CARIBBEAN FOOD CROPS SOCIETY

SOCIETE CARAIBE POUR LES PLANTES ALIMENTAIRES

25

Twenty fifth
Annual Meeting 1989

25e CONGRES ANNUEL

Guadeloupe

Vol. XXV

ASSESSMENT OF ACEROLA POLLEN CHARACTERISTICS FROM A BARBADOS PLANTING

S. MICHELINI (1), D. Murray (2) and L.E. Chillery (1)

1. Fruit Experiment Station for the Caribbean, Lascelles, St James.
2. Department of Biology, University of the West Indies, Cave Hill, Barbados.

ABSTRACT

The causes of low fruit set at a mixed cultivar planting of acerola (*Malpighia punicifolia*) in Barbados were investigated. Pollen viability was assessed by in vitro germination and stainability. Maximum germination rates varied amongst the five cultivars, ranging from 3.4 to 73.0 %. Poor correlations between germinability and stainability were found with staining tending to over estimate pollen viability. All of the flowers opening on a day did so before 6 a.m Anther dehiscence did not start until late afternoon in Cv. Florida Sweet whereas it had started by 8 a.m in the other cultivars. Thus in Cv. Florida Sweet self pollen is being shed after the peak stigmatic receptivity which occurs shortly after flower opening. In all cultivars, pollen germinability was highest early in the morning. The pollen load found on stigmas was generally low (<10 grains). The presence of incomptability barriers was investigated by comparing fruit yields from open pollination, with those obtained from self and cross pollination. Some self and cross incompatibility was found amongst the five cultivars. The primary cause of low fruit set in this planting appears to bo the lack of an efficient pollination vector which is compounded by the presence of incompatibility.

RESUME

EVALUATION DES CARACTERISTIQUES DU POLLEN DE CERISIER ANTILLAIS A PARTIR D'UNE PLANTATION DE LA BARBADE

Les causes d'un faible taux de fructification dans une plantation de la Barbade à mélange de cultivars du cerisier (*Malpighia punicifolia*) a été étudié. La viabilité du pollen a été évaluée par germination in vitro et colorabilité. Le maximum de germination varie, allant de 3,4 à 73 % selon le cultivar. La corrélation est faible entre germination et colorabilité, celle-ci

tend à une surestimation. Toutes les floraisons d'un jour ont lieu avant 6 heures. La déhiscence de Florida Sweet ne commence que tard l'après-midi, alors qu'à 8 h elle est commencée chez les autres. Ainsi l'auto-pollen de Florida Sweet n'est dispersé qu'après son pic de réceptivité stigmatique lequel suit rapidement l'anthèse. Chez tous les cultivars la germination est maximale tôt le matin. Il y avait peu de pollen généralement sur les stigmates (<10 grains). La présence de barrières d'incompatibilité a été étudiée en comparant la fructification en pollinisation ouverte et en auto et allopollinisation contrôlées. Quelques auto et allo incompatibilités ont été trouvées. La première cause de faible fructification dans cette plantation semble être l'absence de vecteur de pollinisation efficace renforcée par la présence d'incompatibilité.

INTRODUCTION

The acerola, also commonly known as the Barbados or West Indian cherry and botanically as *Malpighia emarginata*, is a small red fruit found throughout the West Indies. Following Asenjo's report (Asenjo and Guzman, 1946) of its exceptionally high vitamin C content, commercial orchards were planted in Puerto Rico, Florida, and Hawaii. From 1974 until the present, approximately 130 acres of trees have been established in Barbados (R. Marte, personal communication).

Acerola flowers can appear at any time of the year, are perfect and range from 2 to 2.5 cm in diameter. The flowers are produced in short or long-stalked inflorescences originating from terminal or axillary buds. The calyx consist of 5 erect sepals, the corolla has 5 petals, and the pistil consists of an ovary and 3 styles. The 10 erect stamens are equal in length or slightly shorter than the styles and are shorter than the petals. (Ledin, 1958).

While trees fruited well in Puerto Rico (Jackson, 1958), some fruit set problems occured in Florida (Ledin, 1958) and Hawaii (Yamane and Nakasone, 1961a). Low fruit set in spite of abundant flowering has reportedly been caused by dichogamy, low pollen viability or incompatibility (Frankel and Galun, 1977). Dichogamy, pollen being shed when the stigma is not receptive, is common in avocado (*Persea americana Mill.*), where complementary cultivars must be planted for good fruit set (Bergh, 1975). Nonviable pollen occurs in some *Citrus* hybrids, encouraging seedless fruit production (Soost and Cameron, 1975). In banana (*Musa sp*) incompatibility, due to variations in ploidy, is desirable since it results in seedless fruit (Simmonds, 1976).

In Hawaii, studies concluded that poor fruit set in acerola was mainly caused by poor pollination (Yamane and Nakasone, 1961a). To overcome low natural pollination, they sprayed 4-clorophen-oxyacetic (PCA), a plant growth regulator, on flowers and increased percentage of fruit set from 4 % in open pollinated flowers to 72 % for treated flowers (Yamane and Nakasone, 1961b).

In Barbados, flowering was improved using plant growth regulators and stimulated with water application during the dry season (Michelini and Chinnery, 1988b). Fruit set was improved in container grown acerola, c.v. «Florida Sweet» (Michelini and Chinnery, 1988a).

Despite these results, there has been variable fruit set in plantings at the Fruit Experiment Station for the Caribbean (FRESCA) in Barbados. Early flower flushes set much fruit, although some subsequent flowering resulted in very low fruit set. Flower characteristics were investigated to determine the causes.

MATERIALS AND METHODS

Planting of acerola cultivars from Barbados (Jumbo), Florida (Florida Sweet), and Puerto Rico (labelled PR-1, PR-2, and PR-3) were established in 1985.

The time of anthesis for each cultivar was determined by collecting five flowers from each cultivar at 6 a.m, 10 a.m, 1 p.m;, and 4 p.m. The percentage of ruptured anthers was immediately determined using a dissecting microscope. Only flowers with a complete set of 10 anthers were used.

The stigmas of each flower sampled were examined for the amount of pollen present on the three stigmas. The quantity of pollen per stigma was rated as low (<10 grains), medium (between 10 and 20 grains), or high (>20 grains).

Pollen viability was determined by either staining or in vitro germination. Pollen samples were stained with aniline blue in lactophenol, 2 % IKI (iodine in potassium iodide), or 2 % ITC (2,3,5-triphenyl tetrazolium chloride). All stains were prepared using 10 % sucrose stock solution to prevent the pollen grains from bursting. Triplicate samples were collected and left overnight to absorb stain. Predicted viability from aniline blue and IKI was assessed using dark blue absorbtion as positive, and for IIC, red stained grains were considered viable.

A cover slip was lowered onto the pollen to avoid the formation of air bubbles and to prevent the spreading of the lighter dead pollen to the outer edges of the cover slip. This minimized biased counts encountered with randon microscope fields.

Pollen was germinated to test the correlation between stained-predicted and in vitro viability. The optimal osmotic concentration for germination was determined using a stock solution of 100 ml distilled H2O, 0.01 g H3BO3 ; 0.03Cac(NO3)-2.4H2O ; 0.02MgSO4.7H2O ; and 0.01 KNO3 in 100 ml distilled H2O. Sucrose was added to obtain concentrations of 8 %, 10 %, and 12 %.

For the germination studies, a drop of medium was place onto a depression slide and anthers of a flower were then macerated in the medium with a large pin releasing the pollen grains. A density of approximately 200 grains per microscope field (X40 ocular) was found to offer good germination spacing without the inhibiting influence of clumped grains.

Each preparation was checked under the microscope for density and even dispersion. Particles of debris were then removed using the pin point. The pollen was left to germinate overnight (15 hours) at room temperature without a cover slip, since free movement of air is required for proper germination (Ho and Sziklai, 1979). The petri deshis were lined with moist paper contained replicates for each germination trial.

A randomly chosen microscope field was selected for germination counts. Grains with tubes greater than the diameter of the pollen grain were considered viable. The count was recorded shortly after germination as the presence of long tubes reduced counting accuracy.

The percentage germination were compared with stainability.

Open, selfed, cross, and reciprocal croos pollinations were conducted on the five cultivars. Flower peduncles and
surrounding leaves were marked with india ink to facilitate later counts. Open pollinations were performed on April 4th, with selfed and crossed pollinations made on the day of anthesis. Pollen was collected from stored, mature flowers and transferred using a soft thistle brush. A total of 2,243 flowers were sampled, with reciprocal crosses taking place between all cultivars. Fruit set was recorded at 13 days post anthesis.

Observation of insects possible involved in pollination were made whenever the orchard was visited.

RESULTS :

Time of anther dehiscence was variable by cultivar, with Jumbo and PR-2 exibiting the earlier (6 a.m) beginning and completing dehiscence by 1 p.m. (Table 1.). PR-1 began two hours later, and Florida Sweet was the latest of the cultivars with first anther dehiscence recorded at 4 p.m. PR-3 did not flower during this trial.

Stigmas generally showed low pollen deposits (Table 2). No honey bees were observed visiting flowers, despite the presence of two strong hives less than 100 m from the orchard. PR-1 showed high pollen amounts, observed to have been transferred by crawling insects.

Staining with aniline blue and IKI predicted high viability (Table 3), while TTC predicted germination rates much lower.

The optimum sucrose concentration for pollen germination varied amongst the cultivars (Table 4). In these preliminary test, three cultivars, Florida sweet, Jumbo, and PR-3 had maximum germination rates of under 7 %, while PR-1 and PR-2 had maximum rates of 73 % and 26 % respectively.

Staining did not correlate well with in vitro germination. Aniline blue and IKI had correlation coefficients of -0.83 and- 0.08, respectively, while TTC had an «r» value of 0.36.

Open pollination resulted in fruit set ranging from 0 to 18.6 % (Figure 1). Average fruit set combining all pollination methods were found to be 20.1 % and 16.1 % in PR-2 and PR-1, respectively, significantly better than the other cultivars. PR-2 achieved the highest fruit set of any cultivar when selfed ; 30.7 %. The single best combination was Jumbo (pollen parent) x PR-1, which gave 40 % fruit set.

Insects visiting flowers were restricted to aphids (*Aphis sp.*), stink bugs (*Nezara sp* ., and a variety of crawling insects. Honey bees (*Apis sp.*) were not found visiting flowers.

Table 1 : Another dehiscence for acerola cultivars by hour of days

Cultivar	Time (hour)				
	6 am	8 am	10 am	1 pm	4 pm
Florida Sweet	0	0	0	0	40
Jumbo	30	60	80	100	100
PR-1	0	10	80	96	96
PR-2	30	44	50	100	100
PR-3	-	-	-	-	-

PR-3 did not flower during the trial

Table 2 : Pollen grain ratings on flower stimas by hour of day

Cultivar	Time (hour)				
	6 am	8 am	10 am	1 pm	4 pm
Florida Sweet	Low	Low	Low	Low	Low
Jumbo	Low	Med	Low	Low	Med
PR-1	Low	Low	Low	High	High
PR-2	Low	Low	Low	Low	Low
PR-3	-	-	-	-	-

PR-3 did not flower during this trial
Ratings of pollen, Low = <10; 10<=Med=<20; High = >20

Table 3 : Staining of acerola cultivar pollen grains by three dyes

Cultivar	Percentage of pollen stained		
	IKI	Analine	TTC
Florida Sweet	59,8	40,5	10
Jumbo	12,9	37,7	0
PR-1	40	4	8,5
PR-2	46	25,6	2,7
PR-3	-	-	-
*R	-0,08	0,83	0,36

PR-3 did not flower during the trial
*R is pearson's correlation coefficient

Table 4 : Percentage of pollen germination of acerola cultivars at variable sucrose concentration

Cultivar	Sucrose concentration		
	8 %	10 %	12 %
Florida Sweet	1,8	4,8	2,6
Jumbo	6,1	4,8	5
PR-1	2,5	34	73
PR-2	3,6	26,6	4,6
PR-3	-	-	-

PR-3 did not flower during this trial

DISCUSSION :

Low fruit set is directly attributable to inadequate pollination. Wind pollination appears to be inefficient in Barbados, as was found in Hawaii (Yamane and Nakasone, 1961 a). The absence of bees and low numbers of other insects visiting cherry flowers suggests that the lack of efficient insect pollinators is contributing to low fruit set.

Aphids were observed to transfer pollen, but this only occured on the few trees that were infested and aphids usually stay on one tree, facilitating selfing, not cross fertilization.

Efficient pollinators transferring large quantities of viable pollen should produce moderate yields (Visser, 1984). Increased pollen availability improves the chances for successful pollination. It has be reported that large numbers of low viability spores can stimulate fruit set, as the pollen grains may contain small amounts of growth promoting factors which leach out onto the stigma (Frankel and Galun, 1977).

Pollen availability and stigma receptivity coincided with all cultivars except Florida Sweet. This allowed for adequate fruit set with sufficient pollen transfer among compatible cultivars, such as occured with crosses involving PR-1 (Figure 1). Large plantings of seedlings or mixed cultivars usually fruit well in Barbados and Puerto Rico.

The three pollen stains were unreliable in predicting pollen viability. The poor correlations compared with standard in vitro germinations indicate that until other stains are tested, in vitro germination should be continued.

Pollen viability decreased throughout the day, most likely through desiccation (Jhori and Vasil, 1961). This may help explain observations that light rain or overhead irrigation during flowering increases fruit set (J. Bravo, personal communication).

In every cultivar, when pollen supply was at its maximum, pollen viability was nearly zero. There appears to be an inherent problem with the cultivars tested in supplying large amounts of viable pollen, at a time when the stigmas are most receptive. This can be minimized with systemactic selection of cultivars with the requisite pollen characteristics and compatibility.

Maximum germination rates correlated relatively well (+0.65) with the fruit set observed in the field for the five pollen treatments. Some level of incompatibility is suggested by the variation in fruit set as exhibited by figure 1.

Due to the present public perception of «natural» being desirable, it is recommended that producers rely on non-chemical means of improving fruit set. This will include finding cultivars which provide timely production of large amounts of viable pollen, finding cultivars with female flowers receptive early in the morning, at which time pollen viability is highest, and the identification and promotion of pollinating insects. With careful observance of all pollination factors, combined with present information on flower promotion by water application, yields can be improved for greater acerola production.

REFERENCES

ASENJO, C.F. and de GUZMAN, A.R.F. (1946). The high ascorbic acid content of the West Indian Cherry. Science 103, 2191.

BERGH, B.O., (1975). Avocados (in) Advances in Fruit Breeding, Jules Janick and James Moore, Eds. Purdue Univ. Press, West Lafayette, Indiana, pp. 541-567.

FRANKEL, R., and GALUN, E. (1977). Pollination mechanism, reproduction and plant breeding. Springer-Verlag Berlin, Heidelberg, New York.

HO, R.H. and SZIKLAI O. (1971). Pollen germination of Tsuga heterophylla in vitro. Can. J. Bot. 49, 117-119.

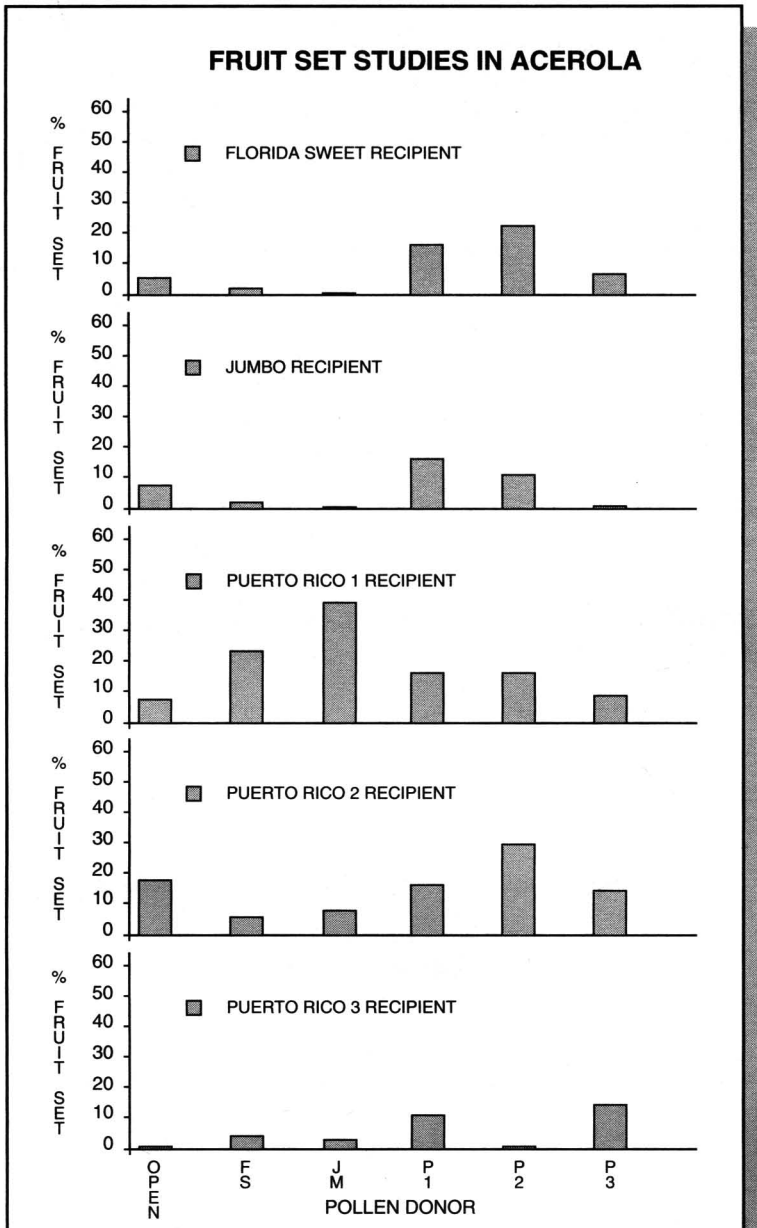
JACKSON, G.C. and PENNOCK, W. (1958). Fruit and vitamin C production of 5 and 6 year old acerola trees. J. agric. Univ. Puerto Rico 42 196-205.

JHORI, B.M. and VASIL, I.K. (1961). Physiology of pollen. The Botanical Rev. 27, 326-368.

LEDIN, R.B. (1958). The Barbados or West Indian cherry. Bull. Univ. Florida Agric. Expt. Stn. 594.

MICHELINI, S.A. and CHENNERY, L.E. (1988a). Enhanced muitiple cropping in Paclobutrazol treated acerola. ACTA Horticulturae (in press).

MICHELINI, S.A. and CHINNERY, L.E. (1988b). The use of plant growth regulators and irrigation to control flowering of the acerola or Barbados Cherry, (Malpighia glabra L.). Proceedings of the Inter-American Society for Tropical Horticulture (in press).



Percentage fruit set by each cultivar

SIMMONDS, N.W. (1976). Bananas (in) Evolution of Crop Plants, N.W. Simmonds, ed. Longman Group Ltd, London 211-215.

SOOST, R.K. and CAMERON, J.W. (1975). Citrus (in) Advances in Fruit Breeding, Jules Janick and James Moore, Eds. Purdue Univ. Press, West Lafayette, Indiana, 507-540.

VISSER, T. and MARCUCCI M.C. (1984). The interaction between compatible and self-incompatible pollen of apple and pear as influenced by their ratio in the pollen cloud. *Euphytica* 33, 609-984.

YAMANE, G.M. and NAKASONE, H.Y. (1961a). Pollination and fruit set studies of acerola Malpighia glabra L. in Hawaii. Proc. Am. Soc. Hort. Sci. 78 141-148.

YAMANE, G.M. and NAKASONE, H.Y. (1961a). Pollination and fruit set studies of acerola Malpighia glabra L. in Hawaii. Proc. Am. Soc. Hort. Sci. 78 141-148.

YAMANE, G.M. and NAKASONE, H.Y. (1961b). Effects of growth regulators on fruit set and growth of the acerola (Malpighia glabra L.). Tech. Bull. Hawaiian Agric. Expt. Stn. Univ. Hawaii 46.