

## TRADE-OFFS IN SEED DISPERSAL STRATEGIES ACROSS RIPARIAN TREES: THE HOW MATTERS AS MUCH AS THE WHEN

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## ABSTRACT

Riparian *Salicaceae* are prolific producers of short-lived seeds that require very restrictive hydro-geomorphic conditions for establishment. It is generally assumed that if floods are able to create nursery sites timed with seed dispersal, recruitment will occur. Other spatial and temporal seed dispersal patterns besides the dispersal period have historically received little attention. However, seed dispersal patterns can be highly variable between regions, species and over the years. In this paper, we report the seed dispersal patterns of three dominant riparian *Salicaceae* trees in Europe: *Populus alba*, *P. nigra* and *Salix alba* to suggest possible trade-offs between seed dispersal patterns, germinability, longevity and establishment. Seed rain of the three species was monitored in 33 glue-coated traps for three months yearly from 2006 to 2008 in an 8-km stretch of the Middle Ebro River (N Spain), which has a pluvio-nival regime. *P. alba* dispersed seeds earlier during a shorter time period and with a fewer number of seed release pulses compared with *P. nigra*, and especially with *S. alba*. With overlapping seed dispersal periods, the two latter species occupy similar landform units but rarely compete with *P. alba*, usually at higher elevations, as shown in a previous study in the same study area. The three species had very high germinability immediately after release (>90%), but longevity in *S. alba* was eight times shorter than that of its two *Populus* counterparts. We suggest that *S. alba* has compensated its lower seed quality with a more spaced seed release in several pulses of similar magnitude. With similar seed dispersal patterns and germinability but a higher longevity, *P. nigra* had a much higher density of individuals than *S. alba* in the recruitment zones of the study area. We hope that our results may inform river managers about how to optimize river flows to promote sexual regeneration of these species. Copyright © 2015 John Wiley & Sons, Ltd.

KEY WORDS: black poplar; seed germination and longevity; seed rain components; seed rain peaks; seed rain pulses; seed rain sharpness; white poplar; white willow

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## INTRODUCTION

The *Salicaceae* family, with its main genera cottonwood or poplar (*Populus*) and willow (*Salix*), is the dominant family of riparian trees in the Northern Hemisphere (Karrenberg *et al.*, 2002). Most riparian *Populus* and *Salix* are dioecious pioneer species that recruit seedlings on the new alluvial deposits formed during fluvial-geomorphic events and exposed as floodwaters recede (Cooper *et al.*, 2003). The conditions for seedling colonization and establishment are nevertheless very restrictive. First, germination must occur immediately after seeds land on moist sites, given that seed viability after seed release is very high (>90%), but seed longevity is limited to only days or a few weeks, which prevents formation of long-term seed banks (van Splunder *et al.*, 1995; Braatne *et al.*, 1996; Karrenberg and Suter, 2003). Seeds need only contact with water for germination,

which normally occurs when seeds are dispersed by floodwaters before being deposited in the recruitment zone (van Splunder *et al.*, 1995; Karrenberg *et al.*, 2002; Johnson, 2000). Second, in order to survive, seedlings must be positioned at elevations high enough to avoid being scoured by subsequent floods, buried by sediments or suffer from permanent anaerobic conditions and low enough to maintain contact between the growing roots and the falling water table and associated moisture zone (Stromberg *et al.*, 1991; Mahoney and Rood, 1998). With these restrictions, it is frequent that establishment of new cohorts of *Salicaceae* in floodplains fail for years or even decades (Rood and Mahoney, 1990; Mahoney and Rood, 1998; Johnson, 2000). In fact, seedling establishment has been traditionally considered as the demographic ‘bottleneck’ for the sexual regeneration of riparian *Salicaceae* (Lytle and Merritt, 2004; Stella *et al.*, 2010; Stella and Battles, 2010; Guilloy *et al.*, 2011) and is probably the topic that has received the most attention in the literature related to riparian vegetation.

As a single mature female *Salicaceae* can produce annually thousands or even millions of seeds (Karrenberg and

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Suter, 2003), it has been generally assumed that timing of floods and their capacity to generate nursery sites are the most determinant factors to trigger seedling establishment rather than the arrival of seeds to the recruitment zones (Karrenberg *et al.* 2002). In other words, if floods creating nursery sites are well timed with the seed dispersal period, it is accepted that there will be always enough seeds for colonization. This assumption is typical of riparian *Populus* population models (e.g. Lytle and Merritt, 2004; Harper *et al.* 2011; Morrison and Stone, In Press). A direct consequence of this supposition is that other spatial and temporal patterns of seed dispersal besides the seed dispersal period have been traditionally neglected in the specialized literature. However, the few studies reporting seed dispersal patterns have shown that the actual number of seeds being dispersed can be very variable both spatially, between study sites, and temporally, over the seed dispersal period and across years (Cooper *et al.*, 1999; Guillois-Froget *et al.*, 2002; Gage and Cooper, 2005; González *et al.*, 2010a). We hypothesized that these differences may be related to evolutionary processes and identifying them may help us to better understand the regeneration strategies of emblematic riparian tree species.

Thus, understanding variability in seed release patterns is important in a management context, for example when planning environmental flow releases from dams to meet the regeneration requirements of riparian trees (Rood and Mahoney, 2000; Rood *et al.* 2005; Shafroth *et al.*, 2010; Hall *et al.*, 2011). Between *Salicaceae* species, some authors have interpreted differences in the seed dispersal period as based on the hydric conditions required by seedlings immediately after substrate colonization, which ultimately explain the position that those species occupy in the floodplain. For example, Amlin and Rood (2002) showed that *Salix exigua* seed dispersal was two weeks later than *Populus deltoides* and *P. balsamifera*. Those differences could partially explain *S. exigua* preference for lower elevation sites that are exposed later in the growing season as river waters recede from spring floods to summer low water levels.

Different aspects of seed dispersal such as duration, shape of a seed rain curve across the season, number of pulses and synchrony between patches have potentially large evolutionary and ecological implications but have rarely been explored in the literature. Further, such studies rarely consider variability over time and/or between locations. In this work, we measured 11 different dispersal metrics over three years and several populations of three, co-occurring *Salicaceae* species to determine which were most meaningful to distinguish between species and to understand the relationship between seed dispersal and safe-site availability. The dispersal profiles of each species are then considered in the context of seed survival and germinability to explore assumed

energetic trade-offs between reproductive strategies and the adaptive pressure to minimize interspecific competition.

## METHODS

### *Monitoring seed dispersal in the field*

Methods to monitor seed dispersal can focus on the seed release, when observations are made in the tree (e.g. visually; Guillois-Froget *et al.*, 2002) or on the seed rain, when observations are made using seed traps (Cooper *et al.*, 1999; Gage and Cooper, 2005; González *et al.*, 2010a). We did not monitor seed release because in preliminary tests, we found high disagreements between three different observers and it was very time-consuming. Instead, we focus here on the seed rain of *P. alba*, *P. nigra* and *S. alba*, monitored during three consecutive years (2006–2008) using traps installed in the riparian forest of five different meanders along a 8-km segment of the Middle Ebro River near Zaragoza (41°39'N, 0°52'W, Natural Reserve Galachos Alfranca, NE Spain). From here on, we will refer indistinctly to seed dispersal or seed rain.

*Populus nigra* L., *P. alba* L and *Salix alba* L. are three major *Salicaceae* species of western European floodplain forests. They have a broad distribution from the Mediterranean region in the south to the northern ~60° latitudes (Lèfevre *et al.*, 1998; Girel *et al.*, 2003; Roiron *et al.*, 2004). Despite the importance of these three species, there are just a few studies that looked at their seed dispersal patterns (Table I). The Middle Ebro floodplain, where the three species co-exist (González *et al.*, 2010b), was considered a good laboratory to compare their seed dispersal patterns as part of differing strategies for recruitment.

A seed trap consisted of a 625-cm<sup>2</sup> wooden board hung horizontally 1 m above the soil surface by four ropes attached to nearby trees. A 400-cm<sup>2</sup> plastic sheet, coated with adhesive Tanglefoot© (Cooper *et al.*, 1999; Gage and Cooper, 2005; González *et al.*, 2010a), was attached to the upper surface of the board and changed every 3–4 days for seed counting from late-March (before seed release of the earlier species) to mid-July (all fruits of the latest species fallen) for a total of ~35 field visits per year.

Initially in 2006, three traps per species were installed, making a total of nine traps distributed in the five meanders. The number of traps was increased to 11 per species in both 2007 and 2008 (total = 33 traps). When choosing the locations for traps, our objective was to maximize the number of female stems of the target species surrounding the trap, so we could capture seed rain in densities that were high enough to detect clear temporal seed dispersal patterns of each species. For each trap, the number of female individuals of the target species was registered in a radius of 20 m (Figure 1). In preliminary field visits during 2005, we observed that the bulk of seeds from each tree fell directly

Table I. Published information about seed dispersal patterns in three major *Salicaceae* species of western European floodplain forests: *Populus alba*, *P. nigra* and *Salix alba*

Species	Duration of seed dispersal period	Authors	Localisation	Method	Process monitored
<i>Populus nigra</i> L.	9 weeks	Legionnet <i>et al.</i> (1997)	Loire River, France	Visual assessment of flowering and establishment of seedlings	Seed release and rain
<i>Populus nigra</i> L.	9 weeks	Barsoum (1998)	Drôme River, France	Visual assessment of beginning and end of release	Seed release
<i>Populus nigra</i> L.	2–3 weeks	Van Splunder <i>et al.</i> (1995)	Waal River, the Netherlands	NA	NA
<i>Populus nigra</i> L.	2–3 weeks	Foussadier (1998)	Isère and Drac Rivers, France	Visual assessment of beginning and end of release	Seed release
<i>Populus nigra</i> L.	8–13 weeks	Guilloy-Froget <i>et al.</i> (2002)	Garonne River, France	Open catkins count	Seed release
<i>Populus alba</i>	2–4 weeks	González <i>et al.</i> (2010a)	Ebro River, Spain	Tanglefoot© coat	Seed rain
<i>Salix alba</i>	2–7 weeks*	Van Splunder <i>et al.</i> (1995)	Waal River, the Netherlands	NA	NA

The great variability between durations reported can be partially explained by the differences in respective population size, local climates, different years of sampling and the methods used for determining seed dispersal periods. Methods to monitor seed dispersal can focus on the seed release, when observations are made in the tree, or on the seed rain, when observations are made using seed traps. NA information denotes not available in the article. \* denotes calculated from Figure 2 of Van splunder *et al.* (1995).

under the tree canopy. Therefore, we assumed that few seeds would arrive from individuals placed at distances longer than 20 m because the radius of the largest trees canopy was rarely that long. Traps for the same species were installed at 30-m minimum distances between each other to minimize the number of seeds arriving from the same female individuals. Caution was also taken to avoid placing two traps close enough that could receive seeds from individuals sharing a single genotype, given the extraordinary capacity of *Salicaceae* for regenerating asexually (Gom and Rood, 1999), especially *P. alba* by root suckering (González *et al.*, 2010a, 2010b). For these reasons, traps were installed in different forest patches (i.e. group of trees presumably belonging to the same cohort in a singular geomorphic landform). As the seed dispersal period coincides with the period of highest likelihood of flooding, all traps were placed in higher elevation landforms (i.e. >2 m above

water level in the river in summer) and gravel bars were avoided. Forest patches being monitored dated more than 25 years old (i.e. mature and old sites, *sensu* González *et al.*, 2010b). Traps remained at the same position each year.

#### Disentangling seed rain components

Seed counts of each species in each trap were used to calculate a series of eleven variables representing the main components of seed rain at a given year (Figure 2). The (1) *start* and (2) *end* of seed rain were the first and last day of the year having seeds captured in the trap. The (3) *duration* was the number of days from the start to the end of seed dispersal. The (4) *seed dispersal period* includes the calendar dates from the start to the end of seed dispersal. The (5) *intensity* was the mean daily number of seeds registered in the

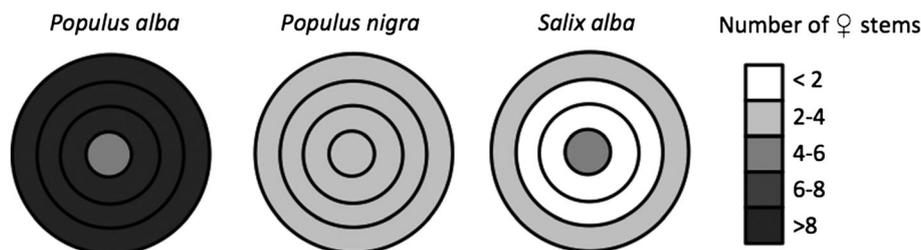


Figure 1. Average number of female stems in a seed trap of *P. alba*, *P. nigra* and *S. alba*. The number of stems per strip of 5 m was obtained by averaging the female stem counts in 11 patches per species. Seed trap was set in the middle of the target. All forest patches were older than 25 years. Stem density of the three species was typically higher in *P. alba* than in *P. nigra* and *S. alba* (mature and old sites in Figure 5 González *et al.*, 2010b)

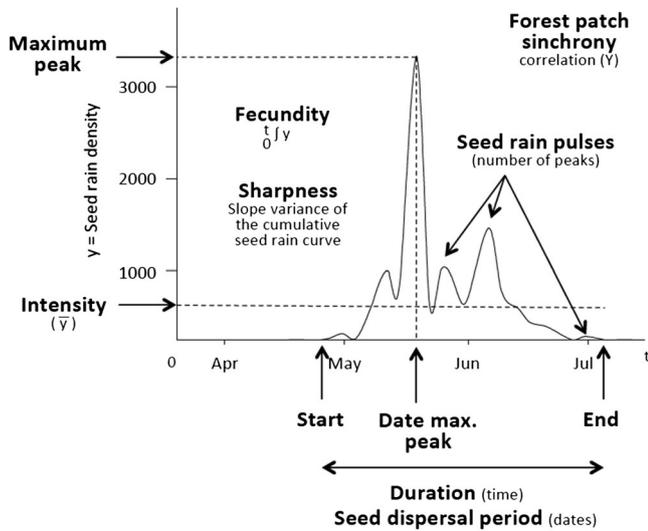


Figure 2. Theoretical eleven seed rain components of riparian tree species with non-dormant seeds

trap. It was expressed as either seeds per day per square meter (*absolute* intensity) and to allow species comparisons and avoid the bias of our criteria that searched for the maximum number of female trees surrounding the traps, seeds per day per female stem (*corrected* intensity). The (6) *maximum peak* was the highest daily number of seeds registered in the trap during the whole period and had the same units as intensity: *absolute* and *corrected*. The day of the year when that maximum peak was registered was also noted ((7) *date of maximum peak*). (8) *Fecundity* was the sum of all seeds registered in the trap (*absolute* for seeds per square meter or *corrected* for seeds per female stem). The next and last three components refer to the shape of the curves: first, the (9) *forest patch synchrony* was the Spearman correlation

between the seed rain curve of the trap and the other 2 and 10 traps of the same species in year 2006 and 2007–2008 respectively. Therefore, it was intended to represent how coordinated the population of each species in the study area was in dispersing their seeds. Second, the (10) *seed rain pulses* was the number of peaks in the seed rain curve observed in the trap (six in the example of Fig. 2). Finally, cumulative seed rain curves were constructed for each species and trap to calculate the slope of the seed rain between two successive sampling dates. The (11) *sharpness* of the seed rain was then calculated as the variance of the slope of those cumulative curves. This parameter indicates whether the *whole* seed rain curve had a pointed or flat shape and represents whether the seeds are homogeneously released during the seed release period or in a short time span, independently of the number of seed rain pulses. The higher the sharpness, the more pointed the curve was. Some seed rain components could not be calculated for *P. nigra* and *S. alba* in 2008 (refer to Table II) because a series of late spring floods submerged most of the traps during May and June.

Preliminary analyses showed that most of our data did not meet the assumptions required by parametric tests and that interactions between year and species existed. Therefore, we used the non-parametric Mann–Whitney *U* test to compare the eleven seed rain components between the three riparian tree species, taking the information of each year independently. Wilcoxon tests were used to assess changes in the parameters per each species over time. Analyses were performed using SPSS 13.0.

*Seed germination and longevity*

In 2007, seeds were collected from natural populations of *P. alba*, *P. nigra* and *S. alba* to perform seed germination and

Table II. Median values (± range) for the eleven seed rain components of *P. Alba*, *P. Nigra* and *S. Alba* computed from seed rain monitored during the period of 2006–2008 in the riparian forest of the Ebro river near Zaragoza (NE Spain)

Year	Species	(4) Seed dispersal period			(5) Intensity		
		(1) Start (date)	(2) End (date)	(3) Duration (days)	Absolute intensity (seed m <sup>-2</sup> day <sup>-1</sup> )	Corrected intensity (seed ♀ <sup>-1</sup> day <sup>-1</sup> )	Absolute maximum peak (seed m <sup>-2</sup> day <sup>-1</sup> )
2006	<i>P. alba</i>	4 Apr ± 0 days <b>b</b>	20 Apr ± 25 days <b>b</b>	16 ± 25 <b>b</b>	1933 ± 573 <b>a</b>	61 070 ± 45 406	3852 ± 6696
	<i>P. nigra</i>	23 Apr ± 0 days <b>a</b>	9 Jun ± 8 days <b>a</b>	47 ± 8 <b>a</b>	1216 ± 1172 <b>ab</b>	63 639 ± 103 181	3000 ± 3175
	<i>S. alba</i>	23 Apr ± 6 days <b>a</b>	16 Jun ± 7 days <b>a</b>	48 ± 7 <b>a</b>	1058 ± 482 <b>b</b>	58 447 ± 10 546	3740 ± 2270
2007	<i>P. alba</i>	9 Apr ± 4 days <b>b</b>	7 May ± 18 days <b>c</b>	24 ± 22 <b>c</b>	802 ± 3081 <b>a</b>	44 384 ± 89 932	2659 ± 13 733
	<i>P. nigra</i>	28 Apr ± 10 days <b>a</b>	12 Jun ± 24 days <b>b</b>	45 ± 30 <b>b</b>	328 ± 727 <b>ab</b>	28 048 ± 149 019	1356 ± 3500
	<i>S. alba</i>	25 Apr ± 10 days <b>a</b>	3 Jul ± 29 days <b>a</b>	66 ± 36 <b>a</b>	268 ± 425 <b>b</b>	38 491 ± 85 448	1368 ± 2293
2008	<i>P. alba</i>	4 Apr ± 4 days <b>b</b>	29 Apr ± 14 days <b>c</b>	25 ± 18 <b>c</b>	255 ± 959	13 307 ± 106 755	933 ± 3893
	<i>P. nigra</i>	22 Apr ± 8 days <b>a</b>	27 Jun ± 20 days <b>b</b>	70 ± 24 <b>b</b>	—	—	—
	<i>S. alba</i>	18 Apr ± 12 days <b>a</b>	12 Jul ± 18 days <b>a</b>	81 ± 22 <b>a</b>	—	—	—

The letters indicate the homogeneous groups after Mann–Whitney pair wise comparisons. No letters were written if significant differences were not found. The number of traps per species was 3 at year 2006 and 11 for the years 2007 and 2008. For a detailed explanation of the seed rain components, refer to the text (methods—disentangling seed rain components). \* denotes the values that were divided by 1000 to facilitate visual comparisons.

longevity tests that could explain potential trade-offs with seed dispersal patterns. Seeds were collected from ripe catkins partially bearing opened seed capsules of three forest stands per species. Three Petri dishes per stand and species (total =  $3 \times 3 \times 3 = 27$  dishes) were scattered with 50 seeds immediately after field collection. The rest of the seeds were kept in paper bags in the dark at ambient temperature and used to reproduce the test on a weekly basis until no further germination was registered. The germinability of the three populations per species was averaged weekly, and the mean weekly average over time was used to calculate seed longevity, expressed as the  $G_{50}$  (i.e. the storage time after which 50% of the seeds were still able to germinate) (Karrenberg *et al.*, 2002) and calculated by adjusting a sigmoidal function to the germination curve using Sigmaplot 9.0.

## RESULTS

### Seed dispersal patterns

*P. alba*, *P. nigra* and *S. alba* showed distinctive seed rain patterns. *P. alba* was characterized by having an earlier and shorter seed dispersal period (from early April to early May) than *P. nigra* (late April to late June) and *S. alba* (late April to early July) (start, end, and duration; Figure 3 and Table II). Accordingly, seed rain of *P. alba* peaked one month earlier than *P. nigra* and *S. alba* (date of maximum peak 2006 and 2007; Figure 3 and Table II). The shorter *P. alba* seed dispersal period, in conjunction with a patch structure characterized by a higher density of female stems (Figure 1), consistent with a high stem density reported in the same study area by González *et al.* (2010b), resulted into a more intense daily seed rain expressed by unit area

compared with *S. alba* (absolute intensity; Figure 3 and Table II). However, when corrected for the number of female stems surrounding the trap, *S. alba* produced more total seeds than either *Populus* spp. in 2007, the year with the greatest replication and with no temporal gaps because of flooding (as was the case for 2008) among the three monitored (corrected fecundity; Figure 3 and Table II).

Seed rain curves were quite similar between traps of the same species (patch synchrony  $\sim 0.80$  in the three species over the three years; Table II). However, the curves of each species were quite different: at least in 2007, *P. alba* had significantly fewer peaks than *P. nigra*, and the latter fewer than *S. alba* (seed rain pulses; Figure 3 and Table II). Furthermore, even although *P. alba* patches peaked a median of two times in 2007 and 2008 (seed rain pulses; Table II), the second peak was usually much lower than the first peak. When significant differences were found between species (2007), the sharpness of the curves represented by the slope variance of the cumulative seed rain curves was significantly higher in *P. alba* than in *S. alba* (sharpness, Table II; cumulative curves not shown).

Over the years, we found a significant decrease in the number of seeds arriving to the traps for *P. alba*, as reflected in Wilcoxon tests for the intensity (2007 versus 2008 observations,  $p = 0.003$ ) and absolute maximum peak (2007 versus 2008 observations,  $p = 0.003$ ) (Table II).

### Seed germinability and longevity

The seed germinability immediately after release was very high ( $>90\%$ ) for the three species (Figure 4). However, germinability decreased dramatically in *S. alba*, with a  $G_{50}$  longevity of only 4 days, and no germination at all occurring

Table II. (Continued)

Year	(6) Maximum peak		(8) Fecundity		Shape of the curve		
	Corrected maximum peak (seed $\text{♀}^{-1} \text{ day}^{-1}$ )	(7) Date of maximum peak (date)	Absolute fecundity (seed $\text{m}^{-2}$ )	Corrected fecundity (seed $\text{♀}^{-1}$ )	(9) Patch synchrony ( $\rho$ )	(10) Seed rain pulses (#peaks)	(11) Sharpness (no relevant units)*
<b>2006</b>	250 639 $\pm$ 342 258	11 Apr $\pm$ 9 days <b>b</b>	32 619 $\pm$ 55 816	2.0 $\pm$ 2.8 $10^6$	0.79 $\pm$ 0.21 <b>c</b>	1 $\pm$ 0 <b>b</b>	177 $\pm$ 713
	162 496 $\pm$ 262 202	17 May $\pm$ 16 days <b>a</b>	52 275 $\pm$ 53 900	3.0 $\pm$ 4.3 $10^6$	0.94 $\pm$ 0.01 <b>a</b>	2 $\pm$ 2 <b>a</b>	82 $\pm$ 119
	200 577 $\pm$ 33 139	13 May $\pm$ 4 days <b>a</b>	56 000 $\pm$ 23 050	3.0 $\pm$ 0.2 $10^6$	0.88 $\pm$ 0.05 <b>b</b>	2 $\pm$ 2 <b>ab</b>	140 $\pm$ 110
<b>2007</b>	132 244 $\pm$ 364 975	18 Apr $\pm$ 10 days <b>c</b>	19 864 $\pm$ 96 356	0.9 $\pm$ 2.6 $10^6$ <b>b</b>	0.81 $\pm$ 0.13	2 $\pm$ 1 <b>c</b>	74 $\pm$ 1541 <b>a</b>
	205 842 $\pm$ 722 695	12 May $\pm$ 28 days <b>b</b>	16 883 $\pm$ 33 174	1.4 $\pm$ 5.4 $10^6$ <b>ab</b>	0.80 $\pm$ 0.27	4 $\pm$ 6 <b>b</b>	12 $\pm$ 99 <b>ab</b>
	203 742 $\pm$ 598 376	20 May $\pm$ 49 days <b>a</b>	17 328 $\pm$ 34 253	2.6 $\pm$ 6.7 $10^6$ <b>a</b>	0.80 $\pm$ 0.23	6 $\pm$ 6 <b>a</b>	5 $\pm$ 24 <b>b</b>
<b>2008</b>	32 098 $\pm$ 425 574	9 Apr $\pm$ 12 days	7401 $\pm$ 34 275	0.3 $\pm$ 3.7 $10^6$	0.87 $\pm$ 0.10 <b>a</b>	2 $\pm$ 2	8 $\pm$ 149
	—	—	—	—	0.83 $\pm$ 0.13 <b>ab</b>	—	—
	—	—	—	—	0.81 $\pm$ 0.16 <b>b</b>	—	—

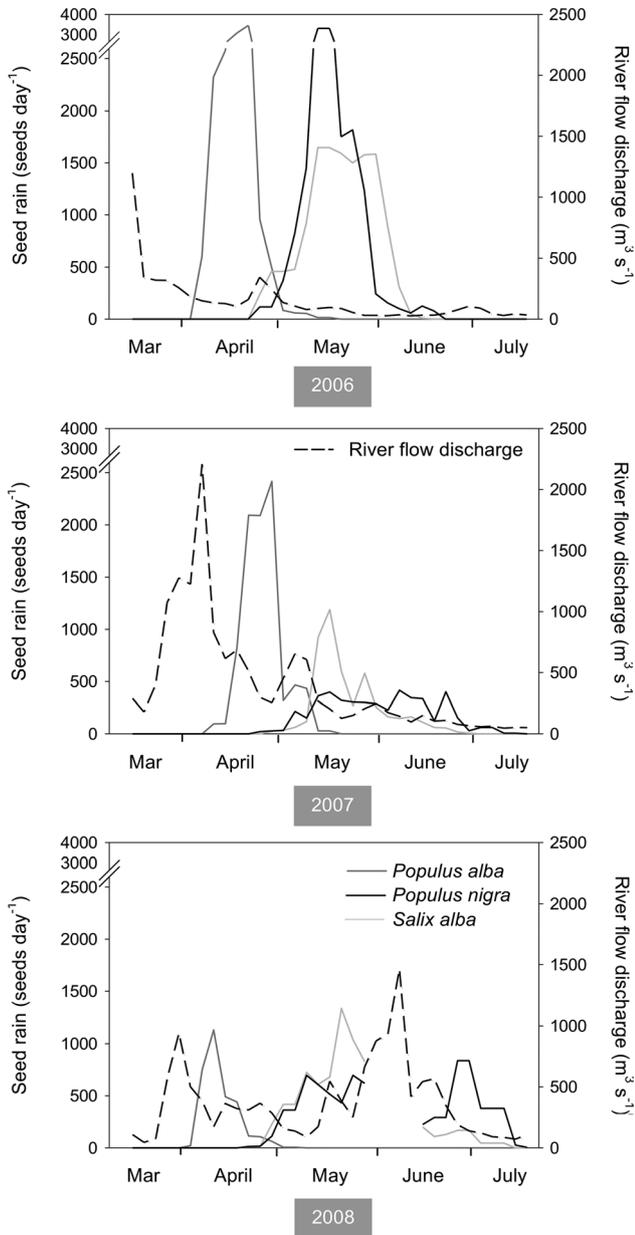


Figure 3. Density of seed rain (seeds  $m^{-2} day^{-1}$ ) in 2006, 2007 and 2008 for three riparian tree species co-occurring in the Middle Ebro River (NE Spain). Each point represents mean of 3, 11 and 11 seed traps installed in different forest patches of five river meanders along a 8-km river segment (2006, 2007 and 2008 respectively). The errors were not represented for visual clarity. Flow discharge of the Ebro River at the gauging station of Zaragoza (A011), data provided by the Ebro River Basin Administration (<http://www.chebro.es>). Note that missing seed rain information for *P. nigra* and *S. alba* in 2008 coincided with a period of spring floods that submerged the seed traps

after only three weeks of release. *P. alba* and *P. nigra* exhibited a relatively similar pattern, with respective median seed longevity ( $G_{50}$ ) of 30 and 32 days. *P. alba* maintained

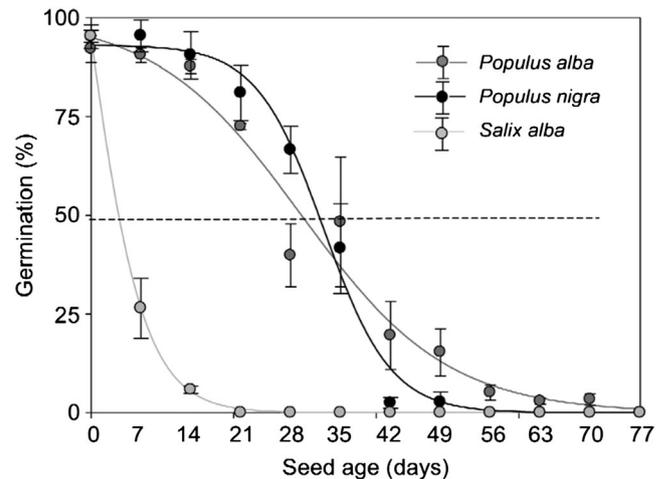


Figure 4. Proportion of germinating seeds stored for longevity tests. For each species, each point represents the mean of three natural populations where seeds were collected. The error bars represent  $\pm 1$  standard error of the mean. Each population mean was obtained from three replicate Petri dishes with 50 seeds each. The line denotes the adjusted sigmoidal function to the germination curve (*Populus alba*:  $y = 100.39 / (1 + \exp[(x - 29.43) / 10.31])$ ; *Populus nigra*:  $y = 93.03 / (1 + \exp[(x - 32.63) / 4.71])$ ; *Salix alba*:  $y = 225.51 / (1 + \exp[(x + 1.29) / 4.11])$ )

potential for survival in dry conditions for three weeks longer than *P. nigra*.

## DISCUSSION

### *Seed dispersal period and zonation of Salicaceae species*

The seed dispersal patterns of three co-occurring dominant riparian tree species differed and may be evidence of different strategies to reproduce sexually. *P. alba* dispersed their seeds much earlier than *P. nigra* and *S. alba*, whose seed dispersal periods almost overlapped. This may partially explain the frequent co-existence of *P. nigra* and *S. alba* in forest patches, which contrasts to an alternative successional pathway dominated by *P. alba* in higher elevation landforms, with almost no presence of the other two species reported by a previous work in the same study area (González *et al.*, 2010b). By dispersing their seeds earlier, *P. alba* would normally occupy land surfaces at higher elevations, provided that the river water level in the Ebro declines from a maximum level during winter and spring floods to a minimum in summer (pluvio-nival regime; González *et al.*, 2010b). In fact, González *et al.* (2010b) found no *P. alba* seedlings colonizing the low-elevation gravel bars of the study area, while there were many young individuals of *P. nigra*, *S. alba* and *Tamarix* spp. Although this does not necessarily mean that *P. alba* seedlings were not obligate phreatophytes. In

greenhouse experiments, survival and growth of young seedlings were heavily controlled by water table levels (González *et al.* 2010a). Meanwhile, the longer seed dispersal period of *S. alba* compared with *P. nigra* could favour the colonization of *S. alba* at slightly lower elevations within the gravel bars, as observed by Barsoum (2002) in the French Drôme River. This hypothesis remains to be tested in our study area, but there is some evidence that suggests that this might be true: González *et al.* (2012) showed that all (seedlings + mature) individuals of *S. alba* exhibited higher mortality and dieback than *P. nigra* (and *P. alba*) at higher elevation sites with deeper groundwater levels and less flooding frequency and duration.

The inter-year variability and significant interaction between site and year in our data suggest that many factors contribute to the sexual reproductive strategy of these species; it is not therefore surprising that the same species could perform differently in different rivers, depending on the particular combination of flood timing and weather conditions during the years of sampling. In particular, cycles of droughts versus high waters and climatic variations over the years would be expected to influence seed production and affect species differently. For example, in contrast to Barsoum (2002), Van Splunder *et al.* (1995) found that *S. alba* in the Dutch Waal River occupied elevations both lower and higher than *P. nigra* but attributed these patterns to abnormally wet conditions in their only year of sampling. Also, contrary to our observations, *S. alba* dispersed the seeds earlier than *P. nigra*. But, like on the Ebro, they also found that *S. alba* seeds had a longer seed dispersal period than *P. nigra*. Longer term monitoring of seed rain concurrently on different rivers would allow us to discern the causes underlying this variation between regions and species. However, we are not aware of any detailed studies on seed dispersal phenology of these species lasting more than three years. The age and health status of the forest patches or the individuals being monitored for seed release is usually not reported and may be other drivers of seed release to consider in future studies.

#### *Trade-offs in the sexual regeneration strategies of Salicaceae*

The high patch synchrony in the seed rain of the three species ( $\geq 0.80$  in the three species over the three years; Table II) was interpreted as evidence that the main factors triggering seed release are operating at spatial levels higher than the study area: photoperiod, atmospheric temperature and precipitation (Mahoney and Rood, 1998; Stella *et al.*, 2006). A higher number of seed rain peaks for *P. nigra* and especially *S. alba* (Figure 3) was seen as a higher capacity of the populations of those species to release their seeds in multiple pulses. In *P. alba*, all trees at each forest patch released their seeds

simultaneously, probably reflecting a clonal origin (González, field observations). *P. nigra* and *S. alba* trees were more likely to have been recruited sexually, with neighbours trees of the same forest patch dispersing seeds within short delays of days and sometimes weeks. *S. alba* were even able to disperse their seeds from different branches at different time intervals (González, field observations).

We also believe that the shape of the curves may reveal the existence of tradeoffs in the sexual regeneration strategies of the three species. There appeared to be at least two distinct strategies for dispersal that related to seed longevity. Overall, *P. alba* dispersal density over time had a much more pointed shape than *P. nigra* and *S. alba*. Sharpness of seed rain was inversely related to the number of peaks, with the flatter of the three species, *S. alba*, having the highest number of peaks, or in other words, the highest number of seed release pulses. These different patterns may be best understood in the context of relative seed longevity. The population of *P. alba*, whose seeds lose viability later ( $G_{50}$ , the time after which 50% of the seeds are still able to germinate was 30 days; Figure 4; González *et al.*, 2010a), dispersed their seeds in an almost single pulse. However, *S. alba*, with a much shorter seed longevity ( $G_{50}=4$  days; Figure 4; Van Splunder *et al.*, 1995), dispersed their seeds along multiple pulses of similar magnitude over the two months of dispersal to compensate for the short viability of seeds. In other words, we suggest the existence of a trade-off between the two components that determine the time frame for recruitment in riparian *Salicaceae*: seed dispersal period and germination capacity (refer to the  $x$ -axis of the box recruitment model in Mahoney and Rood (1998). Curiously, *P. nigra*, with a longevity eightfold longer than *S. alba* ( $G_{50}=32$  days, Figure 4), and a rather flat seed dispersal sharpness as well, had an approximately four times higher density of seedlings (i.e.  $0.3\text{ m} \leq \text{height} < 1.3\text{ m}$  or diameter at breast height  $< 2.5\text{ cm}$ ) than *S. alba* in the recruitment sites sampled by González *et al.* (2010b) in the same Middle Ebro study area, where seeds were collected.

A longer and more punctuated seed dispersal over time could also compensate for a lower quality of seedlings not only for germination but also for establishment, being this a second trade-off in the sexual regeneration strategies that we suggest for these species. Previous studies have reported a higher vulnerability of *Salix* spp. seedlings compared with *Populus* spp. with regard to soil moisture deficits (Stromberg, 1997; Shafroth *et al.*, 1998; Amlin and Rood, 2002; but refer to Stella and Battles, 2010), with some studies focusing particularly on *S. alba* versus *P. nigra* (Van Splunder *et al.*, 1996; Barsoum, 2002; Guillois *et al.*, 2011). All these studies showed that as groundwater declines, *S. alba* seedlings normally perform worse than *P. nigra*. We argue that with a higher number of seed rain

pulses (Figure 3 and Table II), regularly spaced in time and a higher seed production (corrected fecundity, Table II), *S. alba* may be more likely to capture suitable floods, having more opportunities for seedling colonization of nursery sites and counteracting its higher vulnerability of seedlings and its lower seed longevity.

Finally, it is worth mentioning that sexual regeneration and the possible trade-offs between its components among species suggested in this work are not the only factors explaining the persistence and co-existence of riparian tree species in river floodplains. Riparian *Salicaceae* also show a great potential of vegetative propagation that guarantee species persistence when sexual regeneration fails (Legionnet *et al.*, 1997; Gom and Rood, 1999; Barsoum, 1998; 2002; Barsoum *et al.*, 2004; Radtke *et al.*, 2012). Nevertheless, sexual regeneration is essential to maintain and increase genetic variation and resistance of natural populations to disease and environmental changes (Heinze and Lefèvre, 2001; Barsoum *et al.*, 2004). Our observations have shown that seed dispersal is a more complex process than usually considered and that its spatial and temporal patterns deserve to be divided into different components to better understand the regeneration strategies of these key riparian species in other rivers and world regions.

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