Role of pollinators in seed set and a test of pollen limitation hypothesis in the tropical weed *Triumfetta semitriloba* (Tiliaceae)

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Abstract: Two patches of the tropical weed Triumfetta semitriloba Jacq. (Tiliaceae) were studied in Viçosa, Southeastern Brazil, in the 1994 flowering season (March to May). Ten plants per patch, and five flowers per plant were randomly chosen for the following pollination tests: self-pollination; control; bee control; high load of cross-pollen and high load of self-pollen. Fruits were collected to study seeds. Results for role of pollinators and pollen limitation in seed production were different between patches, probably because of differences in patch "quality" (nutrient availability, number of flowers per plant and per patch, pollinator abundance). Seeds initiation was independent of pollinators, but seed initiation and seed set were increased by pollinators. Seed production was limited by pollen, as seed initiation and seed set were increased by high load of cross-pollen, only in one patch. Nevertheless, nutrients may also be limiting seed production, because a high load of cross-pollen did not maximize seed production, in either patch. Fruit abortion was higher in self-pollination and high load of self-pollen treatments, possibly because of selective abortion of self-progeny and fruits with less seeds. As expected for a weed species, *T. semitriloba* has a breeding system where seed production is guaranteed by self-pollination, but the presence of bee pollinators increases seed set, and probably improves seed quality by cross-pollination. Differences between patches showed that this species may experience spatial differences in reproductive success, with patches where seed production is limited by nutrients, patches limited by pollen, and patches where seed production is limited by both.

Key words: Reproductive success, seed set, seed abortion, *Triumfetta semitriloba*, Tiliaceae, weed, pollen limitation.

Self-compatible species my be submitted to differential contribution of pollinators in seed production, specially when flower morphology allows automatic self-pollination. For weed species, defined as colonisers associated with human being disturbance activities (Baker 1965), habitat uncertainty of abiotic and biotic conditions, such as nutrients and the "pool" of pollinators, may select self-compatible and self-pollinated systems, which may guarantee seed production in the absence or prior to pollinator visitation, improving colonising ability (Baker 1965, Abrahamson 1975, 1979, Graumann and Gottsberger 1988).

Additionally, pollinators may play an important role in limiting brood size because of inadequate service, resulting in a low number of initiated seeds, - an effect known as pollen limitation hypothesis (Schemske 1980, Horvitz and Schemske 1988).

However, other hypothesis try to explain brood size and reduction in hermaphroditic plant species (Stephenson 1981, Koptur 1984, Garwood and Horvitz 1985, Sutherland 1987). Resource limitation hypothesis has been mostly evoked as an alternative hypothesis for pollen

limitation. This hypothesis states that a reduction in brood size occurs in low resource supply conditions, because of selective abortion of seeds and fruits containing seeds with lower average expected fitness (Stephenson and Bertin 1983, Haig and Westoby 1988) or sibling rivalry, emerging from genetic differences, leading to inhibition of seeds induced by sink siblings, and a reduction in brood size (Lee 1988, Shaanker, Ganeshaiah and Bawa 1988, Marshall and Folsom 1991). Nevertheless, pollen and nutrient limitation could be considered as complementary hypothesis as long as both may be simultaneously limiting brood size (Casper and Niesenbaum 1993). This simultaneous effect may occur at the same or different levels, such as modules, individuals, and populations or patches. Another hypothesis, the "bet hedging" strategy states that a high number of flowers provide an opportunity to benefit from "windows" of environmental favorability, and would result in a spatial and temporal heterogeneity in seed set (Bawa and Webb 1984, Haig and Westoby 1988). Finally, the improvement of male fitness hypothesis states that the high number of flowers may improve pollinator attraction, increasing pollen donation and seed initiation (Gentry 1974, Augspurger 1980, Haig and Westoby 1988, Bertin 1988). This work aimed to study (1) the role of pollinators in seed production, and (2) to test the hypothesis of pollen limitation in reproductive success of *Triumfetta semitriloba* Jacq. (Tiliaceae), focusing on the spatial variation of seed production within and among patches.

MATERIALS AND METHODS

Study species and site: *T. semitriloba* is a tropical shrub weed species, occurring in well delimited patches in abandoned pastures, secondary forests gaps and road sides in tropical America. In Southeastern Brazil, flowering occurs in autumn (March to May) and buds open sequentially, in the afternoon (Collevatti et al. in prep.), such as a modified Gentry's "steady state" phenological pattern (Gentry 1974). Despite the existence of five floral nectaries around the ovary base, nectar production is negligible. Flower visitors are mainly solitary bee species, although social bee species, beetles, flies, bugs and butterflies have also

been recorded (Collevatti et al. in prep.). Field work was conducted in two patches of abandoned pastures, in Viçosa, Southeastern Brazil (20°45'S, 42°50'W), during the flowering season of 1994 (March to May). Both patches (P1 and P2) were squared shaped areas of 200 m² and 100 m², respectively, occupied by *T. semitriloba* individual plants and other weeds. All individuals in each patch were marked and numbered.

Methods and statistical analysis: The role of pollinators in seed production was verified with the following tests of pollination: (1) automatic self-pollination - bagging buds before anthesis (SP); (2) control - bagging flowers opened for at least 1hr (CT); (3) bee control - bagging flowers after an effective visiting (pollen grain deposition on stigma), by at least one bee pollinator (BC). Ten individual plants were randomly chosen, in each patch, and five flowers in each of these plants were selected for each pollination test. Fruits have three locules with two ovules each one, resulting in a maximum of six seeds per fruit. Ripened fruits were opened and number of follicles that initiated development per fruit (initiated seeds) and number of mature seeds per fruit (seed set) were counted. A tetrazolium test of viability was used to confirm seed viability and abortion. Seed abortion per fruit was defined as the proportion [1-(seed set/initiated seeds)], and fruit abortion per test as [1-(number of fruits with seed set>0/total number of initiated fruits)].

Production of seeds in geitonogamy and xenogamy was not verified since it was impossible to guarantee non-contamination with self-pollen in the pre-anthesis phase, and death of early emasculated flowers. Sporadic fruit and seed development was verified in self-pollination in pre-anthesis, but not in agamospermy (Collevatti, personal observation). The pollen limitation hypothesis of seed production was tested providing flowers with a high load of cross- (HL) or self-pollen (HS). This load was provided just after the opening of flowers, then the flowers were bagged. Ten individual plants were randomly chosen, in each patch, and five flowers, for each test (HL and HS), were selected on each plant. Ripened fruits were treated as in tests for role of pollinators in seed production.An analysis of covariance was used to verify among patch variation

in pollination test results for the role of insects in seed production and pollen limitation hypothesis. A two-way analysis of variance was performed to verify within patch variation (between individual plants) in pollination tests, treating flowers as replications. Mean number of initiated seeds and seed set were compared by an a posteriori Tukey test. Proportion of fruit and seed abortion was analyzed by Pearson's Chi-square considering p=0.05, comparing observed frequencies between tests, within patches, with the expected for equal frequencies.

RESULTS

Role of pollinators: Patch affected seed initia-

tion and seed set in pollination test (Table 1), so the data from the two patches were analyzed separately.

Although individual plants differed in seed initiation on patch P1, there was no difference in seed initiation between pollination tests (N=150, individual plant df=9, F=4.356, p<0.001, pollination test d.f.=2, F=2.784, p=0.065). An opposing result was found for P2 - seed initiation did not differ between individual plants, but pollination tests differed (N=150, individual plants d.f.=9, F=0.624, p=0.775; pollination test d.f.=2, F=5.321, p=0.006,). Therefore, differences were caused only for the lower initiation of seeds on self-pollination test on P2 (Table 2). The same result was obtained for seed set: there was no

TABLE 1

Analysis of covariance (ANCOVA) for patch and pollination test effects on number of initiated seeds and seed set, for role of pollinators on seed production.

Source	Initiated seeds				Seed set			
	SQ	df	F	p	SQ	df	F	p
Patch	71.403	1	67.79	<0.001	4.840	1	12.12	0.001
Test	32.968	3	10.43	<0.001	5.940	3	4.96	0.002
Patch*Test	48.868	3	15.47	<0.001	5.060	3	4.22	0.006
Error	412.860	392			156.520	392		

TABLE 2

Mean number of initiated seeds per fruit, standard deviation (SD), and total number of ripened fruits (N), in each patch, for tests of role of pollinators in seed production.

	Initiated seeds - P1			Initiated seeds - P2		
Test	Mean	SD	N^1	$Mean^2$	SD	N_1
CT	0.360	0.851	50	1.820a	1.101	50
BC	0.560	1.072	50	2.020a	0.892	50
SP	0.160	0.584	50	0.860b	1,069	50

1 Includes fruits with no mature seeds (seed set=0)
2 Means followed by the same letter did not differ by a posteriori Tukey test, p>0,050.

TABLE 3

Mean seed set per fruit, standard deviation (SD), total number of ripened fruits (N), and seed (AS) and fruit (AF) abortion, in each patch, in tests for role of pollinator in seed production.

			Seed set - P1			Seed set - P2			
Test	Mean	SD	N^1	$AS(\%)^3$	$AF(\%)^3$	$Mean^2$	SD	N_1	$AS(\%)^3$	$AF(\%)^3$
CT	0.060	0.240	9	83.3	82	0.460ab	0.734	43	74.7	14
BC	0.260	0.751	12	53.6	76	0.720a	0.948	49	64.4	2
SP	0.100	0.461	4	37.5	92	0.220b	0.465	24	74.4	52

1 Includes only fruits with mature seeds (seed set>0)
2 Means followed by the same letter did not differ by a posteriori Tukey test, p>0,050.
3 Chi-square comparisons, all significative for p=0.05: P1 - AS, df=2, c^2=25.57; AF, df=2, c^2=69.28; P2 - AS, df=2, c^2=16.36; AF, df=2, c^2=72.08.

difference in seed set between tests in P1, but a significant one between individuals (N=150, individual plant d.f.=9, F=2.091, p=0.034, pollination test df=2, F=2.110, p=0.125,) and an opposing result for P2 (N=150, individual plant df=9, F=1.106, p=0.363, pollination test df=2, F=4.094, p=0.019). Bee control did not increase seed initiation, compared to control, but increased seed set in P2, in relation to self-pollination (Table 3). Pollination tests showed differences in seed and fruit abortion (Table 3). Although self-pollination set seed, proportion of fruit abortion was higher, especially in P1. Unexpectedly, seed abortion on self-pollination was lowest in this patch. Bee control was asso-

ciated with the lowest fruit abortion, in both patches.

Pollen limitation hypothesis: Patch affected seed initiation and seed set in pollination test (Table 4), so the data from the two patches were analyzed separately. There was difference in seed initiation between individuals and pollination tests for patch P1 (N=200, individual plant df=9, F=3.959, p<0.001, pollination test df=3, F=6.014, p=0.001), but not for patch P2 (N=200, individual plant df=9, F=1.252, p=0.266, pollination test df=3, F=1.863, p=0.137). Differences in P1 occurred because of the higher seed initiation with high load of cross-pollen (Table 5). Seed set was different

TABLE 4

Analysis of covariance (ANCOVA) for patch and pollination test effects on number of initiated seeds and seed set, for hypothesis of pollen limitation in seed production

| Source | Initiated seeds | | | | Seedset | | | |
	SQ	df	F	p	SQ	df	F	p
Patch	121.000	1	104.41	<0.001	5.523	1	11.09	<0.001
Test	33.420	3	9.61	<0.001	6.687	3	4.48	0.004
Patch*Test	13.460	3	3.87	0.009	4.568	3	3.06	0.028
Error	454.280	392			195.100	392		

TABLE 5

Mean number of initiated seeds per fruit, standard deviation (SD), and total number of ripened fruits (N), in each patch, for hypothesis of pollen limitation on seed production

| | Initiated seeds - P1 | | | Initiated seeds - P2 | | |
Test	Mean[2]	SD	N[1]	Mean[2]	SD	N[1]
CT	0.360[a]	0.851	50	1.820	1.101	50
BC	0.560[a]	1.072	50	2.020	0.892	50
HS	0.280[a]	0.757	50	1.120	1.172	50
HL	1.160[b]	1.462	50	1.800	1.143	50

[1] Includes fruits with no mature seeds (seed set=0)
[2] Means followed by the same letter did not differ by a posteriori Tukey test, p>0,050.

TABLE 6

Mean seed set per fruit, standard deviation (SD), total number of ripened fruits (N), seed (AS) and fruit (AF) abortion, in each patch, for hypothesis of pollen limitation on seed production

| | Seed set - P1 | | | | | Seed set - P2 | | | | |
Test	Mean[2]	SD	N[1]	AS(%)[3]	AF(%)[3]	Mean[2]	SD	N[1]	AS(%)[3]	AF(%)[3]
CT	0.060[a]	0.240	9	83.3	82.0	0.460	0.734	43	74.7	14.0
BC	0.260[ab]	0.751	12	53.6	76.0	0.720	0.948	49	64.4	2.0
HS	0.120[a]	0.435	7	57.1	86.0	0.280	0.573	27	75.0	46.0
HL	0.520[b]	0.953	22	55.2	56.0	0.440	0.705	39	60.8	22.0

[1] Includes only fruits with mature seeds (seed set>0)
[2] Means followed by the same letter did not differ by a posteriori Tukey test, p>0,050.
[3] Chi-square comparisons, all significative for p=0.05: P1 - AS, df=3, c^2=23.98; AF, df=3, c^2=60.64; P2 - AS, df=3, c^2=31.17; AF, df=3, c^2=88.00.

between individual plants and pollination tests in P1 (N=200, individual plant df=9, F=2.216, p=0.023, pollination test df=3, F=4.548, p=0.004), but not in P2 (N=200, individual plant df=9, F=1.252, p=0.266, df=3, F=2.118, p=0.099). Differences in P1 occurred because of the higher seed set with high load of cross-pollen (Table 6), but this treatment was not different from bee control.The proportion of fruit abortion was higher for high load of self-pollen, in both patches (Table 6), and seed abortion was higher for control and high load of self-pollen.

DISCUSSION

Triumfetta semitriloba could be classified as a facultative autogamous species, sensu Cruden (1976), considering reproductive characteristics such as (Graumann and Gottsberger 1988). pollen-ovule ratio (mean of 161.572, SD=10.221), small flowers with low nectar production (Collevatti et al. in prep.), and the observed pattern of seed set in autogamy. Seeds were initiated and matured even in the absence of pollinators, although self-pollination induced a high rate of fruit abortion. Nevertheless, seed initiation and seed set were higher in the presence of bee pollinators, in relation to self-pollination. Differences in seed production within and among patches could be explained by differences in patch "quality" (Haig and Westoby 1988). Patch P1 was the spatially and temporarily more heterogeneous one, situated on a hill. Plants suffered water stress during the dry season (May to September), losing almost all leaves. Therefore, it is possible that a large part of metabolites had been allocated to leaf growth in the summer, before flowering in autumn. Patch P2 was localized along a river-side and plants suffered water stress (there was no leaf fall), so more resources were available and could be allocated to reproduction. These differences could result in a higher production of flowers in P2, which had higher density of plants either (Collevatti et al. in prep.). Patches with higher density of flowers would attract more bees, resulting in a higher rate of pollination (Stephenson 1982, Pyke 1984). This may result in a higher initiation of seeds and seed set at P2, in flowers visited by bees (bee con-

trol). The lack of differences in P1 could be a result of lower patch "quality", regarding nutrient availability and lower rate of bee visitation. The highest fruit abortion occurred with self-pollination, especially for P1. Control showed the highest proportion of seed abortion, for both patches. In control flowers, self and cross-pollen had opportunities to reach the stigma. We hypothesized that these flowers could experience pollen competition, selective abortion (infanticide) and sibling rivalry (fraticide), that could have resulted in a high proportion of seed abortion (Haig and Westoby 1988, Shaanker, Ganeshaiah and Bawa 1988, Marshall and Folsom 1991). Results of pollen limitation on seed production showed that at P1 seed initiation may be limited by pollen, since seed initiation and seed set were increased by high load of cross-pollen. As explained above, this difference, probably occurred because of differences in patch "quality". Patch P1 had less flowers than P2, and probably attracted a lower number of bee pollinators. At P2, there was no increase of seed production - probably seed set is highly limited by nutrients in this patch. However, a high limitation of nutrients is probably occurring at P1, either, since increasing in seed set did not lead to a maximum production of seeds (six), in either patches. The proportion of fruit and seed abortion was higher for high load of self-pollen and control condition, in both patches. By aborting fruits with low seed number, or selectively aborting fruits with self-progeny, plants may improve the average "quality" of their offspring, eliminating fruits resulting from self-fertilization or were there has been less pollen competition for ovules (selective abortion). Thus fruits with few developing seeds, or high number of self-progeny are more likely to be aborted than those with many seeds (Stephenson 1981, Stephenson and Bertin 1983, Becerra and Lloyd 1992). Self-pollination may lead to a low seed initiation, and to higher fruit abortion, as shown by tests for role of pollinators on seed production, and on high load of self-pollen, on pollen limitation on seed production. Our study showed that *T. semitriloba*, as expected for a weed species, has a breeding system where seed production is guaranteed by self-pollination, but the presence of bee pollinators increases seed set, and probably improves seed quality by cross-pollination.

Differences between patches, showed that, for a plant distributed in well delimited patches, this species may experience spatial differences in reproductive success. Probably there are patches where seed production is limited by nutrients, and patches where seed production is limited by pollinators, and patches where seed production is limited by both, as our results showed for P1 and P2. For weed species, which are always invading new patches, there is a spatial and temporal imprevisibility in patch "quality" (pollinator services and nutrient availability) - so the maintenance of high number of flowers, despite the low seed set may have been evolutionary advantageous, as a "bet hedging" strategy, improving seed set and quality in "windows" of environmental favorability.

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