

In the name of Allah

NOTE

بناام خدا

THE EFFECT OF SUPPLEMENTARY HAND  
POLLINATION ON FRUIT SET AND  
POLLEN TUBE GROWTH IN APPLE<sup>1</sup>

اثرگرده افشانی اضافی روی تشکیل میوه  
ورشلوله گرده در سیب

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ABSTRACT

Hand-pollination of open-pollinated flowers of 'Delicious' and 'McIntosh' apple (*Malus domestica* Borkh.) with 'Empire' pollen at full bloom increased initial and final fruit set in 'Delicious' but not in 'McIntosh'. Bagged limbs set only 0.4 to 1 fruit per 100 flowers. Staining with aniline blue made pollen tubes visible in the entire length of 'McIntosh' styles, but none were observed at the base of 'Delicious' styles. Pollen tubes in open- and open-plus hand-pollinated flowers reached the base of 'McIntosh' styles within 4 to 6 days, but almost none reached the base in self-pollinated (bagged) flowers, and most were swollen and terminated in callose plugs.

خلاصه

گرده افشانی بوسیله دست، در گل‌های دیگرگرده افشان سیب قرمزلبنا و مک اینتاش بوسیله دانه گرده سیب امپایر، هنگامیکه قسمت اعظم شکوفه‌ها باز شده بودند، سبب افزایش تشکیل اولیه و نهایی میوه در سیب لبنائی گردید ولی تشکیل میوه در سیب مک اینتاش را افزایش نداد. در شاخه‌هایی که در کیسه قرار داده شده بودند بین ۰/۴ تا یک درصد گل‌ها به میوه تبدیل شدند. رنگ کردن مادگی با محلول آنیلین آبی باعث شدت لوله‌های گرده در کلیه قسمت‌های گل‌های سیب مک اینتاش در زیر میکروسکوپ فلورسنت مشاهده شوند ولی در سیب قرمزلبنا لوله‌های گرده در قسمت پایینی خامه مشاهده نگردیدند. در سیب مک اینتاش گل‌ها نیکه در آنها گرده افشانی هم بصورت طبیعی و هم با دست صورت گرفته بود لوله‌های گرده بعد از ۴ تا ۶ روز بیابا ثین خامه رسیدند حال آنکه در گل‌ها نیکه گرده افشانی آنها بطریقه خودگرده افشانی انجام گرفته بود (شاخه‌ها نیکه در کیسه قرار داشتند) تقریباً

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These results are discussed in relation to the role of "basal gaps" between the stamens in limiting fruit set of 'Delicious'.

هیچکدام از لوله‌های گرده‌بهاثین خامه نرسیدند و اکثراً "متورم شده و در انتهای آنها کالوز تشکیل شده بود. نتایج حاصله در رابطه با فاصله بین پرچمها که در سبب فرمولینائی باعث محدودیت تشکیل میوه میشود مورد بحث قرار گرفته است.

## INTRODUCTION

The beneficial effect of cross-pollination on fruit set of some fruits was recognized as early as 1824 (9). Most of the apple cultivars in the eastern United States are self-incompatible. A biochemical antagonism prevents pollen grains from germinating on, or growing into the stigmas of the same cultivar.

'Delicious' is totally self-unfruitful (3, 5, 6) and cross-pollination is essential for consistent heavy production. Howlett (3) found that limited fruit set in 'Delicious' was due in large part to inadequate pollination.

Roberts (6) reported that the structure of 'Delicious' blossoms permitted honey bees to remove nectar without transferring pollen to the stigma: only about 20% of the bees visiting the flowers crawled over the stigmata. Robinson (7) reported similar "sideworking" of honey bees on 'Delicious' blossoms as a result of "basal gaps" or spaces between the stamens. The maximum width of the tongue (glossa) of a honey bee is about 180  $\mu\text{m}$ . Basal gaps greater than 180  $\mu\text{m}$  were found to be much more abundant in 'Delicious' sports than in 11 other cultivars (7).

Supplementary pollination is a potential method for enhancing fruit set, but the cost would probably be prohibitive. Williams (11) compared the effects of supplementary hand-pollination on fruit set of 'Cox's Orange Pippin' apple in England. The average number of fruits per 100 flower clusters for 21 'Cox' orchards was 33.1 for those receiving supplementary pollination (one flower hand-pollinated per 4 clusters) vs. 25.6 for controls (natural pollination

only). Supplementary pollination increased yield by an average of 29%. Increased fruit set may have been the result of a higher number of pollen grains per stigma as well as more cross-pollinated flowers. Theoretically only a few pollen grains per flower are required for fertilization. However, Forshey (2) suggested that 'Delicious' flowers must be saturated with pollen because a relatively low percentage of pollen tubes actually reach the embryo sac.

The purposes of this study were (a) to evaluate the importance of basal gaps in 'Delicious' flowers by comparing the effects of supplementary hand-pollination on fruit set in 'Delicious' vs. 'McIntosh' (which lacks such gaps), and (b) to compare the rates of pollen tube growth in the styles of these two cultivars.

#### MATERIALS AND METHODS

Three uniform branches were selected on each of the three 'Starking Delicious' and three 'McIntosh' apple trees approximately 40 years old in a commercial orchard at Leslie, MI. One limb on each tree was enclosed in a cheesecloth bag prior to flower opening to exclude insects. At full bloom, all "king" (terminal) and frost-injured flowers were removed from each branch and the remaining lateral flowers were counted. The following treatments were applied, using a randomized block design with trees as blocks: bagged to prevent pollination by insects, open-pollinated, and open-pollinated plus hand-pollinated. All flowers in the last treatment were hand-pollinated at full bloom using pollen collected 48 hr previously from 'Empire' apple flowers. The terminal 75 to 100 flowers on the branch were used to record fruit set and 30 to 50 flowers were collected from each branch 2, 3, 4, 5, and 6 days after full bloom. The flowers were fixed within half an hour of collection in formalin: acetic acid: 80% ethanol (FAA) (1:8:1 by volume), for evaluation of pollen tube growth (4).

The styles were subsequently rinsed with tap water, placed in an aqueous solution of 3N NaOH overnight to clear and soften the tissue, rinsed in tap water for one hour to remove the sodium hydroxide, then held for 18 to 24 hr in a solution of 0.1% aniline dissolved in 0.1 N  $K_3PO_4$  (1 g aniline + 7.072 g  $K_3PO_4$  per liter) at room temperature. The styles were placed on a slide in a few drops of glycerine, covered with a cover slip, and squashed. The prepared slides were viewed under a Nikon-AFM photomicroscope with epi-fluorescence. Ektachrome 200 film was used to record pollen tube penetration into the style.

## RESULTS

### Effect of Supplementary Pollination on Fruit Set

Bagged flowers set very few fruits (Table 1). Supplemental hand-pollination approximately tripled the initial set and doubled the final set in comparison with open-pollination in 'Delicious', but had no significant effect in 'McIntosh'. The 'June' drop was considerably heavier in 'McIntosh' than in 'Delicious' despite similar fruit loads on hand-pollinated limbs.

### Germination of Pollen and Growth of Pollen Tubes in the Style

Pollen grains and pollen tubes fluoresced a brilliant yellow under ultraviolet light following staining with aniline. However, a marked difference between cultivars was evident in the staining of tubes in the basal half of the style. In 'Delicious', no pollen tube was observed in the base of any style, even 5 days after full bloom, despite the fact that fruit set was normal to heavy (Table 1).

In open-pollinated and open- plus hand-pollinated 'McIntosh' flowers, many pollen tubes reached the base of the style within 4 to 6 days of full bloom. Pollen tube growth did not appear to differ in styles of open vs. open- plus hand-pollinated flowers. Few pollen tubes were

Table 1. Effect of supplemental hand-pollination on fruit set of apple.

| Treatment <sup>†</sup>              | No. of flowers | Fruits per 100 flowers |       |
|-------------------------------------|----------------|------------------------|-------|
|                                     |                | Initial                | Final |
| <u>'McIntosh'</u>                   |                |                        |       |
| Bagged                              | 58             | 3b*                    | 1b    |
| Open-pollinated                     | 81             | 60b                    | 20a   |
| Open + hand-pollinated <sup>§</sup> | 88             | 65a                    | 21a   |
| <u>'Starking Delicious'</u>         |                |                        |       |
| Bagged                              | 109            | 1c                     | 0.4c  |
| Open pollinated                     | 136            | 24b                    | 17b   |
| Open + hand-pollinated              | 106            | 68a                    | 41a   |

<sup>†</sup>One limb on each of 3 trees per treatment.

\*Mean separation within columns and cultivars by DMRT, 5% level.

<sup>§</sup>Hand-pollination with 'Empire' pollen on May 6 (McIntosh) or May 8 (Delicious).

evident in the styles of bagged flowers in either cultivar and the few tubes present had highly calloused terminal plugs. No pollen tube reached the base of the style within 6 days after full bloom (Table 2).

#### DISCUSSION

If basal gaps limit the set in 'Delicious', set of open-pollinated flowers should be lower and hand-pollination should have a greater effect in this cultivar than in 'McIntosh', which has fewer such gaps. Data for initial set (Table 1) support this hypothesis, for open-pollinated 'Delicious' flowers set only one-third as many fruits as 'McIntosh' flowers, and hand-pollination had

Table 2. Effects of self- (S)<sup>†</sup>, open- (O), and open-plus hand- (O+H)<sup>§</sup> pollination on pollen germination and tube growth in apple flowers, 1981, as determined by staining with aniline.

| Treatment          | Days after full bloom | Pollen had germinated | % of flowers <sup>¶</sup> in which:                  |     |     |     |
|--------------------|-----------------------|-----------------------|--|-----|-----|-----|
|                    |                       |                       | Pollen tubes had reached indicated % of style length |     |     |     |
|                    |                       |                       | 25   | 50  | 75  | 100 |
| <u>'McIntosh'</u>  |                       |                       |  |     |     |     |
| S                  | 2                     | 40                    | -  | -   | -   | -   |
| O                  |                       | 91                    | 81   | 33  | 12  | 2   |
| O+H                |                       | 73                    | 53   | 33  | 13  | 4   |
| S                  | 5                     | 86                    | 86   | 86  | 86  | -   |
| O                  |                       | 85                    | 85   | 85  | 85  | 70  |
| O+H                |                       | 100                   | 100  | 100 | 100 | 91  |
| S                  | 6                     | 92                    | 92   | 92  | 92  | 4   |
| O                  |                       | 87                    | 87   | 87  | 87  | 87  |
| O+H                |                       | 89                    | 89   | 89  | 89  | 89  |
| <u>'Delicious'</u> |                       |                       |  |     |     |     |
| S                  | 3                     | 36                    | 18   | 6   | 0   | 0   |
| O                  |                       | 50                    | 37   | 6   | 0   | 0   |
| O+H                |                       | 88                    | 76   | 36  | 0   | 0   |
| S                  | 4                     | 67                    | 17   | 4   | 0   | 0   |
| O                  |                       | 90                    | 90   | 61  | 0   | 0   |
| O+H                |                       | 100                   | 100  | 36  | 0   | 0   |
| S                  | 6                     | 68                    | 68   | 0   | 0   | 0   |
| O                  |                       | 100                   | 100  | 9   | 0   | 0   |
| O+H                |                       | 96                    | 96   | 25  | 0   | 0   |

<sup>†</sup>Limbs bagged prior to flower opening.

<sup>§</sup>Hand-pollination with 'Empire' pollen at full bloom on May.

<sup>¶</sup>Approximately 30 to 50 flowers per observation.

a dramatic effect in 'Delicious' but no effect in 'McIntosh'. Data for final set paralleled those for initial set, except that the heavy 'June' drop in 'McIntosh' caused final fruit set on open-pollinated branches to be no greater than in 'Delicious'. It might be concluded that the 'June' drop reflected the overall tree fruit-set rather than the set on the experimental branch units. This provides an explanation for the relatively heavier 'June' drop on 'McIntosh' than on 'Delicious'.

Comparison of pollen tube growth in self- vs. open-pollinated flowers supports previous observations on the growth of incompatible and compatible pollen tubes in apple (8). Swollen and highly callosed terminal plugs together with stoppage of growth in the upper portion of the style, indicated self-incompatibility in bagged flowers. In contrast, pollen tubes in open-pollinated styles had no terminal plugs, grew rapidly, and reached the base of the style in 'McIntosh' flowers within 4 to 6 days. The failure to observe pollen tubes in the base of 'Delicious' styles following open-pollination is difficult to explain in view of the fact that some fruit set occurred. A probable explanation could be that in the samples taken from flowers which were too old, pollen tube callose in the lower part of the styles failed to fluoresce.

#### LITERATURE CITED

1. Cooper, J.R. 1928. The behavior of pollen in self and cross pollination. Proc. Amer. Soc. Hort. Sci. 25: 138-140.
2. Forshey, C.G. 1978. Factors associated with 'Red Delicious' yields. Michigan State Hort. Sci. Ann. Rpt. for 1977. p. 39-44.
3. Howlett, F.S. 1928. Fruit setting in Delicious apple. Proc. Amer. Soc. Hort. Sci. 25: 143-148.
4. Johansen, D.A. 1940. Plant microtechnique. McGraw-Hill Book Co.

5. Overholser, E.L., and F.L. Overley. 1931. Pollination of certain apple bud sports in North Central Washington. Proc. Amer. Soc. Hort. Sci. 28:74-77.
6. Roberts, R.H. 1945. Blossom structure and setting of Delicious and other apple varieties. Proc. Amer. Soc. Hort. Sci. 46:87-90.
7. Robinson, W.S. 1979. Effects of apple cultivar on foraging behavior and pollen transfer by honey bees. J. Amer. Soc. Hort. Sci. 104:596-598.
8. Stott, K.G. 1972. Pollen germination and pollen tube characteristics in a range of apple cultivars. J. Hort. Sci. 47:191-198.
9. Swayne, G. 1824. On fertilizing the blossoms of pear trees. Royal Hort. Soc. Trans. 5:208-212.
10. Wellington, R. 1947. Pollination of fruit trees. Cornell Ext. Bull. 720. p. 8
11. Williams, R.R. 1970. The effect of supplementary pollination on yield. p. 7-10. In R.R. Williams and D. Wilson (eds.). Towards regulated cropping. Grower Books, London.