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Influence of Ethylene on Enzyme Activities and Mobilization of Materials in Pollinated *Arachnis* Orchid Flowers

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Abstract. Pollinated *Arachnis* flowers exhibit morphological changes that are typical of the postpollination phenomena. Associated with these visible changes was the movement of soluble amino nitrogen and phosphorus from the perianth to the column and ovary. A rapid increase in protease and acid phosphatase activities was observed in the perianth, column, and ovary. However, it appeared that the enhanced enzyme activities were not responsible for the mobilization of materials from the perianth to the ovary, though both were induced by ethylene produced following pollination.

Depending on the orchid species or genus, pollination can cause a variety of developmental, physiological, and biochemical events. These include anthocyanin formation or destruction, stigmatic closure, senescence of the perianth, swelling of gynostemium, changes in enzyme activity, hydrolysis of macromolecules in petals, and ethylene production (Arditti 1969, Arditti and Flick 1974, Arditti and Knauft 1969, Arditti et al. 1971a,b, 1973, Burg and Dijkman 1967, Gessner 1948, Goh et al. 1985, Hsiang 1951a,b, Nair 1984, Ringstrom 1968). Among all these, ethylene evolution following pollination has attracted the most interest. Though ethylene has been implicated in the control of several postpollination processes (Akamine 1963, Arditti et al. 1973, Burg and Dijkman 1967, Chadwick et al. 1986, Hsiang 1951a,b, Nair 1984), its effect on postpollination processes remained unresolved (Chadwick et al. 1980).

By comparison, one area of postpollination phenomena in orchids that has received relatively less attention is the mobilization and transport of materials between the floral parts. Pollination causes an increase in fresh weight and dry weight of gynostemium and ovary with a concomitant decrease in perianth (Hsiang 1951, Arditti and Harrison 1979). Movement of sugar, protein, and

phosphate from perianth to ovary following pollination has also been reported in *Cymbidium* and *Cattleya* flowers (Gessner 1948, Hsiang 1951a, Harrison and Arditti 1976, Oertli and Kohl 1960). However, information on the temporal and control aspects of mobilization and transport of materials following pollination remains fragmentary. This paper examines the influence of ethylene on the activities of hydrolytic enzymes such as protease and phosphatase and the mobilization of materials in pollinated *Arachnis* flowers.

Materials and Methods

Uniform *Arachnis* Maggie Oei flower spikes were selected. For each spike, only one mature flower was self-pollinated, and the spike was allowed to remain on the plants. At specific time intervals, the pollinated flowers and the unpollinated control flowers were harvested, and the fresh weight, dry weight, total nitrogen, and amino nitrogen and phosphorus contents in the perianth, column, and ovary were determined. The total nitrogen was determined by the modified Kjeldahl method (Umbreit et al. 1957), and amino nitrogen was measured as described by Yemm and Cocking (1955). Phosphorus was determined according to Fiske and Subbarrow (1925).

For the study of protease and acid phosphatase, 2 g of various floral parts was homogenized with 15 ml of isolation medium containing 0.05 M Tris-HCl buffer at pH 7.2, cysteine-hydrochloride $MgCl_2$ (1 mM), KCl (10 mM), EDTA (1 mM), 2 ml 5% Triton X-100, and 0.5 g PVP. The homogenate was centrifuged at 9000*g* for 20 min, and this crude extract was used for the enzyme assay. The protease activity was determined according to Beevers (1968), and acid phosphatase measurement was made as described by Lowry (1957).

Ethylene production was measured with a Hewlett-Packard gas chromatograph (model 5890A). Each flower was cut underwater and placed in a separate vial filled with water and kept in a sealed bottle. Air sample (1 ml) was removed at specific intervals using a 22-gauge needle and a disposable syringe.

The concentration of ethylene was determined using a flame detector and a Porapak column. Nitrogen was used as the carrier gas at a flow rate of 35 ml min^{-1} with an oven temperature of 100°C (isothermal), injection temperature of 150°C, and detector temperature of 200°C. All determinations were done in triplicate.

Results

Changes in Fresh and Dry Weight of Various Floral Parts

Pollination of *Arachnis* flower brought about wilting of the perianth, closing of the stigma, and swelling of the column and ovary. The fresh weight of the column increased steadily following pollination; on day 15, it was double that

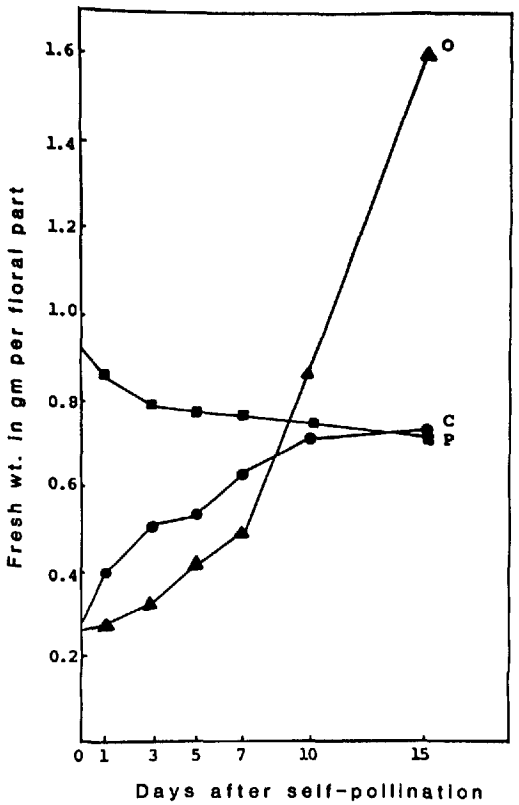

Fig. 1. Changes in fresh weight in floral parts after pollination. (P, perianth; C, column; O, ovary.)

of day 0 (Fig. 1). A dramatic increase in the fresh weight of the ovary was observed on day 7 after pollination. The fresh weight of the perianth decreased gradually with time. Similar changes were observed in dry weight. In the unpollinated control, the fresh and dry weight of the various parts remained fairly constant over the same operation interval.

Changes in Chemical Composition of Flowers

The changes in total phosphorus in floral parts after pollination were very similar to that of the dry weight (Fig. 2). The changes in total nitrogen and α-amino nitrogen were slightly different (Figs. 3, 4). There was no appreciable change in total nitrogen and α-amino nitrogen at early stages of pollination. However, on day 7, the total nitrogen in the perianth declined gradually, but there was a rapid increase in the nitrogen content in the column and ovary. Changes in α-amino nitrogen were not apparent in the perianth and column. A rapid increase in α-amino nitrogen was observed in the ovary at day 10.

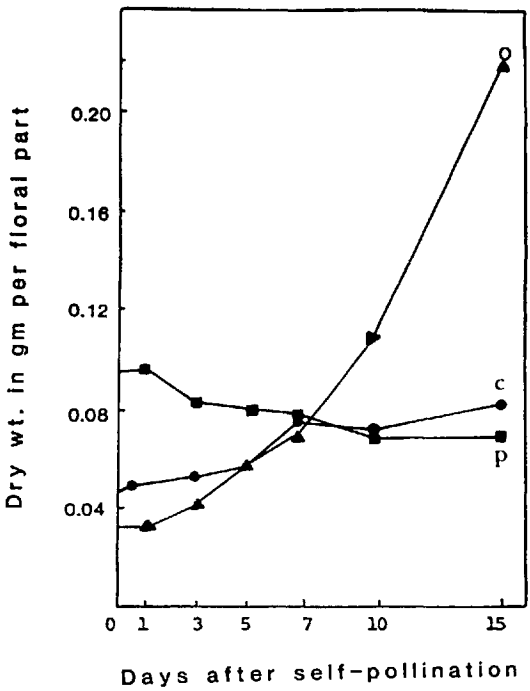

Fig. 2. Changes in dry weight in floral parts after pollination.

Changes in Protease and Acid Phosphatase Activities

In the perianth, the protease activity increased very rapidly, reaching a peak 24 h after pollination. This was followed by a gradual decline (Fig. 5). The same was observed in the column and ovary.

The changes in acid phosphatase activity in various floral parts are shown in Fig. 6. A rapid increase in enzyme activity was observed immediately following pollination in the ovary and the perianth.

Ethylene Production

There was no appreciable production of ethylene in the unpollinated control (Fig. 7). In the pollinated flower, rapid ethylene production was observed, and it reached a peak 24 h after pollination (Fig. 8). A second peak was observed at about 40 h.

Discussion

Like any other orchid flowers, pollinated *Arachnis* flowers undergo remarkable changes that are typical of postpollination phenomena (Arditti 1979). There was an increase in the fresh weight and dry weight in the column and

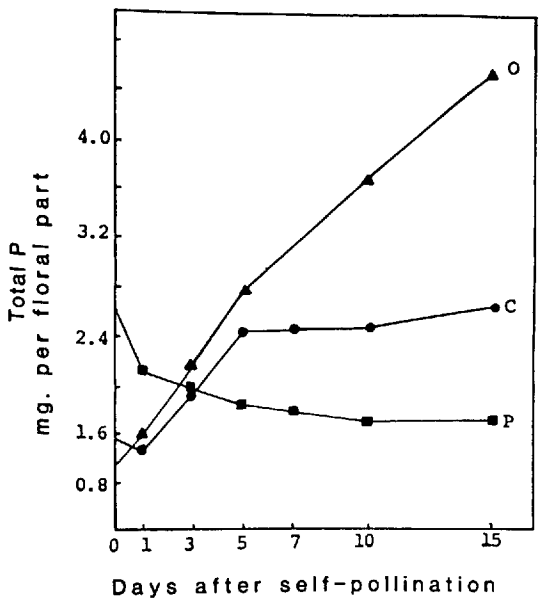

Fig. 3. Changes in total phosphorus in floral parts after pollination.

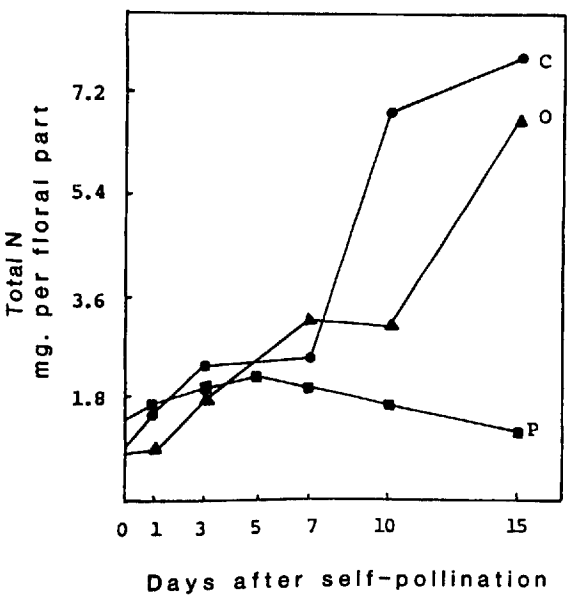

Fig. 4. Changes in total nitrogen in floral parts after pollination.

ovary with a concomitant decrease in the perianth. The increase in fresh weight in the column and ovary has been attributed to active water uptake (Hsiang 1951a, Arditti and Harrison 1979). Associated with these visible changes in the column and ovary was a movement of materials (nitrogenous compounds and phosphorus) from the perianth to the column and ovary. Har-

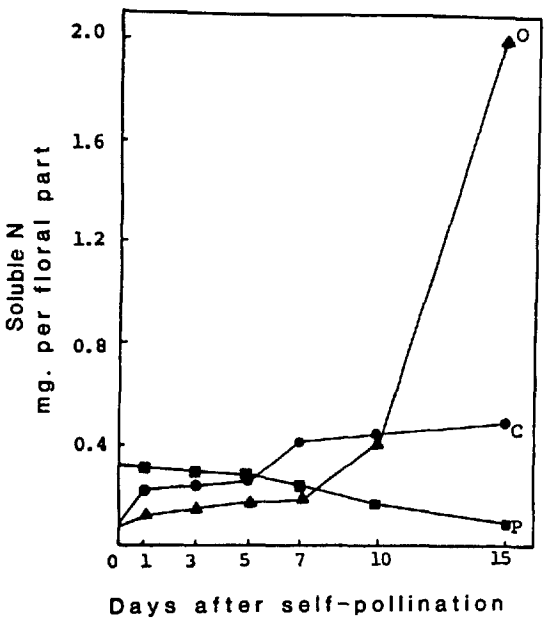

Fig. 5. Changes in soluble and amino nitrogen content in floral parts after pollination.

rison and Arditti (1976) have studied the phosphate movement in pollinated *Cymbidium* flowers. They found that radioactive phosphate applied to labellum, dorsal sepals, or stigma of *Cymbidium* was transported to all parts of the flower. Pollination caused the accumulation of ^{32}P in the gynostemium and ovary. Translocation characterics correlated with vascular anatomy of *Cymbidium* flowers. In pollinated *Cymbidium* and *Cattleya*, the nitrogen content of perianth segment decreased, and that of the gynostemium and ovary increased (Hsiang 1951b, Gessner 1948). However, the nature of the nitrogenous substances in the perianth segments, column, and ovary was not known.

The movement of nitrogenous compounds and phosphorus in pollinated *Arachnis* flower was apparently dictated by the relative sink activities of the floral parts. The materials moved first to the column and then to the ovary, following the sequence of swellings in these floral parts. It is interesting to note that the gains in nitrogenous compounds and phosphorus in column and ovary were very much greater than the actual loss from the perianth. A substantial amount of materials must have been supplied by the leaves adjacent to the spike and/or from other flowers in the same spike, as the pollinated flowers were still attached to the plant until harvest.

The dramatic rise in the activities of protease and acid phosphatase in pollinated *Arachnis* flowers is in agreement with those reported for catalase (Hsiang 1951b), polyphenol oxidase (Tan and Hew 1973), and peroxidase (Trippi and Tran Thanh Van 1971). However, it is doubtful whether the enhanced activity of protease and phosphatase is responsible for the hydrolysis and mobilization of materials in pollinated *Arachnis* flowers, because the developmental pattern of the activities of these two enzymes did not closely associate with the changes of total nitrogen and phosphorus contents in the

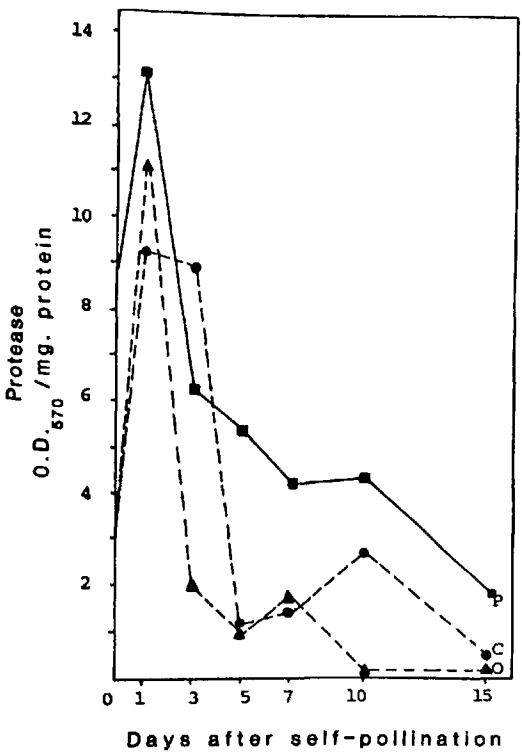

Fig. 6. Protease activity in floral parts after pollination.

column and ovary. Also, the dramatic rise in activity of these two enzymes occurred simultaneously in the perianth, column and ovary. The increase in enzyme activities followed closely that of ethylene production, suggesting a close relationship between them.

The stimulatory effect of ethylene on the activity of a number of enzymes has been described (Abeles 1985). Burg and Dijkman (1967) believed that pollination caused the transfer of auxin to the stigma, the spread of growth hormone to the column, the induction of ethylene formation, and the diffusion of gas to adjacent tissues, where it induced further ethylene production and tissue fading. There is evidence indicating that the rapid senescence of carnation flower following pollination was a response to 1-aminocyclopropane-1-carboxylic acid (ACC) translocated from the stigma to the rest of the flower (Nichols et al. 1983). The ACC or ethylene produced was seen as a result of wound response following the penetration of the stigma tissues by the growing pollen tubes (Boller and Kende 1980, Chadwick et al. 1986, Nichols et al. 1983, Strauss and Arditti 1984, Yang and Pratt 1978, Yu and Yang 1980).

Wound-induced increase in ACC synthase level (Boller and Kende 1980, Yu and Yang 1980) is in agreement with reports of increased ACC content in pollinated flowers of *Phalaenopsis* and *Dendrobium* (Lizada and Rimando 1985, Nair and Tung 1987). Mobilization of materials from perianth into column and ovary following pollination might well be a result of an increased cell activity

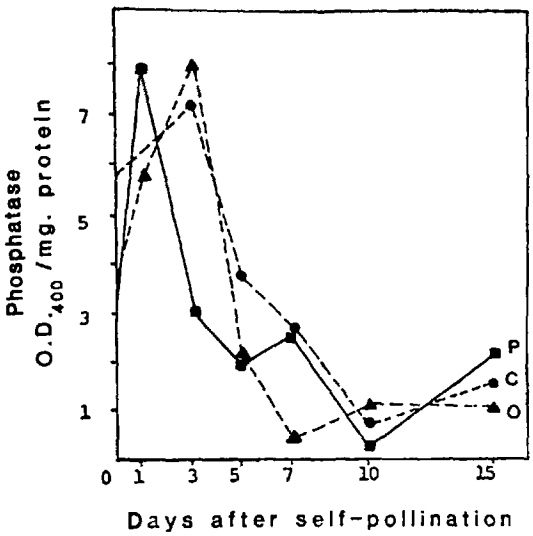

Fig. 7. Acid phosphatase activity in floral parts after pollination.

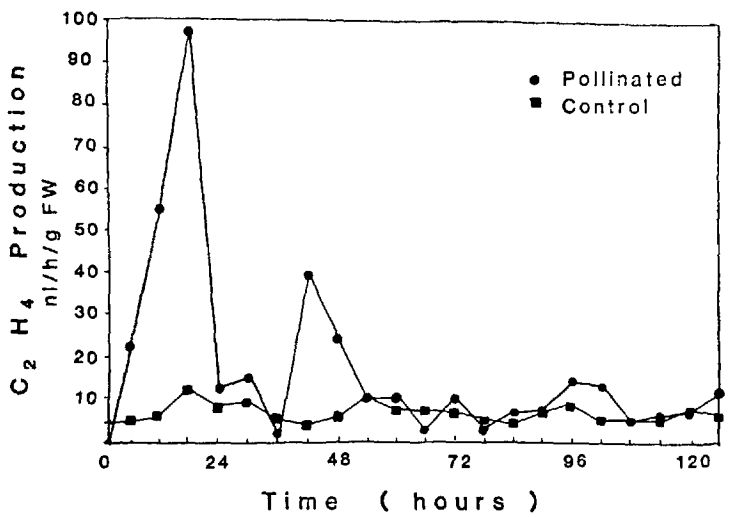

Fig. 8. Ethylene production by pollinated *Arachnis* flowers.

induced by ethylene. Histological examination of the ovary walls from ethylene-treated carnations showed that the cells had enlarged (Nichols 1976). The same had been reported for pollinated orchid flowers (Arditti 1979). The increased cell activity in the ovary following the action of ethylene could serve as an active sink for the influx of materials to the ovary. The manner in which inorganic and organic materials move out of the petal is not known. It seems that the movement of materials may be the result of increased permeability of cell membrane caused by ethylene (Hanson and Kende 1975).

Our results show that pollination caused ethylene production in *Arachnis* flower as has been reported previously for other orchid flowers (Burg and

Dijkman 1967, Goh et al. 1985, Nair 1984). However, the increased activities of protease and phosphatase and mobilization of materials to the ovary are processes induced independently by ethylene. Our observations support the suggestion made by Arditti et al. (1973) that postpollination phenomena, although all initiated by pollination, are each controlled in different ways.

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