**Examensarbeten** **2009:7**

Institutionen för skogens ekologi och skötsel

# Infection rate of pine twisting rust (*Melampsora pinitorqua*) in Scots pine (*Pinus sylvestris*) regenerations with retained aspens (*Populus tremula*)

— Evaluation of the importance of large aspen trees compared to aspen sprouts

*Photo Åsa Fjellborg*

**Åsa Fjellborg**

Sveriges Lantbruksuniversitet — Skogligt magisterprogram/Jägmästarprogrammet
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Examinator: Erik Valinger, SLU, Inst för skogens ekologi och skötsel — Umeå 2009

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

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This report presents an MSc/BSc thesis at the Department of Forest Ecology and Management, Faculty of Forest Sciences, SLU. The work has been supervised and reviewed by the supervisor, and been approved by the examinator. However, the author is the sole responsible for the content.

# Content

# Sammanfattning

Rostsvampen knäckesjuka (*Melampsora pinitorqua*) är en allvarlig skadegörare i tallungskogar i Sverige. Svampen värdväxlar mellan inhemsk tall (*Pinus sylvestris*) och asp (*Populus tremula*). De tydligaste symptomen är böjda eller avbrutna skott i de övre grenvarven i tallarnas krona. Detta kan resultera i reducerad tillväxt och försämrad timmerkvalitet. Väl medvetna om riskerna med knäckesjuka har skogsägare i Sverige under lång tid kämpat med att utrota aspen både i tallföryngringar och i plantskolor. Nuförtiden har attityden gentemot asp förändrats i och med att naturvård implementerats i skogsskötseln i högre grad. Detta har resulterat i en ökning av antalet aspar som lämnas kvar i naturvårdssyfte på föryngringsytorna. Syftet med studien var att utvärdera betydelsen av kvarlämnade naturvärdesträd av asp, jämfört med självföryngrat aspsly, som spridningskälla för knäckesjuka i unga tallbestånd. Huvudhypotesen var att löv från aspsly utgör en större smittokälla än löv från vuxna aspar. Fältstudien utfördes i Västerbottens län. Elva tallungskogar mellan 5-15 år valdes slumpmässigt ut bland bestånd infekterade av knäckesjuka samt innehållande naturvärdesaspar. Förekomst och omfattning av årets angrepp av knäckesjuka registrerades för varje träd inom 18 GPS märkta provytor i varje bestånd. Även antalet rotskott av asp räknades på provytorna. Samtliga stora aspar i bestånden registrerades med GPS för analys av avstånd mellan aspar och provytorna. Resultatet påvisade en högst signifikant korrelation mellan antalet rotskott av asp och infektionsgraden på de unga tallarna. Ingen av de två variablerna avstånd till närmsta stora asp eller antalet stora aspar inom 100 m från provytorna var signifikant korrelerade med infektionsgraden på tallarna. Aspträdens roll i spridningen av knäckesjuka verkade först och främst vara att skjuta rotskott. Det var en signifikant positiv korrelation mellan tallens toppskottslängd och angreppsgraden av knäckesjuka. Välväxande tallar föreföll mer mottagliga för angrepp än mindre produktiva tallar. GIS-kartor producerades för att visualisera den rumsliga distributionen av knäckesjukeangrepp i relation till täckningsgraden av aspsly och förekomsten av stora aspar. Risken med att lämna asp som naturvärdesträd i tallföryngringar kan anses låg så länge som preventiva skogsskötselåtgärder verkar för att hålla nere mängden aspsly i produktiva högriskområden.

## Abstract

Pine twisting rust (*Melampsora pinitorqua*) is a serious fungal pathogen that in Sweden infects young Scots pine (*Pinus sylvestris*) stands and has European aspen (*Populus tremula*) as alternate host. Pine twisting rust (PTR) infects the pine's annual shoots. Main symptoms are bent or broken shoots in the upper crown of young Scots pines which can result in reduced growth and timber quality. Well aware of the risk of PTR Swedish foresters has fought to eliminate aspen, both in Scots pine regenerations and in nurseries. Today, the attitude towards aspen trees has changed with the increased implementation of environmental consideration in Swedish forest management. This has resulted in an increased number of mature aspens retained for conservation purposes in pine regenerations. The aim of this study was to evaluate the importance of mature aspen trees compared to young aspen sprouts as causal agents of PTR in young Scots pine stands. The main hypothesis was that leaves of aspen sprouts carry more infection compared to leaves on mature aspen trees. The field study took place in the county of Västerbotten in northern Sweden and eleven 5-15-year-old Scots pine stands were randomly chosen for the study based the occurrence of PTR and large aspen trees. The occurrence and severity of current-year PTR infection on each pine was registered within 18 GPS-positioned sample plots per stand. Also the number of aspen sprouts was counted. Furthermore, all large aspen trees growing in the stand were GPS-positioned. The result showed a highly significant correlation between the number of aspen sprouts and infection rate on Scots pine saplings. None of the two variables concerning the distance- or the numbers of large aspen were significantly correlated to PTR infection rate. It appeared that the adult aspen's role in the spread of PTR was primarily by producing aspen sprouts. There was a significant positive correlation between leader length and PTR infection rate and hence, productive pines with vigorously growing shoots appeared more susceptible to the pathogen than less productive trees. GIS-maps were created to visualize the spatial distribution of PTR infection in relation to coverage of aspen sprouts and occurrence of large aspens. The risk of leaving aspen trees as green tree retention could be considered as low as long as preventive management practices are used in order to keep the number of aspen sprouts low in fertile, high risk areas.

# Introduction

Pine twisting rust (*Melampsora pinitorqua*) is a serious fungal pathogen in young Scots pine (*Pinus sylvestris*) stands in Sweden. The main symptoms are bent or broken annual shoots in the upper crown of young Scots pines which can result in reduced growth and timber quality (Sylvén 1917, Lagerberg 1945, Kardell 1966). Some of the earliest reports of infected pine stands in Sweden are from the late 19$^{th}$ century (Wilke 1874). Pine twisting rust (PTR) is also a common disease in other parts of Europe. It infects different tree species of the genus *Pinus* (Klingström 1963) and has species of the genus *Populus* as alternate hosts (Eidmann & Klingström 1976). In Sweden, the alternate host of PTR is European aspen (*Populus tremula*).

## *Description of the pine twisting rust*

### Life cycle

The different fungi that cause rust diseases are categorised as obligate parasites and, thus, require living hosts for their life cycle (Manion 1991). The PTR infection on Scots pine shoots is caused by airborne basidiospores which are formed and released during early summer from dead aspen leaves on the ground (Sylvén 1917, Klingström 1969). In general basidiospores of different rust diseases serve as a host-transfer step in the lifecycle of the pathogens. The basidiospores germinate and a small yellow necrosis (aecia) is later developed on the shoots (Sylvén 1917, Manion 1991). If the weather is warm and dry during the shoot elongating period, the wound can heal and leave only a small scar in the bark (Eidmann & Klingström 1976). Contrastingly, if the weather is cool and rainy during the same period, the infection may be severe (Klingström 1969) and causing the shoot to bend or even break (Sylvén 1917). Aeciospores spread from the aecia and infects nearby aspen leaves. The growing hyphae penetrate the stomata of the aspen leaves. The developing urediniospores can be seen as bright yellow dots on the underside of the aspen leaves (Lagerberg 1945). The urediniospores spread the infection between the aspens and works as an intensification of the infection during summer (Sylvén 1917, Lagerberg 1945, Manion 1991). In autumn, between August and September, teliospores are produced. These spores spend the winter on dead aspen leaves and are visible like small dark-coloured clusters on the underside of the aspen leaves (Sylvén 1917).

### Forest damage

Pine twisting rust infects the Scots pine’s annual shoots in early summer (Desprez-Loustau & Wagner 1997a), while the lodgepole pine (*Pinus contorta*) seems to be resistant (Eidmann & Klingström 1976, Martinsson 1985). The infection is most common in young Scots pine stands and predominantly on terminal leaders (Sylvén 1917, Klingström 1963, Kurkela 1973). Because the shoot elongation is not finished at the time of infection, the shoot will become more or less S-shaped when the growth will continue only on the healthy, none infected, side of the shoot (see picture 1 in Appendix). This is one of the pathogens characteristic symptoms (Sylvén 1917). A severe infection often kills the shoots and hence, causes the infected leader to weaken and break at the site of the wound resulting in the formation of multiple leaders. This gives the tree a bushy appearance (Lagerberg 1945, Kardell 1966). Younger pines suffer a greater risk of lifelong damage or, in rare cases, death (Kardell 1966, Martinsson 1985). Long-term damage on the Scots pines are low-quality butt-logs (Sylvén 1917, Kardell 1966) and reduction of tree growth (Jalkanen & Kurkela 1984). The earlier the infection occurs on the pine shoots during the growing season, the larger the reduction in growth. Furthermore, younger, smaller trees are more exposed for PTR infections on the uppermost part of the crown, and hence, PTR may have a large negative effect on the growth of the small trees (Martinsson 1985). Despite this, PTR is generally not considered as a known serious problem in the Swedish forestry. Even if it is a common pathogen the damage it causes is mainly on a local scale (David Rönnblom, personal communication).

### Infection risk on pines

The growing pine shoots are only sensitive to infection during a limited time of the shoot growth (Sylvén 1917, Klingström 1963, Eidmann & Klingström 1976). As early as the needles have developed and start to show on the shoot the major risk for infection is over (Klingström 1963). As for many fungal diseases, water and humidity is very important for spore formation and dispersal (Manion 1991). Moisture is a basic requirement for the teliospores to germinate and form basidia and basidiospores. Therefore damp weather and pine shoot elongation have to take place at the same time for a severe infection to occur in a pine stand (Klingström 1963, Eidmann & Klingström 1976).

Pines are most receptive to PTR infection up to an age of 15 years (Sylvén 1917, Lagerberg 1945, Kardell 1966, Martinsson 1985). Thus, the damage is mainly concentrated to the lowest and most valuable part of the stem as the tree grows older (Lagerberg 1945). Studies have shown that also older pines can be infected during years with favourable conditions for the fungi. However, the older the tree is at the time of infection the greater chance it has to survive and fully recover from the infection (Kardell 1966).

A probability model based on different risk factors was developed by Mattila (2005). The risk for PTR infection is generally lower for naturally regenerated stands than planted or sown stands (Mattila 2005). Several studies have indicated that vigorously growing pines are at greater risk of infection by PTR in comparison to slower growing pines (Klingström 1969, Kurkela 1973, Desprez-Loustau et al 1997a). Soil fertility and the presence of aspen are the main factors affecting the incidence of PTR in a Scots pine stand (Mattila et al 2001).

There have been few studies dealing with risk distances between aspens and pines regarding the spread and infection of basidiospores from PTR. Sylvén (1917) made an observation that it was the pines closest to the aspen trees that were most severely infected. According to Eidmann & Klingström (1976), a recommendation for pine nurseries is to eliminate aspens within a range of 200 meters from the pine seedlings. It is believed that the basidiospores rarely spread longer distances (Eidmann & Klingström 1976, Manion 1991).

## *The importance of aspen in nature conservation*

For almost 60 years, clear-felling has been the dominating forest management system in Sweden (Jäghagen & Sandström 1996). The main idea is to have rather even-aged forest stands with one dominating tree species. Today the challenge is to preserve and restore the biodiversity in a forest landscape that is intensively managed.

The latest Swedish Forestry Act (1994) has a distinguishing characteristic by two equal goals: one production goal and one environmental goal (Skogsstyrelsen 2003). In the 30 § of the Forestry Act, dealing with nature conservation, it is stated that both single- and groups of trees are meant to be retained after harvest and that broadleaved tree species should be preserved in coniferous forests. Dead and dying trees are also meant to be preserved in the landscape. Besides the Forestry Act there are also forest certification standards that act as an agreement between the forestry and the nature conservation (Niklasson & Nilsson 2005). The Swedish standard of the Forest Stewardship Counsil (FSC) forest certification states that the forest management should promote favourable conditions for biodiversity associated with broadleaved trees and that naturally present broadleaved tree species should be retained in the forest stands. Furthermore, trees valuable for nature conservation purposes, such as large aspen trees in conifer dominated stands, are meant to be preserved and protected during cleaning and thinning operations to facilitate their future existence in the stands (FSC 2000).

Aspen is a pioneer tree species that is favoured by different disturbances in the forest such as forest fires and natural gap dynamics. Root suckers develop from the aspen roots and aspen clones can under certain situations cover an area of several hectares (Nitare 2000). In the presence of aspens, young artificially regenerated Scots pine stands or fire-razed areas suffers a great risk of PTR infection due to the aspen's ability to sprout (Lagerberg 1945). Well aware of the risk of PTR the Swedish forestry has fought to eliminate aspen, both in Scots pine mono-cultures and nurseries, for almost a century. There have been several methods used in order to kill the aspens, ranging from girdling the trees to the use of herbicides (Delin 2004). The decreasing numbers of natural forest fires and the big population of moose complicate the establishment of aspens. Browsing reduces the aspen sprouts chance to develop into large trees (de Jong et al 1999). Today, the attitude towards aspen trees has changed since the forestry has implemented environmental consideration into their forest management. Hereby, the aspens have developed from being considered as a weed by the forestry into a tree with a high conservation value.

Today, there are few large aspen trees in the managed forest land (Delin 2004) and leaving individuals or groups of aspen trees, and other deciduous trees, after harvest is a method used in the managed forest land to increase the biodiversity (de Jong et al 1999). The species that utilizes the aspens varies depending on the age of the tree (Hazell 1999). Several species of lichens, bryophytes, fungi and insects are associated with, and often dependent on, aspen (Almgren 1990, de Jong et al 1999, Niklasson & Nilsson 2005). Furthermore, several species of woodpeckers are dependent on aspen as a food source and as nesting trees. A Norwegian study stated that managing the forests, towards a greater number of large aspen trees, will benefit the survival of threatened woodpeckers like the white-backed woodpecker (*Dendrocopos leucotos*) (Gjerde et al 2003). Evidently, the aspen is an essential tree species for the species conservation in the boreal zone (Delin 2004). It is thus a delicate task for forest managers to balance the risk of PTR infection and the conservation value of leaving aspens as green tree retention in Scots pine regenerations.

### *Objectives*

The aim of this study was to evaluate the importance of mature aspen trees and the coverage of aspen sprouts in young Scots pine stands with regards to pine twisting rust (PTR). The question to answer was if large aspen trees or aspen sprouts are the main source of PTR infection. The main hypothesis was that leaves of aspen sprouts carry more infection compared to mature aspen trees and, thus, that coverage of aspen sprouts is more important for disease incidence compared to the number of- or distance to large aspen trees. Another aim of the study was to analyse if trees with longer leaders tend to be more infected by PTR. The results will help foresters in their preventive actions against PTR in high risk areas.

## Materials and methods

### *Study sites*

The field study took place in the county of Västerbotten in northern Sweden during five weeks in the autumn of 2008. Eleven young Scots pine stands were randomly chosen for the study based on the occurrence of PTR and large aspen trees (Table 1). Nine of the stands were located in the area of Rönnäs, north of Sävar, and two east of Nordmaling. Apart from the requirement of large aspens and PTR, the stands were chosen based on a mean age limit of 5-15 years and a mean height limit of 0.5- 2 meters. Both private- and company-owned forests were included. The stand selection was supported by the use of forest stand data from: satellite picture assessment (kNN), the Swedish Forest Agency and the forest company "Holmen skog". For two of the stands, stand selection was assisted by a private forest owner in Nordmaling. All potential stands were visited in the field in order to include or exclude them from the study based on the different requirements.

The inventoried stands were named A-J. The stands named E and EF were in reality considered part of the same management unit. However, due to the large size of the stand and the concentrated distribution of large aspen trees towards different edges, it was treated as two different stands in the analyses.

*Table 1. Stand descriptions and geographic location of the study sites.*

| Site | Area (ha) | Age | Mean height (dm) | Aspen trees (number) | Regeneration method | Latitude/Longitude (WGS 84) |
|---|---|---|---|---|---|---|
| A | 17 | 8 | 11.9 | 17 | Sown | 64°00'N / 20°34'E |
| B | 9.4 | 10 | 13.9 | 22 | Planted | 63°58'N / 20°39'E |
| C | 10 | 10 | 15.6 | 20 | Planted | 63°59'N / 20°34"E |
| D | 4.7 | 10 | 15.7 | 15 | Planted | 64°00'N / 20°34'E |
| E | 11 | 11 | 15.6 | 6 | Sown, planted | 64°01'N / 20°34'E |
| EF | 11 | 11 | 14 | 25 | Sown | 64°01'N / 20°33'E |
| F | 1.5 | 8 | 11.9 | 45 | Sown | 63°56'N / 20°34'E |
| G | 4.1 | 6 | 14.1 | 5 | Planted | 63°58'N / 20°38'E |
| H | 21 | 12 | 14.4 | 39 | Sown | 63°57'N / 20°39'E |
| I | 7 | 8 | 7.3 | 14 | Sown | 63°30'N / 19°42'E |
| J | 4 | 8 | 14.9 | 20 | Sown | 63°29'N / 19°41'E |

## Data collection

Eighteen circular sample plots (radius = 3.5 m) were systematically laid out in each of the 11 stands (A-J), totalling 198 sample plots. Each sample plot centre was positioned with a GPS to be able to perform spatial analyses in GIS. The sample plots were systematically placed in rows of 3 x 6 plots with a distance of 20 meters. The plot positions were randomly chosen by laying the first sample plot at the first located aspen tree in the stand and thereafter position the rest of the sample plots along the estimated lengthwise direction of the stand. To eliminate edge effects the distance to surrounding forest was at least two tree-lengths. All pines within the sample plots were registered. Thereafter, the height of the trees and the length of their terminal leader shoots were measured. Occurrence of current-year PTR infection was registered for each individual tree by counting the number of infected branch terminals. Furthermore, the terminal leader was classified as either infected (1) or not infected (0). The trees probability for future development was assessed as either yes (1): the tree can develop into an economical valuable tree, or no (0): the tree can probably not develop due to PTR infection. This was estimated with reference to terminal leader infection and total severity of

PTR damage. If the leader shoot had been severely attacked this growing season, meaning that lesions covered more than half of its circumference, the tree was classified as having a low probability to develop into a tree of economical value. If the terminal shoot was healthy, or only had a small lesion caused by PTR, the probability to develop into a quality tree was considered high. The ground vegetation was estimated by using the vegetation scheme developed at the Swedish University of Agricultural Sciences (Hägglund & Lundmark 1999). This was done to get an estimation of the ground's nutrient status at a local scale. The vegetation was then categorised into three different classes: grass-, berry- and heather type. Grass type contains thick- and thin grass types, berry type includes blueberry (*Vaccinium myrtillus*) and lingonberry (*V. vitis-idaea)* and the heather type represents heather (*Calluna vulgaris*) and crowberry (*Empetrum spp.*). All aspen sprouts in each sample plot were counted and all large aspen trees growing in the stand or in the adjacent forest edge were GPS positioned.

An additional stand, named K (planted, mean height 8.5 dm), located west of Robertsfors (64°11' / 20°38'E), was inventoried because it contained ten high stumps of aspen trees and high density of aspen suckers (see picture 2 in Appendix). This enabled a separate study to investigate the level of damage in a stand with very few mature aspen trees (3) but with a large quantity of aspen sprouts. The sample plots were laid out with the same systematically procedure as for the other stands but due to the small size (one hectare) together with the shape of the stand only 11 sample plots was used.

## *GIS analyses*

The spatial analysis of the large aspens around the sample plots was carried out in the GIS program ArcMap 9.2. All coordinates from the sample plots and the large aspens for each stand were added to ArcMap and converted into different shape files. For each stand, all large aspens within a distance of 100 meters from the sample plots were counted and the distance from each sample plot to the nearest large aspen was estimated. The distance was estimated by determining the distance from each sample plot in the "Input Features" to the nearest aspen in the "Near Features".

To visualise the spatial distribution of PTR infection in the stands, in relation to the number of aspen sprouts and distribution of large aspens, maps were created in ArcMap for four different stands. Two of the stands had a high level of PTR infection (stand G and I) whereas the other two had a relatively low level of infection (stand D and EF).

## *Statistical analyses*

All statistical analyses were preformed using the Minitab 15 package. The calculations were performed with a confidence interval of 95%.

### Analyses on sample plot level

To study the effect of large aspens and aspen suckers on the occurrence and level of PTR damage in young pine regenerations, analysis of variance (ANOVA, GLM) was performed on the sample plot level (N=198), using the model 1.

Model 1: $y = \mu$ + site + veg class + aspen sprouts + dist large aspen + number large aspens + leader length + e
($\mu$=grand mean, e=random stochastic factor)

Three different response variables (y) were tested; proportion of trees with infected current-year shoots, number of infected shoots per tree and proportion of trees with an infected leader shoot. The variables site and vegetation class were included as random and fixed class variables, respectively. The variables number of aspen sprouts, distance to closest large aspen, number of large aspens within 100 meters and terminal leader length were included as covariates in the model. A Tukey simultaneous test, with pairwise comparisons among levels, was performed to investigate differences in the proportion of infected trees between vegetation classes.

To study the specific effect of large aspen trees on the occurrence and level of PTR damage, the number of aspen sprouts was omitted from the ANOVA model above. The analysis was performed on the sample plot level, using the model 2.

Model 2: $y = \mu$ + site + dist large aspen + number large aspens + e
($\mu$=grand mean, e=random stochastic factor)

The effect of large aspen trees on the incidence and quantity of aspen sprouts was tested using model 2, with number of aspen sprouts as the response variable (y).

### Analyses on tree level

To examine if there was a significant difference in leader length between infected and healthy trees on the tree level, 2-sample t-tests were used.

### Analyses on stand level

The percentage of PTR-infected pines and the number of aspen sprouts per hectare was calculated for each stand. To study eventual relationship between the level of infection and the number of aspen sprouts per hectare a linear regression was performed on the stand level.

## *Questionnaire*

In order to get more information about the level of knowledge- and the severity of PTR damage in Sweden, a questionnaire was sent to forest managers in different parts of Sweden. The questions were concerning if PTR is a common and/or serious pathogen in the forest and if there was any adaptive forest measures used in order to prevent PTR infection. Five forest managers answered; three of them worked at the Swedish Forest Agency and two represented the forest companies “Sveaskog” and “Holmen skog”.

## Results

There was a highly significant correlation between number of aspen sprouts and infection rate on Scots pine saplings (all three response variables) in the sample plots (Table 1-3). Correspondingly, regression analysis showed that the proportion of infected pines per site was higher in areas with a large amount of aspen sprouts (Fig. 1). None of the two variables “distance to closest large aspen” or “number of large aspens within 100 m” was significantly correlated to PTR infection rate (Table 1-3). If the number of aspen sprouts was omitted from the ANOVA, the variable “distance to closest large aspen” fell out as significant (P=0.017, $R^2$(adj)=55.06 %) for the response “proportion of trees with infected current-year shoots”.

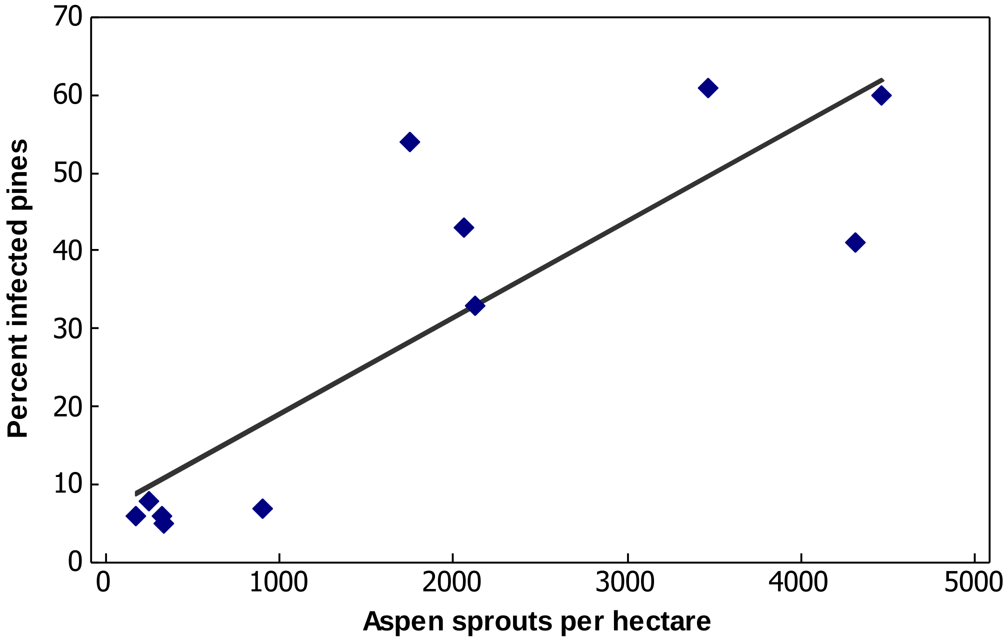


Fig 1. The percentage of infected pines per site in relation to the number of aspen sprouts per hectare. The regression equation is; y = 6.82 + 0.0124*Aspen sprouts/ha, p = 0.001, $R^{2}$(adj) = 70.6%.

There was also a significant positive correlation between leader length and PTR infection rate (Table 1-3, Fig. 3). The vegetation class fell out significantly only for the variable “proportion of trees with infected current-year shoots” (Table 1). The proportion of infected pines was significantly higher (ANOVA (Tukey’s test), P<0.05) for the vegetation class grass compared to the vegetation class heather (Fig. 2).

A significant negative correlation was shown between the number of aspens sprouts and the distance from the closest large aspen (ANOVA, P< 0.001).

Table 1. Analysis of variance for proportion of trees with infected current-year shoots, using adjusted SS for tests. Calculation performed as a general linear model with a confidence interval of 95%. $R^2$(adj) = 63.67%

| Source | DF | Seq SS | Adj SS | Adj MS | F | P |
|---|---|---|---|---|---|---|
| Site | 10 | 94475.3 | 62949.7 | 6295.0 | 19.71 | 0.000 |
| Leader length | 1 | 2000.4 | 3449.5 | 3449.5 | 10.80 | 0.001 |
| No.aspen sprouts | 1 | 12701.1 | 7978.9 | 7978.9 | 24.99 | 0.000 |
| Distance to closest aspen | 1 | 248.9 | 164.7 | 164.7 | 0.52 | 0.474 |
| No.aspens within 100 m | 1 | 247.1 | 90.1 | 90.1 | 0.28 | 0.596 |
| Vegetation class | 2 | 2333.1 | 2333.1 | 1166.6 | 3.65 | 0.028 |
| Error | 175 | 55882.3 | 55882.3 | 319.3 | | |
| Total | 191 | 167888.3 | | | | |

Table 2. Analysis of variance for number of infected current-year shoots, using adjusted SS for tests. Calculation performed as a general linear model with a confidence interval of 95%. $R^2$(adj) = 48.97%

| Source | DF | Seq SS | Adj SS | Adj MS | F | P |
|---|---|---|---|---|---|---|
| Site | 10 | 77.5593 | 50.1606 | 5.0161 | 10.89 | 0.000 |
| Leader length | 1 | 1.5498 | 3.4298 | 3.4298 | 7.44 | 0.007 |
| No.aspen sprouts | 1 | 11.5916 | 9.2319 | 9.2319 | 20.04 | 0.000 |
| Distance to closest aspen | 1 | 0.0640 | 0.1384 | 0.1384 | 0.30 | 0.584 |
| No.aspens within 100 m | 1 | 0.0060 | 0.0025 | 0.0025 | 0.01 | 0.942 |
| Vegetation class | 2 | 1.0365 | 1.0365 | 0.5183 | 1.12 | 0.327 |
| Error | 175 | 80.6250 | 80.6250 | 0.4607 | | |
| Total | 191 | 172.4322 | | | | |

Table 3. Analysis of variance for proportion of infected leader shoots, using adjusted SS for tests. Calculation performed as a general linear model with a confidence interval of 95%. $R^2$(adj) = 48.88%

| Source | DF | Seq SS | Adj SS | Adj MS | F | P |
|---|---|---|---|---|---|---|
| Site | 10 | 29818.5 | 17502.5 | 1750.2 | 9.20 | 0.000 |
| Leader length | 1 | 1306.9 | 2629.9 | 2629.9 | 13.82 | 0.000 |
| No.aspen sprouts | 1 | 6218.3 | 5566.5 | 5566.5 | 29.26 | 0.000 |
| Distance to closest aspen | 1 | 11.1 | 32.1 | 32.1 | 0.17 | 0.682 |
| No.aspens within 100 m | 1 | 37.7 | 53.4 | 53.4 | 0.28 | 0.597 |
| Vegetation class | 2 | 390.1 | 390.1 | 195.1 | 1.03 | 0.361 |
| Error | 175 | 33290.0 | 33290.0 | 190.2 | | |
| Total | 191 | 71072.7 | | | | |

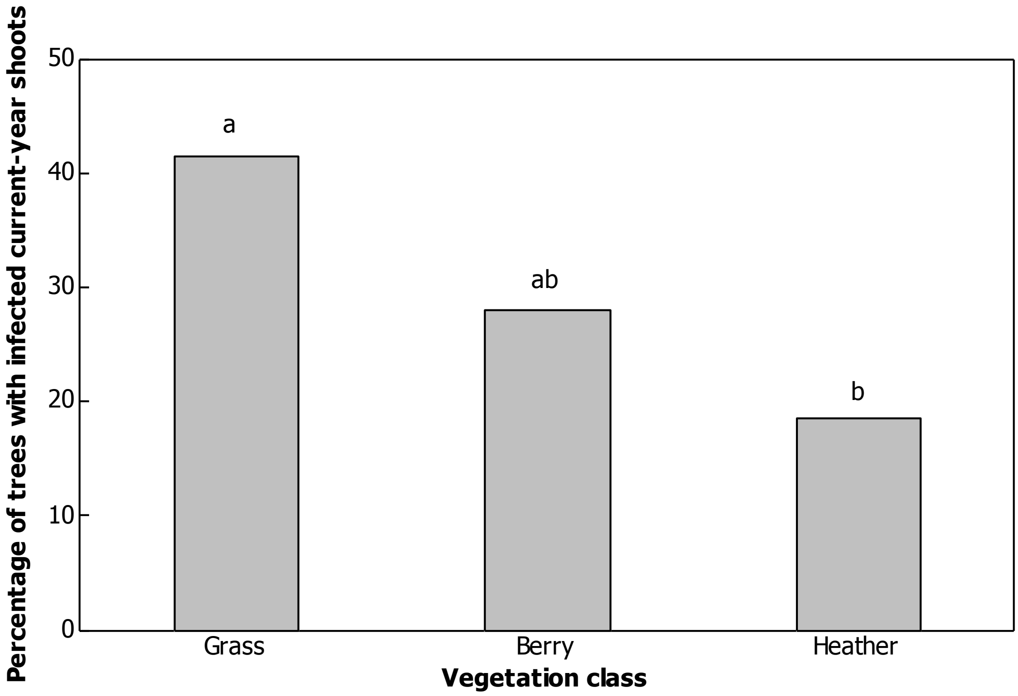


Fig 2. Percentage of trees with infected current-year shoots in each vegetation class. Different letters indicate significant differences between vegetation classes.

More than half of the infected pines (51 %) had a terminal leader that was infected by PTR. Infected leaders were significantly longer than healthy leaders (T-test, P<0.001), 30.8 cm compared to 24.5 cm, respectively (Fig. 3).

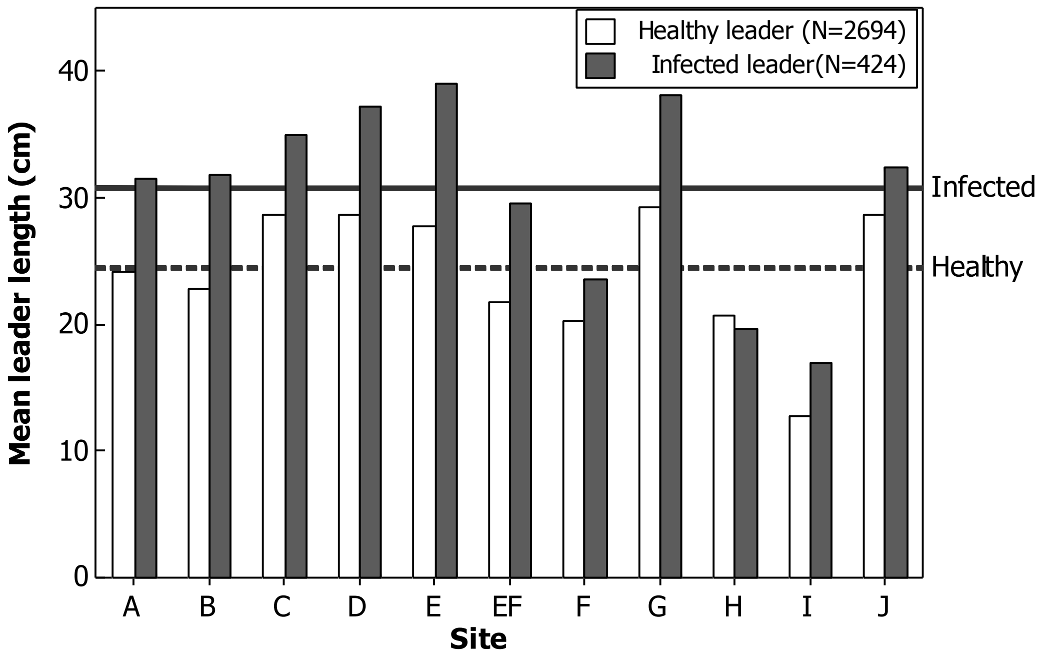


Fig 3. Mean leader lengths for pines with healthy and infected leader shoots on different sites. White bars show healthy leaders and black bars leaders with symptoms of PTR infection. Horizontal lines mark the mean leader lengths for all healthy and infected leaders, respectively.

Pines with infected current-year shoots also had significantly longer leaders than healthy pines (T-test, P<0.001) (Table 4). This was independent of if the leader was infected or not.

Table 4. Mean leader length (cm), mean tree height (dm) and standard deviation (±) for healthy trees (0) and for trees with shoots infected by PTR (1)

| Infected trees | N | Mean leader length (cm) | Mean tree height (dm) |
|---|---|---|---|
| 0 | 2290 | 24.1 (±10.6) | 13.7 (±5.6) |
| 1 | 828 | 29.0 (±11.4) | 14.4 (±5.4) |

In total, 27 % of the 3118 assessed pines were infected by PTR (Fig. 4). The infected pines had a mean number of 2 infected shoots per tree and the range of infected shoots per tree was 0-16. Of the infected pines, 42 % were classified to have a low probability for future development, which concerned the pines possibility to develop into economic valuable trees in regard to damage caused by PTR.

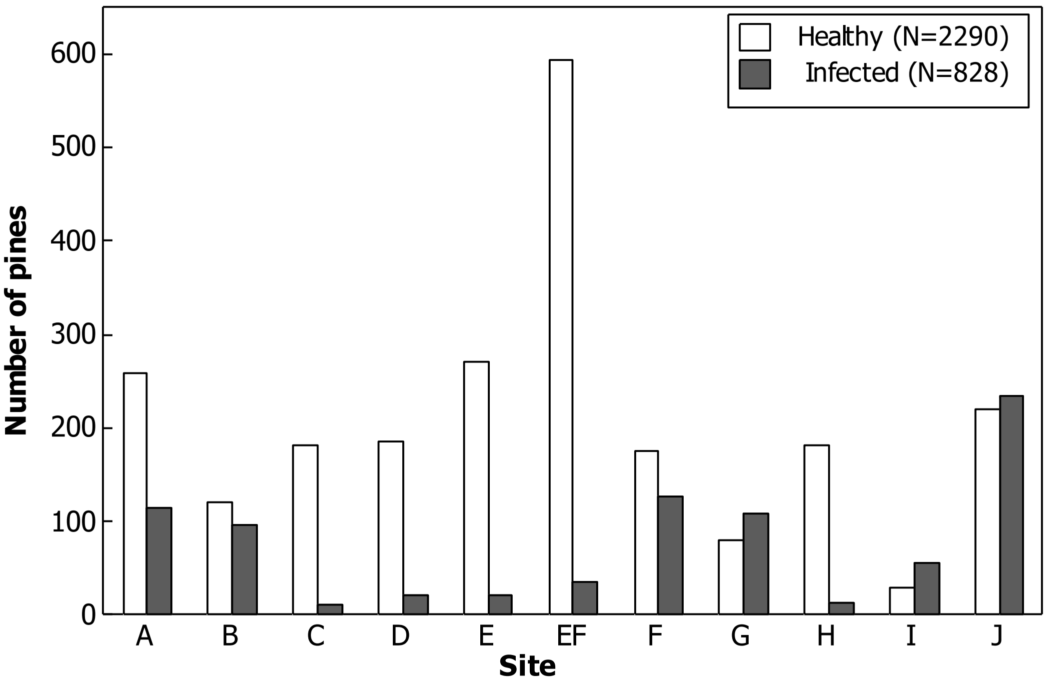


Fig. 4. The number of healthy and PTR infected pines per site.

In the additional stand K, all of the assessed pines (80) had current-year infection by PTR. Ninety percent had an infected leader and 79 % of the trees were classified to have a low probability for future development due to PTR. The number of aspen sprouts was estimated to 13 500 per hectare.

The answers from the Swedish forest managers were that severe PTR damage was mainly local and even though PTR is a common pathogen, it was generally not considered as a large threat in the forestry. There were rarely any adaptive forest measures used to prevent PTR infection but in the most risky areas, alternative tree species like Norway spruce (*Picea abies*) or lodgepole pine (*Pinus contorta*) could be used instead of Scots pine. If was stand was severely infected, the damaged pines could be removed at the first thinning operation.

The maps created in ArcMap, for the stands named I, G, D, and EF, visualises the PTR infection in relation to the number of aspen sprouts and large aspen trees (Fig. 5-8). Stands with a larger number of aspen sprouts showed a great infection level (Fig. 5-6). The aspen sprouts were concentrated to the area closest to the large aspen trees (Fig. 5-7). Stand EF contained several aspen trees but a small number of aspen sprouts (Fig. 8), here the number of infected pines in relation to healthy pines was low (Fig. 4).

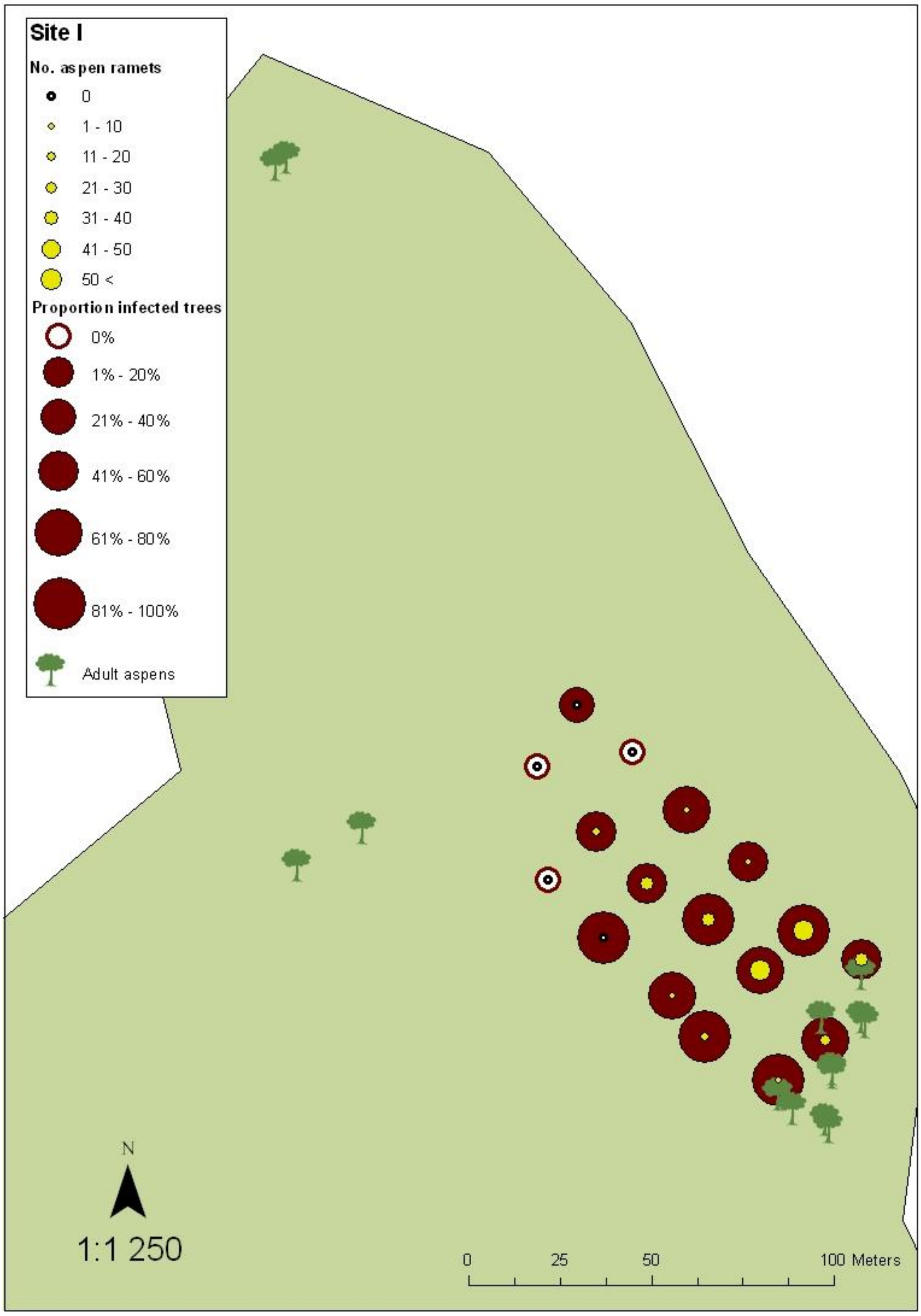


Figure 5. Sample plots within site I, Nordmaling. 60 % of the inventoried pines were infected by PTR The size of the red circles reflects the proportion of pines infected by PTR per sample plot. The sizes of the yellow circles represent the numbers of aspen sprouts. The tree symbols represent large aspen trees in the stand.

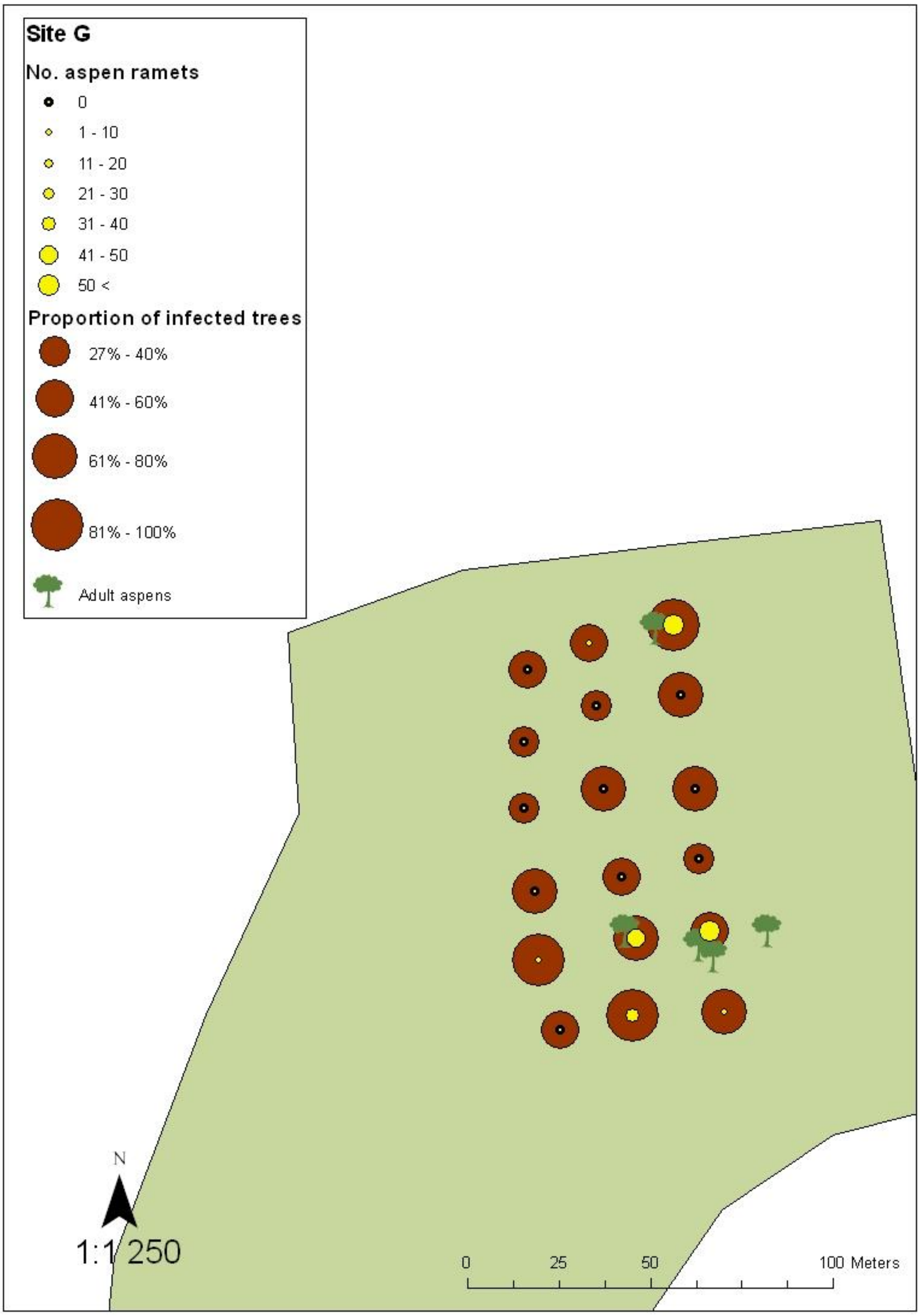


Figure 6. Sample plots within site G, Rönnäs. 61 % of the inventoried pines were infected by PTR. The size of the red circles reflects the proportion of pines infected by PTR per sample plot. The sizes of the yellow circles represent the numbers of aspen sprouts. The tree symbols represent large aspen trees in the stand.

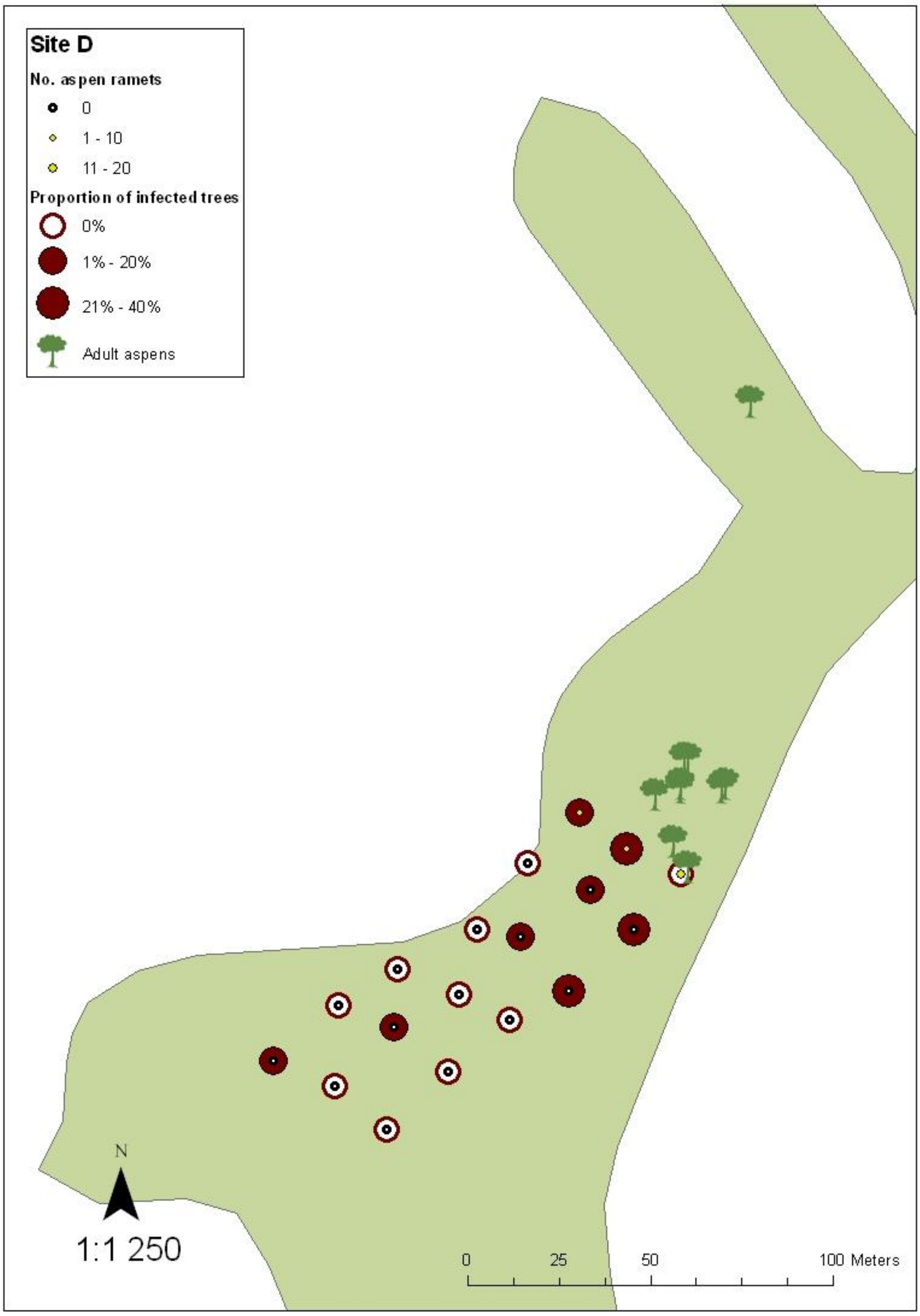


Figure 7. Sample plots within site D, Rönnäs. 8 % of the inventoried pines were infected by PTR The sizes of the red circles reflects the proportion of pines infected by PTR per sample plot. The sizes of the yellow circles represent the numbers of aspen sprouts. The tree symbols represent large aspen trees in the stand.

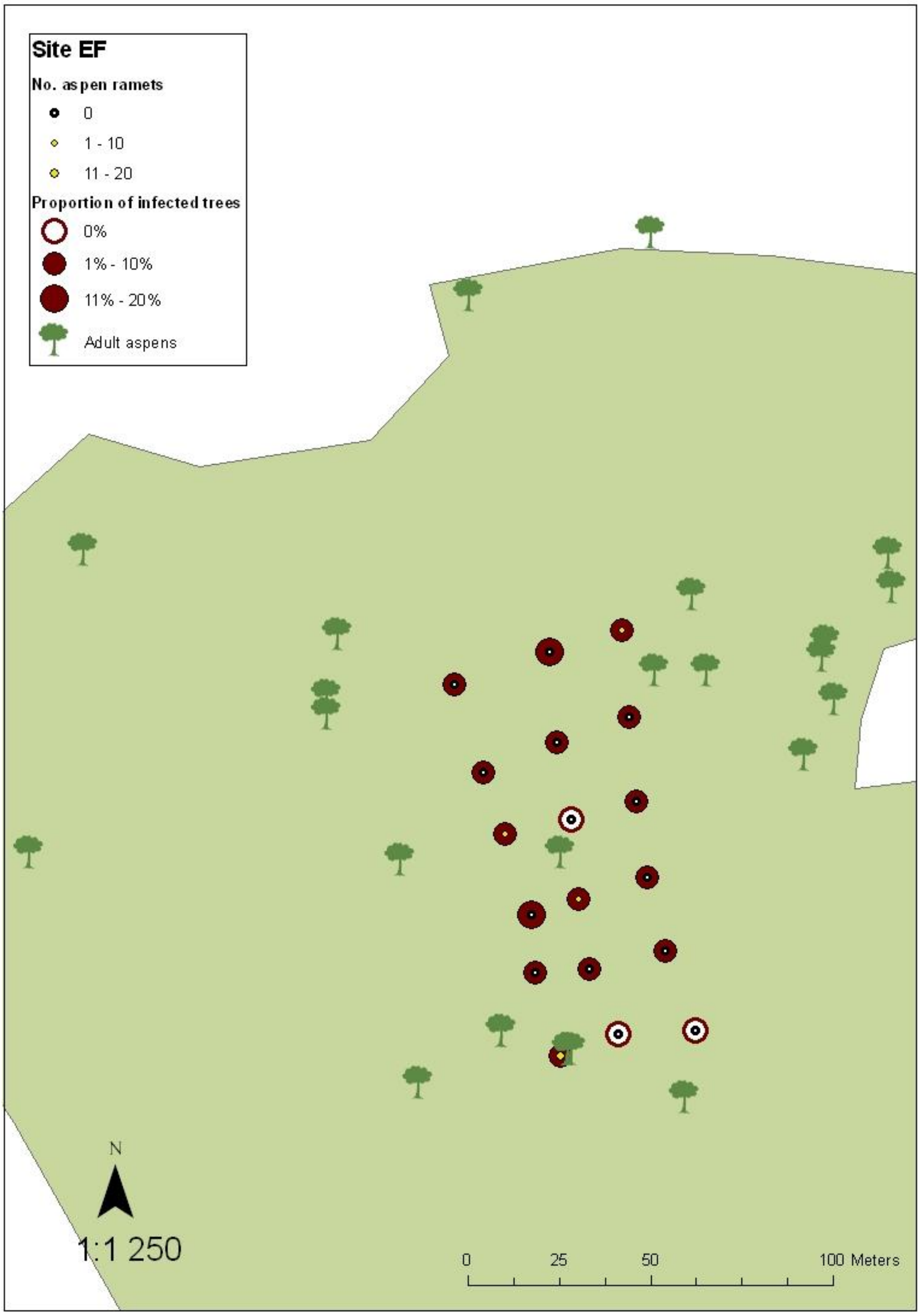


Figure 8. Sample plots within site EF, Rönnäs. 5 % of the inventoried pines were infected by PTR The sizes of the red circles reflect the proportion of pines infected by PTR per sample plot. The sizes of the yellow circles represent the numbers of aspen sprouts. The tree symbols represent large aspen trees in the stand.

# Discussion

The results of this study confirmed the stated main hypothesis that aspen sprouts are more important than large aspen trees for the spread of pine twisting rust (PTR). The highly positive correlation between the infection rate on young pines and the number of aspen sprouts agrees with previous findings by Lagerberg (1945). Although previous studies confirm that the risk of PTR outbreaks increase with the presence of aspen (Lagerberg 1945, Mattila et al 2001, Mattila 2005), the present study elucidated and distinguished the importance of aspen sprouts, in comparison to aspen trees, as a main source in the spread of PTR.

In the present study the distance to the large aspen trees was negatively correlated to the proportion of infected pines only when the aspen sprouts were omitted from the analysis of variance. Thus, assuming a constant-coverage of aspen sprouts on the sample plots, neither the distance to the closest aspen tree nor the number of aspen trees within 100 meters had any effect on disease incidence on the pine saplings. Furthermore, the number of aspen sprouts decreased with increased distance away from the aspen trees. This indicated that the adult aspen's role in the spread of PTR, is primarily by producing aspen sprouts. These findings are supported by a recent study on the occurrence of PTR infection on aspens of different age (Löfstrand unpublished), where it was shown that the occurrence of urediniospores on the leaves was significantly higher on aspen sprouts compared to large aspen trees. Even if the main focus of the present study was towards the infection level on pines in relation to the number of adult aspens and aspen sprouts, the finding by Löfstrand (unpublished) may provide a reasonable explanation for why the infection rate on pines was higher in areas with a greater number of aspen sprouts. Regardless of whether it is the aeciospores, spread from the pines to the aspens, or the urediniospores, which spread in between the aspens, that caused the large number of spores on the aspen sprouts, it is evident that aspen sprouts have a significant role as source of PTR spores.

More than half of the PTR-infected pines had an infected terminal leader and infection rate was significantly correlated with the length of the terminal leader. This agrees with earlier reports that the fungus predominantly infects the terminal leaders of the pines (Sylvén 1917, Klingström 1963, Kurkela 1973) and that productive trees with vigorously growing shoots are more susceptible than less productive trees (Kurkela 1973, Desprez-Loustau & Wagner 1997a and 1997b, Mattila et al. 2001). This fact was additionally shown in the present study by the division of sample plots into vegetation classes. The proportion of infected pines was highest for the vegetation class grass and lowest for the vegetation class heather. In addition, the results that the terminal leaders on infected pines were significantly longer than terminal leaders on healthy trees, contribute to the fact that productive trees are more receptive to the pathogen. A similar relationship, between site productivity and disease occurrence, has been found for other fungal diseases on pine, e.g. resin top disease (*Cronartium flaccidum*) (Sveriges lantbruksuniversitet 2008) and Scleroderris canker (*Gremmeniella abietina*) (Witzell & Karlman 2000). The increased risk of PTR infection in more fertile areas is an important aspect that oppose with the forestry's ambition towards a high productivity.

Approximately one fourth of the total number of assessed pines showed symptoms of PTR infection. Fifty percent of these were estimated to have a low probability for future development due to the severity of the infection. However, the majority of the stands were not severely infected. This is in line with the forest managers' replies to the questionnaire, where PTR was said to be a common pathogen but generally not considered as a large threat since severe damage mainly occurs on a local scale. However, the additional study in stand K clearly exemplifies what could happen if the risk of PTR infection is not considered and incorporated in the forest management. Here, the aspen trees had been cut to high stumps, resulting in an enormous quantity of aspen sprouts and a 100% infection rate on the pine saplings. Furthermore, the current-year PTR infection in the stand was severe since 79 percent of the pines were classified to have a low probability for future development. It would be interesting if this stand was studied further in order to investigate the future growth development after such a severe infection.

In terms of leaving aspens as green tree retention after harvest, the questionnaire stated that no or very little concern is taken to the risk of PTR. The answers implied that there was a lack of knowledge if there were stands within the management area that were severely infected by the pathogen. Furthermore, the local knowledge of previous infections and/or presence of aspens often determine if a preventive action is necessary after a final felling. The preventive recommendations appeared well adapted to prevent severe infections in known risk areas. In high risk forest sites alternative tree species like Norway spruce or lodgepole pine were used. In general, the best recommendation is to avoid planting Scots pine on rich spruce sites where aspen often is frequent (Mattila et al 2001). Overall, all responding foresters were very positive towards that studies about PTR were carried out and everyone wanted to take part of the results.

The maps created in ArcMap showed the proportion of infected pines, on the sample plot level, in relation to the number of aspen sprouts and the distribution of aspen trees in the stand. This was made for four different stands with different level of PTR infection in an attempt to visualize the spatial distribution of PTR infection. It appear as the level of PTR was low in stands where the amount of aspen sprouts was low  independent of the number of large aspen trees (figure 5, 7-8). The map over the proportion of PTR infection in stand EF (figure 8) showed that despite many large aspen trees the infection rate was low. Here, one plausible explanation could be that none or very few aspen trees had been cut and hence, the number of aspen sprouts was low. This further indicates the importance of aspen sprouts for the spread of PTR. For site D and I, higher infection rates were found closer to the large aspens. However, also the largest number of aspen sprouts was found close to the aspens.

Since there are few aspen trees left in the managed forest land it is necessary, from the biodiversity point of view, to favour the development of new aspen trees in the forest management, e.g. in pre-commercial thinning. One way to reduce the risk of PTR incidence in pine regenerations is to direct aspen development into spruce stands. In young pine stands, the aspen sprouts should be removed in cleaning operations in order to reduce the risk of severe PTR infection, especially in more fertile areas.

To improve future studies, the stand selection could focus on that the large aspen trees should be located towards one edge of the stand. Implementing this as a criterion in the stand selection could further clarify the role of aspen trees in the spread of PTR. In the present study, the aspen trees were often spread throughout the stands because there was no criterion in the stand selection based on the position of the aspen trees. Moreover, the distance between the sample plots could be alternated with the size of the stands. In the present study, a fixed distance was used independent of the size of the stands. To only use one fixed distance instead of alternate it with the size of the stand has given the study a limitation to one single distance gradient. It could have been an advantage in adjusting the distance after the size of the stand because larger stands may provide a greater spread of the basidiospores due to the winds turbulence.

## *Conclusions and management applications*

The results of this study confirmed that aspen sprouts are more important than large European aspen trees for the spread of pine twisting rust (PTR). The percentage of infected Scots pines was highest in areas with a large number of aspen sprouts, whereas neither the distance to the closest aspen tree nor the number of aspen trees was significantly correlated to PTR infection rate. Hence, it appeared that the adult aspens' role in the spread of PTR is primarily by producing aspen sprouts.

The infection rate on pines was significantly correlated with the length of the terminal leader and productive trees appeared more receptive to the pathogen. Thus, in the most fertile stands alternative tree species could be an option as a preventive action after a final felling. This is especially important in areas with a history of severe PTR infections.

Management practices in young Scots pine stands should incorporate the removing of aspen sprouts in order to reduce the risk of severe PTR infection on the pine saplings. This is especially important in more fertile areas which suffer a greater risk for infection. If the management towards a greater number of aspen trees could be directed mainly towards spruce stands it could mitigate the future infection risk in pine regenerations.

The important role of aspens in the species conservation is a major argument for why the aspen trees should be left as green tree retention also in Scots pine regenerations. This study indicates that the risk of leaving aspen trees could be considered low as long as preventive management practices are used to reduce the number of aspen sprouts.

## Acknowledgements

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# Appendix

*Photo Åsa Fjellborg*

**Picture 1**. Pine shoot severely infected by PTR.

*Photo Åsa Fjellborg*

**Picture 2**. Overviews picture from the additional stand, K.

## SENASTE UTGIVNA NUMMER

| | | |
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