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Short Communication

Pollination-Induced Corolla Wilting in *Petunia hybrida* Rapid Transfer through the Style of a Wilting-Inducing Substance

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ABSTRACT

Pollination or wounding of the stigma of *Petunia hybrida* flowers led to the generation of a wilting factor and its transfer to the corolla within 4 hours. This was concluded from the effects of time course removal of whole styles. In this 4-hour period, pollen tubes traversed only a fraction of the total distance to the ovaries. Both pollination and wounding of the stigma immediately resulted in an increase of ethylene evolution. Accelerated wilting, however, occured only when treated styles remained connected with the ovaries, and not when they were detached and left in the flower. A wilting factor was found in eluates collected from the ovarian end of the styles, only in the case of previous pollination or wounding. In such eluates, the level of the ethylene precursor 1-aminocyclopropane-1-carboxylic acid was below detection.

These observations suggest a material nature of the wilting factor in *Petunia* flowers, which rapidly passes through the style to the corolla, but which is different from 1-aminocyclopropane-1-carboxylic acid.

Wilting of the corolla of *Petunia* flowers is considerably enhanced after injury of the styles or after pollen tube penetration (2). Accelerated corolla abscission occurs in *Digitalis* upon pollination (8). In this species, excision of styles at intervals after pollination has revealed that a stimulus is formed in the style, which reaches the corolla basis well before pollen tubes have traversed the style (8). The nature of this stimulus has not been elucidated, thus far, nor has its transport through the style been determined.

Plant hormones have been reported to be involved in post-pollination phenomena. Working with orchids, Burg and Dijkman (1) found that application onto the stigma of IAA, a naturally occurring substance in pollinia, can mimic the effects of pollination. This IAA, however, does not move into the gynostemium, and an intermediate transmitter has been postulated (10). This intermediary might well be ethylene or its precursor ACC,[2] as ethylene is also produced in pollinated orchid flowers (1). In many other plant species too, ethylene is produced as a direct result of pollination, particularly in the gynoecium (4, 6), sometimes shortly after pollen deposition (9, 11).

The present study reports on a possible role of ACC or ethylene in the transfer of the wilting stimulus from stigma to corolla.

MATERIALS AND METHODS

Plant Material and Flower Wilting. Plants of *Petunia hybria* clone W166H, were grown in a greenhouse at 20 to 24°C during the day and at 18°C during the night, with supplemental light (16 h) from October until March. To prevent precocious self pollination, flowers were emasculated before anther dehiscence. This treatment did not affect flower longevity. In the longevity experiments, groups of 10 flowers per treatment were used. Three stages of corolla wilting were distinguished: (a) not wilted; (b) partially wilted; and (c) completely shrivelled, adding a value of 0, 0.5, and 1 to the number of wilted flowers, respectively (see Ref. 3; Fig. 1). The W_{50} value, indicating the time lapse in hours until 50% of the flowers had wilted, was obtained by graphical interpolation.

Measurement of Ethylene Production. Flowers were detached and individually placed in small tubes of water in water-locked 125-ml beakers at 22°C. After letting wound ethylene production subside for at least 2 h after detachment, flowers were either pollinated, their stigmas wounded, or left untreated. Ethylene production *in vitro* will resemble the production *in situ*, as long as assay periods do not exceed several hours, in order to prevent interfering effects of accumulated ethylene. At 1-h intervals, 1-ml gas samples were withdrawn through an orifice in the beaker, which was sealed with a silicon rubber septum, and injected into a gas chromatograph equipped with an alumina column and a flame ionization detector. All incubations were performed in the light.

ACC Assay. Eluates collected from the ovarian end of styles, or 25 mg pollen were mixed with 2 ml of 5% sulfosalicylic acid and sonified for 20 min at room temperature. After centrifugation of the mixture for 10 min at 10,000*g*, the concentration of ACC in the supernatant was determined by chemical conversion into ethylene according to Lizada and Yang (5).

RESULTS AND DISCUSSION

Wounding of stigma or style greatly accelerates corolla wilting (2). In contrast, pulling the entire style out of the flower only slightly stimulated wilting. The comparatively mild effect of removal of whole styles allows for a kinetic analysis of the transfer of the wilting stimulus from stigma to corolla. Figure 1 shows the effect of complete removal of styles at intervals after wounding (A), and pollination (B). Variation in wilting sensitivity and flower longevity originated from effects of season and plant age. Style removal, more than 4 h after either pollination or wounding of the stigma, was ineffective in preventing the accelerated corolla wilting. It is evident that transmission of the stimulus was largely

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[2] Abbreviation: ACC, 1-aminocyclopropane-1-carboxylic acid.

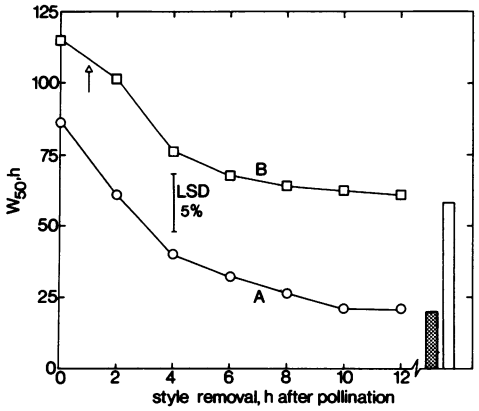

FIG. 1. Effect of style removal at various intervals after wounding of the stigma (A), or self pollination (B) on flower longevity, expressed as the time lapse (h) until 50% of the flowers had wilted (W_{50}). The arrow marks the massive penetration of pollen tubes. The unshaded and shaded histograms represent the W_{50} values of the nonexcised controls of the pollination and wounding treatment, respectively.

Table I. *Effect of Detachment or Removal of Whole Styles, Directly after Self Pollinaton or Wounding of the Stigma, on Ethylene Production in Vitro and Corolla Wilting in Situ (W_{50}, h)*

Detached styles were immediately placed back in the corolla. Ethylene evolution was measured on cut flowers in sealed containers, and is given in $nl \cdot h^{-1} \cdot flower^{-1}$ evolved between 1 and 4 h after the treatment. Data are averages of two independent experiments.

Treatment of the Flower	C_2H_4 Production	W_{50}
	$nl \cdot h^{-1} \cdot flower^{-1}$	*h*
Unpollinated	0.11	118
Unpollinated + style removal	0.74	95
Self pollinated	6.98	66
Self pollinated + style detachment	6.85	97
Stigma wounding	3.66	54
Stigma wounding + style detachment	3.28	91

Table II. *Flower Longevity (W_{50}) as Affected by the Application onto the Stigma of Concentrated Eluates Collected from the Ovarian Ends of Styles, Previously Wounded (Stigma), Self Pollinated, or Unpollinated*

The 4-μl droplets applied, contained the eluates from eight styles each (in experiment 1 also from 16 styles).

Application onto Stigma	W_{50}				
	Exp. 1	Exp. 1 (2× concn.)	Exp. 2	Exp. 3	Exp. 4
		h			
None	169	140	194	142	133
Self pollinated		96	128	94	85
H_2O	174	138	215	155	143
Eluate from wounded styles	125	61	176	122	107
Eluate from pollinated styles			167	122	107
Eluate from unpollinated styles	163	124	187	142	131

completed within this 4 h period. In this period, pollen tubes had just penetrated the stigmatic cells, but had not grown further than about 2 to 3 mm (3). As the style measures about 30 mm, pollen tubes themselves cannot function as carriers for the stimulus. The site of this rapid generation, therefore, is most likely located in the stigma.

As pollen tube growth causes wounding of the stigmatic cells, wound ethylene formation might be one of the early events after pollination. Table I shows that the ethylene production of cut flowers in closed containers is considerable in the first 4 h after pollination, as it is after wounding of the stigma, irrespective of possible style detachment. However, this ethylene cannot be the factor responsible for accelerated corolla wilting as style detachment, immediately after pollination or wounding, considerably postponed wilting (Table I). Thus, the stimulus inducing wilting is not transported in a volatile form but, in stead, requires an unimpeded connection between style and ovary for its proper transmission.

Bioelectrical potential changes occur as a result of pollination, and were suggested to be responsible for the initiation of various post-pollination phenomena (7). In the case of the wilting stimulus, this would mean that its transfer is not mediated by a compound of material nature. In an attempt to investigate this, eluates from batches of 110 excised whole styles were collected from their cut (ovarian) ends in 1.5 ml water during 20 h at 22°C, concentrated by freeze drying, and reapplied as 4-μl droplets onto 10 stigmas. Different batches of styles were previously pollinated, wounded, or left unpollinated. Table II shows accelerated corolla wilting, particularly in the case of application of eluates derived from pollinated and wounded styles. This strongly indicates a material nature of the nonvolatile factor.

Since the wilting process of *Petunia* flowers is effectively postponed by compounds having ethylene trapping properties (unpublished results), the wilting substance might be a compound involved in the synthesis of ethylene, such as its direct precursor, ACC. Whitehead *et al.* (11) recently suggested a role for the leachable ACC in pollen, in starting off autocatalytic ethylene synthesis. This would be the more likely for *Petunia* pollen, as it contains the extremely high level of about 700 nmol $ACC \cdot g^{-1}$ dry pollen (Hoekstra *et al.*, Abstract 646, 11th International Conference on Plant Growth Substances, Aberystwyth, 1982). Assuming that pollination is normally brought about by about 0.1 mg pollen, the intrinsic ACC can account for the evolution of, at most, 1.7 nl C_2H_4. This amount is small in comparison with the C_2H_4 produced in the first 4 h after pollination (Table I). Moreover, Gilissen (3) demonstrated that *Petunia* pollen, killed by x-ray irradiation (1 Mrad), does not significantly accelerate corolla wilting. Our measurements of ACC in such irradiated pollen indicated the presence of 694 $nmol \cdot g^{-1}$ dry pollen. Even the direct application onto the stigma of 70 times more ACC than is usually present in 0.1 mg *Petunia* pollen, failed to accelerate wilting, although an extensive ethylene evolution was the result. On the contrary, tube penetration is the prerequisite for accelerated wilting, even when this is brought about by foreign pollen (2). Analyses of the style eluates for the occurrence of ACC gave negative results, notwithstanding the fact that internal standardization with ACC indicated its full recovery from such eluates. We conclude, therefore, that the compound produced rapidly after pollination or wounding, is different from ACC.

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