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HAND POLLINATION TESTS AND FIELD EVALUATION OF POLLINATORS FOR CITRUS

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ABSTRACT

Standard hand pollination techniques were not always adequate for determining the effectiveness of pollinators for commercial plantings. A modified hand pollination technique consisting of applying counted numbers of pollen grains of various varieties was found useful for comparing the potential capacity of the pollen variety to induce seed and fruit development. Currently, the only completely satisfactory means of evaluating pollinators is with actual field plantings.

From a comprehensive survey of commercial groves, several varieties were found suitable as pollinators for 'Orlando' tangelo and 'Robinson' tangerine. Less data were obtained for 'Lee', 'Osceola', 'Page' and 'Nova' varieties.

INTRODUCTION

In recent years there have been large increases in plantings of interspecific hybrids of grapefruit and tangerines. Some of these varieties are commercially classified as tangelos and some as tangerines. Many of these hybrids are sexually self-incompatible and only weakly parthenocarpic. Therefore, they fruit erratically unless suitable pollinators are used to induce fruiting through the stimulus of seed production (2, 6, 12) or unless their parthenocarpy is strengthened through girdling (7) or spray applications of gibberellic acid (4, 5). The use of pollinators results in the most consistent fruiting and the resulting seedy fruit are larger than

the seedless parthenocarpically produced ones (3).

Unfortunately, the self-incompatible varieties were released before their pollinator requirements were known (9, 10, 11). Thus, the grower has been faced with the dilemma of selecting pollinators with insufficient information.

Extensive research with both hand pollination and field evaluations of pollinators for 'Orlando' tangelo has been reported (4, 6); however, much less information is available for selecting pollinators for such varieties as 'Robinson', 'Lee', 'Osceola', 'Page' and 'Nova'; moreover, most of the information on these varieties is based on standard hand pollination techniques or caging pollinators with bees (2). Krezdorn and Robinson (6) in Florida and Oppenheimer (8) in Israel reported standard hand pollination techniques to at times be inconsistent with performance of pollinators in the field. Thus, some of the current information is questionable.

The purpose of this work was to more fully investigate the reliability of hand pollination techniques as the sole basis for selecting pollinators, to evaluate the effectiveness of currently used pollinators in commercial plantings and to develop an *in vivo* method of comparing the effectiveness of various pollen sources in causing seed development and fruiting in self-incompatible varieties.

MATERIALS AND METHODS

Standard hand pollination tests as a basis for selecting pollinators.—Pollen of several varieties (Table 2) was transferred to the stigmatic surface of pistils of 'Robinson' and 'Orlando' by brushing them with freshly dehisced anthers of the pollen variety. This procedure results in the transfer of hundreds of grains of pollen to the self-incompatible flowers.

The shoots were carefully selected for good vigor and flowers were the leafy bloom type,

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thinned to 1 flower per shoot. Flowers were emasculated and depetaled just prior to opening of the flower and anthesis.

Each pollen treatment was applied to a total of 100 flowers. A localized area on 1 side of a tree constituted a statistical block, with blocking designed to eliminate variability of position on the tree. Within this block, each treatment was applied to 5 flowers. Thus, a randomized block experiment with 5 flower plots and 20 replications was developed and statistically analyzed.

Effectiveness of pollinators in commercial plantings.—Commercial groves of fruiting age trees of self-incompatible varieties were located throughout the citrus area and the pollinating variety, if any, noted. Growers were questioned as to whether any adverse pest or environmental condition drastically influenced the bloom or early fruit development. Any groves with such problems were eliminated. Emphasis was placed on the 2 most important self-incompatible varieties, 'Orlando' tangelo and 'Robinson' tangerine, but several others were also investigated.

Fruiting was estimated by counting the fruit within a 2 x 2 foot frame placed at a height of 3 feet and against the foliage of the tree to be evaluated. Forty-five frame counts were made per grove and the mean count per frame calculated. Frames were placed against trees selected at random, throughout the groves in which pollinators were absent. In groves with pollinators, trees selected at random in rows adjacent to pollinators were used and the arithmetic mean or average number of fruit per frame was calculated.

Thirty fruit were randomly harvested from the trees used and the seeds counted. In some plantings there were so few fruit no samples were taken for seed counts.

Comparison of known quantities and kinds of pollens.—Known quantities of different varieties of pollen were placed on 'Orlando' and 'Robinson' flowers respectively that had been thinned to 1 per shoot, depetaled and emasculated.

Known quantities of pollen were obtained from freshly opened anthers of the desired variety with sharpened dissecting needles, a method suggested by Alkamine and Giorlomi (1). This was done with the aid of dissecting microscopes located in the field adjacent to the experimental trees. Assistants transported the pollen on the needle tips to the flowers and carefully wiped it off onto the stigmatic surfaces of the pistils.

Examination of the needle points indicated all the pollen was transferred.

Two experiments were conducted with 'Orlando'. In one, 10, 25, 50, and 100 grains of 'Temple' pollen were transferred to the stigmatic surfaces of 'Orlando' flowers and compared with flowers to which the standard massive amounts of pollen had been transferred by brushing freshly opened anthers of 'Temple' over the stigmatic surfaces of 'Orlando' until the latter turned yellow.

The experimental design consisted of 5 flowers per plot replicated 10 times. Thus, each quantity of pollen was applied to 50 flowers. Analysis of variance and differences between means were calculated.

A second experiment with 'Orlando', compared 25 grains of 'Hamlin', 'Valencia', 'Duncan', 'Dancy' and 'Temple' varieties, with 'Temple' being considered the standard. Otherwise, the procedures were similar.

With 'Robinson', experimental procedures were similar except 10, 20, 40, and 80 grains and massive amounts of 'Hamlin' and 'Temple' pollen respectively were compared and each treatment contained 35 flowers.

In all experiments the fruit were harvested and counted about 1 month prior to maturity.

RESULTS AND DISCUSSION

Standard hand pollination tests as a basis for selecting pollinators.—It is clearly evident that 'Robinson' and 'Orlando' are self-incompatible and unfruitful in the absence of cross-pollination and that a wide range of pollen sources are equally effective in inducing seed formation and fruitfulness, Table 2. This is in agreement with previous work (2, 4, 6, 8, 12). There were no statistical differences due to pollen source, other than selfing. In assessing this data, it is pertinent that the shoots were carefully selected and every effort made to develop precision. The % fruit set was high because of the careful selection of shoots and the removal of flowers from the area adjacent to those pollinated.

On the other hand, some pollinator varieties were less effective in commercial plantings where the honey bee was the pollinating agent, Table 3. For example, 'Hamlin' was almost completely ineffective as a pollinator for both 'Robinson' and 'Orlando' even though hand pollination tests had indicated the reverse. 'Pineapple' was slightly better but certainly less than adequate. 'Parson

Table 1.--Plant material used.

Cultivar	Species or hybrid name
Sweet orange	
Hamlin	<i>Citrus sinensis</i>
Parson Brown	<i>C. sinensis</i>
Pineapple	<i>C. sinensis</i>
Valencia	<i>C. sinensis</i>
Grapefruit	
Duncan	<i>C. paradisi</i> Macf.
Tangerines and hybrids	
Dancy	<i>C. reticulata</i> Blanco
Lee	<i>C. reticulata</i> x (<i>C. paradisi</i> x <i>reticulata</i>) (Clementine x Orlando)
Minneola	<i>C. paradisi</i> x <i>C. reticulata</i> (Duncan x Dancy)
Murcott	<i>C. reticulata</i> x <i>C. sinensis</i> (?)
Nova	<i>C. reticulata</i> x (<i>C. paradisi</i> x <i>reticulata</i>) (Clementine x Orlando)
Orlando	<i>C. paradisi</i> x <i>C. reticulata</i> (Duncan x Dancy)
Osceola	<i>C. reticulata</i> x (<i>C. paradisi</i> x <i>reticulata</i>) (Clementine x Orlando)
Page	(<i>C. paradisi</i> x <i>reticulata</i>) x <i>C. reticulata</i> (Minneola x Clementine)
Robinson	<i>C. reticulata</i> x (<i>C. paradisi</i> x <i>reticulata</i>) (Clementine x Orlando)

Brown' and 'Valencia' were likewise unsatisfactory for 'Orlando'. One planting of 'Robinson' with 'Valencia' and one with 'Parson Brown' were fruiting well but the erratic results with these varieties as pollinators for 'Orlando' (6) suggest the same may be true with 'Robinson'.

Comparison of known quantities and kind of pollen.—It was concluded from data in Table 5 that 50 grains of pollen was approximately the

Table 2.--The influence of hand-applied pollen¹ on the yield and seed content of 'Robinson' and 'Orlando' fruit.

Pollinator variety	Yield (% fruit matured) ²		No. seeds per fruit ³	
	Robinson	Orlando	Robinson	Orlando
Robinson	0 b	91 a	--	27 a
Orlando	81 a	1 b	19 a	0 b
Temple	77 a	94 a	17 a	28 a
Lee	80 a	90 a	20 a	28 a
Hamlin	81 a	91 a	18 a	30 a
Valencia	82 a	90 a	21 a	29 a
Pineapple	79 a	91 a	18 a	30 a
Duncan	--	92 a	--	29 a

In each column, means followed by unlike letters are significantly different at the 0.01 level.

¹Massive amounts of pollen were applied by brushing freshly dehisced anthers over the stigmatic surfaces of pistils.

²Mean % of fruit resulting from 100 pollinated flowers.

³Mean or average number of seeds per fruit.

Table 3.--Evaluation¹ of pollinators in commercial citrus plantings.

Pollinator variety	Variety pollinated	Varying pollinated					
		Robinson	Orlando	Lee	Osceola	Page	Nova
Robinson	Fruit	<1 (14)	19 (9)	17 (1)	<1 (1)	27 (2)	--
	Seed	3	11	19	2	3	
Orlando	F	26 (11)	4 (25)	16 (1)	--	16 (1)	15 (3)
	S	17	3	16	--	7	20
Lee	F	22 (1)	20 (2)	--	18 (1)	9 (4)	--
	S	16	14	--	10	6	
Osceola	F	<1 (1)	--	--	<1 (1)	--	--
	S	3	--	--	2	--	
Page	F	24 (2)	26 (1)	10 (3)	--	10 (8)	--
	S	12	14	13	--	<1	
Nova	F	--	4 (4)	--	--	--	2 (9)
	S	--	11	--	--	--	<1
Temple	F	16 (6)	23 (25)	--	--	5 (1)	--
	S	13	24	--	--	10	
Dancy	F	15 (2)	13 (15)	--	--	--	--
	S	15	20	--	--	--	
Hamlin	F	4 (3)	2 (10)	--	--	--	--
	S	6	<1	--	--	--	
Parson Brown	F	16 (1)	6 (10)	20 (1)	--	13 (1)	--
	S	12	5	14	--	1	
Pineapple	F	7 (2)	6 (10)	--	--	--	--
	S	13	7	--	--	--	
Valencia	F	14 (2)	3 (10)	--	--	--	--
	S	5	2	--	--	--	

¹The average number of fruit per 2 x 2 feet of fruiting area.

²Average seed content was obtained from 30 fruit at each location.

³Figures in brackets indicate the number of locations evaluated.

minimum needed to develop maximum fruiting under the conditions of this experiment; however, 25 grains was also fairly effective.

The number of seed per fruit increased with each increase in quantity of pollen and the differences in seed content between the 50 and 100 grains and saturation treatments were significantly different even though the number of fruit produced was not. Thus, a "luxury" number of seeds were produced. The data is insufficient to conclude 19 seeds is the absolute number needed. This would probably vary with the physiological condition of the shoots involved.

Table 6 contains the results of applications of 25 grains of pollen of each of 5 varieties to 'Orlando' flowers. 'Temple' was much more effective on a per grain basis than the others in setting fruit; however, the seed content did not differ as widely as had been expected. For example, 'Duncan' pollinations resulted in only 4 less seeds per fruit than those of 'Temple' but a large difference in number of fruit was produced. However, from the data in Table 5 it is evident that small differences in seed content are more influential at the lower end of the scale. The difference between the 10 and 25 grain treat-

ments was 3 seeds per fruit but a 16% increase in yield resulted. A difference of 4 seeds per fruit between the 50 and 100 grain treatments existed with only a 4% increase in number of fruit.

The poor showing of 'Dancy' and, to some extent, 'Duncan' was surprising since 'Dancy' and 'Duncan' have been effective in the field when their bloom periods overlap the 'Orlandos'. The 'Dancy' and 'Duncan' pollen came from 4-year-old trees and the other pollens from mature ones but there is no evidence this makes a difference. However, this experiment was designed only to establish a principle and precise comparisons of varieties will require more data.

It was concluded that the same principles established for 'Orlando' hold for 'Robinson', Table 7. 'Hamlin' was much less effective than 'Temple' pollen on a per grain basis but the difference was masked when massive amounts of both were used. The number of grains of 'Temple' needed for maximum yield was about 40, which agreed closely with the 'Orlando' data.

As in the case of standard pollination tests involving massive applications of pollen, these tests alone are not sufficient to delineate those varieties which are good pollinators. They do not take into account species or variety preference by the bees, the amount of pollen carried by bees, the number of visits bees make to citrus flowers and the amount of pollen produced by flowers of given varieties, none of which have received significant study in citrus. However, these tests are more accurate than standard tests in determining the comparative ability of various pollen varieties to induce fruiting and seed formation. Further research with this method can lead to a more thorough understanding of the pollination problems in citrus and assist in overcoming them.

Evaluation of pollinators in commercial plantings.—Evaluation of pollinators in commercial plantings is the only sure way of determining their effectiveness. Moreover, a 1-year evaluation of a grove or 2 will not suffice. The peculiarities of the honey bees, the time of bloom of various varieties, erratic or alternate blooming characteristics and age of tree are all involved. Observations have been made of several pollinators in 'Orlando' plantings for 11 years; however, little has been reported on the reciprocal performance of the relatively new varieties ('Robinson', 'Lee', 'Osceola', 'Page' and 'Nova') as commercial pollinators.

Table 3 contains the data obtained in a 1-year

survey of pollinators in commercial groves. The data for some of the varieties are very limited and recommendations are therefore tentative. The number of pollinators and their spacing varied tremendously and this greatly influenced the frame count values obtained. Thus, the quantitative data were tempered with observations made in careful inspections of the plantings.

The seed content is particularly coarse and nearly always skewed because highly parthenocarpic varieties produce some seedless fruit even with pollinators, thereby reducing the average seed content. Thus, the seed number was used only to determine whether the pollinator was causing seediness.

Tentative pollinator recommendations are given in Table 4 and discussed below.

For 'Robinson', 'Orlando', 'Lee', 'Temple', and 'Page' satisfactorily induced fruiting. 'Orlando' caused such heavy fruiting that severe limb breakage was common. This may also become a problem with 'Lee', for which only limited data were obtained. Spacing the 'Orlando' at wider intervals might prevent this but no background of experience is available on which to modify the currently recommended plans. 'Page' appeared to satisfactorily induce fruiting in 'Robinson' but 'Osceola' was virtually valueless in this respect, which agrees with hand pollination tests (12).

Table 4.--Tentative pollinator recommendations^{1,2} for self-incompatible tangerine hybrids.

Pollinator variety	Variety pollinated					
	Robinson	Orlando	Lee	Osceola	Page ²	Nova
Robinson	US	FA	FA	US	SA (L)	--
Orlando	SA	US	SA (L)	SA (L)	SA (L)	SA (L)
Lee	SA (L)	SA (L)	--	SA (L)	SA (L)	--
Osceola	US	--	--	US	--	--
Page ²	SA (L)	SA (L)	SA (L)	--	SA	--
Nova	--	US (L)	--	--	--	US
Temple	SA	SA	--	--	--	--
Dancy	ER	ER	--	--	--	--
Murcott	ER	ER	--	--	--	--
Hamlin	US	US	--	--	--	--
Pineapple	PO	PO	--	--	--	--
Valencia	PO	PO	--	--	--	--
Duncan	ER	ER	--	--	--	--

¹These recommendations are judgments based on hand pollination tests, frame counts, responses of growers, and general evaluation of the trees and existing conditions. No recommendation was made unless at least 1 field location was evaluated. Hand pollination tests are available for some varieties not included here; for these, the reader is referred to references (2, 12).

²The key to symbols is: US, completely unsatisfactory as a pollinator; SA, satisfactory as a pollinator; FA, fair (would probably be SA in a 50-50 ratio); PO, poor (gives some help but probably not enough except in unusual situations); ER, erratic (SA as a pollinator when bloom is sufficient and overlaps the variety to be pollinated, but bloom is erratic); (L), the data is very limited and more will be needed before final conclusions can be drawn; --, no field plantings were evaluated.

No 'Nova'-'Robinson' plantings of fruiting age were located. 'Robinson' in solid blocks were very unfruitful.

The 'Orlando' was nearly always used as the pollinator in 'Orlando'-'Robinson' plantings so most of the trees were 'Robinson'. This was very satisfactory, but it seems unlikely the full fruiting potential of 'Orlando' would be reached with less than a 50-50 mix of 'Orlando' and 'Robinson'. In the 2 blocks located, 'Lee' was effective as a pollinator for 'Orlando' and limited data with 'Page' indicated it too would be satisfactory. 'Nova' appeared to be ineffective as a pollinator for 'Orlando', even where 2 rows of each were alternated. In all cases, trees were fruiting for the first time; however, since the same 'Orlando' was causing fruiting of the 'Nova', tree age was not limiting. 'Temple' continued to cause heavy fruiting of 'Orlando', as previously reported (6). Solid 'Orlando' plantings are well established as unfruitful (3, 4, 6) and were so found in this survey. However, they do produce large, seedless crops on occasion, particularly on better soils, on some rootstocks, and in certain years.

The 'Lee' is not widely planted and in several blocks there was such a mixture of varieties, the effectiveness of each could not be sorted out. In the 2 blocks located, 'Orlando' served well as a pollinator. 'Page' also appeared satisfactory. Neither solid plantings of 'Lee' nor plantings of this variety with 'Nova' or 'Temple' were located.

'Osceola' plantings are few in number. Limited data indicate 'Robinson' is not satisfactory as a pollinator for 'Osceola' and this is substantiated by reported hand pollination tests (12). At 1 location, a small planting of 'Lee', 'Osceola' and

Table 5.--The influence of the number of grains of 'Temple' pollen on the yield and average seed content of 'Orlando' tangelos.¹

No. Temple pollen grains	% fruit developed	No. seed/fruit
10	26.0 a	7.4 a
25	42.0 b	10.4 a
50	56.0 c	19.1 b
100	60.0 c	25.2 c
Saturation	60.0 c	31.9 d

In each column, means followed by unlike letters are significantly different at the 0.01 level.

¹Each treatment applied to 50 flowers.

Table 6.--The influence of 25 grains of each of 5 pollen varieties on the yield and average seed content of 'Orlando'.

	Pollen source				
	Temple	Hamlin	Dancy	Valencia	Duncan
% fruit ^{1,2}	42 a	2 b	4 b	6 b	18 c
Seeds/fruit ^{1,3}	10	2	5	6	6

¹From 50 flowers per variety.

²Means in this line not followed by like letters are significantly different on the 0.05 level.

³No statistical analysis was made due to the very small number of fruit in some treatments.

'Robinson' were mixed. No frame counts were made but all varieties were fruiting well. Since 'Robinson' is not satisfactory, it follows that 'Lee' was furnishing the proper pollen. In another case, 'Osceola', 'Robinson' and 'Orlando' were mixed and all fruited well. Thus, 'Orlando' must have induced the fruiting in 'Osceola'.

'Page' reportedly is self-incompatible and highly parthenocarpic (2). This was found true at 8 locations. In fact, fruiting in solid blocks was almost as heavy as where pollinators were present. An exception was 2 plantings in which 'Robinson' and 'Page' were alternated. The rootstocks of 'Page' were also alternated, every other one being 'Carrizo' citrange or 'Cleopatra' mandarin. The 'Page' on 'Cleopatra' were virtually fruitless and those on 'Carrizo' fruited heavily but had very few seeds, less than 3 per fruit. Thus, the fruiting was due to rootstock rather than to the pollinator. The data in Table 3 are from the trees on 'Carrizo'. At a single location, 'Orlando' appeared effective as a pollinator.

'Nova' plantings with pollinators were in their first year of fruiting. At 3 locations the 'Orlando' appeared very effective in increasing fruiting. No other combinations were available. Nine plantings of 'Nova' without pollinators were

Table 7.--The influence of the number of grains of 'Temple' and 'Hamlin' pollen on the yield and seed content of 'Robinson' tangerine.

	No. pollen grains									
	Hamlin					Temple				
	10	20	40	80	Sat. ¹	10	20	40	80	Sat. ¹
% fruit ^{2,3}	0 a	0 a	11 b	29 c	83 e	31 c	49 d	74 e	77 e	86 e
Seeds/fruit ² (av.)	--	--	4.8 a	5.3 a	21.2 e	3.8 a	6.6 a	9.6 b	12.5 c	23.6 d

¹Saturated consist of applying massive amounts of pollen by brushing freshly opened anthers over the stigmatic surfaces.

²From a total of 35 flowers per treatment.

³Means in this line not followed by like letters are significantly different at the 0.05 level.

unfruitful, even though they were the oldest and largest trees found of this variety.

Multiple pollinators may be needed for 'Nova' and 'Osceola' if it develops they will not successfully induce fruiting in the variety pollinating them. For example, a 'Nova'-'Orlando'-'Robinson' planting would result in fruiting of all 3 varieties while 'Nova' with 'Orlando' would result in only 'Nova' fruiting. As of this date, the limited data reported (2) indicates 'Nova' may be a poor pollinator for other self-incompatible varieties. 'Temple', which is self-fruitful, might prove successful for pollinating 'Nova' but its tenderness to cold is objectionable.

Other varieties may be satisfactory in certain combinations but have faults that make their use questionable. 'Dancy' was effective with 'Robinson' and 'Orlando' but in many years it blooms little or none at all and its bloom period is often short and late. 'Murcott' is often an effective pollinator for some varieties but its bloom period is frequently later than varieties requiring cross-pollination. 'Duncan' and other seedy grapefruit produce little bloom in certain years and the general use of arsenic on grapefruit also poses a problem since spray drift to the variety being pollinated would render it unmarketable. Some sweet oranges might be satisfactory pollinators, as indicated from data in Table 3, but their per-

formance is erratic and their cultural requirements are sufficiently different to make management a problem.

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PERFORMANCE OF CLOSELY SPACED TREES

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ABSTRACT

High-density plantings offer a way for citrus growers to meet the challenge of rising costs of land, production, and harvesting. Results of an experiment initiated in 1960 clearly indicate that earlier economic returns may be realized from closely spaced trees. In the 1968-69 season, trees in a 10' x 15' spacing produced 619 boxes of 'Pineapple' oranges per acre. This was nearly twice the per acre yield of trees at a 15' x 20'

spacing and almost 3 times that of a 20' x 25' spacing.

Frequent pruning was required to maintain the vigorous trees in the closest spacing within their allotted space. This further stimulated excessive vegetative growth at the expense of fruit producing wood. Water requirements have also been higher for the closer spacings.

INTRODUCTION

Citrus growers are approaching an economic situation where drastic changes may be needed if groves are to be profitable in the future. Increasing land values and taxes, a decreasing availability of desirable citrus land, rising costs of materials and equipment, higher harvesting costs, and an uncertain labor supply are causing