Effect of Pollen Source and Stigma Receptivity on Seed Production in Ten Cultivars of *Dianthus plumarius* L.

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ABSTRACT

Dianthus plumarius L. is usually propagated vegetatively, but seed propagation is an option. However, erratic and often poor seed setting can be a problem in *Dianthus* spp. Pollen viability, stigma receptivity and the effect of pollen source on seed set were therefore investigated in ten "Hammett" *D. plumarius* cultivars. Pollen viability was high (≥87%) in all ten cultivars. Hand pollination showed stigma receptivity was either nil or very low on the first day of flower opening, and generally peaked between the fourth and sixth days after flower opening, depending on cultivar. Pollen source determined the number of seeds produced, in that for some cultivars as pollen providers seed set was nearly double that when the same cultivars were pollen receivers. Seed set averaged 39% for all cultivars, but was greatest (64%) for cv. Far North when selfed, and lowest (14%) for cv. Spot On, also when selfed. Six of the cultivars were equally receptive to both self and cross pollination, but two (Far North and Neat and Tidy) produced more seeds after self pollination, while the other two (Spot On and Double North) produced more seeds after cross pollination. Reasons for the variability in seed setting are still to be determined.

Additional index words: garden carnation, pink, pollen viability, hand pollination, seed set, seeds per capsule.

INTRODUCTION

The garden pink (*Dianthus plumarius* L.) is also variously known as dianthus, pink and grass pink (Allwood, 1954). *D. plumarius* belongs to the family Caryophyllaceae, which is characterised by a spicy clove-like fragrance, and conspicuous flowers of different colours.

Dianthus spp. are usually propagated by cutting and layering, although their rooting ability may be poor (Hobbs, 1997), and the maintenance of virus free stocks by vegetative propagation is difficult (Arthur, 1984). Seed propagation greatly reduces virus transmission (Hobbs, 1997) and there is thus an increasing interest in *Dianthus* seed production (K.R.W. Hammett, pers. comm., 1996). However, erratic and often poor seed setting has been a problem in *Dianthus* spp. Shafi Bhat, John and Abdullah (1991), for example, reported that more than 50% of the flowers of *D. caryophyllus* L. are sterile, while in *D. plumarius* ovule fertility is believed to depend on cultivar and pollen source (K.R.W. Hammett, pers. comm., 1996).

Information on pollination and seed setting in *Dianthus* is limited (Shafi Bhat *et al.*, 1991) and although internationally there is some commercial seed production (K.R.W. Hammett, pers. comm., 1996), such information is usually kept "in house" due to the highly competitive nature of the industry (Hampton and Phetpradap, 1992).

The objectives of this study were therefore to examine pollen viability, ovule number, time of stigma receptivity and pollen source influence on seed production of *Dianthus plumarius* using ten cultivars bred in Auckland, New Zealand by Dr. K.R.W. Hammett.

MATERIALS AND METHODS

Individual plants of ten "Hammett" dianthus (*Dianthus plumarius* L.) cultivars (Far North, Far Out, Double North, Counterpart, Cross Over, Spot On, Neat and Tidy, Mary, Royal Velvet and Cloud Nine) were purchased from Seaview Nurseries Ltd., Manurewa. Upon receipt, plants were placed in a glasshouse at 19±3°C and grown on until shoots had 10-12 pairs of leaves.

A rooting medium (60% peat, 40% pumice, plus 300 g dolomite, 100 g lime, 100g 14:16:18 + trace elements per 100 litres medium) was prepared (Hewage, 1998) and placed into plug trays (4.5 x 5.0 cm plug holes). Cuttings were taken, the basal cut of the stem wounded, dipped in 8% Seradix 1 (Rhoune-Poulanic Ltd.), and planted in the rooting media. The trays were then transferred to a misting chamber for 20 days before being placed on an open bench in the glasshouse for another 14 days. Plants were then potted into 15 cm diameter plastic containers containing the same medium as used for the cuttings.

Pollen fertility and stigma receptivity

Three similarly sized plants from each cultivar were randomly selected and located at random on an ambient temperature glasshouse bench. Each plant was considered a replicate. Plants were watered as required without wetting the foliage. At flowering they were covered with polyethylene mesh (9 holes cm^{-2}) to exclude pollinating insects.

Flowers were emasculated before anthesis (24 h prior to the flower opening), and flowers were labelled on the day they opened. These dates were recorded. Flowers were then hand pollinated using fresh pollen taken from another plant of the same cultivar on each day from one to seven days after flower opening, and allowed to set seeds. At each time, five to six

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flowers were pollinated from each plant. The mesh was removed once hand pollination was completed.

To estimate pollen stainability, which is an assessment of pollen fertility, three to four anthers selected at random from plants of each cultivar were dehisced over a glass slide to which a drop of 2% acetocarmine was added. The material was then covered with a coverslip. After 10 minutes of staining the percentage of plump, fully stained grains was determined.

A further fifteen flower buds from each cultivar were randomly selected and emasculated before the anthers had matured. A small paper bag was then tied over each flower. For another six randomly selected flowers from each cultivar the ovaries were dissected under a binocular microscope, and the number of ovules per ovary counted.

Seed capsules which developed were harvested by cutting them from the stem between 28-32 days after pollination. Each capsule was then carefully opened, and the number of fully developed seeds (black and generally > 3mm in diameter; Hewage, 1998) recorded.

The effect of pollen source

Ten plants from each cultivar were selected randomly and arranged at random on the glasshouse bench. Immediately before flowering all flower buds except the top one were removed. These top bud flowers were emasculated 24 h before anthesis and plants covered with pollinator exclusion cages. Flowers were pollinated on the fourth day after flower opening, after first checking that stigmas were free of pollen. Each cultivar was pollinated with pollen taken from itself and every other cultivar. Pollen was obtained from extra plants grown in the glasshouse and was collected immediately after the anthers had opened.

The pollinator exclusion cages were removed once pollination was completed. Seeds were harvested between 28-32 days after pollination as already described.

RESULTS

Pollen fertility and stigma receptivity

All ten cultivars produced highly viable pollen (Table 1), the range being from 87% in cv. Neat and Tidy to 100% in cv. Far North, Mary and Cloud Nine. However, ovule number varied widely, ranging from 69 per ovary in cv. Mary to 131 per ovary in cv. Far Out (Table 1). None of the cultivars produced seeds when their flowers were emasculated before flower opening.

Stigma receptivity differed among the cultivars. Stigmas of cv. Far North, Double North, Mary and Cloud Nine were receptive on the first day of flower opening (Table 2), producing a mean of 12.5, 9.0, 7.0 and 12.2 seeds per capsule, but the remaining cultivars were not receptive to pollen until the second day after flower opening (Table 2). The greatest number of seeds per capsule was produced after pollination on the third day for cv. Cloud Nine, fourth day for cv. Counterpart, Cross Over, Double North, Far Out and Royal Velvet, fifth day for cv. Far North and Neat and Tidy, and sixth day for cv. Mary and Spot On (Table 2), but differences among days within a cultivar were often not significant. In general, the stigmas of all cultivars were most receptive to

Table 1. Pollen viability, ovule number and maximum seed set in ten cultivars of *Dianthus plumarius* L.

Cultivar	Pollen viability (%)	Number of ovules per ovary	Maximum seed set (%)[1]	
			self	cross
Far North	100	84ef[2]	64	46
Far Out	97	131a	44	35
Double North	97	115c	23	34
Counterpart	90	126b	30	24
Cross Over	93	113c	26	39
Spot On	92	78f	14	26
Neat and Tidy	87	79f	57	44
Mary	100	69g	-	-[3]
Royal Velvet	99	89e	37	37
Cloud Nine	100	99d	53	48

[1] *Calculated using the number of seeds per capsule data from Table 4 for self and cross pollination.*

[2] *Means within columns with the same letter are not significantly different at P<0.05.*

[3] *Data not available, although for selfing (Table 2) maximum seed set was 54%.*

Table 2. Effect of time of hand pollination on seed number per capsule in ten *Dianthus plumarius* cultivars.

Pollination time (days after flower opening)	Mean number of seeds per capsule									
	Far North	Far Out	Double North	Counterpart	Cross Over	Spot On	Neat and Tidy	Mary	Royal Velvet	Cloud Nine
1	12.5b[1]	0.0b	9.0c	0.0c	0.0b	0.0b	0.0c	7.0b	0.0b	12.2c
2	15.2b	8.0b	25.8b	5.4c	2.4b	0.1b	5.4c	15.2b	3.0b	24.2bc
3	30.0a	15.8ab	30.6b	27.4ab	34.4a	1.2b	21.6b	16.2b	20.2a	45.0a
4	35.2a	34.0a	42.2a	37.8a	37.6a	7.8a	35.4ab	31.8a	29.2a	40.8c
5	36.8a	21.4ab	29.8b	18.2b	34.8a	7.4a	41.3a	34.4a	2.8b	39.8ab
6	23.4ab	18.6ab	29.0b	30.3ab	37.6a	8.4a	20.4bc	37.6a	4.0b	43.6a
7	23.2ab	14.6ab	22.0b	15.2c	35.6a	8.2a	32.4ab	33.2a	4.8b	44.8a

[1]*Means within columns with the same letter are not significantly different at P<0.05.*

Table 3. Effect of pollen source[1] on seed number per capsule in ten *Dianthus plumarius* cultivars.

Pollen donor (♂)	Pollen receiver (♀)									
	Far North	Far Out	Double North	Counterpart	Cross Over	Spot On	Neat and Tidy	Mary	Royal Velvet	Cloud Nine
Far North	**54.2a**[2,4]	55.0ab	36.0cd	29.4cd	65.0a	18.6bc	44.2ab	33.0ab	43.8ab	66.8a
Far Out	47.6ab	**57.0ab**	39.4bc	31.4c	38.0c	16.4cd	31.8f	-[3]	30.4c	50.6bc
Double North	21.6f	34.0de	**26.8e**	28.0cd	27.4d	3.0e	20.2g	28.2bc	13.4d	37.6de
Counterpart	26.1ef	31.2e	29.2e	**33.8b**	24.6de	16.2cd	34.2def	-	14.4d	49.2bcd
Cross Over	32.6de	43.8cd	31.2de	16.6e	**29.4cd**	24.8ab	37.0cde	20.8d	31.2c	31.8e
Spot On	39.0cd	39.2cde	49.0a	24.0d	16.6e	**11.2d**	33.2ef	22.4d	27.2c	42.0cde
Neat and Tidy	41.2bc	59.0a	32.0de	31.6c	57.6ab	29.6a	**45.4a**	-	43.0ab	47.4bcd
Mary	54.6a	44.4cd	46.0a	38.2b	57.6ab	22.8b	38.6cd	-	50.4a	56.2ab
Royal Velvet	38.8cd	47.0bc	46.4a	45.2a	54.2b	21.7bc	48.8bc	24.2cd	**33.0c**	49.6bcd
Cloud Nine	48.4a	60.6a	44.8ab	30.8c	58.4ab	29.6a	36.0def	37.4a	41.4b	**52.6bc**

[1]*Hand pollination in a glasshouse.*
[2]*Data in bold are the combinations for self pollination.*
[3]*No data because flowers not available.*
[4]*Means within columns with the same letter are not significantly different at P<0.05.*

Table 4. *Dianthus plumarius* cultivar comparison.

Cultivar	Seeds per capsule			
	As a pollen receiver (♀)	As a source of pollen (♂)	After cross pollination	After self pollination
Far North	40.4abcd[1]	45.9a	38.8abc	54.2a
Far Out	47.1ab	38.0abc	46.0a	57.2a
Double North	38.1bcd	23.6d	39.3abc	26.8e
Counterpart	30.9d	28.8cd	30.6c	38.1cd
Cross Over	42.9abc	30.9cd	44.4ab	29.4e
Spot On	19.4e	31.3bcd	20.3d	11.2f
Neat and Tidy	36.1cd	43.0 a	35.1bc	45.4bc
Mary	-[2]	45.4a	-	-
Royal Velvet	32.8d	41.9ab	32.8c	33.0de
Cloud Nine	48.4a	44.7a	47.9a	52.6ab

[1]*Means within columns with the same letter are not significantly different at P<0.05.*
[2]*No data because flowers were not available.*

pollen somewhere within the time frame of three to seven days after flower opening.

The effect of pollen source

The crossing combination was important in determining the number of seeds produced (Table 3). For example, the crossing combination of cv. Cross Over (♀) and Far North (♂) resulted in 65 seeds per capsule, whereas the same plants in the opposite combination (Cross Over (♂) and Far North (♀)) produced only 33 seeds per capsule. Similarly cv. Far Out (♀) x Neat and Tidy (♂) produced 59 seeds per capsule whereas cv. Neat and Tidy (♀) x Far Out (♂) produced only 32 seeds per capsule (Table 3).

Cv. Spot On was the poorest seed producer among all the cultivars (Tables 3 and 4), producing only 3 seeds per capsule when cv. Double North was the pollen source. However, the performance of cv. Spot On as a pollen source was considerably better (Table 4; eg. the reverse combination produced 49 seeds per capsule).

Many of the cultivars were equally receptive to both self and cross pollination (Table 4) but some (eg. cv. Far North and Neat and Tidy) produced more seeds after self pollination, while others (eg. cv. Spot On and Double North) produced more seeds after cross pollination.

Seed set differed with cultivar and pollen source (Table 1), although when meaned for all cultivars seed set did not differ between self (39%) or cross (37%) pollination. The greatest seed set (64%) was for cv. Far North when selfed, while the lowest (14%) was for cv. Spot On, also when selfed.

DISCUSSION

Plants of all ten cultivars produced highly viable pollen, and pollen tubes were successfully produced once pollen reached the stigma (Hewage, 1998). Therefore the potential for fertilisation was high.

The fact that none of the cultivars produced seeds when their flowers were emasculated before flower opening suggests that there is no mechanism for self pollination before this time in *D. plumarius* (although *D. silvestris* Wulf. produces cleistogamous flowers that do not bloom but produce seeds; Erhardit, 1988). Most of the *D. plumarius* cultivars studied were either not receptive to pollen, or only partially receptive to pollen at the onset of flower opening. Receptivity to pollen increased with stigma maturation, and stigmas were most receptive at, or after, the fourth day after flower opening. *Dianthus* spp. are normally considered to be cross pollinated (Buell, 1952) and the plant achieves this by maturing the anthers before the stigmas become receptive (Shafi Bhat *et al.*, 1991). Hewage (1998) also found that for cultivars Spot On and Double North, stigma receptivity did not change with pollen source (ie. cross or self), although the number of seeds per capsule was greater following self pollination in these two cultivars than when they were crossed with each other, and the greatest number of seeds was produced following pollination on the fourth to sixth day after flower opening.

While cultivars differed significantly in the number of ovules per ovary, this was not necessarily a factor in the number of seeds produced per capsule, which depended more on pollen source. For example when cultivar Far North supplied the pollen over 50 seeds per capsule were recorded from cultivars Far Out, Cross Over and Cloud Nine, but when cultivar Cross Over supplied the pollen less than 32 seeds per capsule were recorded from cultivars Double North, Counterpart, Spot On, Mary, Royal Velvet and Cloud Nine. The female parent also had a significant effect on the success of seed production, with cultivar Spot On producing a lower number of seeds than most other cultivars irrespective of the pollen source. Reasons for this variable response may include some degree of incompatibility between the parents, the failure of the pollen tube to reach the embryo, slow pollen tube growth, fertilisation failure or abortion of fertilised ovules, but as this was not determined in this study further investigation is necessary.

Aizen, Searcy and Mulcahy (1990) found that *D. chinensis* produced more seeds when cross pollinated than self pollinated, and suggested that this was because of inter-stylet resistance which makes self pollen grow more slowly through the style, resulting in lower seed set. However, in the ten *D. plumarius* cultivars only three (Double North, Cross Over and Spot On) appeared to produce more seeds after crossing than selfing, while cultivars Far North and Neat and Tidy produced more seeds after selfing than crossing. These results do not indicate any self-incompatible mechanism in *D. plumarius* as all the cultivars were, to varying extents, self compatible. Self compatibility is a general trait in the Caryophyllaceae (Erhardit, 1988).

Maximum seed set varied from 14 to 64% depending on cultivar and pollen source, and therefore seeds per capsule varied from 3 to 67. These results were obtained after careful hand pollination in a controlled environment. There is obviously considerable scope for further examination of the factors determining seed set in *D. plumarius*.

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