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Short Communication

Ethylene and Auxin Participation in Pollen Induced Fading of Vanda Orchid Blossoms

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Blossoms from several varieties of orchids fade prematurely when their pollinia are removed or disturbed (2, 3, 7), and also if the flowers are gassed with ethylene (8, 9, 10), pollinated (3, 7), or treated with an auxin (3, 7). During natural fading as well as that induced by removal of the pollinia, ethylene is evolved (2, 3, 9, 10), and therefore it has been inferred that the gas is the causative agent. Ethylene evolution also is stimulated when plant tissues are exposed to auxin (1, 4), and we now present evidence that this response is the basis for flower fading induced by pollination or auxin application.

Blossoms from *Vanda Petamboeran* or *Vanda Rose Marie* were placed with their cut ends in a beaker of water, and sealed under a 200 ml bell jar having a ground glass base and a side arm closed with a rubber vaccine cap. Other flowers were pollinated, emasculated (pollinia removed), self-pollinated, or treated with 5 mM IAA in lanolin or 0.1 mM carboxyl labeled ^{14}C-IAA in 0.8 % agar (specific activity = 8 mc/mmole) applied to the stigmas, and then the flowers were sealed under a bell jar. Air samples, removed with a hypodermic syringe, were analyzed by gas chromatography to determine the ethylene content (4), and at least every 10 hours the chambers were completely aerated. Ethylene evolution by isolated sepals and petals, columns or lips was measured by incubating each tissue in a 50 ml flask fitted with a vaccine cap, and sampling the air in the flask at hourly intervals. Cutting the floral parts induced a wound response, lasting about 1 hour, during which time 1.5 mμl of ethylene per gram of cut control tissue was produced. Treated cut tissue was only considered to produce ethylene if the total evolution exceeded this value, and if the extra evolution continued at a nearly constant rate for at least 4 hours. Intact blossoms were also placed in desiccators and gassed for 3 to 24 hours with 10 ppm ethylene. In such cases the flowers were aerated for at least 1 hour before ethylene production was measured in order to remove any ethylene contained within the tissue. The spread of ^{14}C-IAA from the stigmas of the flower was determined by excising the floral parts from representative blossoms after 3, 6, and 24 hours and dividing these into pieces as illustrated in figure 3. Each piece was ground with ethanol on a planchet, dried, and counted with a 21 % efficient gas flow counter.

When blossoms of *V. Rose Marie* were emasculated, ethylene evolution began after a 10 hour lag period (fig 1), and fading became obvious after an additional 8 to 12 hours. A similar time course of ethylene production and fading has been reported for *V. Miss Agnes Joaquim* blossoms after removal of their pollinia (2), whereas *V. Petamboeran*, a semiterete of heavy texture, fades after a considerably longer lag (fig 2). That removal of the pollinia per se is not responsible for floral fading is indicated by experiments with *Phaleonopsis Pamala* (7) which have shown that disturbance of the connection between the rostellum (the structure supporting the pollinia) and the upper surface of the sticky disk with which it is in intimate contact, suffices to induce the response. There is no indication that this type of fading involves a release or production of auxin, for it does not result in swelling of the column whereas both auxin application and pollination bring about such an effect.

Self pollination induces ethylene formation within 1 hour in *V. Rose Marie*, and flower fading within 8 to 10 hours (fig 1). A similar time course was observed with *V. Petamboeran* even when the flower was pollinated with its own pollinia intact (fig 2). This response was duplicated by applying enough IAA (5 mM) in lanolin paste to fill the stigmatic cavity (fig 2), and exactly the same result was obtained using 0.1 mM ^{14}C-IAA in 0.8 % agar. Other cases in which the fading response caused by pollination is stimulated by auxin have been reported (3, 7), and led to the suggestion that the large quantity of auxin contained in the pollen is responsible for the pollination effect (7). As auxin stimulates ethylene evolution in these blossoms and other plant tissues within an hour, and since ethylene causes floral fading, it is logical to conclude that this is the mechanism involved. Auxin induced ethylene formation must also influence the growth of the column for within 2 hours after application of pollinia or auxin this structure

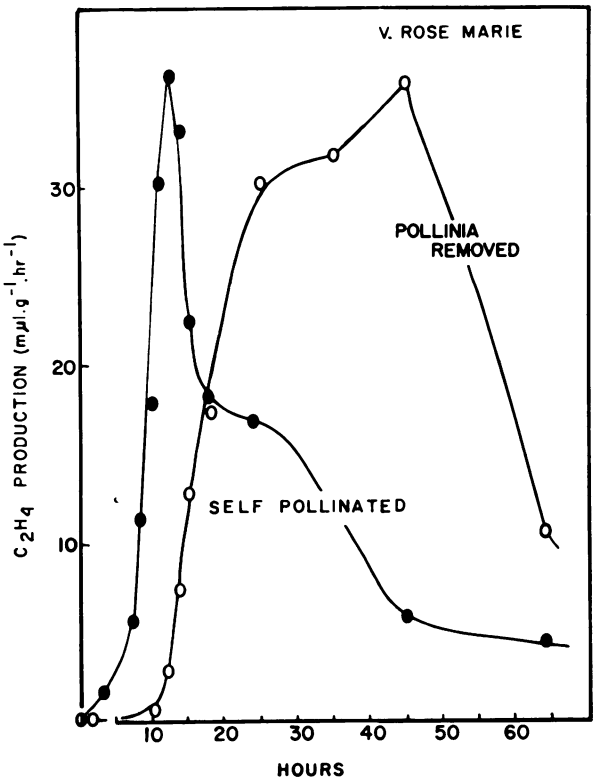

FIG. 1. Ethylene evolution by blossoms of *V. Rose Marie* after self-pollination or removal of pollinia. Control blossoms stayed fresh for about 1 week and produced no ethylene during that time. Fading of the lower petals first became evident after 8 to 12 hours in self pollinated blooms, and after about 24 to 30 hours in emasculated flowers.

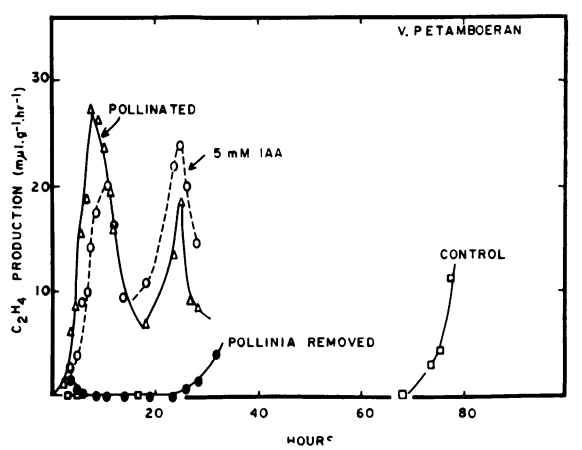

FIG. 2. Ethylene evolution by blossoms of *V. Petamboeran* after pollination (with pollinia intact), application of 5 mM IAA in lanolin to the stigmas, or removal of the pollinia. The lower petals of blossoms which were pollinated or treated with IAA began to fade after 8 to 10 hours, those of emasculated flowers after about 35 hours, and controls after about 80 hours. Removal of the pollinia caused an initial transient production of ethylene, perhaps a wound response, which subsided within 6 hours.

swells extensively, and growth of this type is a characteristic response to ethylene (4).

The time course for fading in IAA treated blossoms of *V. Petamboeran* is similar to that for ethylene production in floral parts cut from treated flowers. For example 150 minutes after application of IAA the column produces ethylene at a rate of 10.8 $m\mu l \cdot g^{-1} \cdot hr^{-1}$, whereas neither the lip, petals nor sepals evolve any ethylene. After 6 hours the sepals and petals produce only a trace of the gas, but by 24 hours they evolve ethylene at a rate of 1.2 $m\mu l \cdot gm^{-1} \cdot hr^{-1}$, and the lip and column at rates of 81 and 50 $m\mu l \cdot gm^{-1} \cdot hr^{-1}$ respectively. Thus the petals and sepals begin to produce ethylene between 6 and 24 hours after pollination, which correlates approximately with the time at which they fade. This result also suggests that the peak in ethylene formation at 10 hours is caused by evolution from the column, whereas the second maximum at 24 hours must be due to the lip, sepals and petals (fig 2).

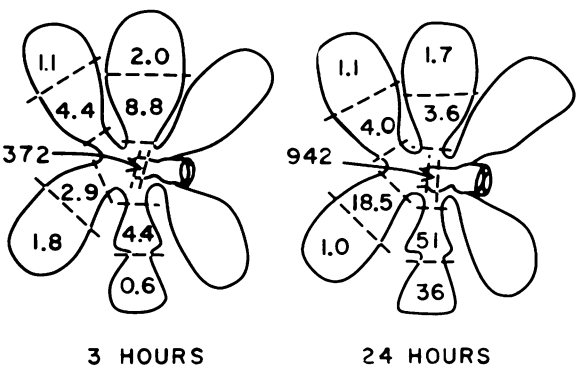

FIG. 3. Spread of ^{14}C-IAA from the stigmas of *V. Petamboeran* to the floral appendages. About 20,000 cpm of carboxyl labeled ^{14}C-IAA (8 mc/mmole) at a concentration of 0.1 mM in 0.8% agar was applied to the stigmas of each of several flowers, and after either 3, 6, or 24 hours the flowers were dissected as indicated (dotted lines). Thus the petals, sepals, and lip were excised and cut into outer and inner halves, and a 1 mm thick cross section was cut from the base of the floral column. Values are total cpm radioactivity in the indicated tissues. The figures at 6 hours (not shown) were only slightly higher than those at 3 hours.

Applied ^{14}C-IAA spreads slowly from the stigma, mainly to the column and lip, and hardly at all to the sepals and petals (fig 3), so there is a close correlation between the quantity of ethylene produced and the amount of auxin reaching each part of the flower. However, even after 24 hours less than 0.1 μM ^{14}C-IAA is present in the lip, and still less in the sepals and petals. As there are no reported cases in which IAA concentrations lower than 10 $m\mu M$ stimulate ethylene evolution, it seems unlikely that the observed response can be accounted for solely in terms of auxin spreading to the floral appendages. An alternate explanation is afforded by experiments in which blossoms of *V. Petamboeran* were gassed with

10 ppm ethylene for 3 to 24 hours. Flowers removed from ethylene after 3 hours did not fade prematurely or produce ethylene, whereas those gassed for 24 hours displayed advanced fading by that time and produced large amounts of the gas. Ethylene was evolved primarily by the column (25 $m\mu l \cdot gm^{-1} \cdot hr^{-1}$) and lip (15 $m\mu l \cdot gm^{-1} \cdot hr^{-1}$) and to a lesser extent by the sepals and petals (1.1 $m\mu l \cdot gm^{-1} \cdot hr^{-1}$). These results indicate that ethylene evolution is autocatalytic in flowers as it is in fruits, and suggest that at least in part the fading response spreads outward from the column because each cell triggers its neighbor to produce ethylene by gassing it with the hormone. Thus the events following pollination which lead to fading of Vanda blossoms may be envisioned as including transfer of auxin from the pollen to the stigma, spread of growth hormone to the column and lip, induction of ethylene formation in these tissues, diffusion of ethylene from its site of production to adjacent tissues where the gas stimulates its own formation, and finally floral fading as a consequence of the endogenous ethylene.

Many types of flowers other than orchids produce ethylene (9, 11) and several of these are affected by the gas (6, 9, 12, 13, 14). Moreover, ethylene induced and natural fading both may be retarded by CO_2 (9, 12, 13), a competitive inhibitor of ethylene action (5). These reports suggest that ethylene might participate in the fading response of flowers other than orchids, and if so the results with Vanda orchids would be applicable in these cases.

Acknowledgments

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While this paper was in press a note by I. V. Hall and F. R. Forsyth appeared in the Canadian Journal of Botany 45: 1163–66, reporting that IAA and pollination stimulated ethylene production in blossoms of lowbush blueberry and the cultivated strawberry.

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