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H-L. Pasonen · M. Käpylä · P. Pulkkinen

Effects of temperature and pollination site on pollen performance in *Betula pendula* Roth – evidence for genotype-environment interactions

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Abstract We studied whether the differences between genetically different pollen donors (*Betula pendula* Roth clones) with respect to pollen-tube growth rate were consistent under different thermal conditions during pollen germination in vivo and in vitro. We conducted a single-donor hand-pollination experiment with same pollen donors and recipients in a plastic house seed orchard and at an outdoor clone collection. The prevailing daily mean temperature during pollen germination was 13°C higher in the plastic house than outdoors. The pollen-tube growth rate of each pollen donor was additionally determined in vitro on agar medium at five temperatures (10°, 15°, 22°, 30° and 35°C). A significant interaction between paternal clone and pollination site as well as between paternal clone and temperature was found, which provides evidence for genotype-environment interactions. Genotype-environment interactions can have evolutionary significance in maintaining the variation in pollen-tube growth rates. At seed orchards, genotype-environment interactions can cause deviations from the expected genetic composition of the seed crop depending on the prevailing environmental conditions during pollen-tube growth.

Key words Pollen-tube growth rate · Pollen competition · Temperature · Seed orchard · *Betula pendula* Roth (silver birch)

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H-L. Pasonen (✉) · M. Käpylä
University of Jyväskylä,
Department of Biological and Environmental Science,
P.O.Box 35, FIN-40351 Jyväskylä, Finland
e-mail: hapasone@dodo.jyu.fi
Fax: +358-14-2602321

P. Pulkkinen
The Finnish Forest Research Institute
Haapastensyrjă Breeding Centre,
Karkkilantie 247, FIN-12600, Läyliäinen, Finland

Introduction

According to the pollen competition hypothesis, pollen grains have to compete for access to the ovules when there are more pollen grains on the stigmas than there are ovules in the ovary. Under conditions of pollen competition only the fastest growing pollen grains are assumed to achieve fertilization (Mulcahy 1979). In addition to the genotypes of the pollen donors and recipients, pollen competitive ability is affected by several external factors (Stephenson et al. 1992). The competitive ability of pollen donors has been an object of numerous studies (e.g. Marshall and Ellstrand 1986; Johnston 1993; Björkman et al. 1995; Snow and Spira 1996; Hormaza and Herrero 1998), but the consistency of the competitive status under different environmental conditions has received less attention.

Changes in pollen competitive ability caused by environmental or weather conditions might have unexpected consequences at seed orchards that have usually been founded to improve the seed production of commercially important tree species. Seed orchards of coniferous tree species have been founded at sites of favourable conditions for seed production, quite often far away from the original growing places of the seed orchard trees. In open-pollinated seed orchards, each seed orchard genotype is expected to be approximately equally represented in a seed crop, i.e. it is assumed that male fertilities are equal and pairwise mating probabilities are identical (Schoen and Stewart 1987). Seed orchards of *Betula pendula* have been established in plastic houses to isolate them from outside pollen sources and to increase seed production. Similarly, fertilizations are assumed to be random, and the reproductive outcome of each clone is expected to be fairly equal.

In previous studies we have obtained evidence for significant differences between seed orchard clones with respect to pollen-tube growth rates and for the fact that the pollen-tube growth rate can be a determinative factor controlling the paternity of the seeds (Pasonen et al. 1999). If pollen donors with the fastest growing pollen tubes sire most of the seeds and environmental conditions significantly affect pollen-tube growth rate, the reproductive outcome of seed orchard clones is not equal, and it can vary depending on the prevailing environmental conditions during pollen-tube growth. In the present study we examined whether the competitive ability (pollen-tube growth rate) of six pollen donors varied depending on the pollination environment and prevailing temperature during pollen-tube growth in *Betula pendula* Roth. We discuss the possible effects of genotype-environment interactions on pollen competition and on the functioning of plastic house seed orchards.

Materials and methods

Species and study site

Betula pendula Roth is an anemophilous, self-incompatible and monoecious tree ranging throughout most of Europe from Norway to Sicilia (Tutin et al. 1964). Its male and female flowers occur in separate inflorescences. There are 200–300 male flowers in each catkin (Dahl and Fredrikson 1996) and, on average, 600 female flowers (personal observation) in each pistillate inflorescence. One female flower consists of a single two-locular ovary with two linear, dry stigmas and two ovules of which only one develops into a mature seed (Sulkinoja and Valanne 1980; Dahl and Fredrikson 1996).

The study was carried out in a plastic house seed orchard at Haapastensyrjä Breeding Centre in Läyliäinen (lat. 60° N, long. 24° E) and at an outdoor clone collection in Kangasniemi (lat. 61° N, long. 26° E), southern Finland. The clones were originally selected for the seed orchards on the basis of superior vegetative growth. Those used in the present study originated from rather a small area, between latitudes 60°30′ N and 62°30′ N.

Hand-pollinations

Four maternal and six paternal clones were selected from a group of *Betula pendula* seed orchard clones for a single-donor, hand-pollination experiment. The same clones were used in a plastic house seed orchard and at an outdoor clone collection. Prior to the onset of flowering, branches with at least three female inflorescences were isolated from each maternal plant by bagging with paper bags to prevent uncontrolled pollinations. Once receptive, maternal plants were hand-pollinated with pollen from the six paternal clones. Frozen pollen was used because male and female flowering occurs almost synchronously in *Betula pendula*, and there would have been no time to both collect fresh pollen and test the germination percentages of the pollen before the hand-pollinations. The germination percentages of frozen pollen from several donors were determined in vitro on agar medium (1% agar, 0.01% boric acid, 0.5 *M* sucrose) (Käpylä 1991). Six paternal clones with germination percentages over 50% and as similar as possible were chosen for the experiment. All the hand-pollinations (4×6 crosses in both places) were carried out in the plastic house seed orchard on 5 May, 1997, and at the outdoor clone collection on 19 May, 1997. The temperature during pollen-tube growth varied substantially between these two sites. In the plastic house, the maximum daily temperature was 28°C, the minimum temperature 11°C and the daily mean temperature 17°C; at the clone collection the temperatures were 8°C, 2°C and 4°C, respectively. The volume of pollen used in the hand-pollinations was kept as equal as possible in each cross, because it is known that the density of pollen grains on the stigma may influence pollen-tube growth rate (e.g. Cruzan 1990; Holm 1994).

Pollen-tube growth-rate measurements

Three female inflorescences were analysed and, on average, 65 randomly chosen pollen tubes (from several flowers) per maternal/paternal combination were measured by UV fluorescence microscopy. For details, see Pasonen et al. (1999). Pollen from the same donors that were used in the hand-pollination experiment (the same pollen samples) was germinated on agar medium (Käpylä 1991) at five different temperatures (10°C, 15°C, 22°C=RT, 30°C, 35°C) for 12 h to study the effect of temperature on pollen-tube growth rate. On average, 70 randomly chosen pollen tubes per sample were measured.

Data analysis

A mixed-effects ANOVA was performed to test the effect of the maternal and paternal parent (random effects) and pollination site (fixed effect) on pollen-tube growth rate. In addition, a *t*-test was performed separately for each paternal clone to test the differences in pollen-tube lengths between two pollination sites on two maternal plants. Pollen-tube lengths were measured only on two maternal plants because the other two recipients had suffered from frost during the night prior to the hand-pollinations (frost damage could only be observed when the female inflorescences were analysed under a microscope). The pollen-tube lengths were square root-transformed to normalize the data. To study whether the ranking orders of the pollen donors changed between the pollination sites, we calculated a Spearman rank correlation coefficient (r) separately for two maternal plants. A random-effects ANOVA was performed to test the effect of pollen-donor genotype and temperature on pollen-tube length in vitro. In addition, one-way ANOVA was performed to test whether the differences between the pollen donors with respect to pollen-tube growth rate were significant at different temperatures. To test whether the rankings of the pollen donors changed across four different germination temperatures in vitro, we calculated a concordance coefficient (*W*) (Sokal and Rohlf 1981, p 609).

Results

Mixed-effects ANOVA revealed significant interactions between maternal and paternal clones and the pollination site (F=3.43, P<0.01) (Table 1). Due to these interactions, maternal and paternal effects on pollen-tube growth rate were tested separately in both places (Table 2a, b), and the differences in pollen-tube growth rates between pollination sites were tested separately for each pollen donor on two maternal plants (Table 3). Significant interaction between maternal and paternal parents with respect to pollen-tube growth rate was detected at

Table 1 Mixed-effects ANOVA of the effects of maternal and paternal clones and pollination site (plastic house vs. outdoors) on pollen-tube length in *Betula pendula* Roth

Source of variation	*df*	MS	*F*	*P*
Maternal clone	1	1.62	123.44	<0.001
Paternal clone	5	0.56	42.67	<0.001
Pollination site	1	0.92	20.35	<0.01
Interactions				
Mother×father	5	0.05	4.10	<0.01
Mother×site	1	0.21	16.11	<0.001
Father×site	5	0.24	18.20	<0.001
Mother×father×site	5	0.05	3.43	<0.01
Error	1532	0.01		

Table 2 Random-effects ANOVA of maternal and paternal effects on pollen-tube length

a) At the outdoor clone collection:

Source of variation	*df*	MS	*F*	*P*
Mother	1	0.26	3.34	0.13
Father	5	0.20	2.41	0.18
Mother x father	5	0.08	4.74	<0.001
Error	610	0.02		

b) In the plastic house:

Source of variation	*df*	MS	*F*	*P*
Mother	1	2.05	329.91	<0.001
Father	5	0.66	107.41	<0.001
Mother×father	5	0.01	0.60	0.701
Error	922	0.01		

the outdoor clone collection ($F=4.74$, $P<0.001$) (Table 2a) but not in the plastic house ($F=0.60$, $P>0.05$) (Table 2b). Pollen-tube growth rate of four out of six pollen donors on the first maternal plant (clone V 5788) and three out of six pollen donors on the other maternal plant (clone V 5718) differed significantly depending on the pollination site (Table 3). A Spearman rank correlation coefficient (r) between two pollination sites on the maternal clone V 5788 was 0.60 ($P>0.05$) and on the maternal clone V 5817, –0.06 ($P>0.05$), indicating that there was no significant correlation in the rankings of the pollen donors between two pollination sites.

After 12 h of germination, the optimum germination temperature in vitro appeared to be 30°C for most of the clones (Fig. 1). Pollen from none of the pollen donors germinated at 10°C. The random-effects ANOVA revealed a significant interaction between pollen-donor genotype and temperature ($F=29.85$, $P<0.001$) (Table 4). There were also significant differences between the pol-

Table 3 The average lengths of the pollen tubes of six pollen donors on two maternal plants in the plastic house and outdoors (number of pollen tubes measured), and Student's t-test

Maternal plant	Pollen donor	Pollen-tube length (mm)		*T*	*P*
		Plastic house	Outdoors		
V 5788	E 9427	0.35 (87)	0.21 (65)	–6.66	<0.001
	E 9563	0.29 (79)	0.22 (67)	–3.89	<0.001
	E 1970	0.40 (86)	0.29 (64)	–5.06	<0.001
	V 5035	0.20 (76)	0.17 (74)	–1.96	0.053
	V 5232	0.25 (69)	0.22 (65)	–1.48	0.142
	V 5794	0.26 (82)	0.20 (60)	–2.97	<0.01
V 5817	E 9427	0.25 (99)	0.16 (55)	–7.09	<0.001
	E 9563	0.19 (88)	0.19 (16)	–0.25	0.802
	E 1970	0.27 (38)	0.16 (38)	–5.03	<0.001
	V 5035	0.12 (87)	0.12 (58)	–0.88	0.383
	V 5232	0.15 (71)	0.25 (34)	6.60	<0.001
	V 5794	0.18 (72)	0.16 (26)	–0.86	0.395

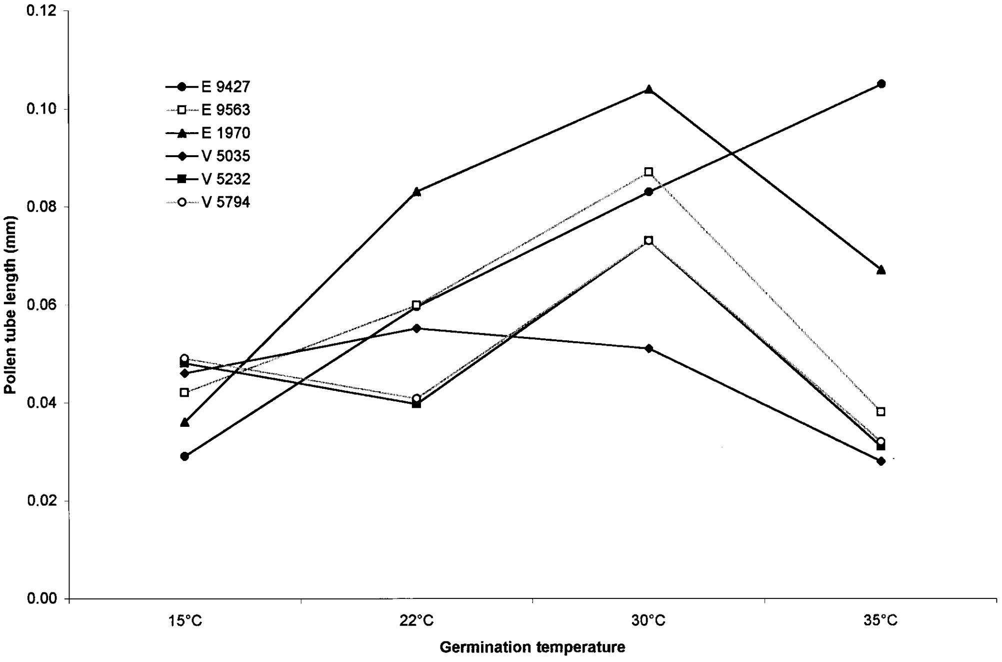

Figure 1 Average lengths of the pollen tubes of six pollen donors after 12 h of germination at four different temperatures in vitro

Table 4 Random-effects ANOVA of the effects of pollen donor and temperature on pollen-tube length

Source of variation	*df*	MS	*F*	*P*
Pollen donor	5	4.85	5.11	0.012
Temperature	3	1.14	1.20	0.354
Temperature ×donor	15	1.01	29.85	<0.001
Error	1217	0.03		

Table 5 One-way ANOVA of the effect of pollen-donor genotype on pollen-tube length at four different temperatures in vitro

Temperature (°C)	*df*	MS	*F*	*P*
15	5	0.40	13.05	<0.001
22 (RT)	5	0.55	16.15	<0.001
30	5	0.61	16.33	<0.001
35	5	2.77	85.19	<0.001

len donors with respect to pollen-tube length at four different temperatures (Table 5). The concordance coefficient (*W*) for the six pollen donors at four temperatures was 0.27 ($P > 0.05$), indicating that there was no significant concordance in the rankings of the pollen donors across different germination temperatures.

Discussion

Among deciduous tree species, pollen germination in different temperature regimes has been studied in several commercially important species including almond (Weinbaum et al. 1984), pear (e.g. Mellenthin et al. 1972; Vasilakakis and Porlingis 1985), walnut (Luza et al. 1987) and pistachio (Polito et al. 1988). Temperature ranges and optima for pollen germination are known to vary among species (Luza et al. 1987; McKee and Richards 1998), but they can also vary among clones (Luza et al. 1987) and morphs (McKee and Richards 1998) within the same species. Although gametophytic selection in general has been the subject of many studies by evolutionary biologists (e.g. Stephenson and Bertin 1983; Mulcahy and Mulcahy 1987; Snow 1994; Stanton 1994; Willson 1994) and plant breeders (see Hormaza and Herrero 1992, 1994, 1996), differential temperature preferences among individuals within a same population with respect to pollen-tube growth rate have not explicitly attracted much attention. Many external factors are known to affect pollen-tube growth rate (see e.g. Stephenson et al. 1992). However, it is the order of the pollen donors and the consistency (or inconsistency) of the competitive status of the donors under different external conditions that matters (Charlesworth et al. 1987).

In the present study, significant pollen donor-environment interaction was detected, and the ranking orders of the pollen donors changed depending on the pollination environment and prevailing temperature during pollen germination, indicating that different donors can be selected under different conditions during pollen-tube growth. Given that pollen-tube growth rate can be a determinative factor controlling the paternity of the seeds (Pasonen et al. 1999), donors that sire most of the seeds might differ depending on whether they are in a plastic house or outdoors, and the genetic composition of seeds from a plastic house might differ from that of seeds from outdoors. Furthermore, the reproductive outcome of seed orchard clones can vary from year to year depending on the weather conditions during pollination and pollen-tube growth.

Many studies document a decreased frost hardiness of coniferous seedlings originating from seed orchard seeds when the seedlings are transferred to harsher growing places (e.g. Johnsen et al. 1995). These kind of influences of weather conditions during sexual reproduction on the adaptive properties of the seedlings are called after-effects (see e.g. Johnsen et al. 1995, 1996). The after-effects have only been documented among coniferous tree species but observations on decreased frost hardiness among *B. pendula* seedlings originating from seed orchard seeds has been made. Johnsen et al. (1996) suggested that some reproductive stages during female flowering may be sensitive to conditions of the female flowering environment, leading to a decreased frost hardiness of the progeny. It is also possible that the effects of greenhouse conditions on gametophytic selection can partly explain the after-effects. Although the potential effect of gametophytic selection on after-effects in *Picea abies* is rather limited due to the low number of pollen grains in each pollen chamber (Johnsen et al. 1996), the situation in *Betula pendula* is quite different. In *Betula pendula*, the number of pollen grains on the stigmas usually exceeds the number of ovules in the ovary during mass pollination in a plastic house (personal observation), and pollen competition is thus likely to occur. If pollen donors that outcompete the other donors in warm greenhouse conditions sire offspring with a decreased frost hardiness, there should be a negative correlation between pollen-tube growth rate and frost hardiness among seedlings originating from seeds produced in plastic houses. Further studies are needed to gain some insight into relationships between pollen-tube growth rate and frost hardiness of the progeny.

Intense pollen competition and gametophytic selection should, in theory, lead to a decrease in the genetic variation in pollen-tube growth rate (e.g. Walsh and Charlesworth 1992). Many studies have reported a substantial variation in pollen tube growth rates within a population (Sari-Gorla et al. 1975; Mulcahy 1979; Ottaviano et al. 1980; Cruzan 1990; Snow and Spira 1991; Björkman et al. 1995) which has led to speculations over the mechanism that might retain the variability in pollen performance. However, the question on the degree of genetic versus phenotypic variation in pollen-tube growth rates has remained largely unanswered in many studies (Havens 1994). In some species the microenvironment in which a plant grows may be more important in determining its mating success than its genotype (e.g. Havens 1994). However, it is usually assumed that at least part of the observed variation in pollen-tube growth rate is genetic, and one possibility to maintain this variability is genotype-environment interactions (Gillespie and Turelli 1989; Delph et al. 1997). Alleles which are favoured by selection in one environment can be at a disadvantage in another environment (see Mulcahy et al. 1996). Although we do not know as yet just how much of the variation in pollen-tube growth rate in *Betula pendula* is actually genetic, the results of the present study provide evidence for genotype-environment interactions in pollen-tube growth rate. In addition to have evolutionary significance in maintaining the variation in pollen-tube growth rate, genotype-environment interactions can cause unexpected deviations from the expected genetic composition of the seed crop at seed orchards depending on the prevailing environmental conditions during pollen-tube growth.

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