

Original Research

# Etiology of Premature Needle Shedding in Geographically Diverse *Pinus sylvestris* Populations

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## Abstract

At the end of July 1997 a premature shedding of one- and two-year-old foliage of Scots pine (*Pinus sylvestris* L.) was observed in central Poland. We examined the etiology and physiological consequences of this needle shedding event in a 15-year-old Scots pine plantation with diverse populations originating from Sweden, Russia, Latvia, Poland, Germany and France. On average, trees lost 20% of two-year-old foliage, with a local population from Poland having the highest needle loss (28%) and the lowest in a population from France (13%). However, differences among populations in needle loss were only marginally significant ( $p = 0.1$ ). Phytopathological observations excluded biotic factors as responsible for needle loss. Analysis of thermal conditions in 1997 suggest that premature needle shedding may originate from the combination of winter physiological drought and unusually high (up to 35°C) air temperatures and low precipitation in late spring. We found that winter drought significantly affected the foliage by reducing its water content and concentration of nonstructural carbohydrates. High summer temperatures increased water stress and as a consequence led to reduction in crown density. Our data indicated that the needle shedding may be also related to root system damage due to low soil temperatures. Marginally significant differences among populations in needle shedding may indicate a weak genetic control over premature needle-fall among European Scots pine populations.

**Keywords:** foliage abscission, winter drought, high temperature, chlorophyll, nonstructural carbohydrates.

## Introduction

Partial loss of foliage by trees due to abiotic and/or biotic stress may alter CO<sub>2</sub> exchange, carbon balance and nutrient cycling of trees [1-4]. In most instances premature needle shedding in forest trees can be linked to winter frost or spring physiological drought, when sunny and warm or windy days desiccate needles of trees rooted in soils that are too cold for adequate water uptake [5-8]. Frost injury usually results from desiccation, rapid needle cooling and/or freezing injury caused by a reduction in cold hardiness due to solar heating [5]. Studies have

shown that severe winter desiccation in conifers may often be due to prior freezing injury, increased as a result of exposure to direct solar radiation and photo-oxidative damage to chlorophyll and other cellular components [6, 9]. Winter and spring drought may affect trees by reducing the amount of fine roots and inducing long-term water stress [10].

The long-term effects of high temperatures can induce foliage abscission due to increased transpiration and desiccation that can exacerbate premature foliage shedding [11]. High temperature and drought stress can also lead to a decrease in needle length [12], reduced growth [13] and an increased susceptibility to infectious diseases [14]. Many studies have been made on the effects of drought [15, 12, 16, 17, 18]. However,

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Table 1. The origin of seeds of *Pinus sylvestris* used in the study. Provenances are ordered by latitude of origin.

Population no.	Country	Latitude (N)	Longitude (E)	Altitude (m)	Precipitation (mm)	Temperature (°C)
15	Sweden	60.18	15.87	185	608*	4.5
3	Russia	58.83	29.12	80	616	5.0*
4	Latvia	55.75	26.67	165	619	5.4
7	Poland	51.60	20.20	160	575	7.4
12	Germany	49.50	8.50	97	645	9.6
14	France	48.80	7.78	150	850	10.0

‡See [51] for origin. \*Calculated from WorldClimate (www.worldclimate.com); otherwise, data provided by the participants of the SP-IUFRO-1982 experiment (see [19] for details).

very little is known regarding possible intraspecific differences in premature needle shedding among diverse populations of trees. To address this issue we studied the effects of abiotic and biotic factors on foliage shedding in Scots pine populations from different geographic origins, utilizing a common-garden experiment in western Poland. Our study was designed to reveal factors responsible for premature shedding of Scots pine needles and to explore possible differences among ecotypes within the species European range. The broad range of the seed sources and common garden conditions with replicated blocks and plots, uniform soil and common environmental factors enabled us to explore these issues.

## Material and Methods

### Plant Material and Study Site

Seeds of Scots pine (*Pinus sylvestris* L.) were collected between 1978 and 1980 in 20 locations in Europe as a part of an international collaborative experiment established under the auspices of the International Union of Forestry Research Organisations [19]. (Detailed information about this experiment has been presented elsewhere [20-21]. In April 1984, two-year-old seedlings of 19 populations of Scots pine were planted in a permanent site in the experimental forest, Zwierzyniec, near Kórnik in central Poland (52°15' N and 17°04' E, altitude 70 m). The soil at this site is a light gray-brown podzol. Its chemical properties have been described in detail in [23]. The site consists of seven blocks. Each provenance was planted in three to seven replicated plots (one per block), 7.2 m x 5.2 m; each with 48 plants (4 rows x 12 plants). The original spacing was 0.6 m within and 1.3 m between rows, and the original stocking was 12,834 trees ha<sup>-1</sup>. In 1994, a thinning was conducted and about 60% of the trees were removed. Premature needle shedding was observed at the end of July and beginning of August of 1997.

### Environmental Conditions

The climate of the region is transitional between maritime and continental. Mean annual precipitation is 526 mm

and mean temperature 7.7°C, with a mean growing season length of 220 days, calculated as the number of days with mean temperature  $\geq 5^{\circ}\text{C}$ . Meteorological data were obtained from a local meteorological station approximately 2 km from the experimental forest. This station operates in the state network of meteorological stations in Poland.

Observations at the experimental site were conducted over a three-year period, 1996-98. The studied years differed in climatic patterns. In 1996 the mean annual air temperature was 7.1°C (0.6°C below normal). In contrast 1997 and 1998 had mean temperatures of 9.1 and 9.7°C, which were 1.4 and 2.0°C warmer than the long-term average. Mean annual precipitation was 526 mm in 1996, 516 mm in 1997 and 634 mm in 1998.

### Sampling and Observations in the Field

Needle shedding was studied on six geographically diverse populations from the continuous part of the European range of Scots pine in Sweden, Russia, Latvia, Poland, Germany and France (Table 1). Defoliation was assessed as a residual amount of needles in the trees by two independent observers on all trees (between 20 and 47) of selected populations in two blocks. There were no statistical differences in needle shedding estimates between the observers (data not shown). Therefore, in further analyses, average values of the two observers were used.

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The possible effect of biotic factors on needle shedding was assessed on shoots taken from 15 trees per population from different crown positions. One and two-year-old needles were evaluated for pathological symptoms such as (1) discoloration, (2) needle tip desiccation and necroses, (3) wounds and injuries due to insect grazing, and (4) anatomical anomalies. The presence of fungal pathogens

and insect egg deposits was assessed on dead needles. Foliage litter was analyzed for the presence of the needle cast fungus *Lophodermium seditiosum*, which was observed at this and other sites of the Scots pine IUFRO-1982 experiment in the past [24]. Using a binocular microscope, foliage litter was examined in 1997 and 1998 for ascomata and conidiomata.

### Measurements of Nonstructural Carbohydrates

Total nonstructural carbohydrate (TNC) concentrations were determined by a modification of the method described by Haissig and Dickson [25] and Hansen and Møller [26]. Sugars were extracted from oven-dried

(65°C, 48h) tissue powder in methanol-chloroform-water, and tissue residuals were used for determination of starch content. Soluble sugars were determined colorimetrically with anthrone reagent at 625 nm within 30 min. Starch in the tissue residual was then gelled and converted to glucose with amyloglucosidase. Glucose concentrations were measured with glucose oxidase by mixing the sample with the reagent peroxidase-glucose oxidase-o-dianisidine dihydrochloride. Absorbance was measured at 450 nm after a 30 min. incubation at 37°C. Soluble carbohydrate concentrations were calculated from standard curve linear regression equations using glucose standard solutions and expressed in percent of tissue dry mass. Data are means of two replications consisting of one composite sample from each of two sampled blocks.

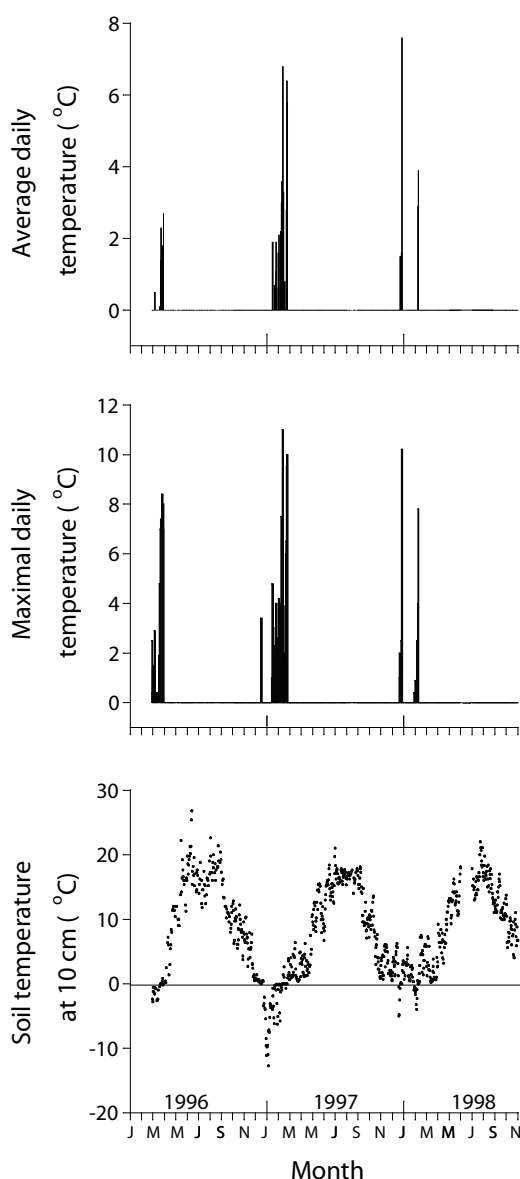


Fig. 1. Pattern of average and daily maximum air temperatures during the days when soil remained frozen (top two panels) and soil temperature at 10 cm depth (lower panel). Soil temperatures below -10°C can damage root systems of Scots pine (see [37]).

### Chlorophyll Extraction and Water Content Measurements

Leaf tissue (20 mg) from two needle cohorts (two trees in each of two blocks per provenance) were cut into small (2 mm) pieces and placed in a vial with 2.5 ml of dimethylsulfoxide (DMSO) and incubated for 4 hours at 60°C. The chlorophyll extract was transferred to a cuvette and absorbances were determined at 470, 648 and 665 nm (DMSO) on a spectrophotometer (Beckman 6400, USA). Chlorophyll concentrations were calculated using Barnes et al. [27] equations:  $Chl_a = 14.85A_{665} - 5.14A_{648}$ ;  $Chl_b = 25.48A_{648} - 7.36A_{665}$ ;  $Chl_{a+b} = 7.49A_{665} + 20.34A_{648}$ ;  $Car = (1000 A_{470} - 3.27 * Chl_a - 104 Chl_b) / 229$ .

The water content in foliage, woody shoots, and buds was determined by subtracting the fresh mass of different organs from its dry mass (after drying at 65°C for >48 hrs).

For all variables, statistical differences among provenances were calculated by analysis of variance (GLM procedures). All statistical analyses were conducted with JMP software (version 3.2.2, SAS Institute, Cary, NC, USA).

### Results

Meteorological conditions from the neighbouring meteorological station were studied both in winter and summer to investigate the effects of drought on needle shedding. Analysis of air and soil temperatures in winter 1997 indicated that during 39 consecutive days (from 15 January to 23 February), maximum daily temperatures varied between 4 and 11°C while soil temperature remained below 0°C (Fig. 1). Both in the previous and subsequent years, such temperature conditions lasted for no longer than 10 days. In addition at the beginning of 1997, the average daily soil temperature at 10 cm depth declined to < -10°C (Fig. 1).

Sunshine duration recorded with the Campbell-Stokes heliograph recorded ca. 105 hours of bright sunshine during the same period, indicating the prevalence of clear

Table 2. Average concentrations of chlorophyll, carotenoids ( $\text{mg}\cdot\text{g}^{-1}$ ), starch, soluble and total nonstructural carbohydrates (TNC, %) in foliage of two needle-age cohorts and water content (%) in foliage, buds and woody twigs before (23 December 1996) and during physiological drought (21 January and 17 February 1997).

Trait	Needle age	Before drought	During drought	ANOVA effects P>F
Chlorophyll a	1-year-old	2.98	3.02	0.7
Chlorophyll b		1.05	1.22	0.005
Chlorophyll a+b		4.04	4.21	0.3
Carotenoids		1.06	1.04	0.5
Chlorophyll a/b		2.9	2.5	<0.0001
Starch		0.47	0.49	0.2
Soluble carbohydrates		10.9	9.9	0.04
TNC		11.4	10.4	0.04
Water content		58.6	56.9	0.0003
Chlorophyll a		2-year-old	2.51	2.46
Chlorophyll b	0.82		0.91	0.03
Chlorophyll a+b	3.36		3.37	0.9
Carotenoids	0.84		0.85	0.4
Chlorophyll a/b	3.1		2.8	0.007
Starch	0.46		0.58	0.2
Soluble carbohydrates	10.9		8.8	<0.0001
TNC	11.4		9.41	0.0002
Water content	60.6		59.4	0.04
Woody twigs	1-year-old		56.4	58.3
	2-year-old	58.5	58.3	0.7
Buds		45.9	46.0	1.0

sunny days. The effect of winter drought stress on trees was studied by comparing physiological parameters such as concentrations of soluble sugars, starch, total nonstructural carbohydrates (TNC) and chlorophyll in one and two-year-old foliage and water content in foliage, woody twigs and buds both before (23 December 1996) and during the winter drought episode (data averaged for the two sampling dates in 21 January and 17 February 1997; Table 2). We did not find significant differences among populations in the studied metabolites ( $p > 0.05$ , data not shown). Therefore, the presented data were analyzed for all studied populations combined.

The physiological drought also decreased the chlorophyll a/b ratio significantly by increasing  $\text{Chl}_b$  concentration by 10 and 14% in one- and two-year-old needles, respectively ( $p \leq 0.03$ , Table 2).  $\text{Chl}_b$ ,  $\text{Chl}_{a+b}$  and carotenoid concentrations remained unchanged during the winter drought ( $p \geq 0.3$ ). At the same time, the concentration of soluble carbohydrates declined by ca. 20% in one-year-old and by 10% in two-year-old foliage ( $p \leq 0.04$ ). Water content declined from 60.6 to 59.4% in one-year-old and

from 58.6 to 56.9% in two-year-old needles ( $p \leq 0.04$ , Table 2). Only small and marginally significant declines in water content of woody twigs and no changes in bud water content were detected.

The meteorological conditions preceding premature needle shedding were unfavourable for plant growth. The mean monthly air temperature in June reached  $20.4^\circ\text{C}$ ,  $2.9^\circ\text{C}$  higher than the previous record for the region set in 1964 [28]. The maximum daily temperature reached  $35^\circ\text{C}$  on June 30, 1997 (Fig. 2). On the same day, a severe storm dropped 54 mm of precipitation, equal to the average monthly total for June. Shortly after the storm, air temperature dropped by  $17.5^\circ\text{C}$ . A few days after that episode a dramatic decline in needle water content occurred, followed by needle yellowing and shedding.

On average, trees lost  $\approx 20\%$  of their foliage, with a local population from Poland exhibiting the highest needle loss (28%). The lowest needle loss was observed in the population from France (13%). However, differences among populations in shedding intensity were only marginally significant ( $p = 0.1$ , Table 3). Statistically signifi-

Table 3. Pathological symptoms (percentage of needle surface affected) in one- and two-year-old needles of Scots pine populations at the SP-IUFRO-82 experiment during premature needle shedding. n=100 shoots per population.

Population	Discolorations (%)		Necroses (%)		Insect wounds (%)		Needle shedding (%)
	2-yr-old	1-yr-old	2-yr-old	1-yr-old	2-yr-old	1-yr-old	2-yr-old
3	62.0	11.3	1.3	0.8	17.5	2.0	23.1
4	63.8	30.0	2.8	2.5	3.0	24.3	17.5
7	7.5	10.0	1.5	2.5	13.8	26.3	28.1
12	39.5	4.0	0.8	0.5	7.8	10.0	22.4
14	76.5	0.0	3.0	0.0	1.0	11.3	13.1
15	91.3	17.5	1.5	4.8	0.0	29.0	19.7
Average	56.8	12.1	1.8	1.9	7.2	17.2	20.3
P>F	0.01	0.3	0.6	0.4	0.4	0.1	0.1

cant differences among populations were noted in discoloration of 2-year-old foliage, with the highest values noted for the population from Sweden (91%) and lowest for the population from Poland (7.5%, Table 3). On average, more than 56% of two-year old needles showed discolorations and only 12% of one-year-old needles (Table 3). An opposite pattern was found in the incidence of insect damage. More than 17% of the two-year-old and only 7% of the one-year needles showed symptoms of insect damage ( $p = 0.02$ , Table 4).

Observations of needle litter indicated the presence of the following taxa of fungi: *Lophodermium pinastri*, *Cyclaneusma* sp., *Phoma* sp. and *Sclerophoma pythiophila*. On some needles, egg deposits of *Nuculaspis abietis*, *Brachonyx pineti* and *Cinara pinea* were found. However, none of these species of fungi or insects were responsible for premature needle shedding. Foliage litter analyses (performed twice, in 1997 and 1998) also excluded the needle cast fungi (*Lophodermium seditiosum*) as a potential factor contributing to the observed phenomenon.

## Discussion

Comparison of effects of winter drought made on all six Scots pine provenances showed marginally significant

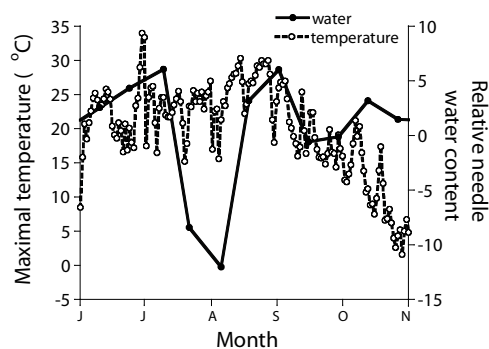


Fig. 2. Seasonal pattern of maximum air temperature and relative needle water content expressed as deviation (in percent) from the long-term trend.

differences ( $p = 0.1$ , Table 3). This result corresponds with the absence of significant differences among populations in physiological response to winter drought and likely indicates only weak genetic control over premature needle-fall and winter drought injury among European Scots pine populations.

Larger population differences were noted only in the discoloration of older needles. The comparatively low incidence of needle discoloration in the local Polish population suggests that it is better adapted to the environmental conditions that induced such changes. However, in cases of other needle symptoms, no advantage of the local population or clear geographical pattern among populations was found (Table 3). Our observations did not support prior findings of a lower susceptibility of northern populations to the combination of biotic and abiotic factors [29].

A higher frequency of insect damage to younger needles (Table 4) is most likely related to their higher contents of water and nitrogen and lower content of phenolics, usually linked to higher foliage attractiveness for herbivores [30-34].

Although the needle cast fungus (*Lophodermium seditiosum*) is usually observed on second-year needles on young trees and is associated with needle abscission and the death of the young plants [35, 36], our observations did not support the notion that the needle cast fungi were responsible for needle shedding. That fungus was previously noted at this plantation [24]; however, repeated surveys failed to find it in 1997 and 1998. Also, none of the pathogenic fungi or insect herbivory recorded on Scots pine needles in the studied site were associated with premature needle shedding. However, their presence may indicate the weakening of the trees after the relatively harsh winter and late winter physiological drought.

Our data indicated that the needle shedding may be related to several incidents of severe weather conditions. The winter of 1996-97 was harsh, with soil temperatures falling below  $-10^{\circ}\text{C}$  (Fig. 1). In controlled environments such temperatures affect white root tips [37]. We found that the damage to the root system and/or needle shedding significantly lowered carbohydrate reserves in fine roots [38]. Low winter temperatures and physiological stress may have exacerbated water deficit in early summer. In

Table 4. Results of a two-way analysis of variance test of the effects of Scots pine population and needle age (one- or two-year-old needle cohorts were analyzed) on foliage discolorations, necroses and insect damage symptoms at the SP-IUFRO-82 experiment during premature needle shedding in 1997.

Symptom	Source of variation	DF	SS	F	P>F
Discolorations	Population (P)	5	11138.7	4.3	0.004
	Needle cohort (NC)	1	23896.7	46.0	<0.0001
	P*NC	5	8648.2	3.3	0.01
Necroses	Population (P)	5	36.7	0.9	0.5
	Needle cohort (NC)	1	0.02	0.002	1.0
	P*NC	5	41.9	1.0	0.4
Insect damage	Population (P)	5	976.9	1.0	0.4
	Needle cohort (NC)	1	1190.0	6.0	0.02
	P*NC	5	2408.4	2.4	0.05

addition, our study provided evidence that winter physiological drought of 39 consecutive days with positive air and negative soil temperatures severely affected foliage water relations (Table 2). During such weather conditions, water lost through cuticular and stomatal transpiration cannot be resupplied from frozen roots [39, 15]. These conditions can induce browning of pine needles and are considered a key factor in the location of the alpine tree line [40].

Winter desiccation can also disrupt water transport in stems leading to foliage drying [41]. During incidents of winter physiological drought, xylem water can thaw; however, its volume can be insufficient to sustain CO<sub>2</sub> assimilation and transpiration [42]. Often when drought-induced water deficits develop in a branch, the stems tend to cavitate, inducing air embolisms and subsequent leaf drop [16, 43]. A significant reduction in needle water content during late-winter physiological drought (Table 2) is often observed in northern conditions. For example, Havas and Hyvärinen [44] showed a ca. 15% decline in needle water content in Scots pine grown in Northern Finland at 65°N.

An additional unfavourable factor that may have contributed to the January/February 1997 physiological drought was the bright sunshine and its duration (>105 hours). Hadley and Amundson [6] have shown that winter needle mortality in *Picea rubens* was increased by exposure to direct solar radiation as a result of photo-oxidative injury, accelerated winter desiccation, and reduced cold tolerance due to heating of sun-exposed needles. The significant decrease in the chlorophyll a/b ratio and increased concentration of chlorophyll b (Table 2) may indicate a compensatory response to photooxidation. During the 39 days of physiological drought, no snow or rime deposition that may have mitigated injury by reducing the frequency or intensity of needle temperature fluctuations was observed [13].

Despite the negative effect of the long-lasting winter drought on Scots pine foliage, we did not observed any

shoot or massive foliage decline. This observation supports prior reports of a relatively low susceptibility of Scots pine to winter drought, high solar radiation and/or combinations of those factors [9].

The physiological drought in January-February 1997 induced large-scale damage to foliage and roots of many trees in western and northwestern Poland [14]. However, it is not clear whether premature needle fall observed in July/August 1997, more than four months after winter physiological drought is directly linked to that phenomenon. Nonetheless, it is possible that, weakened by winter drought, the trees became more vulnerable to summer drought and a heat wave with a maximum daily temperature of 35°C (Fig. 2) that preceded needle fall. It is also known that the antioxidant system of pine needles is temperature-dependent and that needle temperatures exceeding 25°C may result in impairment of antioxidant metabolism [45].

Shedding of expendable organs is a common strategy adopted by plants to survive during droughts [17, 46]. Our observed needle shedding did not induce tree mortality or shoot dieback. Similar premature needle shedding after unusually hot and dry summers were observed in Scots pine plantations in central Russia [18]. The significantly lower starch reserves accumulated in fine roots following the partial defoliation observed at this site [38] may reflect a shortage of carbohydrates on a whole-tree basis. A decrease in starch concentration in roots following defoliation was also shown in deciduous and coniferous trees [47-49]. It is possible that lower autumn carbohydrate reserves in roots may have an adverse effect on root growth in the following spring, when the carbon demand by the growing shoots prevents its allocation to roots. A lower root carbohydrate concentration also has a dampening effect on soil microbial activity [50].

In summary, the results of our study indicate that prolonged winter physiological drought significantly weakened Scots pine trees by reducing foliar water content and

nonstructural carbohydrate reserves in foliage (Table 2) and roots [38]. Late winter stress in combination with high summer temperatures lead to premature needle shedding and crown reduction. Differences among Scots pine populations in needle shedding were marginally significant, indicating only a weak genetic control over stress-induced needle loss.

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