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Pollen Viability in Lychee

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Abstract. **The lychee (*Litchi chinensis* Sonn.) has two types of pollen-releasing flowers—M_1 and M_2. We compared the morphology and viability of these two pollen types, mainly for the two commercial cultivars in Israel: 'Mauritius' and 'Floridian'. Observation by scanning electron microscope did not reveal any consistent morphological differences between the two pollen types. However, M_2 pollen was found to have a consistent and significant advantage over M_1 pollen in in vitro germination tests. M_2 pollen from 'Mauritius', 'Floridian', 'No Mai Chee', 'Wai Chee', and 'Early Large Red' had a much higher germination rate at 15, 20, 25, 30, and 35 °C than M_1 pollen from those same cultivars. The optimal incubation temperature for in vitro pollen germination was 30 °C for M_2 pollen of all five cultivars studied; adequate germination rates were also found at 35 and 25 °C. The optimal temperature for M_1 pollen germination was also 30 °C for 'Mauritius' and 'No Mai Chee', but was not well defined for the other three cultivars. No pronounced advantage of M_2 pollen-tube growth could be discerned 48 h after hand pollination. However, final fruit set was consistently and significantly higher after hand pollination with M_2 pollen, relative to M_1 pollen. Hot (32/27 °C) and warm (27/22 °C) regimes during flower development had a pronounced detrimental effect on pollen viability compared to a cool (22/17 °C) regime. 'Floridian' was much more susceptible than 'Mauritius' in this respect.**

The lychee (*Litchi chinensis*) has three types of flowers: one female and two males (M_1 and M_2), which are all functionally unisexual (Joubert, 1986; Tindall, 1994). As a rule, the three types are found on all inflorescences, coming into anthesis in three distinct waves: the first consists of the male (M_1) bloom, the second is the female bloom, and the third is a pseudohermaphroditic (M_2) bloom (Galan-Sauco and Menini, 1989; Stern et al., 1993b; Stern and Gazit, 1996). Few studies have dealt with the morphology and viability of pollen from the two male types.

Under a light microscope, the lychee pollen grain is elongate, ≈10 µm wide and 20 µm long, with three germination pores (Mustard et al., 1953; Singh, 1962). Normal grains are triangular, whereas abnormal ones are quadrangular (Liu, 1954). In these studies, no distinction was made between the two pollen types.

Lychee pollen viability has been determined in vitro in several studies, using the hanging-drop technique (Costes, 1988; Mustard et al., 1953; Singh, 1962). Singh (1962) found a higher germination rate of M_1 as compared to M_2 pollen (62% and 48%, respectively) in 'Calcutta'. He did not find any differences in pollen-tube growth between the two pollen types. In contrast, Mustard et al. (1953) and Costes (1988), reported higher germination rates for M_2 pollen.

The poor productivity of lychee in Israel prompted us to examine potentially responsible factors (Stern et al., 1993a, 1995, 1996, 1997; Stern and Gazit, 1996). We describe the viability of pollen from M_1 and M_2 flowers and the effect of temperature on that viability. Most of the work was carried out with the two commercial cultivars in Israel: 'Mauritius' and 'Floridian'.

Materials and Methods

Morphology of pollen grains, SEM study. Pollen from open M_1 and M_2 anthers was scattered on stubs, critical-point dried for 90 min with liquid CO_2 (Hayat, 1978), and gold-coated. Examination was carried out in a Jeol JSM 35C SEM.

In vitro pollen germination. Pollen collected from small plants kept in the phytotron and from mature orchard trees was germinated by the hanging-drop technique (Singh, 1962) in a medium composed of 0.3 M sucrose plus 100 ppm boric acid (Shalem-Galon, 1980). Each treatment consisted of five petri dishes, with five drops per dish. Each drop contained ≈50 pollen grains.

In vivo pollen germination. Two-year-old flowering 'Mauritius' and 'Floridian' plants were taken on 1 Apr. at their female flowering stage (Joubert, 1986) from a nursery and placed in the Faculty of Agriculture phytotron under one of four temperature regimes: 17/12 °C, 22/17 °C, 27/22 °C, or 32/27 °C (Stern et al., 1996). Two-day-old female flowers were hand pollinated with

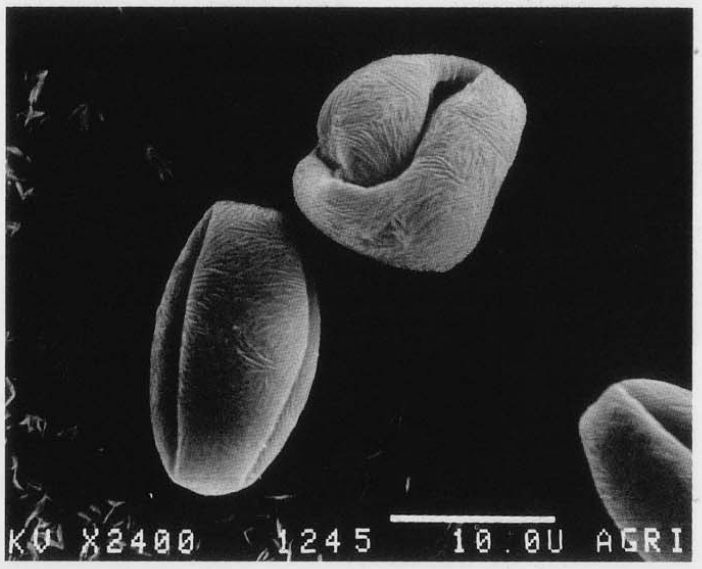

Fig. 1. Scanning electron micrograph of normal (**bottom**) and degenerate (**top**) 'Mauritius' lychee pollen grains (×2400).

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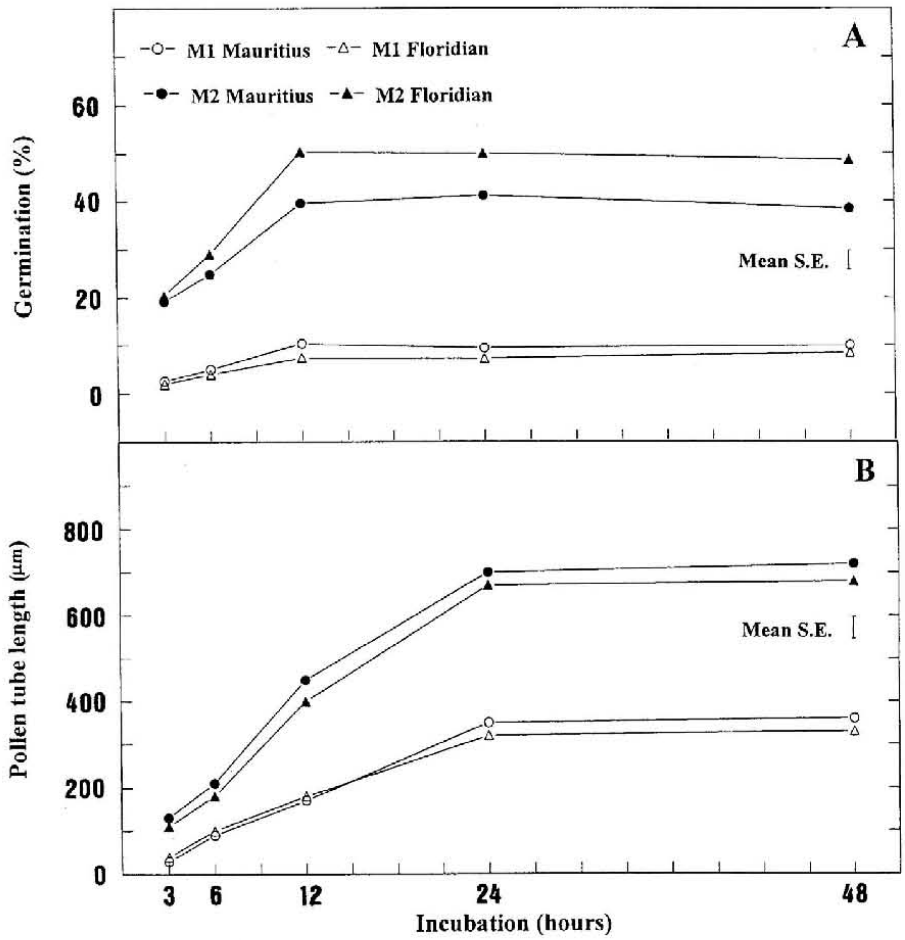

Fig. 2. In vitro germination of M_1 and M_2 pollen from 'Mauritius' and 'Floridian' after incubation at 3, 6, 12, 24, and 48 h at 25 °C. (**A**) Percentage of germinating pollen grains. (**B**) Pollen-tube length. Data are the means of ≈50 pollen grains per drop × 25 drops for germination and ≈50 pollen grains per drop × 5 drops for tube length.

dehisced M_1 and M_2 'Mauritius' stamens by touching the stigma with a pollen-laden anther. The pollinated flowers were picked after 48 h, fixed in 2 ethanol : 1 acetic acid, and later examined under a fluorescent microscope for pollen germination and pollen-tube growth (Stern and Gazit, 1996).

Effect of temperature regimes during flower development on pollen viability. Two-year-old 'Mauritius' and 'Floridian' plants with emerging inflorescences were placed in the Faculty of Agriculture's phytotron on 1 Feb. under one of three temperature regimes: 22/17 °C (cool), 27/22 °C (warm), or 32/27 °C (hot). Flowering began ≈2 months later. Pollen from M_1 and M_2 flowers was germinated in vitro at five incubation temperatures (15, 20, 25, 30, and 35 °C).

Fruit set after hand pollination. Four inflorescences on each of six mature 'Mauritius' and 'Floridian' trees were covered with paper bags. At peak female bloom, 50 (1989) and 100 (1990) 2-day-old flowers were hand pollinated per inflorescence and all nonpollinated female flowers were removed. Each of the four inflorescences was pollinated uniformly with one of the following pollen types: M_1 or M_2 from 'Mauritius' or 'Floridian'.

Statistical analysis. All data on pollen viability (germination and pollen-tube growth) and fruitlet survival were analyzed using the general linear model (GLM) procedure of SAS. The effect of incubation time on germination and pollen-tube length for different pollen types and cultivars was analyzed by analysis of covariance. Duncan's multiple range test or Fisher test were used to compare pairs of treatments when

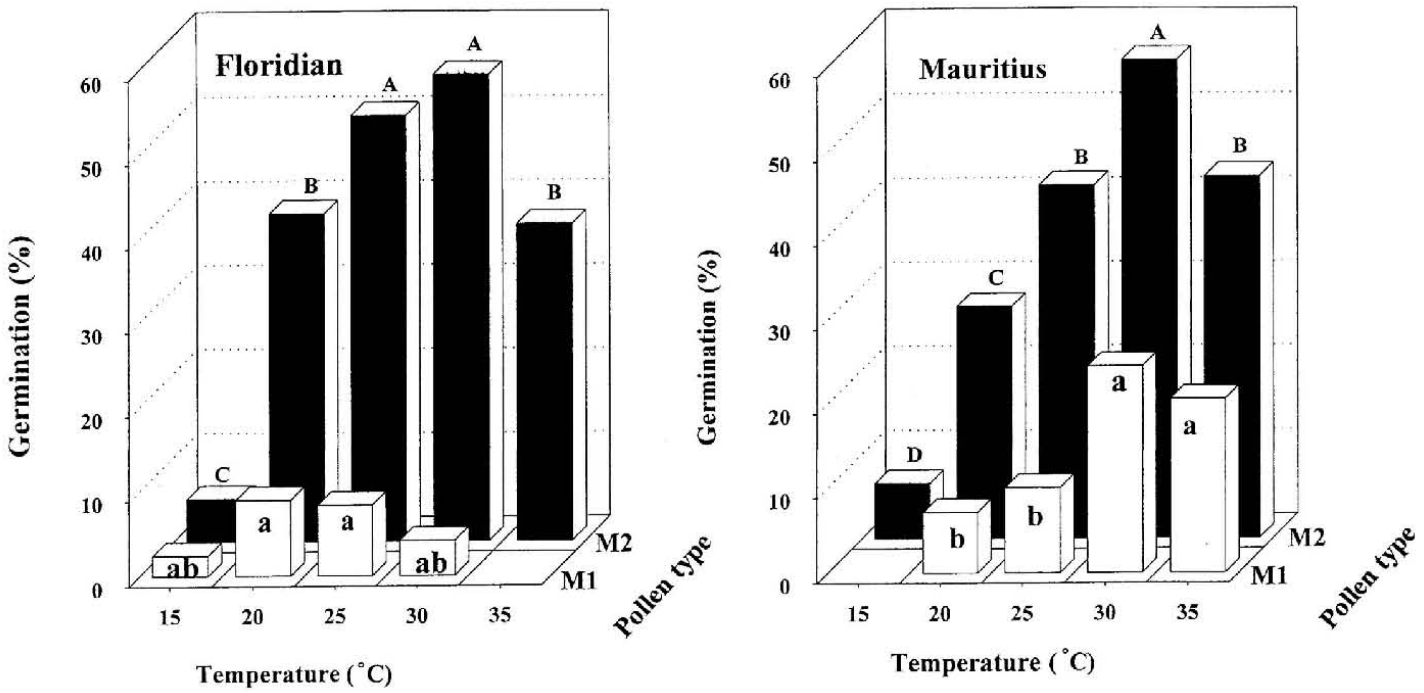

Fig. 3. Effect of five incubation temperatures on in vitro germination of M_1 and M_2 pollen from 'Mauritius' and 'Floridian'. Germination was determined after 24 h. Results from the same pollen type marked with different uppercase (M_2) or lowercase (M_1) letters differ significantly by Duncan's multiple range test, $P = 0.01$.

ANOVA showed significant differences among the means. Percentage data were subjected to arcsin transformation before analysis to provide for a normal distribution.

Results

Pollen-grain morphology. 'Mauritius' and 'Floridian' pollen from M_1 and M_2 flowers was examined by SEM at magnifications of 1000 to 10,000×. No noticeable differences were found between pollen grains from the two cultivars or the two flower types. Typical normal and degenerate pollen grains are shown in Fig. 1. The normal pollen grain was elongate; its length was 20 ± 2 μm and its width ≈10 ± 1 μm. It had three elongate germination pores. Degenerated pollen grains appeared at a low rate.

In vitro pollen germination. 'Mauritius' and 'Floridian' pollen from M_1 and M_2 flowers was incubated at 25 °C. Germination rate and pollen-tube length were determined after 3, 6, 12, 24, and 48 h. The maximal germination rate occurred after 12 h, whereas maximal pollen-tube length occurred after 24 h (Fig. 2 A and B, respectively). In all further in vitro pollen germination tests, an incubation time of 24 h was used. Germination rate and pollen-tube length were consistently and significantly higher for M_2 pollen (Fig. 2 A and B, respectively). No significant differences in these values were found between the two cultivars.

Effect of incubation temperature on in vitro pollen germination. 'Mauritius' and 'Floridian' pollen from M_1 and M_2 flowers was incubated at 15, 20, 25, 30, and 35 °C. No noticeable differences in germination rate were found between pollen collected from the phytotron (22/17 °C) or from the orchard; therefore, Fig. 3 contains the mean pollen germination rates from these two sources. In both cultivars, germination rate of M_2 pollen was consistently greater ($P = 0.01$) than that of M_1 pollen. The optimal incubation temperature for 'Mauritius' M_1 and M_2 pollen was 30 °C. 'Floridian' M_2 pollen germinated well at 25 and 30 °C, whereas M_1 pollen germinated best at 20 and 25 °C.

In a similar experiment, we studied the effect of the same incubation temperatures on germination of M_1 and M_2 pollen from 'Mauritius', 'Floridian', 'No Mai Chee', 'Wai Chee', and 'Early Large Red'. The results for 'Mauritius' and 'Floridian' were nearly identical to those presented in Fig. 3.

The germination rate of M_2 pollen was consistently greater ($P = 0.01$) than that of M_1 pollen in 'Wai Chee', 'No Mai Chee', and 'Early Large Red' (Fig. 4). The maximal germination rate reached 55% to 59% for M_2 pollen, but only 8% to 19% for M_1 pollen. The optimal temperature for M_2 pollen germination, was 30 °C in all cases (Fig. 4). The same temperature was also optimal for the germination of 'No Mai Chee' M_1 pollen, whereas the germination of 'Wai Chee' and 'Early Large Red' M_1 pollen was greatest at 25 °C.

Effect of temperature on in vivo pollen germination and pollen-tube growth. Due to the great similarity between the results for the two cultivars, mean values are presented in Table 1. In all pollinated flowers, pollen germinated and pollen tubes reached the base of the style. However, the hot temperature regime (32/27 °C) had a pronounced detrimental effect on further pollen-tube growth (Table 1). Pollen tubes reached the ovary in ≈20% of the flowers and in no flower did they reach the ovule. Under the cold regime (17/12 °C), pollen tubes reached the ovary in all flowers, but did not proceed any further. However, in this case, the

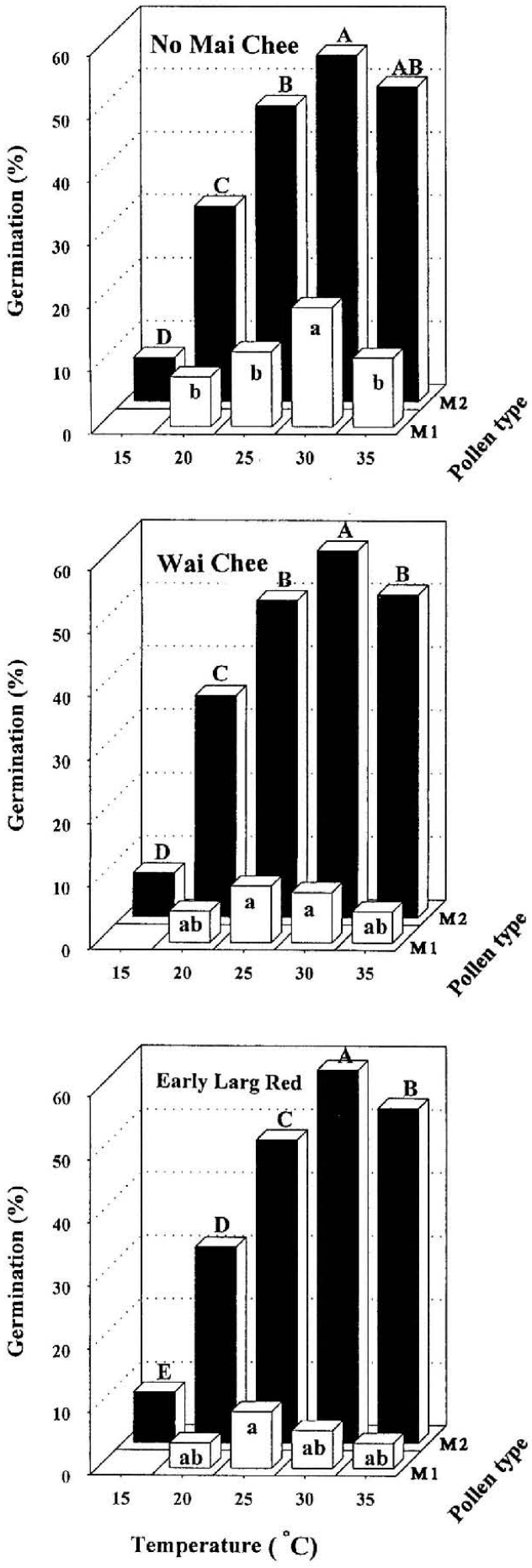

Fig. 4. Effect of incubation temperature on the in vitro germination of M_1 and M_2 pollen from 'No Mai Chee', 'Wai Chee' and 'Early Large Red'. Germination was determined after 24 h. Results from the same pollen type marked with different uppercase (M_2) or lowercase (M_1) letter, differ significantly using Duncan's multiple range test, $P = 0.01$.

Table 1. Pollen-tube location 48 h after hand pollination of 'Mauritius' and 'Floridian' flowers with M_1 and M_2 'Mauritius' pollen, at four temperature regimes. Data are means of the two cultivars, 50 flowers per cultivar per treatment (five trees).

Treatment		Percentage of flowers with pollen tubes reaching the		
Temp regime (°C)[z]	Pollen type	Ovary	Ovule	Micropyle
17/12	M_1	100	0	0
	M_2	100	0	0
22/17	M_1	100	38	0[y]
	M_2	100	34	4
27/22	M_1	100	34	1[y]
	M_2	100	35	6
32/27	M_1	23	0	0
	M_2	17	0	0

[z]Day/night cycle (16 h/8 h).
[y]The *P* value for the difference between M_1 and M_2 in the same temperature regime was 0.06 in each case, using the Fisher test.

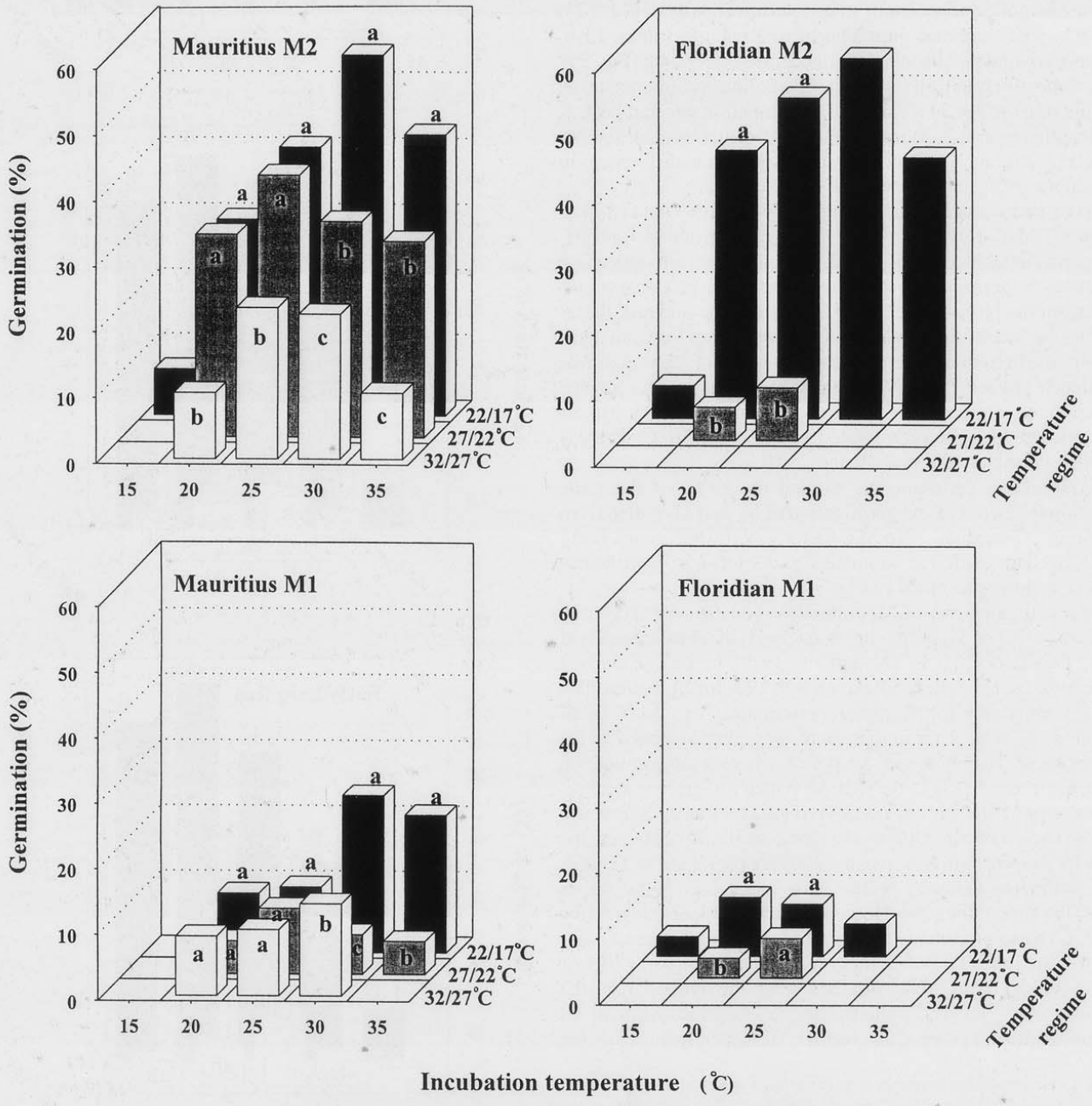

Fig. 5. Effect of three temperature regimes during flower development (February and March) on the in vitro germination of M_1 and M_2 pollen from 'Mauritius' and 'Floridian'. Germination rate was determined after a 24-h incubation at five different temperatures. Results within the same incubation temperature marked with different letters differ significantly using Duncan's multiple range test, $P = 0.05$.

Table 2. Fruit set percentage after hand pollination with M_1 and M_2 pollen from 'Mauritius' and 'Floridian'. For each treatment 50×6 flowers were pollinated in 1989, and 100×6 in 1990.

Pollen source		Pollinated cultivar			
		Mauritius		Floridian	
Cultivar	Type	1989	1990	1989	1990
Mauritius	M_1	0.7 B[z]	1.0 B	0.7 B	1.3 B
	M_2	3.3 A	3.7 A	1.5 A	2.3 A
Floridian	M_1	1.0 B	1.7 B	0.3 B	0.7 B
	M_2	4.0 A	4.0 A	1.5 A	2.0 A
Mean		2.5 a[z]		1.3 b	

[z]Results within a column followed by different uppercase letters and results within a row followed by different lowercase letters differ significantly by Duncan's multiple range test, $P = 0.05$.

fact that no pollen tube reached the ovule in 48 h may only reflect the slow growth rate at this cold regime.

Pollen-tube growth rate was similar under the cool (22/17 °C) and warm (27/22 °C) regimes (Table 1). Tubes reached the ovary in all flowers and reached the ovule in ≈35% of the flowers. In a few flowers, pollen tubes reached the micropyle of the embryo sac. It is only at this advanced stage that M_2 pollen performed better than M_1 pollen, the difference between the two was close to significance ($P = 0.06$).

Effect of temperature regime during flower development on pollen viability. The temperature regime during flower development had a pronounced effect on pollen germination rate (Fig. 5). The cool regime (22/17 °C) gave the best results, whereas the warm (27/22 °C) and hot (32/27 °C) regimes had a detrimental effect on pollen viability. 'Floridian' was especially affected, and under the hot regime M_1 and M_2 pollen lost the ability to germinate. Under the warm regime, germination occurred only at incubation temperatures of 20 and 25 °C, and the rate did not exceed 6% and 8% for M_1 and M_2 pollen, respectively. The viability of 'Mauritius' pollen decreased moderately with increasing temperature during flower development. Even under the hot regime, pollen germinated at moderate rates. There were differences between the two cultivars in the germination rate of pollen that developed under warm and hot regimes.

Fruit set after pollination with M_1 and M_2 pollen. Final fruit set was greater after pollination with M_2 pollen (Table 2). Cross-pollination almost always resulted in greater set than self-pollination; however, the differences were not significant.

Discussion

Lychee has two types of pollen-releasing flowers—M_1 and M_2. Although we could not find any morphological differences between the two, we did find a consistent and usually significant advantage of M_2 over M_1 pollen. This advantage was pronounced when pollen from five cultivars was germinated in vitro under five different temperature regimes (Figs. 2–4). Our findings concur with those of Mustard et al. (1953) and Costes (1988), each for one cultivar, and Fivaz et al. (1994) for four cultivars. To date, an advantage for M_2 over M_1 pollen has been found in six distinct cultivars, whereas no difference was found by Fivaz et al. (1994) in 'Bengal'. An advantage for M_1 over M_2 pollen was found by Singh (1962) in 'Calcutta'. Pollen-tube growth rate in vitro was also faster for M_2 than M_1 pollen (Fig. 2B). Thus, we can conclude that M_2 lychee pollen is usually more viable than M_1 pollen.

No pronounced advantage of M_2 over M_1 pollen was obvious in an in vivo study of pollen-tube growth rate (Table 1). Some advantage for M_2 pollen was found only at the last stage of pollen-tube growth. However, in this particular experiment, copious amounts of pollen were placed on the stigma, and only the location of the fastest pollen tube was recorded. The conclusive test for pollen viability is fruit set. In both cultivars studied, pollination with M_2 pollen resulted in a significant increase in fruit set relative to that obtained with M_1 pollen (Table 2).

The great difference in viability between the two pollen types is surprising. Genetically they are identical, so the difference must be phenotypic. We did not find any differences in the size or shape of the pollen grains. Thus, we assume that the M_2 pollen grain has higher levels of essential nutrients. M_2 flowers secrete greater amounts of nectar and sugar than M_1 flowers (Stern and Gazit, 1996). This phenomenon indicates that the M_2 flower is a much stronger sink, and this may also manifest itself in a better supply of nutrients to its developing pollen.

We found a pronounced detrimental effect of high temperature regimes (27/22 °C and particularly 32/27 °C) during flower development on pollen viability (Fig. 5) and gynoecium normality (Stern et al., 1996). The lychee originated in southern China. Its center of cultivation is the Guangdzhou region, where temperatures during flower development (February through March) are ≈20/10 °C (Groff, 1921). Thus, the detrimental effect on pollen viability of much higher temperatures could be expected in light of similar effects found in other fruit crops such as tomato (*Lycopersicon esculentum* Mill.) (Rudich et al., 1977), avocado (*Persea americana* Mill.) (Sedgley, 1977), mango (*Mangifera indica* L.) (Issarakraisila and Considine, 1994), and others (Levit, 1980).

'Floridian' was much more susceptible to high temperature than 'Mauritius', particularly during flower development (Fig. 5), but also with respect to M_1 pollen germination in vitro at 35 °C (Fig. 3). On the other hand, 'Floridian' M_1 pollen germinated at 15 °C, whereas 'Mauritius' M_1 pollen did not (Fig. 3). The high temperature regime also had a more severe detrimental effect on gynoecium development in 'Floridian' than 'Mauritius' (Stern et al., 1996). This difference between the two cultivars in their response to temperature may reflect the climate in their place of origin. 'Floridian' is related to 'Brewster' (Degani et al., 1995), which is probably identical to the known Fujian cultivar 'Chen Zee' (Cobin, 1954; Groff, 1948). 'Chen Zee' is cultivated mainly in Putian (25°N Lat.), whereas 'Mauritius', which is apparently identical to the Chinese cultivar 'Tai So', is cultivated mainly in the southern parts of Fujian and Guangdong (22–23° N Lat.) (Gazit and Goren, 1997).

The greater viability of M_2 pollen (Figs. 2–5, Table 1), coupled with the greater attractiveness of M_2 flowers to the honeybee (Stern and Gazit, 1996), make the M_2 bloom more effective for pollination than the M_1 bloom. Thus, when a pollenizer is needed to increase the productivity of a certain lychee cultivar, we should select a pollenizer whose M_2 bloom overlaps with the female bloom of this cultivar.

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