Effect of temperature regime on pollen and the effective pollination of ‘Kent’ mango in Israel

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Abstract

‘Kent’ mango (Mangifera indica L.) flowers were sampled in an orchard in the coastal plain of Israel during the 1997 flowering season. Effective pollination rate was determined at two stages of the fertilization process: (a) pollen germination on the stigma; (b) pollen-tube penetration into the ovule. Pollination rates were negligible during the first part of the flowering season (31 March to 18 April), reaching a high value only at the end of the season (21 May). The same phenomenon also occurred, albeit with consistently higher rates of effective pollination, when detached flowers, taken from the orchard during the flowering season, were pollinated and incubated for 24 h at the presumed optimal temperature of 30°C, indicating that the reproductive organs were not fully functionally viable. At the start of the flowering season, all the pollen was deformed, and later (16 April 1997), when pollen grain shape was normal, only 23% of it was found to be viable by Alexander’s staining. The functional viability of the pollen and pistils of orchard- and phytotron-grown (22/27°C, night/day) flowers was determined in detached flowers. At the beginning of the flowering season, both orchard pollen and pistils tended to be defective. Orchard pollen germinated poorly, even on phytotron adapted stigmas. Ovule penetration was hampered in orchard pistils, even when phytotron pollen was used for pollination. Chilling injury appeared to be responsible for the damage to the reproductive organs of the orchard flowers. The negligible rate of effective pollination found in mango orchards in Israel during a significant part of the flowering season therefore appears to be due to the detrimental effect of cold weather on the pollination and fertilization processes as well as on the functional viability of the male and female reproductive organs. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Mangifera indica; Temperature; Pollen; Pistil; Fertilization process
1. Introduction

Mango is grown mainly in tropical regions with seasonal rains. However, mango is also widely grown in hot subtropical areas such as northern India, Florida, Egypt, Israel, Australia, South Africa, Brazil, and more recently, Spain. Its growth area extends from 39°S in Australia (Crane et al., 1997) to 36°N in Spain (Galan Sauco, 1997).

The main limiting factor for mango tree survival is severe frost; thus, mango is best grown in areas that are frost-free, or that are subject to only occasional light frosts.

Commercial mango orchards are found in Israel from 29.5°N, near the Red Sea, to 33°N, near the Sea of Galilee. The following study was undertaken in Rehovot (~32°N), in the coastal plain.

The winter in Israel is cold enough and long enough to induce a profuse mango bloom every year. The flowering season in the coastal plain usually begins in March and ends in May. However, normal fruit set usually occurs only from mid-April (Homsky, 1997).

Roizman (1984) found effective pollination (as reflected by the presence of germinating pollen grains on the stigma) in mango orchards near Rehovot to be nil or negligible during most of March and April, even on days when the weather conditions are favorable for pollination. Similar findings have been reported from other subtropical regions with relatively cold winters: South Africa (de Wet et al., 1989), Australia (Issarakraisilia et al., 1992; Issarakraisilia and Considine, 1994), Taiwan (Shu et al., 1989), Mexico (Lakshminarayana and Aguilar, 1975), and Florida (Young, 1942; Miner and Zill, 1958; Young and Sauls, 1979).

The reproductive organs of plants are usually the most sensitive to chilling (Larcher and Bauer, 1980). Low temperature during flower development has been found responsible for the occurrence of defective reproductive organs in different agricultural crops: almond (Egea and Burgos, 1995), grape (Ebadi et al., 1995), persimmon (Fukui et al., 1990), and white clover (Pasumarty et al., 1995). Tropical and subtropical plants are prone to this damage when flower development occurs at suboptimal temperatures (Polowick and Sawhney, 1985; Mercado et al., 1997). Chilling injury has been implicated in damage to mango pollen (Roizman, 1984; Issarakraisilia and Considine, 1994) and probably also to mango female organs (Young and Sauls, 1979; Issarakraisilia et al., 1992).

The present study was conducted with the commercial mango cultivar ‘Kent’ (Campbell, 1992), which comprises 13% of the mango growing area in Israel (Homsky, 1997). ‘Kent’ is relatively tolerant to low temperature (Miner and Zill, 1958; Young and Sauls, 1979). Our aims were: (a) to elucidate the relationship between effective pollination rate and temperature regime during the flowering season; (b) to study the seasonal changes in pollen and pistil viability by determining pollen ability to germinate and reach the ovule.
2. Materials and methods

2.1. Plant material

Flowers were sampled from: (a) four mature ‘Kent’ trees at the Faculty of Agriculture’s farm in Rehovot; (b) 2 year-old ‘Kent’ plants which were introduced into the phytotron (at 22/27°C, night/day) about a month before the beginning of flowering. For the effective pollination survey, 2–3 day-old flowers were sampled.

2.2. Pollen viability

Pollen was observed under a stereomicroscope (×80) and in a scanning electron microscope (SEM). Viability of normally shaped pollen grains was determined by their reaction to Alexander’s stain (Alexander, 1969).

2.3. Determination of pollen germination and pollen-tube growth in the pistil

Flowers were fixed in ethanol:acetic acid (2:1, v:v). Pistils were detached and cleared in 8 N NaOH for 24 h, rinsed thoroughly in distilled water, and stained in 0.1% (w/v) aniline blue dissolved in 0.1 N K₂HPO₄. Germinating pollen grains and pollen tubes in the pistil were detected under a fluorescence microscope (Kho and Bear, 1968).

2.4. Pollination of detached flowers

Flowers were detached in the morning, a short time after anthesis and before anther dehiscence. The flower pedicel was inserted into a 2% agar layer in a petri dish, and kept for 1–2 h under a table lamp, until dehiscence. Released pollen was used to pollinate the flowers under a stereomicroscope. At least 10 pollen grains were placed on each stigma (de Wet et al., 1989). The pollinated flowers were put in a plastic box with a wet paper towel and incubated for 24 h at 30°C. After incubation, pistils were detached, fixed, and prepared for examination by the above-described method.

2.5. Experimental design

For all experiments, flowers were sampled from four ‘Kent’ trees in the orchard, four ‘Kent’ plants in the phytotron, or both, 10–20 flowers per tree/plant. The statistical significance of the treatments was tested by analysis of variance with individual plants as replicates and means were compared using Tukey’s test.
3. Results

3.1. Survey of effective pollination in the orchard

Hermaphrodite ‘Kent’ flowers were sampled eight times throughout the 1997 flowering season, at the Faculty of Agriculture’s farm in Rehovot. A pronounced seasonal change was found in effective pollination rate (Table 1). From 31 March to 18 April, <10% of the flowers had germinating pollen grains on the stigma (1.0–1.2 per stigma) and no pollen tubes were found in the ovules. In mid-bloom, 24 April to 9 May, a pronounced increase was found in all these parameters. A large and statistically significant increase was found at the end of the bloom (21 May): 88% of the stigmas had germinating pollen grains, with an average of 4.9 grains per stigma, and 56% of the pistils had a pollen tube penetrating the ovule.

3.2. Pollen viability

‘Kent’ pollen was examined under a microscope during the 1996 and 1997 flowering seasons. During the first few weeks, all pollen grains were shrunken and deformed. Only after 3–4 weeks did most of the pollen grains have a normal shape. In a survey conducted during the 1997 flowering season, all of the pollen collected on 31 March was shrunken. On 16 April, some anthers had normal-shaped pollen and on 10 May, almost all of the pollen was normal in appearance.

The viability of normal-shaped pollen was determined by Alexander’s staining (Alexander, 1969). The percentage of stained pollen grains increased significantly, from 23 on 16 April, to 95 and 96 on 10 and 16 May, respectively (Table 2).

Table 1
Seasonal changes in the percentage of ‘Kent’ mango flowers in the orchard with germinated pollen and a pollen tube in the ovule, and in the number of germinated pollen grains per stigma (Rehovot, 1997)

<table>
<thead>
<tr>
<th>Sampling date</th>
<th>Flowers with germinated pollen (%)*</th>
<th>Flowers with pollen tube in the ovule (%)*</th>
<th>Germinated pollen grains per stigma (n)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>31 March</td>
<td>1c</td>
<td>0b</td>
<td>1.0b</td>
</tr>
<tr>
<td>4 April</td>
<td>9bc</td>
<td>0b</td>
<td>1.1b</td>
</tr>
<tr>
<td>11 April</td>
<td>9bc</td>
<td>0b</td>
<td>1.2b</td>
</tr>
<tr>
<td>18 April</td>
<td>1c</td>
<td>0b</td>
<td>1.0b</td>
</tr>
<tr>
<td>24 April</td>
<td>30b</td>
<td>7b</td>
<td>2.5b</td>
</tr>
<tr>
<td>1 May</td>
<td>29b</td>
<td>7b</td>
<td>1.8b</td>
</tr>
<tr>
<td>9 May</td>
<td>35b</td>
<td>9b</td>
<td>1.9b</td>
</tr>
<tr>
<td>21 May</td>
<td>88a</td>
<td>56a</td>
<td>4.9a</td>
</tr>
</tbody>
</table>

*Results within a column followed by different letters differ significantly by Tukey’s test, P < 0.05.
3.3. Pollination of detached flowers

Detached mango flowers have been used successfully to study pollen germination (de Wet et al., 1989). We employed a similar technique to determine pollen germination and pollen-tube penetration into the ovule.

First we confirmed 30\(^\circ\)C to be the best temperature of four tested for ‘Kent’ pollen germination and found that it was also best for the penetration of the pollen tube into the ovule (Table 3). At 15\(^\circ\)C, pollen germination rate was still quite high (60%); however, although in most pistils pollen tubes penetrated the ovary, none had reached the ovule at the end of the 24 h incubation period.

Throughout the 1996 flowering season, ‘Kent’ flowers were taken from the orchard and pollinated in the laboratory with fresh ‘Kent’ pollen sampled from the orchard trees. A consistent and significant increase was found in the percentage of flowers with germinated pollen, from 2% on 5 April to 78 and 57% on 3 and 10 May, respectively (Table 4). A consistent increase was also found in the ovule penetration, from 0% on 5 April to 16% on 10 May. During the second

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### Table 2

Seasonal changes in ‘Kent’ mango pollen viability in the orchard, as determined by Alexander’s staining (Rehovot, 1997)

<table>
<thead>
<tr>
<th>Sampling date</th>
<th>Viable pollen grains (%)(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16 April</td>
<td>23b</td>
</tr>
<tr>
<td>1 May</td>
<td>82a</td>
</tr>
<tr>
<td>10 May</td>
<td>95a</td>
</tr>
<tr>
<td>16 May</td>
<td>96a</td>
</tr>
</tbody>
</table>

\(^a\) Results within a column followed by different letters differ significantly by Tukey’s test, \(P < 0.05\).

### Table 3

The effect of four incubation temperatures on effective pollination rate in detached ‘Kent’ mango\(^a\)

<table>
<thead>
<tr>
<th>Incubation temperature ((^\circ)C)</th>
<th>Flowers with germinated pollen (%)(^b)</th>
<th>Flowers with pollen tube in the ovule (%)(^b)</th>
<th>Germinated pollen grains per stigma ((n))(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15.0</td>
<td>60a</td>
<td>0c</td>
<td>4.3ab</td>
</tr>
<tr>
<td>22.5</td>
<td>76a</td>
<td>45a</td>
<td>5.7a</td>
</tr>
<tr>
<td>30.0</td>
<td>80a</td>
<td>54a</td>
<td>4.5ab</td>
</tr>
<tr>
<td>37.5</td>
<td>69a</td>
<td>13b</td>
<td>3.1b</td>
</tr>
</tbody>
</table>

\(^a\) Flowers and pollen were collected from plants kept in the phytotron under an optimal temperature regime (27/22\(^\circ\)C). Flowers were hand-pollinated with ‘Kent’ pollen from the same plants and kept for 24 h at the experimental temperature.

\(^b\) Results within a column followed by different letters differ significantly by Tukey’s test, \(P < 0.05\).
part of the 1997 flowering season, a similar survey was conducted. From 25 April to 9 May, the percentage of stigmas with germinated pollen increased from 59 to 90% (Table 4).

On 12 April 1996, when effective pollination rates were found to be low in detached orchard flowers pollinated with orchard pollen (Table 4), we pollinated detached flowers from the orchard and the phytotron (22/27°C, night/day) with orchard and phytotron pollen. Both pollen and pistils from the orchard were significantly inferior to their phytotron counterparts (Table 5). Pollen germination was influenced mainly by pollen source: orchard pollen was consistently inferior to phytotron pollen. In contrast, pollen-tube penetration into the ovule was

<table>
<thead>
<tr>
<th>Date</th>
<th>Flowers with germinated pollen (%)</th>
<th>Flowers with pollen tube in the ovule (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 April 1996</td>
<td>2b</td>
<td>0a</td>
</tr>
<tr>
<td>12 April 1996</td>
<td>16b</td>
<td>1a</td>
</tr>
<tr>
<td>19 April 1996</td>
<td>17b</td>
<td>4a</td>
</tr>
<tr>
<td>3 May 1996</td>
<td>78a</td>
<td>5a</td>
</tr>
<tr>
<td>10 May 1996</td>
<td>57a</td>
<td>16a</td>
</tr>
<tr>
<td>25 April 1997</td>
<td>59b</td>
<td>19a</td>
</tr>
<tr>
<td>5 May 1997</td>
<td>71a</td>
<td>16a</td>
</tr>
<tr>
<td>9 May 1997</td>
<td>90a</td>
<td>41a</td>
</tr>
</tbody>
</table>

Results for the same year within a column followed by different letters differ significantly by Tukey’s test, P < 0.05.

Table 5

Effect of environmental regimes (orchard and phytotron) under which ‘Kent’ mango flowers developed on pistil receptivity and the ability of the pollen to germinate and reach the ovule (12 April 1996)

<table>
<thead>
<tr>
<th>Pollen source</th>
<th>Pistil source</th>
<th>Flowers with germinated pollen (%)</th>
<th>Flowers with pollen tube in the ovule (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orchard</td>
<td>Orchard</td>
<td>16b</td>
<td>1b</td>
</tr>
<tr>
<td></td>
<td>Phytotron</td>
<td>30b</td>
<td>12b</td>
</tr>
<tr>
<td>Phytotron</td>
<td>Orchard</td>
<td>36a</td>
<td>5a</td>
</tr>
<tr>
<td></td>
<td>Phytotron</td>
<td>57a</td>
<td>34a</td>
</tr>
</tbody>
</table>

Pairs of results within a column followed by different letters differ significantly by Tukey’s test, P < 0.05.
influenced mainly by the pistil source, with orchard pistils being consistently inferior to phytotron ones.

The seasonal changes in orchard pistil receptivity were determined in 1997 by using viable phytotron pollen to pollinate orchard and phytotron flowers. There was no significant difference in pollen germination between orchard and phytotron flowers (Table 6). In contrast, a pronounced and significant difference was found in ovule penetration: on 7 and 12 April it was only 6% in orchard flowers, compared to 50 and 75%, respectively, in phytotron flowers; on 19 April it reached 31% in orchard flowers, not significantly lower than the 41% found for phytotron flowers.

Table 6
Seasonal changes in the percentage of detached ‘Kent’ mango flowers from the orchard and phytotron with germinated pollen and a pollen tube in the ovule, after hand-pollination with viable ‘Kent’ pollen from the phytotron (Rehovot, 1997)

<table>
<thead>
<tr>
<th>Date</th>
<th>Flowers with germinated pollen (%)a</th>
<th>Flowers with pollen tube in the ovule (%)a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Flowers from phytotron</td>
<td>Flowers from orchard</td>
</tr>
<tr>
<td>7 April</td>
<td>80a</td>
<td>72a</td>
</tr>
<tr>
<td>12 April</td>
<td>90a</td>
<td>60a</td>
</tr>
<tr>
<td>19 April</td>
<td>83a</td>
<td>53a</td>
</tr>
</tbody>
</table>

a Results within each horizontal pair followed by different letters differ significantly by Tukey’s test, $P < 0.05$.

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4. Discussion

The phenomenon of a very low rate of effective pollination in ‘Kent’ mango during a large part of the early flowering season (Table 1) confirms Roizman (1984) observation and appears to be typical for mango grown in subtropical countries with relatively cold winters (Young, 1942; Miner and Zill, 1958; Lakshminarayana and Aguilar, 1975; Young and Sauls, 1979; de Wet et al., 1989; Shu et al., 1989; Issarakraisilia et al., 1992; Issarakraisilia and Considine, 1994). During the first part of the ‘Kent’ flowering period, the pollen tube did not reach the ovule at all (Table 1), explaining the absence of normal fruit set during this period (Homsky, 1997).

The observed low rate of effective pollination may result from concurrent environmental factors, particularly inadequate pollinator activity and suboptimal temperatures. It may also result from the degeneration and low viability of the reproductive organs — the pollen and the pistil. The experimental use of hand-pollinated detached flowers that were at the presumed optimal temperature for
pollen germination and pollen-tube growth (Table 3) enabled us to determine the viability of the reproductive organs (Tables 4–6). Using pollen and pistils which had developed under a highly suitable temperature regime (22/27°C, night/day), we were able to check the viability of orchard pollen and pistils separately (Tables 5 and 6).

The ultimate proof of reproductive organ viability is pollen ability to germinate, reach the ovule and perform the fertilization required for normal fruitlet set. In the past, most viability tests of mango reproductive organs examined the morphology and anatomy of the pistil (Young, 1942; Sturrock, 1969; Issarakraisilia et al., 1992), the reaction of the pollen to vital stains (Randhawa and Damodaran, 1961; Singh, 1961; Issarakraisilia et al., 1992; Issarakraisilia and Considine, 1994), and its germination in artificial media (Popenoe, 1917; Young, 1958). The only in vivo studies were limited to pollen germination on the stigma (de Wet et al., 1989; Issarakraisilia et al., 1992). In such studies, negative results are usually ample proof of degeneration or lack of viability. On the other hand, positive results do not prove functional viability (Dafni, 1992). We determined both pollen germination and ovule penetration by the pollen tube, and showed that pollen germination by itself is not enough, in that it does not guarantee pollen tube ability to reach the ovule (Tables 1 and 4–6). Pollen-tube penetration into the ovule is a much more reliable measure of functional viability than pollen germination, even though it still falls short of providing the ultimate proof of such viability.

At the beginning of the flowering season, we found all of the ‘Kent’ pollen to be severely defective, as has been found for other mango cultivars in Israel (Roizman, 1984) and for ‘Kensington’ in Australia (Issarakraisilia et al., 1992; Issarakraisilia and Considine, 1994). Later, when pollen shape was normal, 77% of the pollen grains were still not viable (Table 2). During this latter period, effective pollination in the orchard was negligible and no pollen tube reached the ovule (Table 1). Our findings (Tables 5 and 6) show that both pollen and pistil are responsible for the low rate of effective pollination in detached flowers during a considerable part of the flowering season (Table 4).

Low pollen viability was the main factor behind the low rate of pollen germination on stigmas (Table 5). Indeed, when viable pollen from flowers grown in the phytotron was used, a high rate of germination occurred in pollinated orchard flowers, even in early April (Table 6). In contrast, low pistil viability was the main factor responsible for the low rate of ovule penetration (Tables 5 and 6). Overall, it seems that during a large part of the flowering season in the coastal plain of Israel, the functional viability of both the pollen and pistils of ‘Kent’ mango flowers is seriously impaired.

Concurrent environmental factors were also involved in the low rate of effective pollination during most of the flowering period. Effective pollination of orchard flowers (Table 1) was consistently higher when detached orchard flowers
were pollinated and then kept at the presumed optimal temperature of 30°C (Table 4). Our experiments were not designed to separate the positive effects of hand-pollination and optimal temperature. We speculate that each of these two factors has a considerable favorable effect.

Damage to mango reproductive organs appears to occur during flower development, as a result of suboptimal temperatures (Popenoe, 1917; Roizman, 1984; Issarakraisilia et al., 1992; Issarakraisilia and Considine, 1994). The temperature data for February–May 1997 (Fig. 1) show that until 20 April, the daily minimum was usually below 10°C, occasionally dropping to below 5°C. Only after 12 April was there consistent warming, with night temperatures staying above 12°C. Our findings support Issarakraisilia and Considine’s (1994) formula which is based on the assumption that the detrimental effect of a cold spell on pollen viability (as determined by staining) ends after 155 ‘degree–days’ (above 10°C) after the cold spell. From the cold spell on 11 and 12 April (Fig. 1) to 1 May, when pollen viability reached 82% (Table 2), about 155 ‘degree–days’ had indeed elapsed. However, because the staining method used tends to overestimate functional viability values, we assume that the recuperation period to full functional viability is longer.

‘Kent’ pollen germinated well at 15°C (Table 3) and pollen tubes even reached the ovary, though not the ovule. Had the incubation period been longer than 24 h, the pollen tubes might in fact have reached the ovule. de Wet et al. (1989) also
found that ‘Haden’ pollen germinates at 15°C. These results contradict the general conclusion that mango pollen does not germinate at 15°C (Davenport and Nunez-Elisea, 1997). This contradiction may reflect differences among cultivars, ‘Kent’ being less sensitive to low temperature (Miner and Zill, 1958). It may also reflect differences in pollen-germination techniques, with in vitro germination on artificial media being more sensitive to low temperature than in vivo germination on the stigma.

Chilling injury to the mango reproductive organs may be prevented by heating with stoves to prevent excessive temperature drops at night (Lakshminarayana and Aguilar, 1975). However, this method is probably not economically feasible. A more logical solution is to delay the flowering season. In Israel, this has been achieved by destroying the first bloom to induce a second bloom (chemically or by hand). In the coastal plain, this method has been performed successfully in mid-March (Homsky, 1989), the second bloom starting a month later, in mid-April. However, when the spring is colder than usual, as happened in 1997, even the second bloom may sustain considerable chilling damage.

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