POLLEN–PISTIL INTERACTION IN KIWIFRUIT (*ACTINIDIA DELICIOSA*; ACTINIDIACEAE)¹

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The pollen–pistil interaction has been examined in kiwifruit (*Actinidia deliciosa*). In this species a large number of seeds are produced in each fruit and a great many pollen grains germinate and grow to reach the ovules. This growth is assisted by an abundant secretion that is present all along the pistilar tract. At anthesis, the stigma is covered by a secretion where the pollen grains germinate and grow. The stylar transmitting tissue is initially rich in starch reserves, but the starch gradually disappears and, concomitantly, an abundant secretion that stains for carbohydrates appears in all of the intercellular spaces. Pollen tube growth relies on this secretion since it is depleted after pollen tube passage, while in unpollinated flowers it remains unaltered throughout the flower life-span. In the ovary a similar situation occurs. The placental surface, where the pollen tubes grow before reaching the ovules, is covered by a number of obturators. At anthesis, these obturators are rich in starch reserves and have an abundant secretion on their outer surface. As time passes, starch disappears while the secretion increases. It is in this secretion that the pollen tubes grow on their way toward the ovules. These observations are discussed in terms of the support given by the pistil to pollen tube growth to achieve the highly successful reproductive performance of this species.

Key words: *Actinidia deliciosa*; Actinidiaceae; kiwifruit; pollen–pistil interaction; pollen tube growth.

Kiwifruit (*Actinidia deliciosa*) is a dioecious species whose reproductive biology has been much studied, due to its agronomic implications. Thus, information is available on the pollination system (Craig and Stewart, 1988), the structure, viability, and germination of the male gametophyte (Polito and Weibbaum, 1988; Li et al., 1989), and on pollen tube kinetics (Hopping and Jerram, 1979; Harvey et al., 1987). Likewise the pistil has been studied (Harvey et al., 1987) as well as postfertilization events (Harvey and Fraser, 1988). However, there has been little exploration of the pollen–pistil interaction and its practical implications. Reports on the pollen–pistil interaction in other species indicate that, while it has clear implications on the incompatibility reaction (Heslop-Harrison, 1983), in compatible matings (Owens, 1992) a number of changes appear to be devised to assist fertilization (Herrero, 1992a). Pollen tubes have a trophic dependence on the pistil since pollen tube growth in the style is supported at the expense of pistil reserves (Herrero and Dickinson, 1980; Mulcahy and Mulcahy, 1983). In a number of species, cells of the pistil structures are rich in starch that disappears after pollination (Herrero and Dickinson, 1979; Herrero and Arbeloa, 1989), and secretions are produced. These secretions supply nutrition and provide a pathway for the pollen tubes toward the ovules (Sedgley and Scholefield, 1980; Tilton and Lersten, 1981; Tilton et al., 1984). Furthermore, this trophic dependence plays a role in regulating pollen tube kinetics (Herrero

and Arbeloa, 1989) and in reducing the gametophytic population (Herrero, 1992b); it has been suggested that through this interaction mate choice can occur in plants (Marshall and Folsom, 1991) and that particular pollen genotypes could be selected by the pistil (Hormaza and Herrero, 1994).

Despite the attractiveness of these hypotheses the field is mainly speculative and little experimental work has been done. Recent work concentrates on the extracellular matrix where pollen tubes grow and emphasizes the pollen–pistil interaction (Wang, Wu, and Cheung, 1993).

Kiwifruit is a species with a highly efficient reproductive process; it is able to mature a fruit from each flower, and a fruit may have up to 1,400 seeds (Biasi and Costa, 1984). The number of seeds produced is critical since it has a direct bearing on fruit size and thus on the economic value of the kiwifruit crop. This implies the fertilization of a great number of ovules and therefore the growth of at least 1,400 pollen grains in a strictly female flower (Hopping and Jerram, 1979). The aim of this work was to examine the pollen–pistil interaction in kiwifruit to determine how the pistil can support the growth of so many pollen tubes.

MATERIALS AND METHODS

Plant material—Work was conducted on mature, 8-yr-old vines of the female cultivar ‘Hayward’, grafted on ‘Bruno’ seedlings, and grown in northern Spain. Pollinations were performed with pollen from male C selected out of a seedling population for its good pollinator performance (González, Coque, and Herrero, 1994). Pollen was obtained by collecting flowers from the male C 1 d prior to anthesis, removing the anthers, and leaving them to dry on paper at room temperature for 24 h. After this time, the pollen was sieved through a fine mesh, separating it from the dehiscent anthers and other impurities. Female flowers were bagged 1 d prior to anthesis to prevent outside pollination, in all of the

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experiments. Self-pollination is not possible in this species because female cultivars produce inviable pollen. One group of flowers was hand pollinated at anthesis and another one was left unpollinated.

Pollen tube observations—To trace pollen tube growth, flowers were fixed in formalin:acetic acid:70% ethanol (1:1:18; FAA) (Johansen, 1940) at daily intervals for the first 15 d after pollination. Pollen tube growth was monitored in squash preparations after washing out the fixative with water and softening the tissue in 5% Na₂SO₄ warmed to the boiling point (Jefferies and Belcher, 1974). The squashes were stained with 0.1% aniline blue in 0.03 mol/L K₃PO₄ (Linskens and Esser, 1957). Pollen tube growth in the stigma and style was observed in detached stigmatic arms, and pollen tube penetration was followed in dissected ovules using the same technique, but omitting softening.

Histochemical preparations—Five flowers per day and per treatment were fixed in 2.5% glutaraldehyde in 30 mmol/L phosphate buffer (Sabatini, Bensch, and Barnett, 1963) at pH 7.0, dehydrated in an ethanol series, and embedded in Histo-resin (Reichert-Jung, Heidelberg). Sections, 2 µm thick, were stained (1) with 0.5% periodic acid-Schiff reagent (PAS) for carbohydrates (Feder and O'Brien, 1968); (2) with 0.02% toluidine blue for 30 s at 60 °C following a modification of Feder and O'Brien's technique (1968) for phenolic compounds; (3) a combination of 1% safranin in 50% ethanol for 30 s followed by 1% methylene blue for 2 min (Gutmann, 1993) also for phenolic compounds; and finally (4) with a double staining PAS–0.02% toluidine blue combination (modified from Feder and O'Brien, 1968) for a general histological examination.

Preparation for scanning electron microscopy (SEM)—At anthesis and for the 5 d following anthesis, five flowers per day and treatment were fixed in 3% glutaraldehyde in 25 mmol/L phosphate buffer (pH 6.8), dehydrated in an ethanol series, critical point-dried, and coated with gold. Specimens were observed under a Philips P 500 scanning electron microscope.

RESULTS

Pistil anatomy—The kiwifruit flower has a syncarpous gynoecium, with generally over 30 united carpels (Hart, 1974). Each carpel, at the top of the compound ovary, gives rise to a separate style, basically open on the adaxial side. At the base the stylar furrow is closed but upwards it gradually widens, becoming the papillate stigmatic surface (Fig. 1). The stigma is papillar and covered by a conspicuous secretion (Fig. 2). The inner part of the style is occupied by a transmitting tissue, which is made up of loosely packed cells with a rich intercellular substance (Fig. 3). In the ovary, each carpel contains two rows of 10–20 ovules, which are anatropous, unitegmic and tenuinucellate with an axial placentation. Each row of ovules is paralleled by a row of placental humps, one facing each ovule (Fig. 4). The cells at the tip of the integument contain red globules after safranin-methylene blue staining (Fig. 5), and also stain green with toluidine blue, indicating the presence of phenols (Ramalingam and Ravindranath, 1970).

Secretion and pollen tube growth in the style—At anthesis, cells of the transmitting tissue are rich in starch reserves, and the intercellular substance stains positively with PAS (Fig. 6). In unpollinated flowers, these cells gradually lose their starch, while the intercellular substance increases. Two days after anthesis, when little starch remains in the transmitting tissue cells, the intercellular substance stains heavily with PAS (Fig. 7), and the cells show signs of degeneration.

When a flower is pollinated, the pollen grains germinate and penetrate into the intercellular spaces of the transmitting tissue the 1st d following pollination and the tubes reach the base of the style 2 d after pollination (Fig. 8). The transmitting tissue of a pollinated flower has a maturation process similar to that of an unpollinated flower: starch vanishes, while the intercellular substance increases. The situation is drastically different once the pollen tubes have passed, however. The intercellular substance disappears and the space is occupied by pollen tubes (Fig. 9). Thus, if pollinated and unpollinated pistils of old flowers are compared, both present a collapsed transmitting tissue, but the secretion is still present in unpollinated pistils, while it is completely gone from those that were pollinated.

Secretion and pollen tube growth in the ovary—On the placenta facing each micropyle there is a protuberance (Fig. 10) that is very much like an obturator on whose surface the pollen tube grows on its way to the ovule (Fig. 11). Since it both resembles the anatomy of and functions as an obturator, supporting pollen tube growth in the vicinity of the ovule, it will from now on be referred to by that name. The outer surface of the obturators is covered by an abundant secretion that stains profusely with PAS (Fig. 12). This secretion also extends all around the funicle (Fig. 10), thus providing an accumulation of secretion between the micropylar exostome and the obturator.

At anthesis, secretion is already present in the ovary, and the outer cells of the obturator show starch reserves. As time passes, starch disappears from these cells and secretion increases (Fig. 13). This occurs both in pollinated and unpollinated flowers, although the secretion remains unaltered in unpollinated flowers (Fig. 13), while in pollinated flowers it is partially reduced in amount after pollen tube passage (Fig. 14). Pollen tubes penetrate the ovary 2 d after pollination. They approach the ovules by growing on the obturator surface, and the first penetrated ovules (Fig. 15) can be seen 3 d after pollination.

DISCUSSION

Secretion in the pistilar tract appears to have a clear function in supporting pollen tube growth. In kiwifruit, the abundant stigmatic secretion ruptures the stigmatic

Figs. 1–5. Pistil anatomy of *Actinidia*. 1. SEM view of stigmatic arm of pistil at anthesis showing the papillar surface extending along adaxial side and the central furrow (arrowheads). Bar = 400 µm. 2. SEM view of stigma at anthesis showing secretion (s) overlying receptive papillar surface. Bar = 25 µm. 3. Transverse section of style of unpollinated flower; central furrow is occupied by transmitting tissue (tt). PAS stain. Bar = 125 µm. 4. SEM view of ovary showing a row of ovules (ov) with their respective obturators (ob). Bar = 100 µm. 5. Longisection of ovule, showing phenolic inclusions (arrowheads) in cells located at the integuments tip. Safranin-methylene blue stain. Bar = 125 µm.

Figs. 6–9. Secretion and pollen tube growth in style of *Actinidia*. 6. Transverse section of style at anthesis, showing starch (arrowheads) in cells that are surrounded by intercellular substance (is) rich in insoluble carbohydrates. PAS stain. Bar = 15 μ m. 7. Transverse section of style of unpollinated flower 2 d after anthesis. Cells are loosely packed in an abundant intercellular substance (is) but contain little starch. PAS stain. Bar = 15 μ m. 8. Squash of stigmatic arms stained with aniline blue and viewed with fluorescence to locate pollen tubes at base of style 2 d after pollination. Frequency of pollen tubes can be recognized by frequency of callose plugs. Bar = 30 μ m. 9. Transverse section of style of a flower 2 d after pollination. Pollen tubes (arrowheads) have already passed by this area, and the intercellular substance has disappeared. PAS stain. Bar = 15 μ m.

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Figs. 10–15. Secretion and pollen tube growth in ovary of *Actinidia*. 10. Longisection of ovary at anthesis showing ovule (OV), funicle (F), and obturator (OB). Bar = 125 μ m. Secretion occurs on obturator and all around the funicle. PAS stain. 11. SEM view of placental surface in a flower 5 d after pollination. One pollen tube (arrowheads) shows twists and turns on its way to reach ovule. Bar = 240 μ m. 12. Longisection of obturator at anthesis. Outer cells are rich in starch (arrowheads), and secretion (s) is already present. PAS stain. Bar = 50 μ m. 13. Longisection of obturator in an unpollinated flower 5 d after anthesis. Cells lack starch while a conspicuous secretion (s) covers outer surface. PAS and toluidine blue stain. Bar = 50 μ m. 14. Longisection of obturator in a flower 5 d after pollination. Pollen tubes have passed by, and secretion (s) is partially reduced. PAS stain. Bar = 50 μ m. 15. Dissected ovule from a flower 3 d after pollination, stained with aniline blue, showing pollen tube penetration of ovule through the micropyle. On the exostome remnants of twisting and branching of pollen tube before penetration can be observed. Bar = 150 μ m.

cuticle (Harvey et al., 1987) as also occurs in other species (Knox, 1984). This secretion favors pollen adherence, hydration, and germination (Sedgley and Scholefield, 1980).

In the style, secretion plays a major role in supporting pollen tube growth. This is very conspicuous in kiwifruit since the abundant secretion that occurred in the style at anthesis disappears after pollen tube passage, while it remains unaltered in unpollinated flowers. This situation has been previously recorded in such different species as lily (Labarca and Loewus, 1973), petunia (Herrero and Dickinson, 1979), or avocado (Sedgley, 1979) and appears to be associated with the change from an autotrophous to heterotrophous way of life of the pollen tubes when entering the style (Herrero and Dickinson, 1980; Mulcahy and Mulcahy, 1983).

In the ovary, the placenta is covered by a number of humps, interpreted as obturators, one facing each ovule. The pollen tubes crawl on these obturators to approach the ovule. The obturator, was first described by Juel (1918) in the Rosaceae, and has since been described in a number of species from different taxonomic groups as a protuberance facing the micropyle on the surface of which pollen tubes grow (Tilton and Horner, 1980). Secretion on the obturator surface, which is very clear in kiwifruit, was also described in peach (Arbeloa and Herrero, 1987). A major difference exists between these two species, however. While secretion is present throughout the pollen pathway at anthesis in kiwifruit, in peach it appears on the obturator only at a particular time, and pollen tubes can only grow on the obturator surface when secretion is present. The obturator has been postulated as having a role in pollen tube nutrition (Peterson, Scott, and Miller, 1979; Tilton and Horner, 1980; Tilton et al., 1984; Arbeloa and Herrero, 1987). It also could well be involved in pollen tube guidance (Tilton and Horner, 1980). In this sense it is worth mentioning that the secretion produced by the obturator in kiwifruit does not appear to be restricted to it, but also occurs on the base of the funicle, forming a continuous medium lining the pollen tube pathway from stigma to ovular exostome and could be associated with pollen tube guidance. The presence of a secretion covering the placental surface in the ovary has been described for other species as well (Singh and Walles, 1992) and has been associated with pollen tube passage (Tilton and Horner, 1980; Clifford and Owens, 1990; Peck and Lersten, 1991).

The fact that secretion occurs all along the pistilar tract in the kiwifruit flower and persists until flower wilting may be important in the high reproductive success of this species. Secretion is produced independently of pollination since a similar secretion occurs in unpollinated flowers. This situation has been described for *Rhododendron* (Palser, Rouse, and Williams, 1992) where secretion is produced basipetally all along the pistilar tract, including the ovary, both in pollinated and unpollinated flowers. In other species, while some of the changes that take place in the pistil appear to be dependent on pollination (Herrero and Dickinson, 1979; Sedgley and Scholefield, 1980; Sedgley, 1982; Herrero and Gascón, 1987), other processes are independent of it and follow a programmed developmental process (Dickinson, Moriarty, and Lawson, 1982; Arbeloa and Herrero, 1987). Nevertheless, this de-

velopment has an effect on pollen tube performance. Such a situation has been described for kiwifruit, where pollen germination depends on stigmatic papillar integrity (González, Coque, and Herrero, 1995). This phenomenon could be associated with the liberation of some intracellular contents into the stigmatic germination medium. While no information is available on what those contents that impair pollen germination are, the most conspicuous intrapapillar contents are phenolic inclusions. Phenolic inclusions also have been observed here in the micropylar end of the kiwifruit ovular integument. The significance of these inclusions is not clear. They do not appear to be involved in the reproductive process, since they do not change either with pollination or with aging, at least within the range of days observed here. However, they could play a role later in seed coat establishment.

Previous work (Herrero and Arbeloa, 1989) has reported the need for male–female synchrony during the reproductive process. In some species, as in *Prunus persica*, the pistil is not mature at anthesis and maturation occurs basipetally, resulting in accelerations and decelerations of the pollen tubes (Herrero and Arbeloa, 1989). The idea of coordinated timing between male and female development also has been stressed for *Rhododendron nuttallii* (Palser, Rouse, and Williams, 1989). In kiwifruit this requirement seems to be easily fulfilled since starting with anthesis abundant secretions are present along the entire pollen tube pathway from stigma to ovules, thus allowing support for a great many pollen tubes. This secretion clearly appears to be involved in pollen tube nutrition and guidance since it disappears after pollen tube passage. The time when the secretion is present and its quantity may well ensure a large number of fertilized ovules and could explain the high reproductive success recorded for this species.

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