström 1963). The small spores (5-8 µm) are spread in early summer and can travel quite far with the wind to the shoots of young pines (Klingström 1963, Pei & Shang 2005). About 14 days after the infection of the pine shoot, basidiospores germinate and the haploid hyphae spread between the cells. From these hyphae a spermogonia is formed from which spermatia is spread. Spermatia fuse with specialized hyphae and form dikaryotic hyphae and from this, an aecidia with aeciospores are formed on the pine shoot (Manion 1991). At this stage the rust fungus now changes host back to the aspen as the aeciospores spread and infects the aspen leaves at the beginning of the summer (Eidmann & Klingström 1976). When the aeciospores have infected the aspen they germinate and form dikaryotic hyphae from which uredospores (summer spores) are formed on the lower side of the leaves. The asexual uredospores re-infect the same tree individual or neighboring trees of the same species during the summer, which leads to an intensified infection. To complete the cycle, teliospores (winter spores) are formed during the autumn when two nuclei fuse and form a diploid nucleus. It is in the teliospore stage that the rust stays dormant during the winter. When the teliospores germinate in the spring basidia with basidiospores are formed and the life cycle is completed (Manion 1991, Pei & Shang, 2005).

It is only for a limited time during the elongation of the pine shoot that it can be infected; the most critical period is before the needles begin to show on the new shoot (Klingström 1963). During this period moist weather is required to enable the basidia to form and basidiospores to spread from aspen to pines (Klingström 1963, Eidmann & Klingström 1976, Samuelsson & Örlander 2001). At the next stage of development, drying and high temperatures (above 30°C) have a destructive effect on the basidiospores (Klingström 1963, Eidmann & Klingström 1976). Spreading of the infection to pines is controlled by a mechanism in the teliospores that inhibit too early germination of basidia in the spring. However, when mild autumns and winters occur, there is a risk for the teliospores to germinate too early (Eidmann & Klingström 1976).

Symptoms

Infection usually occurs on the top shoot and the shoots in the upper part of the pines (Lagerberg 1945, Samuelsson & Örlander 2001). The rust can be identified on the young pines by the yellow patches (between 1 and 3 cm) on the central part of the shoots. A resin-soaked canker hereafter appears on the shoot and if the damaged tissue covers too big an area, the shoot tissue above the canker will die. If the damage is more concentrated to one side of the shoot, the other side will continue to elongate which will result in an S-shaped bend of the stem which will cause lower quality of the but-log in the future (Lagerberg 1945, Samuelsson & Örlander 2001). The damage on shoots can be mistaken for browsing of moose, if the shoot is broken off, or damage caused by pine shoot moth *(Rhyacionia buoliana)*, if it is bent. Scots pine seedlings up to two years of age might even die if severely infected by pine twisting rust (Skogsstyrelsen 1995). During the rust’s life stages on aspen, the disease can be recognized by the bright yellow to orange uredinia (0.3-0.5 mm) on the lower side of the leaves during summer. These spots turn brown or almost black when the teliospores are formed during fall (Lagerberg 1945).

Young Scots pines are more frequently infected in the leader and the upper lateral branches, which results in a greater reduction in growth compared to older trees (Martinsson 1985). Different saplings are more or less receptive to the disease in different years; an explanation for this can be that the climatic factors controlling sporulation of the rust and the development of the shoot are different (Martinsson 1985). This means that the same tree can be much more susceptible one year than the next at the time for spreading and infection of *Melampsora* spores. Another explanation for the difference in severity of attack between different years on a sapling is the lack of shoot tissue the year after a severe attack (Martinsson 1985). Scots pines are most susceptible to the pine twisting rust under the age of 15 years but can occasionally be infected at any age (Lagerberg 1945, Skogsstyrelsen 1995). Certain Scots pine clones are more susceptible to pine twisting rust (Klingström 1963). Significant differences in rust susceptibility between populations and among families in populations have been concluded by Quencez and Bastien (2001). In their study they found differences in susceptibility correlated to shoot length. They also concluded that populations with later elongation where less susceptible to the rust and that a population less exposed to infection had lower resistance than one that had been more exposed to the disease (Quencez & Bastien 2001).

Stand factors influencing outbreak

Soil fertility and presence of aspen are the primary factors that determine if pine twisting rust is present or absent in a Scots pine stand (Mattila *et al.* 2001, Mattila 2005). Studies from Finland show that the rust occurs even if there is only a low density of aspen within the pine stands, but that there is a significant increase in disease incidence with number of aspen. Scots pine stands on fertile soils show more infection than stands on poor soils. One reason for this seems to be that on fertile soils trees has more susceptible tissue, which means a larger spore catching area. (Mattila *et al.* 2001, Mattila 2005).

The soil fertility seems to have more influence on the degree of infection in a Scots pine stand than number of aspens, given that aspen is present (Mattila *et al.* 2001). The same authors also found more pine twisting rust damage in planted or sown Scots pine stands compared to naturally regenerated stands. This can be explained by a higher growth rate (and thereby more susceptible tissue) as a result of a chosen suitable origin and soil preparation (Mattila 2005). Another sign that is correlated with the risk of pine twisting rust infection in a Scots pine stand is the presence of willows, given there is also aspen present. The number of willows indirectly describes the moisture conditions, which in turn influence the germination of the teliospores on the aspen leaf litter (Mattila *et al.* 2001).

Hybrid aspen

Already in 1939, the first successful crossing of the American trembling aspen and the European aspen was made in Sweden (Johnsson 1955). In this small scale experiment the researchers noticed that the young saplings were not attacked by diseases such as rust fungi, which the European aspen often was. After the Second World War, North American parent trees were picked out and new crossings were made and planted in southern Sweden. It was early concluded that these hybrids had a higher production than both the original aspen species (Johnsson 1955). This trait can at least partly be explained by the hybrid aspen's resistance towards rust and *Venturia* diseases. European aspen often suffer severe damage by these diseases during their first years, which distinctly reduce the growth rate (Johnsson 1957).

Production

For many decades now, hybrid aspen has been considered an interesting choice on former farmland. A comparison with Norway spruce on farmland, performed in the mid 1980s outside Sundsvall, confirms the hybrid aspens' competitiveness as a fast growing tree (Elfving 1986). The result showed a mean increment of 13.2 m^3/ha and year for hybrid aspen, compared to 14 m^3/ha and year for spruce. The hybrid aspen had a higher density at breast height compared to Norway spruce (Elfving 1986).

Today, hybrid aspen planted in southern Sweden may produce 20 m^3/ha and year during a rotation period of 20 to 25 years (Rytter & Stener 2005). To reach high growth rates of hybrid aspen further north as well, it is under development to produce clones more suited for the northern parts of the country. According to Jörgen Hajek, Forestry Research Institute of Sweden, it is probable that clones suited for the climate at the coast of Norrland can produce about 15 m^3/ha and year during a rotation time of 35 to 40 years. Apart from round-wood it is also possible to retrieve 20 to 25 tons of dry matter for bio-fuel at the final cutting (Pettersson Ostelius 2008).

Phenology

In a study on phenology and growth of hybrid aspen compared with European aspen, all four different hybrids tested outperformed the European aspen after five years (Yu *et al.* 2001). There were big differences among the hybrid clones in volume and annual growth rate but the wood production was significantly higher compared to European aspen. The authors found some differences in growth patterns between the two species. The first being that the hybrid aspens had two peaks in growth rate and the European aspens had only one. Secondly, the European aspens reached its highest growth rate earlier than the hybrids and peaked in mid June, while the hybrids peaked in mid July and in mid August. Thirdly, the growth period for the hybrids started earlier in the spring and the defoliation occurred about a month later in fall. This resulted in a 30 to 40 days longer growth period for the hybrid aspen compared to European aspen (Yu *et al.* 2001). The difference in growth period length could be explained by the fact that the European aspen have adapted to the short summer on higher latitudes, minimizing the risk of frost damage. The provenance of the North American parent trees is, on the other hand, of more southern latitudes which explain the later cessation and defoliation of the hybrid aspens (Johnsson 1953, Yu *et al*. 2001). Another difference that may cause the European aspen to defoliate earlier is the higher susceptibility of infection by leaf rust (Yu *et al*. 2001).

The trembling aspen, also known as quaking aspen, has its natural range across the North American continent (Brinkman & Roe 1975). It is a fast growing and short lived tree species that barely reaches pulpwood size on unfavorable soils, but when growing on good sites, produces high quality saw logs. The major use of the aspen is to make chipboard and pulp since its physiological maturity is reached before it reaches saw log size. The most common reproduction is by production of new shoots from existing root systems (suckering) (Brinkman & Roe 1975), which is also the best way of reproducing European aspen and hybrid aspen (Hansson 1989). Only when populating a new area, as for example after a fire, it is reproduced by seedlings. Some regular diseases on trembling aspen are of the kind that attack leaves and shoots (Brinkman & Roe 1975). Compared to the hybrid aspen, the stem shape of the trembling aspen is usually inferior and it is more receptive to diseases. Another advantage of the crossing, from a Swedish perspective, is that the hybrid clones can be adapted to different growing conditions and climates when fast growing trembling aspen is combined with suitable European clones (Johnsson 1953).

Forestry

A more intensive forestry caused a spread and increase of pine twisting rust during the first part of the 20th century (Klingström 1963). In the beginning of the 1960s, a lot of agricultural land in Sweden had been left unused and was turned into forested land, mainly dominated by deciduous species. This lead to an increasing problem of pine twisting rust (Klingström 1963, Eidmann & Klingström 1976). Severe outbreaks of pine twisting rust can occur on locations with high sprouting of aspen root suckers after a clear-cut or fire. To diminish the spreading of the disease, foresters have tried to remove aspen from pine stands in different ways during the years. The eradication of aspen have been carried out by mechanical treatments (which is still used) and chemical treatments. In the 1970s, a common procedure was to spray herbicides from the air (Eidmann & Klingström 1976). According to Eidmann and Klingström (1976), the most imporatant measure to fight pine twisting rust is to remove aspens around nurserys. A zone of 200 meters is suggested to be sufficient since the basidiospores are belived not to spread further than this.

Today, the value of aspen and other decidous trees for biodiversity is common knowledge among foresters and ecologists. Old aspens left in the landscape can be used by many species of many orders (de Jong *et al.* 1999). If old aspens are cut down, the result is extensive sprouting which in turn leads to more rust in the stand. Therefore, girdling is prefered as a complemantary procedure to leaving old aspen for free development. Girdling results in limited sprouting and leads to more standing dead wood which also is rare in the landscape and an important substrate for many threatened species (de Jong *et al.* 1999).

Objective

The objective was to test the hypothesis that hybrid aspen (*Populus tremula x tremuloides*) is more resistant than the European aspen (*P. tremula*) to infection by pine twisting rust (*Melampsora pinitorqua*). The goal is that the outcome of this study can come to practical use when hybrid aspen stands are to be established in the future. The results will simplify the risk assessment procedure when hybrid aspen is to be planted adjacent to plantations of pine.

Material and methods

An assessment of pine twisting rust occurrence was made on plantations with hybrid aspen and compared to the presence of the rust-spores on adjacent naturally regenerated European aspen. The plantations are located in Jämtland and Västernorrland and were established during a three year period, between 2005 and 2007. As a complementary part in this study, three parcels with 15-year old hybrid aspen close to Umeå in Västerbotten were evaluated for pine twisting rust occurrence.

Assessment of rust spores on hybrid and European aspen saplings

A total of 23 plantations of hybrid aspen were planned for establishment with the assistance of Jämtlands läns institut för lantbruksutveckling (JiLU), Bispgården, during 2005 to 2007. Of these, some had not survived or the planting had been postponed, but of the registered 23, 15 were visited and 12 of these were included in the study. Of the twelve plantations, three were established on forest land while the remaining nine were established on former crop land. For a stand to be counted for in the study, the following requirements needed to be met. There had to be European aspen in comparable sizes present in a distance of maximum 200 meters from the hybrid aspen plantation. Furthermore, if presence of the rust could not be directly verified on aspen it had to be confirmed by recent infection on pine.

At each plantation, 20 saplings of hybrid aspen were chosen for the inventory. These were the ones with the most similar prerequisites as the European aspens with which they were compared. For most of the plantations the chosen hybrid aspens were those closest to the European aspen outside the plantation and in these cases the saplings were systematically chosen. For the remaining plantations where European aspens were present among the planted hybrid aspens, the hybrid saplings were randomly chosen. On each sapling of hybrid aspen and sucker of European aspen, the same number of leaves was checked for uredospores. At the younger plantations and plantations where the trees had grown rather slow, 30 leaves per tree were checked. For the older plantations and where it was possible, 50 leaves were chosen. On bigger saplings, leaves were chosen at around breast height, i.e. 1.3 ± 0.5 meters. Occurrence and coverage of spores was visually assessed and registered for each leaf. If spores were detected, a scale of three grades was used for spore coverage assessment. Class 1; <10% of the leaf area covered by spores, Class 2; 10 - 50%, Class 3; >50% (photos see Appendix 3). All leaves without infection were registered as Class 0.

For each plantation, the GPS-coordinates were recorded to get exact location and height above see level. The GPS was also used for measuring distance from the plantation to nearest European aspen. By talking to the landowners some information regarding problems with plant establishment was collected, e.g. damage by rodents, browsing by deer or other factors that might have caused reduced growth or death of saplings. This first visit and data collection was made in mid-September. During two weeks all 23 landowners were contacted and 12 of the plantations were visited and inventoried. A more detailed description of the inventory at each site is presented in Appendix 2, and a map of the locations is presented in Appendix 1. Geographical and stand data from the twelve plantations used in the study is presented in Table 1.

All twelve locations were revisited in mid-November when data concerning height and ground preparation was collected. At each plantation, five sample plots with a radius of 5.64 meters (area=100 m2) were laid out along a transect line with 20 meters spacing. The location of the first sample plot was laid out randomly by walking 10 meters into the stand and from this point throwing a stick behind the back to get a starting point. The sample plots were distributed parallel to the plantation edge and when an edge was encountered, a 90 degree turn was made and the remaining plots were placed in the new direction. Sampling recommendations from the Forestry Research Institute website (Skogforsk 2008) were used. The height of each tree in the plots was measured in even 5 centimetres with a four meter rod. In each sample plot, the proportion of disturbed vegetation layer was recorded in quarters, from 0/4 for no ground cover removal to 4/4 for total ground cover removal.

Table 1. Stand data and geographical location for each inventoried site.

Site no.	Site	Plantation year	Mean height (m)	Spacing (m)	Latitude	Altitude (m)	Distance to European aspen (m)
1	Ammer	2005	2.05	3.2	63°10'	86	<20
2	Aspby	2006	1.46	3.5	63°03'	80	150
3	Binböle	2006	1.37	3.2	62°55'	38	20-30
4	Kullsta	2007	0.95	1.9	63°06'	240	20-30
5	Österede	2007	1.06	4.1	63°02'	213	20-30
6	Ösjö	2006	1.17	3.3	62°52'	237	20-30
7	Oviken	2006	1.75	3.3	63°00'	312	30-50
8	Rödön	2006	1.31	3.3	63°15'	339	<20
9	Hammerdal	2005	1.81	4.1	63°34'	314	<20
10	Sollefteå	2006	1.50	3.3	63°09'	82	30-50
11	Holafors	2005	3.31	3.5	63°33'	249	<20
12	Baggböle	2006	1.16	3.3	62°12'	73	<20

Disease assessment on 15-year-old hybrid aspen

The second field study for this report was conducted in late September at two locations outside Umeå, Spöland and Sävar, where hybrid aspen have been planted among many other non-native tree species in a long-term production trial. The hybrid aspen stands were checked by collecting fallen leaves and examine the presence of pine twisting rust spores.

The trial plantations are part of a joint project between the Swedish University of Agricultural Sciences (SLU) and the Forestry Research Institute of Sweden

(Skogforsk). The plantations are located on former cropland close to the coast in Västerbotten. At the Spöland location, two parcels were planted with hybrid aspen in 1993. Each of the parcels was planted with 18 times 18 cuttings with a spacing of two meters. In Sävar, one parcel was planted with hybrid aspen in 1993. At this location 17 times 17 trees were planted with a spacing of two meters.

At each parcel, between 300 and 350 leaves were collected from the ground, randomly picked all over the parcel area. The leaves were taken inside and examined under a magnifying glass. Occurrence and coverage of spores was visually assessed and registered for each leaf, using the same classes as described above for assessment on saplings.

Plant material

The plant material used on the plantations established between 2005 and 2007 was ordered by JiLU from Svenska Skogsplantor AB. In turn, Svenska Skogsplantor AB had imported the hybrid aspen from Finland. The clone used had been produced at Nurmijärvi nursery, 60°30'N 24°24'E. The seedlings had a mean height of 50 cm, and minimum height of 30 cm when delivered.

The hybrid aspen used in the Spöland and Sävar plantations came from clones produced as a trial in Järved (63°16'N) and Svanögården (63°30'N). Several different clones were planted in all parcels.

Statistical analysis

All collected data was rewritten and compiled in Microsoft Excel. The worksheet with all necessary information was than transferred to Minitab 15 and this program was used for all statistical analyses, using a significance level of 0.05. Initially, mean values were calculated for the response variables "proportion of infected leaves" and "spore coverage" for both hybrid and European aspen. Minimal calculation unit was tree level and transformation from spore coverage class on aspen leaves to mean spore coverage per tree was performed using class midpoints. Healthy leaves (spore coverage class 0) were included in the response variables.

Comparisons of disease incidence between hybrid aspen and European aspen was conducted on tree level (N=438) using analysis of variance (GLM) and the model; $y=\mu+\alpha+\beta+(\alpha\beta)+e$, where μ=grand mean, e=random stochastic factor. The data was tested for significant differences between the two species (α), between sites (β) and for interaction between species and sites ($\alpha\beta$).

Relationships between disease incidence and other variables, such as altitude, latitude, plantation year (age class), ground treatment, tree height and land use class, were analyzed on stand level (N=12) using both analysis of variance (GLM) and linear regression.

The mean height of the hybrid aspens was calculated for each site. An analysis of variance (GLM) with a Tukey's test of comparisons was preformed to show the differences in height between the different plantation years. The mean height was also tested for correlations with the site traits plantation year, latitude, altitude and ground treatment, using multiple linear regressions.

From the data collected from Sävar and Spöland, the percentage of infected leaves was calculated for each of the three parcels and presented in a graph.

Results

Pine twisting rust infection was significantly lower on hybrid aspen than on European aspen, regarding both proportion of infected leaves and spore coverage (Figure 1, Table 2-3). The mean proportion of infected leaves and the average spore coverage was 24 and 78 times higher on European aspen than on hybrid aspen, respectively (Table 4). There was no significant difference between sites, but a significant interaction between site and species regarding disease incidence (Table 2-3).

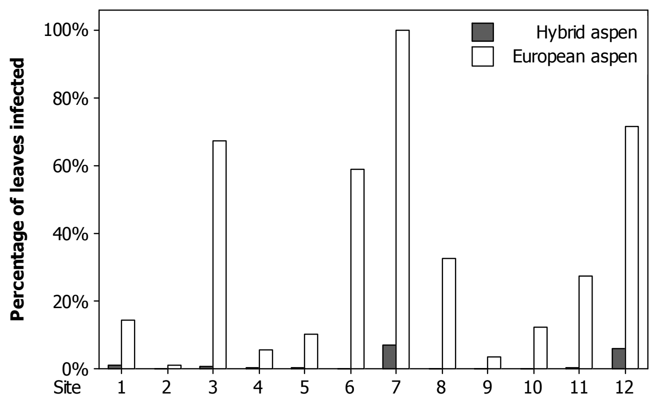


Figure 1. Percent of aspen leaves infected by pine twisting rust on each site.

Table 2. Analysis of variance for proportion of infected leaves, using Adjusted SS for tests. R^2adj = 79.78%.

Source	DF	Seq SS	Adj SS	Adj Ms	F	P	
Site	11	12.831	12.957	1.178	1.260	0.365	Not sign.
Species	1	12.008	10.749	10.749	12.180	0.005	Sign.
Site*Species	11	10.316	10.316	0.938	46.610	<0.001	Sign.
Error	414	8.329	8.329	0.020			
Total	437	43.485					

Table 3. Analysis of variance for spore coverage, using Adjusted SS for tests. R^2adj = 68.92%.

Source	DF	Seq SS	Adj SS	Adj Ms	F	P	
Site	11	0.710	0.710	0.065	1.050	0.472	Not sign.
Species	1	0.371	0.317	0.317	5.460	0.039	Sign.
Site*Species	11	0.679	0.679	0.062	34.800	<0.001	Sign.
Error	414	0.735	0.735	0.002			
Total	437	2.494					

Table 4. Proportion of infected leaves and spore coverage for hybrid aspen and European aspen. N = number of aspen saplings.

Variable	Species	N	Mean	SE Mean	StDev
Prop. infected leaves	Hybrid aspen	220	0.014	0.003	0.043
	European aspen	218	0.345	0.026	0.379
Spore coverage	Hybrid aspen	220	0.001	0.000	0.002
	European aspen	218	0.059	0.007	0.099

Regarding hybrid aspen, regression analysis showed no significant correlation between proportion of infected leaves or spore coverage and latitude of site ($p>0.05$, R^2adj=12.2% and 16.4%, respectively). However, when the most protruding observation (site 7) was excluded from the data set, there was a significant decrease in disease with increasing latitude of the site ($p<0.05$, R^2adj= 45.5% (proportion infected leaves) and 45.6% (spore coverage)).

Regarding European aspen, regression analysis showed a significant reduction in proportion of infected leaves with increasing latitude of site (Figure 2). If the protruding value (site 7) was removed, the level of explanation increased from 31.6% to 67.4%. The same analysis for spore coverage showed no significant correlation unless site 7 was removed from the data and if this site was removed, the level of explanation increased from 4.0% to 41.9%.

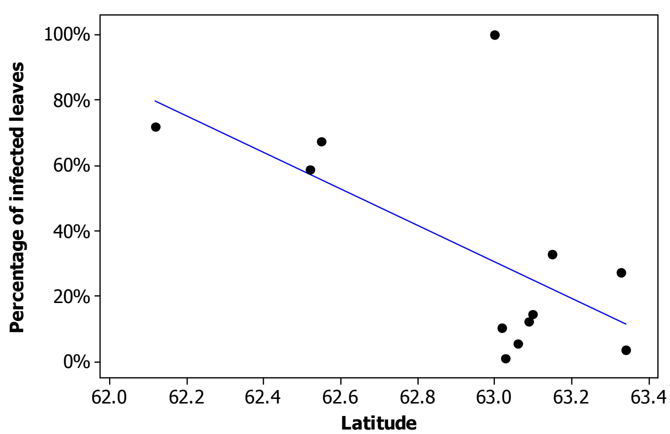


Figure 2. Percentage of infected leaves on European aspen plotted against latitude.
y = 35.4 – 0.557*Lat., p = 0.033, R^2adj = 31.6%.

The multiple regression analysis, including both latitude and altitude, also showed a significant reduction in proportion of infected leaves of European aspen with increasing latitude ($p=0.035$, R^2adj=41.9%). There was no correlation between disease incidence and altitude for neither hybrid nor European aspen.

Regarding hybrid aspen, analysis of variance showed no significant difference in disease incidence between different plantation years or different land use classes.

Mean values are shown in table 5-6. Furthermore, regression analysis showed no significant correlation between disease incidence and either of the continuous variables mean height and ground treatment.

Table 5. Proportion of infected leaves and spore coverage of hybrid aspen for the different years of plantation. N = number of aspen saplings.

Variable	Year of plantation	N	Mean	SE Mean	StDev
Prop. infected leaves	2005	50	0.0047	0.0022	0.0157
	2006	130	0.0213	0.0047	0.0532
	2007	40	0.0042	0.0021	0.0135
Spore coverage	2005	50	0.0002	0.0001	0.0008
	2006	130	0.0011	0.0003	0.0031
	2007	40	0.0002	0.0001	0.0007

Table 6. Proportion of infected leaves and spore coverage of hybrid aspen for different land use classes. N = number of aspen saplings.

Variable	Land use class	N	Mean	SE Mean	StDev
Proportion infected leaves	Crop land	170	0.0104	0.0028	0.0366
	Forest land	50	0.0280	0.0081	0.0573
Spore coverage	Crop land	170	0.0006	0.0002	0.0023
	Forest land	50	0.0014	0.0004	0.0029

Regression analysis showed a significant increase in tree height with increasing time since planting ($p<0.001$, R2adj=49.34%) (Figure 3). Furthermore, Tukey's test of comparisons showed a significant difference between all combinations of years paired. The difference in height growth was 4.2 meters between the largest and smallest tree planted during the summer of 2005.

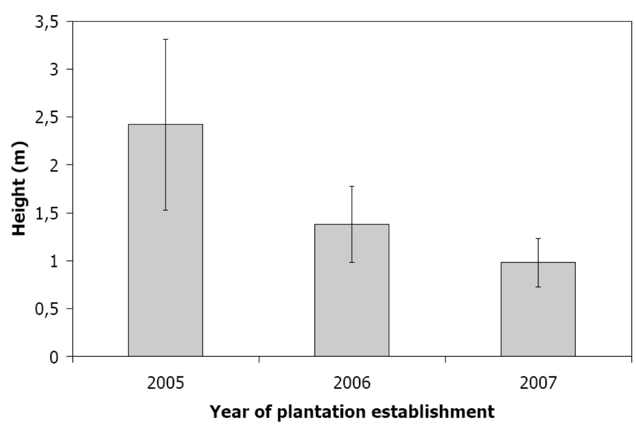


Figure 3. Average height of hybrid aspens of different age (year of plantation establishment). Error bars show standard deviation of the mean.

Regarding hybrid aspen, no significant correlation was found between mean height and either of the variables latitude, altitude or ground treatment.

The results for proportion infected hybrid aspen leaves in the Sävar and Spöland plantations are presented in Figure 4. The mean proportion of infected trees for the sites was 4.3%. All leaves on which spores were detected were rated as Class 1.

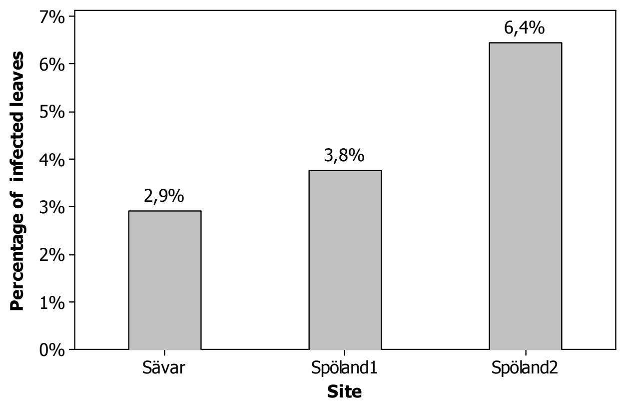


Figure 4. Percentage of leaves infected by pine twisting rust on the three sites planted with hybrid aspen in 1993.

Discussion

The results support the hypothesis that hybrid aspen is more resistant than the European aspen to infection by pine twisting rust. The level of infection was several times higher on European aspen than on hybrid aspen on all sites included in this study. Nevertheless, the results confirm that hybrid aspen is susceptible to pine twisting rust. However, since the level of uredospores on hybrid aspen is only a small fraction of what can be found on European aspen, the hybrid aspen will not likely be the source of any severe outbreak of the pathogen. Natural sprouting of European aspen is more likely to cause problems by spreading of pine twisting rust spores.

According to Bergman (1953), hybrid aspen is a less suitable alternative host of pine twisting rust compared to European aspen. The same author describes the results from a German study in which it was discovered that the amount of uredospores was considerably higher on European aspen. The uredospores did not seem to germinate on hybrid aspen, resulting in only one uredospore generation during one season. Since no proliferation of uredospores happened during summer, much less winter spores (teliospores) were produced and as a consequence of this, less infection occurred on pine. This could explain the results from the present study. The difference in disease incidence was that; the infection on hybrid aspen was very low at all stands, while some of the European aspen stands had very high infection rates. Considering spore coverage, an apparent difference was that no leaf on hybrid aspen was rated as Class 3 (>50% spore coverage). If the theory above is correct, the infection can accumulate during the summer on European aspen by spreading within the stand, while the infection rate on hybrid aspen only reflects the amount of spores coming into the stand from pines in the spring and European aspen during the summer. However, the study described by Bergman (1953) is the only study that have investigated this phenomenon and presented these conclusions. This should be further examined and tested under controlled conditions to secure the accuracy of this statement and to test whether pine twisting rust can not have many summer spore generations on hybrid aspen.

The proportion infected leaves is a measure to easily get an overview of how great the distribution of the rust is in the tree population. The other measure, spore coverage, says more about how serious the infection is. Area of the leaves covered by spores is a better indication if the tree is likely to be affected by the infection and tells more about how much infection can be expected to spread from the tree or population of trees.

Blomberg and others (2007) preformed a study to investigate whether genetically modified hybrid aspen interact differently with pine twisting rust compared to non-modified hybrid aspen. They wanted to test if modification in different traits would result in different responses in the interaction with the fungi. A comparison was made between a non-modified hybrid and hybrids with changed sucrose, pectin or gibberellin biosynthesis. Some different fungi populations were used and in nine of the 30 pathogen-plant combinations tested, a significant difference in susceptibility compared to non-modified hybrid aspen was concluded. No consistent pattern could be concluded since five of the combinations had a significantly higher resistance compared to the non-modified hybrid aspen, and for the remaining four the resistance was significantly lower. However, all pathogen populations were able to infect all hybrids but the aggressiveness of the pathogen differed between different populations (Blomberg *et al.* 2007). This is one of very few recent studies that investigate the interaction between pine twisting rust and hybrid aspen. Even though the study focused on genetically modified hybrid aspens an important conclusion can be drawn that coincide with the hypothesis of the present study: The hybrid aspen can be infected by pine twisting rust if exposed to the disease, even if the susceptibility varies between different hybrids and rust populations. However, we still do not know what or which gene that makes the hybrid aspen more resistant to the rust than European aspen.

In a study by Gallo and others (1985), infection of *Melampsora magnusiana* on trembling aspen, European aspen and their hybrid was investigated. They found a highly significant difference between the three aspen species regarding their receptiveness. There was almost no leaf rust infection on the trembling aspen while the European aspen was highly infected. The hybrid aspen showed an infection level that was in-between the other two species. They concluded that the European aspen grows slower and is more rust susceptible, while the trembling aspen grows faster and is more resistant (Gallo *et al.* 1985). This experiment suggests that there are differences between the European aspen and trembling aspen that result in different responses to rust diseases. More importantly for the present study, there was a difference in receptiveness between European aspen and hybrid aspen, where hybrid aspen was less infected than the European aspen by *M. magnusiana* (Gallo *et al.* 1985). The same response seemed to prevail for *M. pinitorqua* examined in the present study. This result was expected since the two rusts are closely related; both belong to the *M. populina* group.

Testing for patterns between infection rate and geographical traits of the plantation sites, proportion of infected leaves and spore coverage was plotted against latitude and altitude. As for the relation between proportion of infected leaves and latitude, a significant decrease with increasing latitude was found for the European aspen. All the other relations tested against latitude showed no significant correlation. Only when testing for correlation without site number 7 (Oviken), a significant correlation was found, showing that the infection slightly decreases with increased latitude for both hybrid and European aspen. The level of explanation was acceptable, however, a statistical population of 11 sites is rather limited which should be taken into consideration when drawing conclusions from these results. As for the regression between altitude and infection, there was no significant correlation in the statistical population. Including altitude in the regression could however raise the level of explanation in the regression analysis for proportion of infected leaves on European aspen against latitude mentioned above. The decreasing infection rate the further north the plantation was situated at could be a result of a shorter vegetation period, which results in shorter period for the rust to produce uredospores. However, if this were the only explanation there should have been a similar trend for altitude as well, supposing that the altitude span is big enough. It is likely that the overall occurrence of European aspen differs with the geographical traits, but I don't have enough information to be able to analyze this.

The site with the protruding value, Oviken, had a very high density of suckers of European aspen and the distance to hybrid aspen was 30 to 50 meters. The high density of suckers probably caused the extremely high amount of rust infection on European aspen. That was why I chose to remove this site from some of the analyses. Another possible problem at this site was that the majority of the leaves on the European aspen turned yellow, which could have influenced the visual judgement of amount of spores on the leaves. This was the only site at which the leaves had started to turn yellow at the time for the inventory. It seems to be consistent with the theory that high rate of rust infection leads to earlier defoliation (Yu *et al.* 2001).

Considering only the hybrid aspen, many traits were tested for relations with infection rate. For infection versus year of plantation establishment the results show a big difference in mean value but the variance was high and no significant difference could be proven. This result was expected considering the relatively large variations in height growth within the age-classes. In theory, age itself should not affect risk of infection but instead the size of the sapling and the total leaf area. A greater leaf area implies a higher probability to catch spores and therefore a higher risk to get infected. There was no significant difference in infection between plantations established on land formerly used for agriculture and on forest land. A significant difference between the two land use classes was expected based on the assumption that pines in appropriate sizes grow closer to the hybrid aspen on forest land. Only three sites represented hybrid aspen on forest land and this sample is not sufficient to be able to draw conclusions with enough certainty. More replications have to be made with plantations of the same age and with similar prerequisites to confirm possible differences between the land use classes. If it would have been possible, equally many plantations would have been chosen of each land use class for the present study. However, the majority of the plantations were established on former crop land and some of the ones on forest land did not fulfil all other requirements necessary to be included in the study.

Even if there was a big height difference in each age-class of hybrid aspen, there was a significant increase in tree height with increasing time since plantation establishment. This result suggests that the prerequisites are quite similar for all plantations. No other trait than time of planting seems to influence the growth to the extent that overcome the expected difference between the ages of the hybrid aspen. The hybrid aspen planted 2005 had at time of plantation a mean height of 50 cm. In the end of season 2008 the mean height for the three plantations was 2.4 meters, which gives a growth about 0.5 meters per growth season over the four years. The highest production was found in Holafors where the hybrid aspen had grown 2.8 meters in four seasons; 0.7 meters per year. For comparison, hybrid aspen in the south of Sweden has grown about 1.1 meters per year over a four year period after plantation (Johnsson 1953). Another example of height growth during the first years has been presented in a study done near Sundsvall (62°24'N). During the first five growing seasons the hybrid aspen grew 3.6 meters which translates to about 0.7 meters per year (Elfving 1986). The growth development of hybrid aspen in the current study seems to be at a comparable level to results from earlier growth trials. The results suggest that hybrid aspen can reach high growth rates even as far north as Jämtland. It is also important to remember that even the plantation with the highest mean height after four seasons had suffered damage of primarily rodents, which have reduced the growth. Lower productivity is to be expected in "ordinary" plantations compared to growth trials since the latter are usually much more controlled and have as optimal pre-requisites as possible. In the current study, some plantations had suffered from drought, some had been damaged by browsing and others had suffered from competing vegetation.

A possible source of error for the comparison of growth and infection rate on hybrid aspen is that the height measurement was not done on the same tree individuals as inventoried for infection. The reason for this was that the inventories were done at two different times during the fall. Height growth was not measured for European aspen since we can not know the age of the suckers. Therefore, no comparison in growth could be made between the two aspen species.

As established by Longo and others (1976), the timing and intensity of germination in spring is not depending on where the teliospores are formed, but where they have over wintered and the environmental conditions during this period. In the cited study, the start, peak and length of the germination period were different between the studied years. They also saw a higher germination percentage on uncovered soil but the timing of germination seemed not to be affected by this trait (Longo *et al.* 1976). A higher germination percentage should lead to a higher amount of infection in the stand during the following season. That is why the plantations where the ground cover had been removed were expected to have a higher infection rate at the time of the present assessment. This expected correlation however could not be found. A possible explanation could be that where no ground treatment has been made, European aspen ramets have been able to establish among the planted hybrid aspens. The higher density of saplings results in higher infection accumulation.

To compare planted seedlings with root-suckers was not the optimal way of performing this study. However, it was the only possible way to perform a data collection within the timeframe and limitations of a project like this. Problems with very different spacing and density of stems, distances from saplings to big aspen and Scots pine is of course something I have had in mind. In the future, it would be interesting to compare suckers of hybrid aspen with suckers of European aspen, since this is the way the hybrid aspen plantations are to be regenerated in the upcoming rotation periods.

The results from the inventory on mature aspen in Sävar and Spöland showed that pine twisting rust can infect older hybrid aspen and several different clones as well. It was also possible to conclude that teliospores can develop on hybrid aspen leaves. This step in the pine twisting rust life cycle, forming of teliospores from uredospores was recorded on all three parcels. This additional study strengthens the conclusion that the pine twisting rust can infect hybrid aspen, but to much less extent than European aspen. Just over 4 % of the collected leaves in Sävar and Spöland were observed to have teliospores. All of the infected leaves were categorized in class 1, which means less than 10 % of the leaf area was covered with spores.

In conclusion, the results of this study supports the hypothesis that hybrid aspen is more resistant than European aspen to pine twisting rust infection. Even if hybrid aspen becomes much more common in the future, it is unlikely to result in a higher risk of pine twisting rust damage in Scots pine stands.

Acknowledgements

Thanks are due to my supervisors Andreas Bernhold and Per Hansson for all the help and support during the entire course. I am also grateful to my contacts at JiLU, Håkan Schüberg and Per-Olof Nilsson for getting me in contact with the forest owners in "Lövskogsprojektet" and for helping me during my stay in Bispgården. I also wish to thank all forest owners that kindly helped me by giving me information about their hybrid aspen plantations and let me use their plantations for gathering data to my study. Finally I would like to thank Åsa Fjällborg for valuable discussions and support throughout the writing of this report. The study was funded by FORMAS and the Department of Forest Ecology and Management, SLU, Umeå.

Literature

Bergman, F. (1953). Skogsträdens sjukdomsresistens. In: *Årsberättelse – Föreningen för växtförädling av skogsträd.* 1949-1955. 70-98. Föreningen för växtförädling av skogsträd, Norrtälje.

Blomberg, P., Wennström, A., Hjältén, J., Lindau, A., & Ericson, L. (2007). Testing the effect of genetically modified hybrid aspen Populus tremula x tremuloides on the interaction with the non-target pathogen Melampsora pinitorqua. In; Blomberg, P. (2007). *Non-target effects of genetically modified trees*. Akad. avh. Umeå universitet.

Brinkman, K.A., & Roe, E.I. (1975). *Quaking Aspen: Silvics and Management in the Lake States.* U.S. Dep Agric., Agric. Handb. 486, 52 p.

De Jong, J., Larsin-Stern, M. & Liedholm, H., (1999). *Grönare skog*. Skogsstyrelsen, Jönköping. ISBN: 91-88462-45-5

Elfving, B. (1986). Ett försök med åkerplantering av hybridasp och gran nära Sundsvall. *SST 5* , 43-45.

Eidmann, H. & Klingström, A. (1976). *Skadegörare i skogen*. LTs förlag, Borås. ISBN: 91-36-00269-0

Gallo, L., Stephan, B., & Krusche, D. (1985). Genetic variation of Melampsora leaf rust resistence in progenies of crossings between and within Populus tremula and P. tremuloides clones. *Silvae Genetica 34 (6)* , 208-214.

Hansson, P. (1989). Rotskottföryngring av hybridasp. Examensarbete Nr 1 1989. Institutionen för skogsskötsel. Sveriges lantbruksuniversitet.

Johnsson, H. (1953). Hybridaspens ungdomsutveckling och ett försök till framtidsprognos. *Svenska Skogsvårdsföreningens Tidskrift* , ss. 73-95.

Johnsson, H. (1955). *Från Föreningens för växtförädling av skogsträd verksamhet.* Stockholm: Föreningen för växtförädling av skogsträd.

Johnsson, H. (1957). Aktuellt om hybridasp. *Skogen 44 (24),* 798-801.

Karlman, M. (1981). The introduction of exotic tree species with special reference to Pinus contorta in northern Sweden. *Stud. Forest. Suec. 158,* 1-25.

Klingström, A. (1963). *Melampsora pinitorqua* Rostr. – Pine twisting rust. *Stud. Forest. Suec. 6*, 1-23.

Lagerberg, T. (1945). Skoglig mykologi. 3rd ed. Skogshögskolans Studentkårs Kompendieförmedling.

Longo, N., Moriondo, F., & Naldini Longo, B. (1976). Germination of teleutospores of Melampsora pinitorqua Rostr. *Eur. J. For. Path. 6* , 12-18.

Manion, P.D. (1991). *Tree disease concept*. 2nd ed. (Chap 11). Prentice Hall Inc., Englewood Cliffs, N.J.. ISBN: 0-13-929423-6

Martinsson, O. (1985). The influence of pine twist rust (Melampsora pinitorqua) on growth developement of Scots pine (Pinus sylvestris). *Eur. J. For. Path. 15* , 103-110.

Mattila, U. (2005). Probability models for pine twisting rust (Melampsora pinitorqua) damage in Scots pine (Pinus sylvestris) stands in Finland. *Forest Pathology 35* , 9-21.

Mattila, U., Jalkanen, R., & Nikula, A. (2001). The effects of forest structure and site characteristics on probability of pine twisting rust damage in young Scots pine stands. *Forest Ecology and Management 142* , 89-97.

Microsoft® Excel 2002 [computer software]

Minitab 15® Statistical Software for Windows® [computer software]

Pei, M. H., & Shang, Y. Z. (2005). A brief summary of Melampsora species on Populus. In M. H. Pei, McCracken, & A. R., *Rust diseases of willow and poplar* (pp. 51-61). King's Lynn, UK: CAB International.

Pettersson Ostelius, M. Hybridasp ett alternativ också i norr. (2008, 4 April). *Skogsland*, s 8-9.

Quencez, C. & Basien, C. (2001). Genetic variation within and between populations of Pinus sylvestris L. (Scots pine) for suseptiability to Melampsora pinitorqua Rostr. (pine twist rust). *Heredity 86*, 36-44.

Rytter, L., & Stener, L.-G. (2005). Productivity and thinning effects in hybrid aspen (Populus tremula L x P. tremuloides Michx.) stands in southern Sweden. *Forestry vol 78, no 3* , 285-295.

Samuelsson H. & Örlander, G. (2001). *Skador på skog*. Skogsstyrelsen, Jönköping. Rapport 8O.

Sinclair, W. A. & Lyon, H. H. (2005). *Diseases of trees and shrubs.* 2nd ed. (pp. 284-286). Cornell University, New York. ISBN: 978-0-8014-4371-8.

Skogforsk. Forestry Research Institute of Sweden. Kunskap Direkt. [online] (21 November 2008). Available from: http://www.skogforsk.se/KunskapDirekt/Templates/AreaStartPage____11729.aspx [24 November 2008].

Skogsstyrelsen. (1995). *Skador på barrträd*. (s 179-171). Skogsstyrelsen, Jönköping. ISBN: 91-88462-22-6

Yu, Q., Tigerstedt, P., & Haapanen, M. (2001). Growth and phenology of hybrid aspen clones (Populus tremula L. x P. tremuloides Michx.). *Silva fennica 35 (1)* , 15-25.

Appendix 1

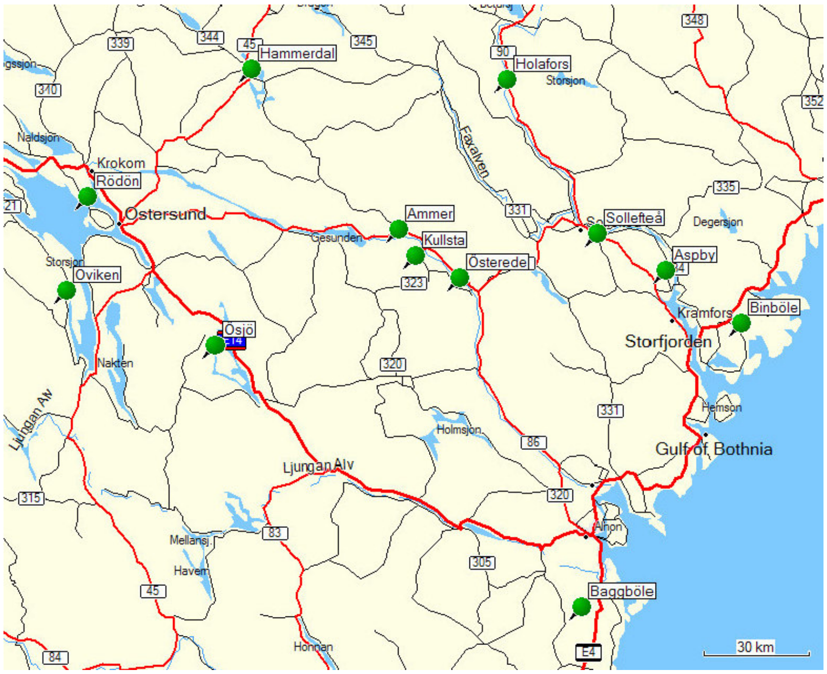


Figure 1. Geographic locations of hybrid aspen plantations in Jämtland and Västernorrland.

Appendix 2

Description of hybrid aspen plantations in Jämtland and Västernorrland.

Ammer. The hybrid aspen was planted in 2005 on cropland in a slope with adjacent forest on one side. In the forest patch, both older aspen and suckers were observed. The row of hybrid aspen (10 saplings) closest to the European aspen was assessed for pine twisting rust damage. To be able to make comparisons, ten saplings of natural aspen at the forest edge were checked for disease.

Aspby. The plantation was established in 2006 on old cropland with some natural regeneration of pine (and other tree species) in the fenced area. Big aspens are growing on both sides of the plantation. The hybrid aspen was inventoried by checking ten saplings in each of the outermost rows. Because of a lot of damage caused by rodents, the healthy trees in these rows were examined. Suckers of European aspen were chosen for comparison in an area about 150 meters from the plantation.

Binböle. The hybrid aspen was planted in 2006. On the location there were two adjacent fields separated by a strip of large aspens in one end. One of the fields were fenced and planted with hybrid aspen. Of these, the saplings closest to the fence and the big aspens were chosen for the inventory. European aspen of corresponding size and at equal distance from the large aspens were chosen for the inventory on the other field.

Österede. The hybrid aspen was planted on old cropland in 2007 and was separated from the natural aspen by a small road. On the forest side of the road, there were saplings growing underneath big aspen. The 20 hybrid aspens that were closest to the road were examined. Because of their small size, planted last summer, 30 leaves per tree were checked.

Kullsta. Hybrid aspen had been planted on cropland in 2007. 20 hybrid aspens were chosen in the lower part of the plantation closest to some big European aspens. For comparison 20 saplings of natural aspen were chosen to be examined between the fence and the big aspens. The hybrid aspen had been planted last year and hade not developed leaves enough to check more than 30 per tree.

Ösjö. The plantation was established on cropland in 2006. Of the hybrid aspens, 10 saplings in each of the two rows closest to the forest were chosen. Only a couple of meters from the fence some big aspens were growing and at the forest edge, 20 saplings of European aspen were chosen. The hybrid saplings were too small to check 50 leaves so the number of examined leaves was reduced to 30 per tree.

Oviken. The hybrid aspen was planted on a clear-cut in 2006. The plantation had cropland on one side and at one corner of the plantation some big aspens were growing with a high density of suckers underneath. From these, 20 saplings in the same size as the hybrid plants were examined. Twenty hybrid aspens closest to the natural saplings were checked. Even though these saplings were planted in 2006, they had not grown enough to make it possible to sample 50 leaves, therefore 30 leaves from each sapling were looked at. A source of error for this particular plantation could be that the European aspen had started to turn yellow, while the leaves on the hybrids were still green.

Rödön. The plantation was established on forest land in 2006. It was difficult to find European aspen in the right size to be able to make the comparison. Therefore only 10 of each species were chosen. The chosen seedlings and the suckers of natural aspen were separated by a forest road.

Hammerdal. A plantation on cropland, planted in 2005, with a lot of big aspens adjacent to the fenced area. Twenty saplings of European aspen were chosen along the fence and 20 hybrids closest to these.

Holafors. The plantation was established in 2005 on cropland surrounded by forest on two sides. Twenty natural suckers of aspen growing right outside the fence were chosen and checked for infection. Along this side, the closest rows of hybrids were inventoried. The planted hybrids that had not been damaged by rodents had grown rapidly and some reached between 3 and 4 meters. This fact resulted in an inventory of 50 leaves per sapling at breast height.

Sollefteå. The hybrid aspen was planted on a clear-cut in 2006. Some European aspens had been cut down which had resulted in natural suckers growing between the planted hybrid aspens. Of the natural aspen included in the inventory, eight were growing inside the fenced area and the remaining twelve just outside, underneath some large aspens. The lower part of the plantation was growing on old cropland, but this part was not used in this study.

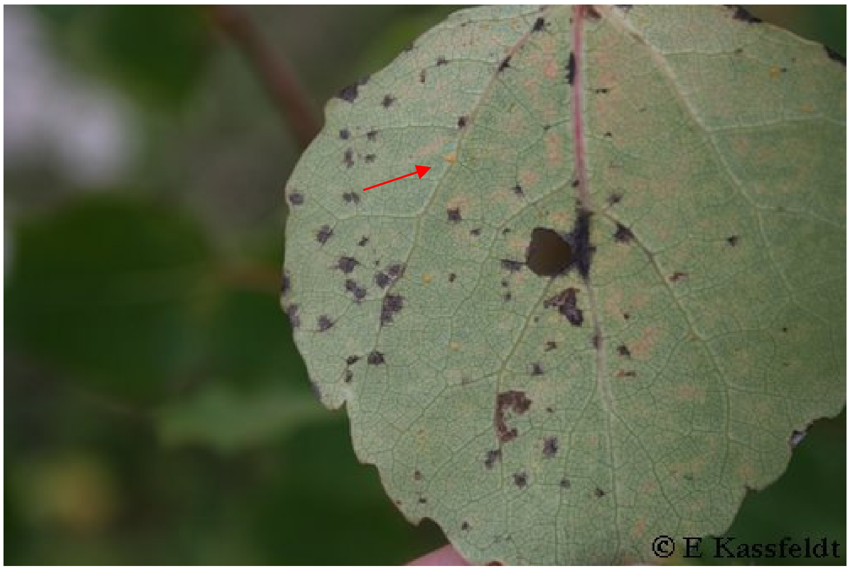
Baggböle. The plantation, which was established in 2006, reached over both cropland and a forested patch. In the area fenced for the plantation, a group of big aspens had been left and underneath these, suckers in the same size as the planted hybrid aspens where found. Therefore, both hybrid and natural saplings underneath and around this group of trees where checked in the inventory. Thirty leaves on 20 saplings of each species were examined, and this was almost all leaves on some of the planted hybrid aspens.

Hemling. The hybrid aspen was planted in 2007 on cropland surrounded by mixed forest. There were no European aspen detected close enough to be able to use this plantation in the comparison-study. However, a survey of the hybrid aspens was done, but no disease was found.

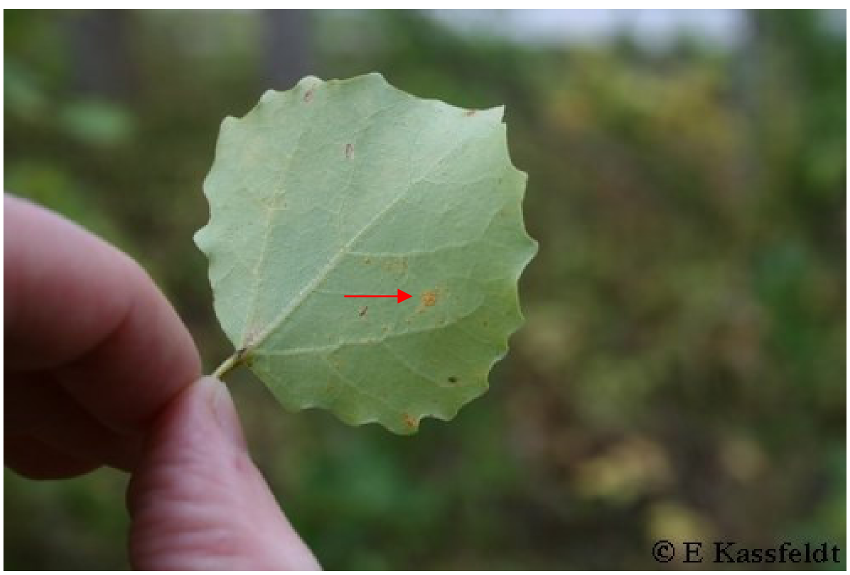
Äspnäs. The plantation was established in 2005 and was surrounded by cropland and forests dominated by birch close to the plantation. Only five European aspens of appropriate size were growing close enough to the plantation for the study and none of these five were carrying *Melampsora*-infection. No infection was detected among the 20 hybrid saplings that were checked.

Ljusdal. No disease was discovered on the hybrid aspen in the plantation, which was established in 2007. Since there were no European aspen in the area close enough to make a comparison, this plantation was not used in the calculations. The hybrid aspens were planted last year and were rather small. The surrounding forests consisted mainly of spruce and birch so the probability for finding *Melampsora*-spores was very little.

Appendix 3



Picture 1. Leaf of hybrid aspen with a few patches of uredospores. Class 1.



Picture 2. Leaf of European aspen, less than 10 % of leaf area covered with spores; class 1.

Picture 3. Leaf of European aspen, more than 10 % of leaf area covered with spores; class 2.

Picture 4. Leaf of European aspen, more than 50 % of leaf area covered with spores; class 3.

SENASTE UTGIVNA NUMMER

2008:23 Författare: Eva Rodriguez
Vegetation history and *Picea abies* L. Karst. establishment in the Härjedalen province (central Sweden)

2008:24 Författare: David Elm
Dikesrensning och skyddsdikning – en fältstudie och utredning av behov i södra Sverige

2008:25 Författare: Ida Dahl
The effects of forest clear-cutting on stream water DOC.

2008:26 Författare: Anders Bergman
Betydelsen av kolkälla och mikrobiell fysiologisk status för temperaturresponsen (Q_{10}) vid nedbrytning av organiskt material

2008:27 Författare: Helena Gustafsson
The effect of moisture, litter and stand age on N fixation in the feathermoss, *Pleurozium schreberi*

2008:28 Författare: Pablo Martin Ortega
Water availability controls nitrogen fixation in the feather moss *Pleurozium schreberi*

2008:29 Författare: Hanna Triumf
Landskapsplanering och konnektivitetsförbättringar inom värdetrakter i Västerbottens län

2008:30 Författare: Pablo Garrido Rodriguez
Local vegetation history of Norwegian spruce (*Picea abies* L.) in central Scandes (Sweden) since the mid-Holocene

2008:31 Författare: Emma Perés
Stabilitet, rot- och stamegenskaper efter plantering med Starpot 50 i jämförelse med Hiko 50 och sådd – Resultat efter 6 - 12 år för tall och contorta i Härjedalen

2008:32 Författare: Pernilla Bärlund
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2009:1 Författare: Marianne Karlsson
Influence of light competition on vitality in old aspen

2009:2 Författare: Frida Carlstedt
Kan risken för spontan contortaföryngring elimineras genom hyggesbränning?

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