Flowering Phenology of *Populus nigra* L., *P. nigra* cv. *italica* and *P.* x *canadensis* Moench. and the Potential for Natural Hybridisation in Belgium

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Abstract

The presence of hybrid and exotic poplar plantations is considered to pose a severe potential threat to the native populations of *Populus nigra* in Western Europe. To evaluate the potential risks associated with the use of exotic or hybrid poplar plantations, particularly plantations of *P.* x *canadensis* and *P. nigra* cv. *italica* (Lombardy poplar), in the surroundings of natural *P. nigra* stands, the flower biology of a collection of *P. nigra*, *P.* x *canadensis* and *P. nigra* cv. *italica*, was assessed. The results of this study showed that, in this stand and over the two years of observation, the flowering time did not constitute a barrier to hybridisation between *P. nigra* and *P.* x *canadensis*. In contrast, it appears that during both springs, *P. nigra* cv. *italica* could not hybridise with native female black poplars and female *P.* x *canadensis* trees in the stand, due to non-synchrony of flowering.

Key words: Populus, flowering period, flowering phenology, hybridisation.

Introduction

The European black poplar (*Populus nigra* L.) is considered as a keystone species of floodplain forests in Europe. The European black poplar is dioecious and a typical, wind-pollinated species. The flowers occur as unisexual, pendulous catkins that appear early in the spring before the foliage develops. Fruits of *Populus* consist of dehiscing capsules grouped in catkins. At maturity, the capsules open and release large quantities of seeds. Seeds are dispersed by wind and water. Today, there is a real interest in the restoration of riparian ecosystems. Existing black poplar stands may act as a source population for recolonisation of floodplains in restoration. Unfortunately, the European black poplar is one of the most endangered indigenous forest tree species in Belgium (VAN SLYCKEN, 1994). Besides the loss of its natural habitat, the presence of many introduced exotic poplar plantations is considered to pose a severe potential threat to the native populations of black poplar in Europe (e.g. CAGELLI and LEFÈVRE, 1995; ARENS *et al.,* 1998). There are fears that the introduced poplars may import foreign genes into the native species (i.e. introgression or introgressive hybridisation). However, low levels of introgression are observed in natural populations that were in the close neighbourhood of adult hybrid poplar plantations (e.g. HEINZE, 1997; BENETKA *et al.,* 1999; FOSSATI *et al.,* 2003). One possible explanation of the low levels of introgression observed in natural populations is non-synchrony of flowering time acting as a barrier for hybridisation. To evaluate the potential risks associated with the use of exotic or hybrid poplar plantations, particularly plantations of *P.* x *canadensis* and *P. nigra* cv. *italica* (Lombardy poplar), in the surroundings of natural Black poplar stands, the flower biology of a collection of *P. nigra*, *P.* x *canadensis* and *P. nigra* cv. *italica*, was assessed.

Material and Methods

The study site was an artificial mixed poplar stand located 50°40'30"N/ 4°00'00"E, 30 km Southwest Brussels (Belgium) and composed of 11, 56 and 19 trees of *P. deltoides*, *P. nigra* (indigenous and foreign origin), and *P.* x *canadensis*, respectively, including 39 indigenous black poplar trees. The origin of the trees is given in ANNEX I. The artificial stand covered an area of 0.4 ha (200 m x 20 m) and was located at the border site of a large poplar plantation covering a total area of 5.25 ha. The plantation was established in 1964 with two-year old grafted ramets of *P. nigra* (indigenous and foreign origin) and *P. deltoides*, and two-year old sets of *P.* x *canadensis*. The original plant distance was 2 m by 2 m. By 1999, spacing within the orchard was no longer uniform, owing to losses from graft incompatibility and thinning operations. A row plantation of mature trees of *P. nigra* cv. *italica* was located outside the stand.

Stage of flowering was observed on standing trees during two successive springs (1999 and 2000). This was done on a regular base (every 3 to 4 days) from March until June by using a telescope with 42 x magnification and resulted in 15 assessments for the first (1999) and 22 assessments for the second spring (2000). For the female clones of *P. nigra*, begin of seed dispersal was observed. Flowering phenology was also observed on several mature trees of *P. nigra* cv. *italica* located outside the mixed poplar stand. Flower phenology and seed production was scored using the scale outlined in *Table 1*. Female clones were considered receptive and males where considered mature when they reached the stage of full elongated catkins. When the development of seed capsules was observed and when the males showed declined catkins, final receptivity or dehiscence was assumed.

DNA was extracted using Dneasy Plant Miniprep Kit (Qiagen, Helden, Germany). Using the microsatellite (SSR) markers PMGC014 (developed by the Poplar Molecular Genetics Cooperative, forward: 5'-TTCAGAATGTGCATGATGG-3'/ reverse: 5'-GTGATGATCTCACCGTTTG-3') and PMS09, PMS014, PMS016, PMS020 (VAN DER SCHOOT *et al.,* 2000; SMULDERS et al., 2001) clonal duplicates were identified

Table 1. – Method used to record flower phenology.

Stage of flowering	Description of stage (M=male, F=Female)
0	M/F: dormant flower buds, no flowering
1	M/F: flowers emerging from the buds
2	M/F: flowers completely emerged (full elongation) M: dispersing pollen F: receptive
3	M: decline of catkins F: capsules are ripening
4	F: seed release

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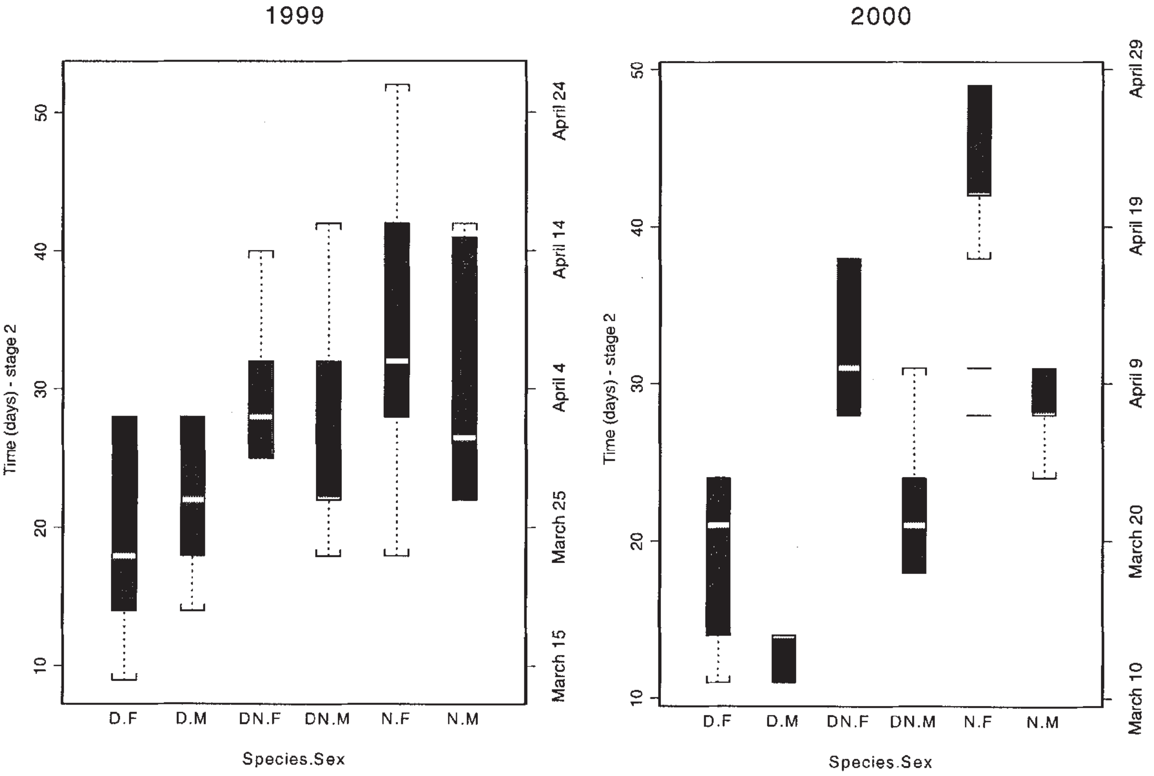

Figure 1. – Distribution of the flowering phenology data of *P. deltoides* (D), *P.* x *canadensis* (DN) and *P. nigra* from Belgian natural settings (N) in 1999 and 2000 in the study stand.

between the ramets originated from indigenous trees of *P. nigra*. Based on the multilocus DNA-fingerprints, the different genets of the stand were identified.

Floral synchrony between and within species was described based on the observational data: the mean duration (in days), the peak day and range of pollen shed/receptivity were calculated for each sex within a given species based on the data of the individual trees. Mean scores were calculated for ramets representing the same genet. Differences in flowering sequence (stage 2) of the individual trees between the two springs was studied by grouping the trees of *P. nigra* and *P.* x *canadensis*, in three precocity classes with equal range, on the basis of the earliest and latest individual flowering tree. Meteorological data were obtained from the Belgian Royal Meteorological Institute.

Results and Discussion

The genus *Populus* is widely studied, however, information on the natural variation in sexual reproductive aspects is very sparse (FARMER, 1996). This is probably due to the inability to determine the exact period of pollen release and receptivity on standing trees in the field (FARMER, 1966; SPIES and BARNES, 1981).

In this study, there is a possible confounding between observer-effect and year-to-year variation. However, the overall results are quite consistent. The proportion of total trees flowering was 93% (80/86) in 1999 and 95% (82/86) in 2000. In both years, the onset of flowering (stage 1) in the poplar plantation started after a temperature peak; in week 11 (March 15) and week 10 (March 10) in spring 1999 and 2000 respectively. Spring floral phenology is known to be regulated by temperature (PAULEY, 1950; PERALA, 1990). Therefore, fluctuations in flowering time from year to year may be considerable.

Interspecific variation

Hybrid clones of *P.* x *canadensis* showed intermediate mean flowering times compared with the parent species. However, a considerable overlap was found to exist in the flowering times of the taxa. *P. nigra* females were receptive during the anthesis of *P. nigra* males as well as during the anthesis of *P.* x *canadensis* males. Also, receptivity of female clones of *P.* x *canadensis* overlapped with pollen shed of *P. nigra* as well as *P.* x *canadensis* males. Protandry is common in *Populus* (e.g. HOUTZAGERS, 1937; FARMER, 1966). However, during the spring of 1999, we observed no systematic differences in mean time of pollen release and mean female receptivity within a taxon (*Figure 1*), although opening of the flower buds began slightly earlier on male trees than on females for *P.* x *canadensis* and *P. nigra* (results not shown). In contrast, in 2000, protandry was clearly

Table 2. – Peak day/period, mean length of time period of pollen release and female receptivity (stage 2) and standard deviation observed in *P. deltoides*, *P.* x *canadensis* and indigenous *P. nigra* for each sex. The number of trees and the time period between the earliest and the latest tree that reached stage 2 (Range) is also given.

		pollen release and female receptivity (stage 2)					
		Peak day/period		Mean length of time period and standard deviation (days)		Range (days)	
		1999	**2000**	**1999**	**2000**	**1999**	**2000**
P. deltoides	M n=5	April 2- 6	March 24- 31	3,0 (SD 3.8)	14,0 (SD 1.4)	17	8
	F n=6	March 29	April 3 - 7	5,1 (SD 4,5)	14,4 (SD 2.0)	19	13
P. x *canadensis*	M n=14	April 6	April 7-10	4,7 (SD 3.5)	16,1 (SD 3.0)	19	10
	F n=5	April 9-12	April 17-21	3,0 (SD 4.2)	14,0 (SD 2,8)	15	10
P. nigra *(origin Belgian natural settings)*	M n=8	April 9	April 10-17	8.7 (SD 10.2	7,4 (SD 2.1)	31	6
	F n=31	April 9-16	April 10-17	6.7 (SD 5.0)	11.1 (SD 8.0)	16	25
P. nigra cv. *italica*	M	March 24-April 4	March 21-March 31	11 (SD 0)	10 (SD 0)	0	0

observed for each taxon. Male flowers generally ripen and shed pollen a few days before females (*Figure 1*), thus insuring that pollen are in the air when the first female trees flower. Protandry is also observed in other forest trees e.g. *Quercus petraea* and *Q. robur* (BACILIERI et al., 1995).

Intraspecific variation

A large variability among individuals in precocity and in mean length of flowering time was observed (*Table 2*). As a result of this great variability, flowering overlap is an important factor in determining mating partners. These differences are likely under strong genetic control (VALENTINE, 1975). However, in this study we observed a significant variation in flowering time within a genet (results not shown). This may be due to the inability to determine the exact period of pollen release and receptivity on standing trees in the field. However, it is likely that also other factors like the location of the tree in the stand (southern border side or inside the stand) may influence

ANNEX 1. – Species, sex, and origin of the trees observed in the study stand.

Species	***SEX***	***Genet***	***# Ramets***	***CLONE_NAME***	***Origin1***	***Origin2***
D	F	D01	2	S.623-7	half-sib	half-sib offspring *P. deltoides* Amana, Iowa/seedling 7
D	F	D02	1	(S.77-6 x S.4-263)2	controlled cross	(*P. deltoides* “S.77-6” (Wapeloo,U.S.A.) x *P. deltoides* “S4-263”)/seedling 2
D	F	D03	1	(S.77-6 x V4)8	controlled cross	(*P. deltoides* “S77-6” (Wapeloo,U.S.A.) x *P. deltoides* “V4” (Minnesota, U.S.A)/seedling 8
D	F	D04	1	(S.71-3 x S.117-5)10	controlled cross	(*P. deltoides* “S.71-3” (Illinois) x *P. deltoides* “S117-5” (South-Dakota))/seedling 10
D	F	D05	1	(S.71-10 x S.1-198)3	controlled cross	(*P. deltoides* “S.71-10” (Illinois) x *P. deltoides* “S1-198”) /seedling 3
D	M	D06	2	S.621-188	half-sib	*P. deltoides* “Ames I”, Iowa/seedling 188
D	M	D07	1	(S.77-6 x V4)2	controlled cross	*P. deltoides* “S77-6” (Wapeloo,U.S.A.) x *P. deltoides* “V4” (Minnesota, U.S.A)/seedling 2
D	M	D08	1	(S.77-6 x V4)26	controlled cross	*P. deltoides* S.77-6 (Wapeloo,U.S.A.) x *P. deltoides* “V4 “(Minnesota, U.S.A)/seedling 26
D	M	D09	1	(S.77-6 x V4)23	controlled cross	*P. deltoides* “77-6” (Wapeloo,U.S.A.) x *P. deltoides* “V4 “(Minnesota, U.S.A)/seedling 23
DN	F	DN01	1	S.682-64B	controlled cross	(*P. deltoides* "S.9-2" x *P. nigra* "Ghoy 3")/seedling 64B
DN	F	DN02	1	S.682-228	controlled cross	(*P. deltoides* "S.9-2" x *P. nigra* "Ghoy 3")/seedling 228
DN	F	DN03	1	S.682-262	controlled cross	(*P. deltoides* "S.9-2" x *P. nigra* "Ghoy 3")/seedling 262
DN	F	DN04	1	S.683-02	controlled cross	(*P. deltoides* "S.9-2" x *P. nigra* "Gibecq") /seedling 2
DN	F	DN05	1	S.683-03	controlled cross	(*P. deltoides* "S.9-2" x *P. nigra* "Gibecq")/seedling 3
DN	M	DN06	1	S.680-14	controlled cross	(*P. deltoides* ""S.9-2" x *P. nigra* "Ghoy 1")/seedling 14
DN	M	DN07	4	S.682-26	controlled cross	(*P. deltoides* "S.9-2" x *P. nigra* "Ghoy 3")/seedling 26
DN	M	DN08	1	S.682-50	controlled cross	(*P. deltoides* "S.9-2" x *P. nigra* "Ghoy 3")/seedling 50
DN	M	DN09	2	S.682-86	controlled cross	(*P. deltoides* "S.9-2" x *P. nigra* "Ghoy 3")/seedling 86
DN	M	DN10	1	S.682-186	controlled cross	(*P. deltoides* "S.9-2" x *P. nigra* "Ghoy 3")/seedling 186
DN	M	DN11	1	S.683-24	controlled cross	(*P. deltoides* "S.9-2" x *P. nigra* "Gibecq")/seedling 24
DN	M	DN12	2	S.684-12	controlled cross	(*P. deltoides* "S.9-2" x *P. nigra* "Ghislenghien")/seedling 12
DN	M	DN13	1	S.684-18	controlled cross	(*P. deltoides* "S.9-2" x *P. nigra* "Ghislenghien")/seedling 18
DN	M	DN14	1	S.686-09	controlled cross	(*P. deltoides* "S.9-2" x *P. nigra* "Gibecq")/seedling 9
N	F	N01	29	Wannebecq-Ostiche 1	natural setting	Belgium (Dender Valley)
N	F	N02	1	Isières 1	natural setting	Belgium (Dender Valley)
N	F	N03	1	Essene1	natural setting	Belgium (Dender Valley)
N	F	N04	2	Lebeny 67	natural setting	Hungary (collecting institute: ERTI,Sarvar, Hungary)
N	F	N05	1	Yougo 4	natural setting	Former Yougoslavia
N	F	N06	2	H.6	natural setting	Hungary
N	F	N07	2	Ede	natural setting	Netherlands (collecting Institute; Alterra, Netherlands)
N	F	N08	1	Terwolde	controlled cross	Netherlands (collecting Institute; Alterra, Netherlands)
N	F	N09	1	Yougo 104		Former Yougoslavia
N	F	N10	1	W.O.I x GYOR 203/2	controlled cross	*P. nigra* “Wannebecq-Ostiches I” x *P. nigra* “Gyor” 203 (Hungary)/seedling 2
N	M	N11	5	Ogy	natural setting	Belgium (Dender Valley)
N	M	N12	1	Papa 321	?	Exact origin not known but not indigenous in Belgium
N	M	N13	1	Chopo canadensis	?	Exact origin not known but not indigenous in Belgium
N	M	N14	1	Lens	natural setting	Belgium (Dender Valley)
N	M	N15	1	Ollignies	natural setting	Belgium (Dender Valley)
N	M	N16	1	Sint_Pieters_Kap	natural setting	Belgium (Dender Valley)
N	M	N17	1	W.O.IxLeb211/1	controlled cross	*P. nigra* “Wannebecq-Ostiches I” *x P. nigra* “Lebeny 211” (Hungary)/seedling 1
N	M	N18	1	W.O.IxLeb211/5	controlled cross	*P. nigra* “Wannebecq-Ostiches I “x *P. nigra* “Lebeny 211” (Hungary)/seedling 5
N	M	N19	1	W.O.I x GYOR 203/11	controlled cross	*P. nigra* “Wannebecq-Ostiches I” x *P. nigra* “Gyor 203 “ (Hungary)/seedling 11
N	M	N20	1	W.O.1 x Gyor203/44	controlled cross	*P. nigra* “Wannebecq-Ostiches I” x *P. nigra* “Gyor 203 “ (Hungary)/seedling 44
N	M	N21	1	W.O.I x Gyor 203 /70	controlled cross	*P. nigra* “Wannebecq-Ostiches I” x *P. nigra* “Gyor 203 “(Hungary)/seedling 70

the flower phenology of a tree. Seed production on indigenous black poplar was obvious higher in 2000 than in 1999, probably due to unfavourable weather conditions during pollination in 1999. April 1999 was characterised by exceptional heavy rainfall with thunder- and hailstorms in the study area. Seed release of female indigenous black poplar started begin June and half of May in 1999 and 2000, respectively. At the end of May 2000, all flowering indigenous female black poplars were releasing seeds.

The cultivated male *P. nigra* cv. *italica* reached the stage of fully elongated catkins during March 24-April 4 and March 21-March 31, in 1999 and 2000, respectively. At that time, all female trees investigated showed closed or opening flower buds. It appears that during both springs, *P. nigra* cv. *italica* could not hybridise with native female black poplars and female *P.* x *canadensis* trees in the stand, due to non-synchrony of flowering.

Comparison between years

Comparisons between 1999 and 2000 revealed that within *P. nigra*, 18% (3/17) of the genets could be classified in the same class in the two years, 53% (9/17) changed from one class to the adjacent one and 29% (5/17) changed from early to late flowering or vice versa. For *P. deltoides* the percentages were 33%, 33% and 33%, respectively. For *P.* x *canadensis*, 30% of the genets could be classified in the same class in the two years while the other 70% of the genets changed one class to the adjacent one. From the observations made over 2 consecutive years, it is possible to state that about 72% of the individuals change precocity class from one year to another, probably under the influence of environmental factors. Variability over years was also found within *Quercus* species by Bacilieri *et al.* (1995). The variability over years inhibits the formation of mating groups that could lower the gene flow and the effective population size (Bacilieri *et al.,* 1995).

Conclusion

The results of this study showed that, in this stand and over the two years of observation, the flowering time did not constitute a barrier to hybridisation between *P. nigra* and *P.* x *canadensis*. As the controlled crossing between *P. nigra* and *P.* x *canadensis* can result in viable seedlings (Vanden Broeck *et al.*, in press a; Vanden Broeck *et al.*, in press b), we can conclude that the cultivated, widely planted *P.* x *canadensis* clones pose a potential threat to *P. nigra* in Belgium. In contrast, non-synchrony of flowering time was observed for indigenous clones of *P. nigra* and the widely planted male clone *P. nigra* cv. *italica.* However, this may be not the case in other regions is Europe.

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