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SOME OBSERVATIONS ON THE
REPRODUCTIVE BIOLOGY OF THREE SPECIES OF
LONICERA L. (CAPRIFOLIACEAE)

LA.U.N. Gunatilleke and C.V.S. Gunatilleke

Department of Botany, University of Peradeniya, Sri Lanka.

ABSTRACT

Breeding experiments with three introduced species, Lonicera deflexicalyx Batl., L. gracilipes Miq. and L. tatarica Reg. reveal them to be partially self-compatible, but the capacity for out-crossing and successful open-pollination to be significantly greater than that for selfing. Stigma receptivity during the entire period of bloom in all three species suggests an exception to the protandrous condition previously suggested for this genus. Interspecific crosses between these species result in fruit yield ranging from 25-91 percent which may account for the phenotypic variation observed among cultivated plants of these species.

INTRODUCTION

Lonicera spp. (Caprifoliaceae), commonly called honeysuckles, are deciduous or more rarely evergreen

ornamental lianes and shrubs grown for their attractive flowers and fruits. While about 25 species occur naturally in North America, over 100 species have been introduced into cultivation there (Rehder, 1940; Ferguson, 1966). These are known to produce natural hybrids in cultivation (Ferguson, 1966) and some of the introduced species, e.g. L. japonica, have become "a ruthless pest" in some parts of the United States (Hardt, 1986). Many species are reported to be protandrous and the cultivated species with long corolla tubes are pollinated by long-tongued insects or by humming-birds while those with short tubes are visited by various Diptera and Hymenoptera (Ferguson, 1966).

ability
Especially because of their ornamental value, to hybridise in nature and reported aggressive "weed-like" growth of some species, we examined the breeding biology of three introduced and now commonly cultivated species of Lonicera growing at the Case Estate of the Arnold Arboretum, Weston, Massachusetts, USA. They are Lonicera deflexicalyx Batl., L. gracilipes Miq. and L. tatarica L., all introduced to the United States from Eastern Asia between 1855 and 1955 (Rehder, 1940). Although none of the individuals selected for study was a horticultural variety (according to records kept at the Arnold Arboretum), their flower and fruit colour showed some degree of intraspecific variation, possibly a result of natural hybridisation while in cultivation as suggested by Ferguson (1966).

MATERIALS AND METHODS

Two individuals each of L. deflexicalyx (Arnold Arboretum accession nos. 29-71, 285-71), and L. gracilipes (102-71A and 102-71B), and one individual of L. tatarica L. (284-71) growing in the shrub collection at the Case Estate were used for phenological observations and breeding experiments. Records of the Arnold Arboretum (Case Estate Nursery Inventory, 1981) indicate that the above individuals were raised from seeds received from Botanic Gardens either in the United States or overseas.

In order to examine the breeding systems of these three species, enforced self- and cross-pollinations were carried out using both emasculated and non-emasculated flowers. Emasculatation was done at late bud stage prior to anther dehiscence and both emasculated and non-emasculated flowers were kept free of contamination from extraneous pollen using pollination bags (Pollen-Tector No. 850/OT). Selfing

was carried out with pollen from different flowers of the same plant (geitonogamy). Presence of agamospermy was examined by enclosing emasculated flower buds without any pollination and observing for seed set apparently without sexual fusion. Examination of pollen tube germination was done using fluorescein microscopy as described in our previous paper on Cornus species (Gunatilleke and Gunatilleke, 1984). The capacity to form interspecific hybrids was examined in these three widely cultivated species by several reciprocal crosses made between them. Regular phenological observations of flowering and fruiting of these species, as well as hourly observations on diurnal visitors during the flowering period, were also made.

An attempt was made to determine the time period of stigma receptivity by using moist peroxidase paper (Perotesto KO) which changes from white to blue upon contact with the respective stigmatic surface. The data from these breeding experiments were analysed statistically using a 2-way ANOVA test (Sokal & Rohlf, 1969).

RESULTS

Floral and fruit morphological features such as size and colour as well as the nectar production in flowers were examined in all three species (Table 1).

There was no fruit set in emasculated and bagged flowers in any of the three species examined (1a in Table 2). In L. deflexicalyx and L. gracilipes, the intraspecific variation in percent fruit set for each of the treatments examined was found to be non-significant ($P < 0.05$). As we had only monitored one individual of L. tatarica in our experimental area, intraspecific crosses could not be made with this species. The capacity of outcrossing determined by fruit-set after cross-pollination treatments in both L. deflexicalyx and L. gracilipes ranged between 40% and 76% (1c and 2c in Table 2) and was significantly higher ($P < 0.05$) than the capacity for selfing 2-30% (Table 2 lines 1b and 2b). Open-pollinated flowers of all three species set 40-90% fruit (1d and 2d in Table 2); this was not significantly higher than the fruit-set in artificially cross-pollinated flowers but significantly higher than that in selfed flowers. However, differences between emasculated and non-emasculated floral treatments in these two species proved to be statistically non-significant at the 5% level when tested by the 2-way ANOVA test. In L. tatarica, fruit-set in open-

pollinated flowers was much higher than in its selfed treatments, irrespective of whether emasculated or non-emasculated flowers were used. As no cross-pollination was done in L. tatarica, the data for this species were not included in the statistical analysis.

In the interspecific pollination experiments done with emasculated flowers, one individual of L. deflexicalyx (no. 29-71) was used as both male and female parent for crosses with L. gracilipes and L. tatarica; the latter two species were not crossed with each other as the additional work involved in emasculations and hand pollinations could not be accommodated within the relatively short flowering period. Reciprocal crosses made between L. deflexicalyx and L. gracilipes or L. tatarica gave a wide variation in the fruit-set; eg. in crosses with L. gracilipes, more fruit set (64%) was observed when L. deflexicalyx was used as female parent than when used as the male parent (25%). However, in crosses with L. tatarica, L. deflexicalyx used as the female parent set less fruit (35%) than when used as the male parent (91%). The results also indicate that L. deflexicalyx (plant no. 29-71) shows a greater compatibility to pollen of L. gracilipes than to pollen of L. tatarica (284-71). On the other hand, pollen of L. deflexicalyx (29-71) seem to be more acceptable to L. tatarica (284-71) than to L. gracilipes (102-71B).

Microscopic examination of the stigmas and styles under fluorescent light revealed no apparent differences in pollen germination and pollen tube growth between selfed and cross-pollinated flowers in each of the species studied. Although both selfed and crossed flowers showed pollen tubes traversing the entire length of the style and entering the ovary, the method employed did not clearly show whether the pollen tubes entered the ovules or not.

When stigma receptivity of the three species was examined using peroxidase (Peroxtesmo KO) paper, it was found that in all of them, the moist peroxidase paper turned blue when placed upon stigmas at different stages of development, from bud to corolla-withering stage. Under the microscope, papillae were also observed on the stigmatic surface in all three species examined. These papillae were tightly packed in the mature bud stage but became more lax and much enlarged as the stigmatic surface broadened with the development of the flower.

All three species produced nectar which was

contained in a small sac on one side, at the base of the corolla tube. Apis mellifera and Bombus species were seen visiting these flowers frequently during the day and foraging both on the nectar and pollen of the flowers from all three species. The bodies of the bees visiting these flowers were profusely covered with pollen and they could well be the most probable pollinators.

The species studied were in leaf at the time of flowering, which commenced about the first week of May and terminated by mid-June 1983 in our experimental plants and the duration of bloom in each species was between 4 and 5 weeks (Figure 1). Longevity of individual flowers (i.e. between opening and withering of the corolla) was found to be 4 to 5 days in these three species. Anther dehiscence in all of them occurred just after anthesis.

Mature fruits of all three species are as ornamental as their flowers and took 6 to 7 weeks to ripen. These fleshy fruits were observed to be eaten by birds.

DISCUSSION

The results of breeding experiments performed indicate that Lonicera gracilipes and L. deflexicalyx are out-crossing species with partial self-compatibility; their potential to outcross is significantly higher than that for selfing (Table 2). The longer duration of stigma receptivity, as determined by the peroxidase paper test, suggests that these flowers have a longer period available for cross-pollination. These species are also capable of self-pollination and eventual but reduced fruit-and seed-set even in the absence of cross-pollination. Although a variation in percent fruit-set in corresponding treatments existed between individuals of the same species, these differences were statistically non-significant. Similarly, the emasculation procedure did not alter the amount of fruit-set significantly in any of the treatments.

Lonicera tatarica shows a capacity for selfing, comparable to that of the other two species, but nothing conclusive could be said about its ability for outcrossing as this was not examined. The higher percentage of fruit-set in open-pollinated flowers (68-71%) of L. tatarica, at least in part, could be due to interspecific hybridisation.

The absence of fruit-set in emasculated and bagged flowers of all three species (1a in Table 2) indicates that no agamosperous or apomictic fruit-set occurs under these conditions. The small percent of fruit-set observed in non-emasculated and bagged flowers (1b in Table 2) comparable to that in selfed treatments in these species suggests that the fruit-set in 1b could either be due to unaided self-pollination inside pollination bags or due to pseudogamy, stimulated by deposition of self-pollen on their stigmatic surfaces.

Similar patterns of pollen tube growth observed in selfed and crossed flowers of Lonicera deflexicalyx and L. gracilipes also provide indirect evidence for partial self-compatibility of these species.

The greater variation observed in percent fruit-set resulting from reciprocal crosses between different species, as compared to reciprocal crosses made within the same species (Table 3) merits further examination of factors that may bring about these differences; some among them could be pollen viability, germination and rate of pollen tube growth on stigmas of reciprocally pollinated flowers in each treatment (unidirectional compatibility?). However, these questions were not addressed in this study. The results obtained nevertheless confirm the view that these species can easily hybridise when brought together in cultivation (Ferguson, 1966). This may indeed have contributed to the morphological variation observed between cultivated individuals of two of the species studied (Table 1).

Peroxidase paper test indicates that stigmas are receptive during the entire period of bloom and even in the bud stage in all three species examined and therefore, these species do not seem to have the protandrous conditions suggested for this genus by Ferguson (1966).

The apparent ease with which these species produce interspecific hybrids and the prolific production of brightly coloured berries effectively disseminated by birds could pose the potential danger of some of these new hybrids becoming more aggressive colonizers than their ancestors who were introduced to the United States a century or so ago.

TABLE 1

FLORAL AND FRUIT CHARACTERISTICS OF
THREE SPECIES OF LONICERA

	<u>L. deflicalyx</u>	<u>L. gracilipes</u>	<u>L. tatarica</u>
Pedicel length	12-20 mm	15-17 mm	15-17 mm
Flower length	15-17 mm	15-17 mm	15-17 mm
Flower colour	White/light pink	Pink	white
Nectar	Present	Present	Present
Fruit	Sessile, ellipsoid berry	Sessile, ellipsoid berry	Sessile ovoid berry
Fruit size	5 x 7 mm	7 x 7 mm	8 x 6 mm
Ripe fruit colour	scarlet red	scarlet red/orange	scarlet red

TABLE 2

PERCENT FRUIT-SET IN DIFFERENT POLLINATION TREATMENTS IN THREE SPECIES OF LONICERA

Plant No.	<u>Lonicera deflexicalyx</u>				<u>Lonicera gracilipes</u>				<u>Lonicera tatarica</u>	
	29 - 71		285 - 71		102 - 71B		102 - 71A		284 - 71	
	No. of flowers	% fruit set	No. of flowers	% fruit set	No. of flowers	% fruit set	No. of flowers	% fruit set	No. of flowers	% fruit set
1a. Flowers emasculated and bagged	110	0	134	0	87	0	98	0	109	0
2a. Flowers non-emasculated and bagged	137	1	114	12	50	8	122	20	145	4
1b. Flowers emasculated, selfed and bagged	95	6	96	30	70	4	78	17	105	10
2b. Flowers non-emasculated, selfed and bagged	120	16	118	18	113	2	88	23	122	8
1c. Flowers emasculated, crossed and bagged	46	70	112	60	122	53	79	67	-	-
2c. Flowers non-emasculated and bagged	105	40	130	58	109	76	144	76	-	-
1d. Flowers emasculated and open pollinated	58	45	116	67	60	40	110	70	89	71
2d. Flowers non-emasculated and open pollinated	88	43	135	52	114	62	197	90	184	68

TABLE 3

PERCENT FRUIT-SET IN INTRA- AND INTERSPECIFIC CROSSES
IN LONICERA SPECIES

		<u>L. deflexicalyx</u> (29 - 71)		<u>L. gracilipes</u> (102 - 71A)	
		As Male Parent	As Female Parent	As Male Parent	As Female Parent
<u>L. deflexicalyx</u> (285 - 71)	As Male Parent	-	70	-	-
	As Female Parent	60	-	-	-
<u>L. gracilipes</u> (102 - 71B)	As Male Parent	-	64	-	67
	As Female Parent	25	-	53	-
<u>L. tatarica</u> (287 - 71)	As Male Parent	-	35	-	-
	As Female Parent	91	-	-	-

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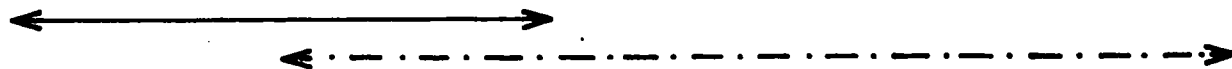
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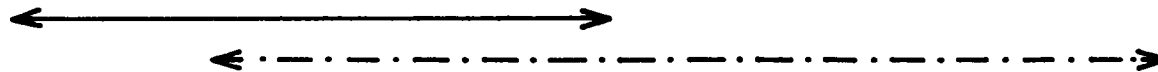
LEGENDS TO FIGURE

Figure 1: Duration of bloom (————) and duration of fruit development (-.-.-.-.-) in Lonicera species at Case Estates of the Arnold Arboretum, USA.

L. deflexicalyx



L. gracilipes



L. tatarica

