Pollen germination and pollen tube growth in *Fraxinus pennsylvanica*

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Abstract: With regard to adaptation of green ash (*Fraxinus pennsylvanica* MARSHALL) to ecological conditions in Croatia, pollen germination and pollen tube length after 2, 4 and 6 hours were examined *in vitro* at 10, 15, 20 and 25 °C during two years 2001 and 2002. Narrow leaved ash (*F. angustifolia* VAHL) pollen served as a control in 2002. The year, time and temperature, and the interaction between time and temperature were significant for both germination percentage and pollen tube length. Interactions year × temperature and year × time were significant for pollen tube length only. The highest germination percentage (17.86% in 2001 and 19.40% in 2002) of green ash pollen was at 15 °C after 6 hours. The pollen tube length was greatest at 20 °C (393.46 μm) in 2001 and 25 °C (899.50 μm) in 2002 after 6 hours. Narrow leaved ash pollen had the highest germination percentage (19.22%) at 20 °C after 6 hours and was significantly reduced at 25 °C. The pollen tube length was greatest at 25 °C (518.90 μm) after 6 hours. It can be concluded that green ash pollen has satisfactory germination in ecological conditions in Croatia and that the optimum temperature for pollen germination is higher than 20 °C.

Key words: green ash pollen, exotic species, ecology, pollen germination, pollen tube growth

Introduction

Green ash (*Fraxinus pennsylvanica* MARSHALL) is naturally spread in North America from Nova Scotia in the northeast to Northern Florida and central Texas in the south. It grows at altitudes of 0 to 900 m, in moderate warm to moderate cold and humid to semiarid climates. Average rainfalls are 300–1500 mm (250–900 mm during vegetation period), average snow height is up to 250 cm, and there are 120–180 frost-free days (MILLER, 1955; GRIMM, 1962; CLAUSEN, 1979; HARLOW et al., 1996; DIRR, 1998).

Green ash has been present in Croatia for more than 130 years. One herbarium sample with twigs and fruits collected in Lipik (Croatia) in 1877, is in the herbarium of Faculty of Sciences, University of Zagreb (Croatia). ETTINGER (1889, 1892) mentioned this species in the Maksimir public garden, and in the list of plants in Botanical Garden of the Royal University of Franz Josef I in Zagreb. Green ash and white ash (*F. americana* L.) were used for afforestation of the lowlands on wet sites where the native narrow leaved ash (*F. angustifolia* VAHL) could not survive. Distribution of green ash in part of lowland forests in Croatia was described by KREMER (2001). The green ash crops regularly in the Botanical Garden of the Faculty of Sciences in Zagreb (JURKOVIĆ, 1988). At present, the green ash plays an important role in the preparation of habitat for the arrival and survival of native narrow-leaved ash, though its economic function is not substantial (KREMER, 2001).

Green ash has been present in the lowland forests in Croatia for a long time. A question of a possible hybridization with the native narrow leaved ash still persists. The possibility of interspecific hybridization in the genus *Fraxinus* L. is unclear (SANTAMOUR, 1981).

As a first step for obtaining hybrids and for the estimation of green ash adaptation to the ecological conditions in Croatia, pollen germination and pollen tube elongation were tested in different time and temperature ranges. It was shown that green ash pollen has satisfactory germination at temperatures prevailing during flowering in the ecological conditions of Croatia.

Material and methods

Pollen was collected when the majority of male flowers had dehisced anthers from 10 randomly selected trees of green ash in 2001 and 2002 in Zagreb (Croatia). The pollen of 10 trees was mixed to obtain random samples. As control, the pollen of 10 narrow leaved ash trees was collected in 2002 in Zagreb.

According to Koppen's classification the climate of Zagreb belongs to the climate type Cfwbx. This is a moderately warm rainy climate without a dry period and with precipitation uniformly distributed throughout the year. The mean temperature of the coldest month (January) does not drop below −0.4 °C, while the mean temperature of the warmest month (July) usually does not exceed 21.4 °C. Annual precipitation amounts to 900 mm (up to 489 mm in the vegetation period), while the annual relative air humidity ranges between 77 and 92% (SELETKOVIĆ, 1996).

Pollen germination was performed in Petri dishes in a medium of 1% agar-agar and 10% sucrose. According to NIKOLAEVA (1962, cited by MIKSCHE, 1976) the pollen of common ash (*F. excelsior* L.) and black ash (*F. ornus* L.) had the best germination on a medium with 15% and 5% sucrose, respectively. So we assumed that green ash pollen might have good germination at sucrose concentrations between these values. Pollen was added to the Petri dishes with the sucrose-agar medium and germination was performed at 10, 15, 20 and 25 °C for 2, 4 and 6 hours. For each temperature × time combination we used three replicates. After achieving the appropriate germination time, further germination was halted with formaline. The germination percentage and pollen tube length were examined with the microscope Carl Zeiss GmbH., Germany at 50× magnification using a measuring scale. Germination was examined in 10 randomly selected places in each Petri dish. Also, twenty randomly selected pollen tubes were measured in each Petri dish. Statistical analysis was performed using SAS Software (SAS Institute, Cary, NC, USA) and the GLM Procedure, and Duncan multiple range test at the $p = 0.05$ level. Before analysis, data were transformed using logarithmic transformation.

Plant growth and development is proportional to biological time, or thermal time, which can be defined as the integral of the product of time and temperature above a certain threshold. This constitutes the concept of units of growing degree days (GDD), calculated as the sum of differences between the temperature of a certain time period and a threshold temperature (base temperature) for each period after a given starting date. The concept is simple and accurate in predicting phenological stages and has been used to forecast the main stages of plant development (OLIVEIRA, 1998), particularly for predicting vegetable maturity in the processing industry, for predicting the completion of rest, predicting bloom, and for predicting harvest dates for tree fruits (RICHARDSON et al., 1975; EISENSMITH et al., 1980). For this reason, GDD were used to explain the effect of year on dehiscence of anthers and pollen germination. They were calculated for the period from 1 February (of the current year) to the phenophase of beginning, culmination and end of flowering. The GDD were obtained using standard method: GDD = $\{(t_{max} + t_{min})/2\}$ – base temperature (PERRY et al., 1986). The temperature of 4 °C was used as the base temperature.

Results

Year, temperature, time and the interaction between temperature and time had a significant effect on both pollen germination and pollen tube growth in green ash. However, interactions between year and temperature, year and time only had a significant effect on pollen tube length. The interaction among year, temperature and time had no significant effect (Tab. 1). The effect of year can be explained by the difference in air temperature for the period before flowering in 2001 and 2002 (Fig. 1, Tab. 2).

Time, temperature, and their interaction had a significant effect on pollen germination and pollen tube length of green ash in 2001. In 2002, temperature and time interaction had no significant effect on pollen tube

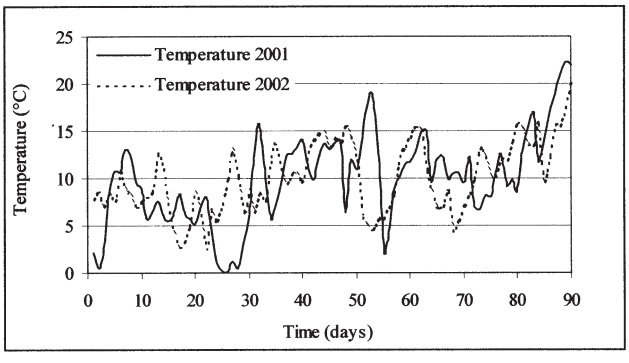

Fig. 1 Mean air temperatures from February 1 to May 1, (period prior to and during the flowering of *F. pennsylvanica*) in 2001 and 2002 in Zagreb, Croatia.

Table 1. Effect of year, temperature and time on pollen germination of *F. pennsylvanica*.

Source of variability	Germination	Pollen tube length
	P	
Year	0.0001	0.0001
Temperature	0.0001	0.0001
Time	0.0001	0.0001
Temperature × Time	0.0001	0.0051
Year × Temperature	0.0810	0.0002
Year × Time	0.1077	0.0003
Year × Temperature × Time	0.5240	0.1256

Table 2. Phenological data for flowering of *F. pennsylvanica* in Zagreb in 2001 and 2002.

Phenophase of flowering	Date	Days from 1st February	GDD
	2001		
Beginning	12. 4.	71	369
Culmination	24. 4.	82	444
End	1. 5.	89	530
	2002		
Beginning	18. 3.	46	229
Culmination	8. 4.	67	360
End	15. 4.	74	393

Note: GDD = growing degree days.

Table 3. Effect of time and temperature on pollen germination of *F. pennsylvanica* in 2001 and 2002 and *F. angustifolia* in 2002.

Source of variability	Germination	Pollen tube length
	P	
	F. pennsylvanica	
	2001	
Temperature	0.0001	0.0001
Time	0.0001	0.0001
Temperature × Time	0.0001	0.0008
	2002	
Temperature	0.0001	0.0001
Time	0.0001	0.0001
Temperature × Time	0.0001	0.712
	F. angustifolia	
	2002	
Temperature	0.0001	0.0001
Time	0.0001	0.0001
Temperature × Time	0.0001	0.0003

Table 4. Pollen germination and pollen tube length in *F. pennsylvanica* after 2, 4 and 6 hours at 10, 15, 20 and 25 ℃ in 2001 and 2002.

Temperature (℃)	Time of germination (h)		
	2	4	6
	Pollen germination (%)		
	2001		
10	0.83Ba	1.41Ca	1.66Ba
15	0.73Bc	10.48Ab	17.86Aa
20	7.16Aa	11.59Aa	13.03Aa
25	5.72Ab	6.39Bb	12.36Aa
	2002		
10	1.33Bb	2.21Ca	2.60Ca
15	1.73Bb	17.38Aa	19.40Aa
20	8.35Aa	10.59Ba	13.31ABa
25	12.87Aa	10.29Ba	11.52Ba
	Pollen tube length (μm)		
	2001		
10	10.97Db	26.68Da	30.12Ca
15	23.58Cb	64.30Cb	145.17Ba
20	40.84Bc	156.57Bb	393.46Aa
25	87.30Ab	353.26Aa	355.75Aa
	2002		
10	14.16Dc	21.13Cb	52.16Da
15	67.62Cc	145.77Bb	305.35Ca
20	100.87Bc	164.54Bb	419.47Ba
25	269.37Ac	446.00Ab	899.50Aa

Note: values marked with the same letter are not significant at $p \leq 0.05$ according to Duncan's multiple range test; capital letter = difference in the column; small letter = difference in the row.

length (Tab. 3). Such results justify performing separate statistical analysis for time and temperature (Tabs 4, 5).

In green ash (Tab. 4) there was a trend of increasing both the germination percentage and pollen tube length with increasing temperature and time. The highest germination percentage was at 15 ℃ after 6 hours in both years (17.86% in 2001 and 19.40% in 2002) and there was no significant difference in comparison to 20 and 25 ℃ in 2001. In 2002 there was a significant difference between 15 and 25 ℃ (Tab. 4). At 10 ℃, the germination percentage was very low in both years.

Time and temperature affected pollen tube length to a greater extent than the germination percentage. The longest tubes (899.50 μm in 2002) in green ash were at 25 ℃ after 6 h. There was a significant difference with all other temperatures after 6 h, except in 2001 when the maximum (393.46 μm) was attained after 6h at 20 ℃, but with no difference at 25 ℃ (Tab. 4).

In narrow leaved ash, temperature, time, and their interaction had a significant effect on pollen germination and pollen tube length (Tab. 3). The highest germination percentage (19.22%) was at 20 ℃ after 6 hours and rapidly decreased at 25 ℃ (Tab. 5). The greatest pollen tube length (518.90 μm) was noted after 6 hours at 25 ℃ without a significant difference at 20 ℃.

Discussion

The optimal temperature for green ash pollen germination is between 20 and 25 ℃ (Tab. 4). Based on pollen tube lengths, the optimal temperature is closer to 25 ℃. As opposed to green ash, narrow leaved ash has an optimal temperature closer to 20 ℃ since its germination percentage was higher at this temperature. Pollen tube length was lower at 20 ℃, though the difference in comparison to 25 ℃ was not significant (Tab. 5). For specifying the optimal temperature of germination, the determination of the pollen tube growth rate could be useful. Measurements should be done on the same pollen grain, however such an approach is connected with technical difficulties and as such, there is no reference for the pollen tube growth rate. There are only a few reports on pollen germination and pollen tube length in the genus *Fraxinus*. After 8 h of incubation at 25 ℃ on the media with 5% sucrose, the average germination percentage of black ash pollen was 31% and pollen tube length was 817 μm (Paoletti, 1992). Ishida & Hiura (1998) achieved satisfactory pollen germination of *F. lanuginosa* Koidz. on a media of 5% and 10% sucrose. The mean pollen germination *in vitro* (after 24 hours at room temperature) for hermaphrodite trees was 0.6% on the media with 10% sucrose and 1.4% on the media with 5% sucrose. For male trees, the

Table 5. Pollen germination and pollen tube length in *F. angustifolia* after 2, 4 and 6 hours at 10, 15, 20 and 25 °C in 2002.

Temperature (°C)	Time of germination (h)		
	2	4	6
	Pollen germination (%)		
	2002		
10	0.65 Cb	2.93 Ba	4.70 Ba
15	12.33 Ab	14.86 Aa	14.86 Aa
20	8.85 Bb	12.65 Aab	19.22 Aa
25	4.93Ba	7.93 Aab	9.49 Bb
	Pollen tube length (μm)		
	2002		
10	17.31 Dc	61.66 Cb	138.38 Ba
15	66.45 Cb	189.75 Ba	207.21 Ba
20	131.67 Ba	228.49 Bb	467.32 Ac
25	196.11 Ac	349.94 Ab	518.90 Aa

Note: values marked with the same letter are not significant at $p \leq 0.05$ according to Duncan's multiple range test; big letter = difference in the column; small letter = difference in the row.

germination percentages were 1.7% and 4.8%, respectively.

Based on the literature data and the results of this study, it can be concluded that ash species have very diverse percentages of pollen germination.

Future research should be extended to other important flowering and fruit set characteristics (longevity of ovule, fruit set, etc.) which have been studied in great detail in fruit trees from the family Rosaceae, (Shuraki & Sedgley, 1994; Egea & Burgos, 1995, 2000; Cerović et al., 2000; Oukabli et al., 2000; Jonke et al., 2003). It is hard to make a comparison of these data with the results for the genus *Fraxinus* (Oleaceae) since these two families have completely different pollination strategies, pollen production and pollen loads to stigmas.

From the results of this study, it can be concluded that the introduced green ash produces pollen of a satisfactory germination for Croatian climate conditions. The optimal temperature for pollen germination is closer to 25 than to 20 °C. The small germination percentage at 10 °C indicates questionable success of hybridization at low temperatures. As such, hybridization should be conducted during the highest daily air temperature in the part of crown, which is exposed to direct sunlight.

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