Journal of Horticultural Science (1994) **69** (4) 697–702

Pollinator selection in kiwifruit (*Actinidia deliciosa*)

By MARIA V. GONZALEZ,[1] M. COQUE[1] and M. HERRERO[2]
[1]Instituto de Experimentación y Promoción Agraria, Apartado 13, 33300 Villaviciosa, Asturias, Spain
[2]S.I.A.-D.G.A., Campus de Aula Dei, Apartado 727, 50080 Zaragoza, Spain

SUMMARY

Pollinator performance was evaluated in kiwifruit to select pollinators for cv. Hayward adapted to the cultural conditions of southern Europe. Flowering time was determined in forty male seedlings. From, these, nine were selected for having a flowering period overlapping with 'Hayward'. In these nine males, as well as in the commercial pollinators 'Matua' and 'Tomuri', the production of pollen was studied over two years, investigating pollen quantity through flower density and pollen production per flower. Likewise, *in vitro* pollen viability and its *in vitro* germination were recorded to study pollen quality. Finally, *in vivo* pollen performance was studied through fruit set and fruit characteristics in controlled hand pollinations. Two males have been selected with a flowering period coincident with 'Hayward', which produce more than twice as much germinable pollen than commercial pollinators. Whilst there were no significant differences in pollen quality or in fruit production, clear differences existed for pollen quantity in terms of both flower density and pollen production per flower. As pollen quantity, together with flowering time, can be easily evaluated at an early stage, this is encouraging for future selection of kiwifruit pollinators.

KIWIFRUIT (*Actinidia deliciosa* (Chev.) Liang and Ferguson) is a dioecious vine; female plants have pistillate flowers with stamens that contain abortive pollen and male plants have staminate flowers with rudimentary pistils (Polito and Grant, 1984). Pollination appears to be a prime factor in kiwifruit production since inadequate pollination leads to small unmarketable fruits, because there is a close correlation between fruit size and seed number (Pyke and Alspach, 1986). Thus the production of female plants requires the introduction of staminate cultivars with enough pollen to assure the formation of 700–1400 seeds per fruit (Jansson and Warrington, 1988). Therefore, the choice of efficient pollinators is essential for the production of commercial fruit from kiwifruit vines.

The main female cultivar is Hayward (Ferguson *et al.*, 1990). Pollination of this cultivar relies on two male clones 'Matua' and 'Tomuri' selected in New Zealand (Zhang and Thorp, 1986). While these male clones appear to be well suited to New Zealand conditions, in Southern Europe, where kiwifruit culture is increasingly developing, these male clones may not perform as well as they do in other parts of the world. Thus, in the north of Spain the flowering period of 'Matua' and 'Tomuri' do not fully coincide with that of the female cultivar 'Hayward' (Coque and Fueyo, 1987). A similar situation has been reported in France (Blanchet and Guirbal, 1984) and Italy where, in addition of a lack of coincidence of flowering, reduced production of pollen by these male cultivars has been reported (Testolin *et al.*, 1990).

This situation had led to a search for good male pollinator clones. Thus a selection programme has been initiated in Italy (Testolin *et al.*, 1990) and in China (Shunwang *et al.*, 1988). However, no results are yet available. Here, work is reported on the selection of male cultivars with a flowering period coincident with 'Hayward', which will produce a good quantity of high quality pollen.

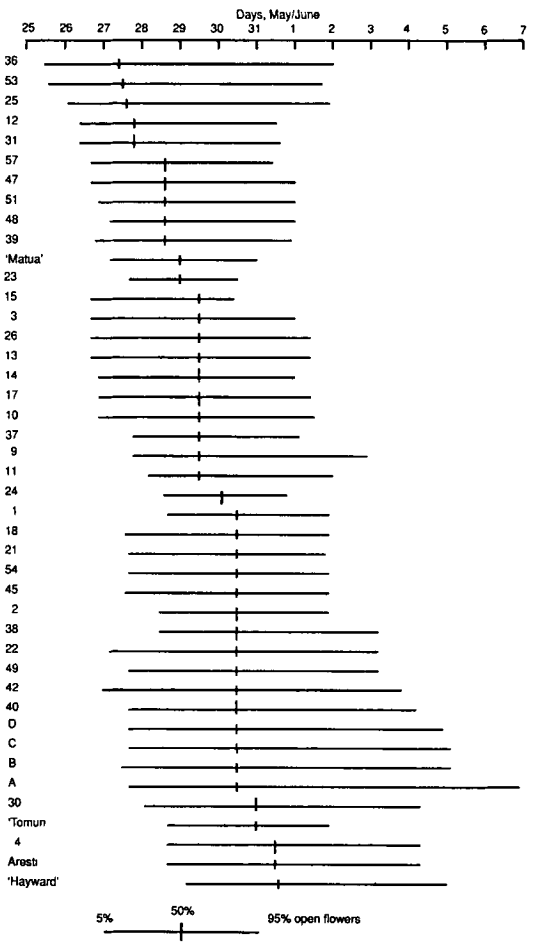

FIG. 1
Flowering time of forty male seedlings in relation to flowering time of 'Hayward'.

MATERIALS AND METHODS

Plant material

In a population of seedlings originated from open pollination in the north of Spain, 40 male vines were selected as being well adapted to these conditions. In these male vines, as well as in the male cvs Matua and Tomuri, and in the female cv. Hayward, flowering dates were recorded over three years. Of this population, on nine preselected staminate vines, flower density, pollen quantity and quality were evaluated for two years and compared with these variates in traditional pollinators. In addition, *in vivo* behaviour was evaluated on eight of those pollinators, but they were not compared with traditional pollinators. Data were analyzed following Duncan's Multiple Range Test (Litell, 1989).

Flower density

In 1990 and 1991 five one-year old canes were selected on each vine. These canes had a variable number of shoots, each of which was measured from its base to the last inflorescence. The flowers and inflorescences were counted. Flower density was expressed as number of flowers or inflorescences per linear centimetre.

Pollen quantity

In each staminate vine, five batches of 15 flowers each were collected just before anther dehiscence; the anthers were removed by combing onto smooth paper, and allowed to dehisce overnight at room temperature (20–25°C). Pollen from each batch was sieved, weighed and stored over silica gel at −18°C (Hopping and Jerram, 1980) for 3–4 months before being used for *in vitro* tests.

To determine the number of pollen grains per flower, counts of pollen grains per gram of dry pollen were made. Five milligrams of pollen from each male were suspended in 1 ml of vegetable oil and drops from each of these placed on either side of a haemocytometer (Church and Williams, 1983). Five examples were counted for each male clone.

Pollen quality

Pollen viability was determined using the fluorochromatic reaction technique with fluorescein diacetate (Heslop-Harrison and Heslop-Harrison, 1970) in 0.4 M sucrose.

Germination was tested using a modification of the liquid medium method of Hopping and Simpson (1982) using 0.4 M sucrose, 0.3 mM boric acid and 1% agar.

In vivo *behaviour*

In two subsequent years, a hundred pistillate flowers from four 'Hayward' vines were pollinated with fresh pollen from each of the eight staminate vines. Flowers were covered with muslin bags to exclude honey bees and pollen transport by wind. At harvest, final fruit set was evaluated and the fruits were weighed and measured. In the first year, seeds from these fruits were also collected and counted.

RESULTS

Flowering time

Flowering time of 'Hayward' lasted about

TABLE I
Flower density of staminate vines. For each column values followed by the same letter are not significantly different. P≤0.01

Pollinator	Inflorescences per cm	Flowers per inflorescence	Flowers per cm
A	0.66 a	2.88 ab	1.68 ab
B	0.66 a	2.19 abc	1.37 b
C	0.61 a	2.94 a	2.01 a
D	0.52 abc	2.91 ab	1.49 ab
4	0.47 abc	1.42 cd	0.67 dc
30	0.29 c	2.15 bc	0.66 cd
40	0.36 bc	1.15 d	0.42 d
42	0.55 ab	2.76 ab	1.50 ab
Aresti	0.71 a	1.62 dc	1.14 bc
'Matua'	0.65 a	1.93 c	1.25 bc
'Tomuri'	0.62 a	1.82 cd	1.09 bc

seven days. That of the 40 male clones studied ranged from 5–12 d (Figure 1). Although in the second year spring came early and flowering time was earlier than normal, relative flowering time was similar in both years. Seedlings 4, 30, 40, 42, A, B, C, D and Aresti were selected for having a flowering period close to 'Hayward'. The other males flowered earlier. In these clones flower density and pollen quantity and quality were evaluated.

Flower density

Differences were recorded between clones for flower density (Table I), both in the number of inflorescences per centimetre (from 0.29 to 0.71) and in the number of flowers per inflorescence (1.15 to 2.94). When these two variates are expressed as the number of flowers per centimetre, these differences were five-fold, ranging from 0.42 to 2.01. The most profusely flowering male was clone C (2.01 flowers per cm), significantly better than the results obtained for 'Matua' and 'Tomuri'. These two pollinators, in our culture conditions, had similar flower densities (1.25 and 1.09 flowers per cm).

Pollen quantity and quality

The quantity of pollen produced per flower by the different clones (Table II) was variable, ranging from 1.81 to 9.01 mg per flower. Expressed as pollen grain number per flower, these values ranged from 451,039 to 2,775,957. Clones A, B and Aresti performed significantly better than the traditional pollinators. This number is directly related to pollen weight per flower since no differences were recorded in the number of pollen grains per milligram of pollen, which averaged 290,748 (± 12,014) for all the clones.

Pollen stored at −18°C for 3–4 months retained high viability, as measured with the fluorochromatic reaction. Clones averaged 73% with no significant differences between clones. Likewise, the pollen used in this study germinated well (Table II), ranging from 64% for male 4 to 78% for male C. Similar results were recorded for 'Matua' and 'Tomuri', with no significant difference between them.

To integrate pollen quantity and quality a method proposed by Church and Williams (1983) to evaluate apple pollinators was followed. Thus, germinable pollen mg cm^{-1} expresses the amount of germinable pollen that a pollinator is capable of producing by calculating pollen weight per flower × flowers per cm × % germination. This yields big differences between clones (Figure 2), ranging from 52 to 1064 mg of germinable pollen. Males A and C produced significant more germinable pollen than 'Matua' and 'Tomuri'.

TABLE II
Pollen quantity and quality in staminated vines For each column values followed by the same letter are not significantly different. P≤0.01

Pollinator	Pollen weight per flower (mg)	Number of pollen grains per flower	Pollen viability (%)	Pollen germination (%)
A	9.01 a	2 675 572 a	73 a	70 bcd
B	7.50 ab	2 449 092 a	74 a	69 bcd
C	6.79 b	1 529 067 bcd	77 a	78 a
D	3.67 cd	1 156 483 d	73 a	65 cd
4	4.26 c	1 368 105 cd	73 a	64 d
30	1.81 d	451 039 e	70 a	69 bcd
40	1.83 d	470 364 e	72 a	69 bcd
42	7.71 ab	1 979 881 b	72 a	73 abc
Aresti	8.44 ab	2 775 957 a	75 a	76 a
'Matua'	4.23 c	1 462 968 cd	70 a	67 cd
'Tomuri'	6.27 b	1 727 319 bc	73 a	71 abcd

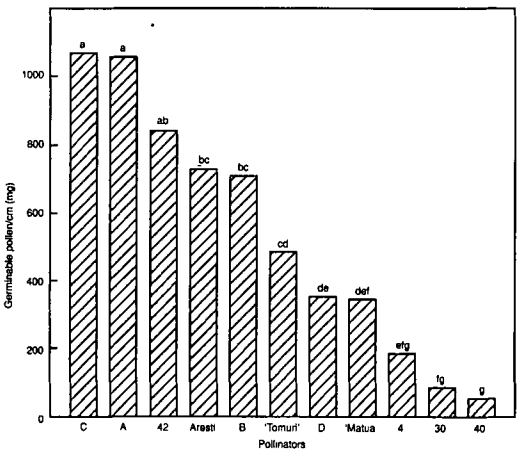

FIG. 2
Germinable pollen (mg/cm) for the different male clones studied. Values with the same letter are not significantly different at $P \leq 0.01$.

In vivo *behaviour*

Fruit set was high after hand pollination, ranging from 88 to 96%. Pollen source had no significant effect on fruit set, fruit weight and major or minor fruit diameter after hand pollination (Table III). However, there were differences in fruit length and seed number where some pollinators appeared to perform better than others.

DISCUSSION

Most of the 40 studied males tended to flower earlier than the female 'Hayward' vines. While in New Zealand 'Matua' and 'Tomuri' showed a significant overlap in flowering time with 'Hayward' (Thorp *et al.*, 1990) under our culture conditions they flowered earlier than 'Hayward'. Thus, 'Tomuri', while having a good covering of the early flowers, finished flowering when 'Hayward' was at about 60% flower opening. Likewise, 'Matua' was in full bloom even before 'Hayward' started flowering. A similar situation had been recorded in Italy where 'Matua' flowers before (Testolin *et al.*, 1990). Perhaps climatic conditions in New Zealand and Southern Europe are sufficiently different to account for the different synchrony of flowering between 'Hayward' and 'Matua' and 'Tomuri' in both areas, since differences in the time of flowering are not uncommon in temperate woody perennials and can be explained by the timing of the flowering period being regulated by spring temperature (Reader, 1975; Rathcke and Lacey, 1985) or specific stimuli such as photoperiod or vernalization (Jackson and Sweet, 1972).

Differences in flower density have been recorded here between clones. Similar differences were reported by Ferguson (1984) who observed that cultivars varied in the number and distribution of flowers along a shoot. While Martens (1985) described 'Matua' as a clone with abundant flowering and 'Tomuri' with sparse flowering, no significant differences were obtained between 'Matua' and 'Tomuri' under our conditions.

Pollen production per flower was very variable between clones, some having had five times the production of others. Results obtained with 'Matua' (4.23 mg per flower) were far lower than those (9.5 mg per flower) reported by Ferguson (1984). This could be due to a poor adaptation to our growing conditions. However, there were no differences in the number of pollen grains per milligram of pollen. This is not surprising, since there were no differences in either pollen size or viability between the males studied. Therefore, since the number of pollen grains per flower is tedious to calculate, for future work, providing that there

TABLE III
Fruit set and fruit characters obtained from 'Hayward' vines pollinated with different males. For each column values followed by the same letter are not significantly different. $P \leq 0.01$

Pollinator	Fruit set (%)	Fruit weight (g)	Diameters (cm)		Length (cm)	Number of seeds
			Major	Minor		
A	89 a	103 a	5 5 a	4.7 a	6 7 b	1 141 abc
B	90 a	110 a	5 5 a	4.8 a	6 6 b	901 c
C	94 a	119 a	5 6 a	4.8 a	7.0 ab	1 142 abc
D	96 a	127 a	5 8 a	4.9 a	6.9 ab	967 bc
4	88 a	120 a	5 6 a	4.8 a	7.0 ab	1 250 ab
30	94 a	124 a	5.7 a	4.9 a	7.0 ab	1 338 ab
40	91 a	116 a	5.6 a	4.8 a	6.9 ab	1 183 abc
42	94 a	137 a	5.8 a	4.9 a	7.5 a	1 344 a

are no differences in pollen size and pollen viability, the amount of pollen is accurately described by pollen weight.

Pollen viability and germination percentages were high and similar for all pollinators. This lack of differences between pollinators agrees with data about pollen fertility of kiwifruit staminate plants reported by Ford (1971) and White (1990). While 'Tomuri' has been reported to have poor pollen viability (Wilson and Bennenbroek, 1988), in our conditions germination and viability were good.

Fruit set following hand pollination was high and similar to that reported by Palmer-Jones and Clinch (1974) for 'Hayward'. While no differences have been recorded between the male clones, in their ability to set fruit or in the weight of the fruits produced, differences have been recorded in seed number. Others have pointed out a correlation between fruit weight and seed number (Ferguson, 1984; Pyke and Alspach, 1986; Galimberti *et al.,* 1988). In this work these two factors appear not to be related; this can be explained by the conditions of this experiment where, in all cases, big fruits were obtained because of hand pollination. On the other hand, observed differences in seed number are not easily understood, considering that the flowers were hand pollinated, and that there were no differences in the viability of pollen from any of the male clones. Snow and Spiro (1991) have shown that in *Hibiscus* differences exist in male competitive ability. A similar situation could exist in *Actinidia* but to evaluate this hypothesis competition experiments would have to be conducted.

The variable germinable pollen quantity per centimetre of shoot, previously used by Church and Williams (1983) to evaluate potential apple pollinators, clearly distinguished between clones and expressed the overall ability of a staminate clone as a pollinator, since this variable integrates quantity and quality of pollen. Among the studied plant material clones A and C produced over twice as much germinable pollen as traditional pollinators. Together with their concurrent flowering time with 'Hayward', this gives them a considerable advantage in our growing conditions.

It is still not clear what is the pollination vector in *Actinidia*. While wind can probably contribute as a pollination agent (Craig and Stewart, 1988), the presence of bees increases fruit set (Palmer-Jones and Clinch, 1974). To evaluate the attractiveness of these clones to insects requires an experimental approach, but no differences have been observed, in this work, in flower morphology. Likewise, the final evaluation of these pollinators should include an estimation of their pollination efficiency in terms of marketable fruit set under natural field conditions. However, the work reported here shows clear differences in reproductive characteristics of the clones examined. While some of the variates analyzed, e.g. fruit set and pollen viability, showed little variability between clones, others, e.g. flowering time and pollen quantity, were highly variable. The fact that these two variates can be easily evaluated at an early stage is encouraging for the future selection of kiwifruit pollinators.

We acknowledge I.N.I.A. (Projects 2193 and 8559) and CICYT (Project 0739) for financial support. I.N.I.A. for the award of a fellowship to M.V.G. We also thank Mr. Ramón Amenabar (Estación de Fruticultura de Zalla, Diputación Foral de Vizcaya, Spain) for providing the male plants of Aresti, 'Matua' and 'Tomuri'.

REFERENCES

BLANCHET, P. and GUIRBAL, M. (1984). Observation sur la floraison et la pollinisation du kiwi (*A. chinensis* Planch) en 1983. *L'Arboriculture Fruitière,* **362,** 29–32.

CHURCH, R. M. and WILLIAMS, R. R. (1983). Comparison of flower numbers and pollen production of several dessert apple and ornamental *Malus* cultivars. *Journal of Horticultural Science,* **58,** 327–36.

COQUE, M. and FUEYO, M. A. (1987). Recomendaciones para el cultivo de la Actinidia en el Norte de España. In: *Colección agricultura práctica, N° 39* (Ministerio de Agricultura Pesca y Alimentación, Eds). Madrid, Spain.

CRAIG, J. L. and STEWART, A. M. (1988). A review of kiwifruit pollination: where to next? *New Zealand Journal of Experimental Agriculture,* **16,** 385–99.

FERGUSON, A. R. (1984). Kiwifruit: a botanical review. *Horticultural Reviews,* **6,** 1–64.

FERGUSON, A. R., SEAL, A. G. and DAVISON, R. M. (1990). Cultivar improvement, genetics and breeding of kiwifruit. *Acta Horticulturae,* **282,** 335–47.

FORD, I. (1971). Chinese gooseberry pollination. *New Zealand Journal of Agronomy,* **122,** 34–5.

GALIMBERTI, P., MARRO, M. and JOSSEF, J. (1988). Periodo utile d'impollinazione in *Actinidia deliciosa. Rivista di Frutticoltura,* **50,** 71–4.

HESLOP-HARRISON, J. and HESLOP-HARRISON, Y. (1970). Evaluation of pollen viability by enzymatically induced fluorescence: Intracellular hydrolysis of fluorescein diacetate. *Stain Technology,* **45,** 115–20.

HOPPING, M. E. and JERRAM, E. M. (1980). Supplementary pollination of tree fruits. I. Development of suspension media. *New Zealand Journal of Agricultural Research,* **23,** 509–13.

HOPPING, M. E. and SIMPSON, L. M. (1982). Supplementary pollination of tree fruits. III. Suspension media for kiwifruit pollen. *New Zealand Journal of Agricultural Research,* **25,** 245–50.

JACKSON, D. I. and SWEET, G. B. (1972). Flower initiation in temperate woody plants. *Horticultural Abstracts,* **42,** 9–24.

JANSSON, D. M. and WARRINGTON, I. J. (1988). The influence of temperature during floral development and germination *in vitro* on the germinability of kiwifruit pollen. *New Zealand Journal of Experimental Agriculture,* **16,** 225–30.

LITELL, R. C. (1989). Statistical analysis of experiments with repeated measurements. *Horticultural Science Journal (Stuttgart),* **24,** 37–42.

MARTENS, L. (1985). Le kiwi, où actinidia de Chine. *Le Fruit Belge,* **412,** 288–94.

PALMER-JONES, T. and CLINCH, P. G. (1974). Observations on the pollination of chinese gooseberries variety 'Hayward'. *New Zealand Journal of Experimental Agriculture,* **2,** 455–8.

POLITO, V. and GRANT, J. A. (1984). Initiation and development of pistillate flowers in *Actinidia chinensis. Scientia Horticulturae,* **22,** 365–71.

PYKE, N. B. and ALSPACH, P. A. (1986). Inter-relationships of fruit weight, seed number and seed weight in kiwifruit. *New Zealand Journal of Agricultural Science,* **20,** 153–6.

RATHCKE, B. and LACEY, E. P. (1985). Phenological patterns of terrestrial plants. *Annual Review of Ecology and Systematics,* **16,** 179–214.

READER, R. J. (1975). Effect of air temperature on the flowering date of dogwood (*Cornus florida*). *Canadian Journal of Botany,* **53,** 1523–34.

SHUNWANG, L., SINGQYAD, X. and YUDAD, W. (1988). A study on selection and utilization of male cultivars of *Actinidia. Proceedings of the International Symposium on Horticultural Germplasm, Cultivated and Wild.* Beijing, China, 53.

SNOW, A. A. and SPIRO, T. P. (1991). Pollen vigour and the potential for sexual selection in plants. *Nature,* **352,** 796–7.

TESTOLIN, R., COSTA, G. and BIASI, R. (1990). Impollinazione e qualità dei frutti nell'actinidia. *Rivista di Frutticoltura,* **10,** 27–35.

THORP, T. G., ZHANG, J. and LAY-YEE, M. (1990). Horticultural characteristics of seven pistillate and three staminate New Zealand cultivars of kiwifruit. *New Zealand Journal of Crop and Horticultural Science,* **18,** 233–40.

WHITE, J. (1990). Pollen development in *Actinidia deliciosa* var. deliciosa: Histochemistry of the microspore mother cell walls. *Annals of Botany,* **65,** 231–9.

WILSON, G. and BENNENBROEK, M. (1988). Some M series males are not much good. *New Zealand Kiwifruit,* **52,** 16.

ZHANG, J. and THORP, T. G. (1986). Morphology of nine pistillate and three staminate New Zealand clones of kiwifruit (*Actinidia deliciosa* (A. Chev.) Liang et Ferguson var. deliciosa). *New Zealand Journal of Botany,* **24,** 589–613.

(Accepted 24 October 1993)