EFFECTS OF INTERSPECIFIC POLLINATION ON STYLAR ETHYLENE PRODUCTION AND FLOWER LONGEVITY IN P E T U N I A H Y B R I D A

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Abstract

In petunia cross and self pollination are known to reduce flower longevity to less than 70% of that of the unpollinated control. Pollen from related species, capable of tube growth in petunia stigmas caused similar reduction in flower life. When foreign pollen tubes did not penetrate the stigma, varying effects on corolla longevity were noticed. Irradiation-killed petunia pollen, however, was unable to significantly accelerate wilting.

Upon pollination with different pollen species, excised styles produced various amounts of ethylene, ranging from 0.1 to 8.9 nl/h/style (measured from the 2^{nd} to the 7^{th} h after pollination). The amount of ethylene produced did not correlate with the rate of wilting. Neither was the endogenous ACC content in the different pollen species correlated with the level of pistillate ethylene production. Thus, apart from ACC, other substances leak from pollen and induce stylar ethylene synthesis. Furthermore no correlation could be established between pollen ACC content and accelerated flower wilting , although coincidentally, the *Solanaceae* pollen tested, had extremely high ACC contents, grew into the stigma, and caused accelerated wilting.

Eluates from a few pollen species accelerated wilting when applied onto the stigma in physiological concentrations. Applied more concentrated, these eluates caused early ethylene synthesis and rapid wilting. The neutral (sugar) fraction particularly, and the amino acid fraction to a lesser extent, were shown to have this wilting-inducing property. Sucrose, the main component in the neutral fraction, but also glucose, fructose and mannitol were effective in concentrations ranging from 0.2-0.6 mg/stigma. Application of IAA to the stigma at concentrations sufficiently high to cause senescence of *Cymbidium* flowers (10 nmol) did not stimulate wilting or ethylene production. The common free amino acid in pollen, proline, was equally ineffective.

We conclude that a high pollen ACC content is not required for stylar ethylene production and flower wilting, but that these processes can be accelerated due to the leakage of a mixture of other substances from pollen.

1. Introduction

Pollination-induced acceleration of flower wilting or abscission has been reviewed in detail (see Nichols et al., 1983; Hoekstra and Weges, in press). Burg (1964), and Hall and Forsyth (1967) were among the first to discover the production of ethylene as a result of pollination. This has since then been confirmed for many other plant species (see Hoekstra and Weges, in press). Involvement of ethylene in flower senescence was evidenced by the favourable effects of hypobaric

storage (Burg, 1973), and after inhibitors became available such as amino-ethoxyvinyl glycine (AVG) (Rando, 1974), silver thiosulfate (Veen and van de Geijn, 1978), and norbornadiene (Sisler and Yang, 1984).

Some pollen species contain considerable amounts of the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) (Hoekstra et al., 1982; Whitehead et al., 1983). It has been suggested that this pollen ACC leaks onto the stigma to subsequently trigger the synthesis of ethylene there (Whitehead et al., 1983). Furthermore ACC was thought to be the mobile wilting factor travelling through the style from stigma to corolla to induce ethylene-mediated corolla wilting (Nichols et al., 1983; Whitehead et al., 1984; Reid et al., 1984). In line with this assumed role of pollen ACC is the observation that ethylene synthesis starts immediately upon pollinating petunia stigmas, prior to pollen tube emergence (Hoekstra and Weges, in press). However, AVG applied onto the stigma 3 h prior to pollination completely prevented this ethylene synthesis, indicating that the ethylene is produced through stigmatic ACC synthase, and not by the conversion of pollen ACC . The authenticity of ACC in pollen was verified by its ability, after partial purification, to stimulate ethylene synthesis in AVG-treated petunia stigmas. The foregoing is indicative of a restricted ACC leakage from pollen, and of an involvement of other pollen-derived substances in the induction of stylar ACC-synthase activity.

Recently Hoekstra and Weges (in press) demonstrated that in petunia, the early pistillate ethylene has no function in the acceleration of the wilting. It is during this early period (4 h) that the wilting stimulus travels from the stigma to the corolla (Gilissen and Hoekstra, 1984). Although Gilissen and Hoekstra (1984) could not detect ACC in stylar eluates, Hoekstra and Weges (in press) deduced from inhibitor studies that ACC nevertheless is the mobile wilting factor.

In this paper we report on the effects of stigmatic application of foreign pollen and their cold water extracts on early pistillate ethylene synthesis and rate of flower wilting in *Petunia hybrida*.

2. Materials and Methods

Plants of *Petunia hybrida* clone W166H were used and grown under conditions as described by Gilissen and Hoekstra (1984). Prior to anther dehiscence flowers were emasculated. Flower longevity *in situ* (W_{50} values) was determined as described in the legend of figure 1.

Pollen of the following species were used: *Pinus sylvestris, Alnus glutinosa, Corylus avellana, Fagus sylvatica, Chenopodium bonus-henricus, Ranunculus ficaria, Papaver rhoeas, Erythrina orientalis, Impatiens glandulifera, Nicotiana alata, Nicotiana tabacum, Petunia hybrida* (W166H), *Aster tripolium, Cosmos bipinnatus* (fresh and old), *Narcissus poeticus* and *Typha latifolia*. Pollen was tested for viability by germination *in vitro* according to standard procedures (Hoekstra and Barten, in press). Only the 10-year old *Cosmos* pollen was not viable. Pollen was stored dry at -20°C until use. Pollen tube growth *in vivo* was analysed by macerating styles in 10N KOH for at least 24 h, followed by extensive washing in water, staining with 0.1% decolorized (K_3PO_4) anilin blue, squashing, and viewing under an UV microscope.

Pollen eluates were made by washing 1 gram pollen in 40 ml cold water for 3 min. Eluates were freeze dried, dissolved in 100% MeOH, filtered, dried under vacuum, dissolved in H_2O, and processed according

to Redgwell (1980) with the aid of Sephadex SP C-25 and QAE column chromatography, to yield separate classes of sugars, amino acids, and organic acids. After freeze drying, the fractions were dissolved in 1 ml water and used for wilting experiments (2-5 µl/stigma). The sugar fraction was analysed by HPLC using a Bio-Sil amino 5S (Biorad) column and refractometer detection, and with acetonitril-H_2O (80:20) as the eluant (30°C, P = 19 atm., flow 2ml/min).

Procedures to collect ethylene from excised styles or detached flowers were as outlined by Hoekstra and Weges (in press). Ethylene and ACC were determined as described earlier (Gilissen and Hoekstra, 1984).

3. Results

Figure 1 shows an example of how self pollination accelerates flower wilting in petunia. The W_{50} value representing the time lapse until half of a group of 10 flowers of one particular treatment has wilted, is obtained by intrapolation. For a number of interspecific pollinations the W_{50} values are given in table 1. Wilting was significantly accelerated when pollen tubes had penetrated the stigmatic tissue, which was commonly observed after pollinations with related species. Wounding of the stigmatic tissue by the penetrating tubes might be responsible, since wilting was not stimulated by petunia pollen unable to form pollen tubes due to a lethal dose of γ-radiation (1 Mrad). However, acceleration of wilting did sometimes occur upon pollination with foreign pollen species without the necessity of tube penetration.

To analyse whether substances leaking from pollen might be inducers of wilting, cold water extracts from different pollen species were applied onto stigmas in physiological amounts (table 1). With some pollen eluates significant acceleration of the wilting was obtained indicating that, indeed, wilting stimuli can be eluted from the grains. Table 1 further shows that the ethylene precursor ACC was high in pollen from *Solanaceae* species, and much lower in the other pollen species tested. The assumed role of pollen ACC in the acceleration of wilting (Whitehead et al., 1983) urged for a comparison between endogenous ACC contents in pollen and W_{50} values. However, accelerated wilting also occurred after pollination with pollen species having minimal amounts of ACC, from which we conclude that substances from pollen other than ACC can evoke rapid wilting. Using the data of table 1 in figure 2 (A), it is evident that pollen having minimal endogenous ACC still allowed for considerable synthesis of early ethylene in the pistils, from which it is concluded that substances other than ACC can leak from pollen and evoke early ethylene synthesis. Similarly, there was no good correlation between early pistillate ethylene synthesis and W_{50} values (Fig. 2B) i.e. considerable ethylene synthesis may occur without consequences for flower life span.

Because of its role in post-pollination phenomena of orchids (Burg and Dijkman, 1967) we have analysed the effect of IAA application onto stigmas. Table 2 shows that IAA had no effect on both early ethylene production and wilting. Similar concentrations were very effective in promoting senescence of *Cymbidium* flowers. On the other hand petunia pollen was unable to evoke senescence in *Cymbidium* flowers. We conclude that IAA in pollen is not the substance that may be responsible for the accelerated flower wilting in petunia.

More concentrated (10x), the cold water extracts from several pollen

species could severely reduce flower life and cause varying levels of early ethylene production upon application to the stigma (table 3). Further purification of the pollen eluates according to Redgwell (1980) gave three fractions, of which the sugar fraction was generally the most effective, and the amino acid fraction to a lesser extent. The composition of the sugar extracts was determined by HPLC (table 4). Sucrose turned out to be the main sugar, accompanied by small amounts of fructose and glucose. The extremely effective sugar extract from stored *Cosmos* pollen contained, in addition, an unidentified carbohydrate. Tests with authentic sucrose, glucose and fructose verified their wilting-inducing properties. Reduction of W_{50} values to 30% of those of the water-treated controls was reached for sucrose at 0.6 mg/stigma, glucose at 0.4 mg, fructose at 0.6 mg, and mannitol, very effectively, at 0.2 mg/stigma.

The main free amino acids in pollen were proline, and to a lesser extent alanine and glutamic acid. Free histidine was specific for *Cosmos* pollen. Application of authentic samples of these amino acids onto stigmas in concentrations up to 30-50 µg/stigma did not significantly accelerate ethylene production and rate of wilting.

4.Discussion

Gilissen (1977) noted that irradiation-killed petunia pollen was unable to accelerate wilting. This author, however, did not measure ethylene synthesis. We found considerable production of ethylene during the first 6 h after pollination with γ-irradiated pollen, possibly triggered by its endogenous ACC content, but without consequences for flower longevity. Application of authentic ACC (2 nmol) on the stigma also caused ethylene synthesis, but no reduction of flower longevity (Hoekstra and Weges, in press). In accordance with Gilissen (1977) we observed that stigmatic penetration by foreign pollen tubes *(Solanaceae)* promoted rapid wilting. On the other hand, we found that some other foreign pollen species, not belonging to the *Solanaceae* and not able to grow into petunia stigmas, were still capable of accelerating wilting. A high level of pollen ACC and an elevated early pistillate ethylene production apparently are not prerequisites to effectuate accelerated wilting. This is in line with the earlier observations that pollen ACC is not readily converted on the stigma, and that early synthesis of pistillate ethylene is not involved in the acceleration of flower wilting (Hoekstra and Weges, in press). In contrast, the ethylene evolving later, from the corolla, does have a role in the wilting.

We demonstrate that substances other than ACC leak from the different pollen species to evoke pistillate ethylene synthesis and/or elicit corolla wilting. It should be undesirable for an efficient fertilization that interspecific pollinations lead to substantial reduction of flower life. Such substantial reduction occurred only after pollination with *Cosmos* pollen stored for 10 years in the deep freezer, in contrast to fresh *Cosmos* pollen (table 1), suggesting that degradation products might have formed during storage which elicit corolla wilting. Because of a possible similarity between such pollen-derived wilting elicitors and the natural wilting substance(s) formed in the stigma upon pollination, we have made cold water extracts from various pollen species. The presence of sugars in the pollen eluate indicates that the integrity of the plasmalemma was lost during the

cold water elution. The harmful effects of cold germination media on membrane integrity of dry *Typha latifolia* pollen was described earlier (Hoekstra, 1984). Despite of their effectiveness as wilting inducers, sugars will not normally leak from pollen. Moreover, effective amounts equalled or exceded the weight of pollen used for pollinations. Whether the sugars exert their effects directly, or merely through osmotically wounding the stigma is not known, although the high effectiveness of mannitol favours the latter explanation. In the eluate from 'old' *Cosmos* pollen part of the carbohydrate fraction was not recovered by HPLC. As the eluate as well as the pollen itself was very effective in causing wilting, we assume that the non-recovered fraction might contain elicitor-like degradation products from cell walls.

Amino acids are better candidates for diffusing from pollen onto the stigma (Linskens and Schrauwen, 1969). Free proline occurs in concentrations up to 2% of the pollen dry weight. However, about 50 µg/stigma, corresponding to 2.5 mg pollen, was not effective in stimulating ethylene production and flower wilting. It cannot be excluded that different amino acids together can act synergistically. As in this paper, IAA was ineffective on flower senescence in *Digitalis* (Stead and Moore, 1979), and carnation (Reid et al., 1984). It is unlikely, therefore, that IAA is involved in the accelerated wilting in petunia.

We conclude that a high content of ACC in pollen is not required for stylar ethylene production and flower wilting, but that these processes can be accelerated due to the leakage of a mixture of substances other than IAA and proline from the pollen.

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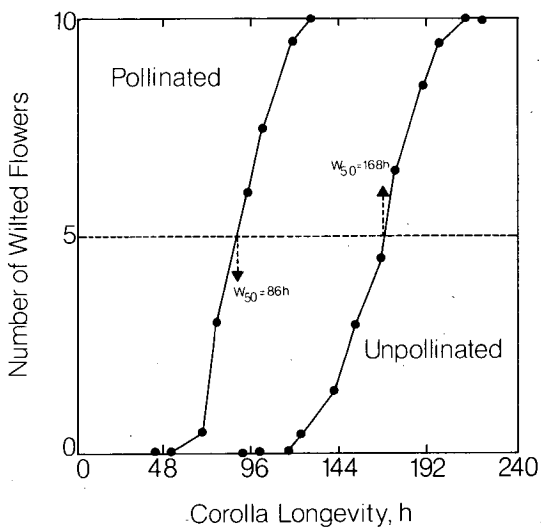

Figure 1. Effect of self-pollination on flower longevity of a group of 10 flowers. Three stages of wilting were distinguished: (a) not wilted; (b) partially wilted; and (c) completely shrivelled, adding a value of 0, 0.5 and 1 to the number of wilted flowers, respectively. The W_{50} value, indicating the time lapse in h until 50% of the flowers had wilted, was obtained by graphical intrapolation.

Table 1 - Effects of pollination with foreign pollen species on pollen tube emergence and growth in the stigma, on ethylene synthesis in excised pistils (measured from the 2nd to the 7th h after pollination) and flower longevity. The effect of cold water eluates (equivalent of 0.1-0.2 mg pollen) per stigma) was also determined. The symbols ++, +, and o stand for excellent, moderate, and absence of tube penetration /growth, respectively. All experiments were performed twice. The C.V. ranged from 1.3-7.9% for the ACC data.

Pollen	Pollination					Extract
Species	Tube Growth	Stigma Penetration	ACC in pollen (nmol/g)	Ethylene (nl/h/style)	W_{50} (h)	W_{50} (h)
Corylus	++	o	8	0.10	121	143
Typha	+	o	10	0.10	112	133
Alnus	++	o	13	0.19	118	147
Impatiens	o	o	4	0.22	126	137
Fagus	o	o	4	0.50	120	152
Narcissus	+	o	5	0.60	112	117
Pinus	o	o	23	0.62	121	152
Chenopodium	+	o	5	0.90	114	146
Papaver	++	o	25	1.16	104	-
Aster	+	o	5	1.45	117	134
Erythrina	o	o	7	3.16	129	-
Nicotiana alata	++	++	1230	3.52	87	-
Ranunculus	++	o	5	3.99	93	-
Petunia(γ-rad.)	o	o	684	5.57	123	-
Cosmos(fresh)	o	o	4	7.70	120	-
Cosmos(old)	o	o	4	8.40	99	98
Nicotiana tab.	++	++	1953	8.46	110	130
Petunia	++	++	704	8.90	87	139
None/water				0.12	133	158
LSD (P= 0.05)					15	20

Table 3 - Effect of the concentrated, crude and partially purified eluates from pollen of *Typha*, *Cosmos* (old), *N. alata* and *Petunia* on early ethylene synthesis of whole flowers (2-7 h after application, nl/h/flower), and on corolla longevity (W_{50},h).

Extract or Fraction	Source							
	Typha		*Cosmos*		*Nicotiana*		*Petunia*	
	W_{50}	C_2H_4	W_{50}	C_2H_4	W_{50}	C_2H_4	W_{50}	C_2H_4
Control (water)	140	1.0	146	1.1	144	1.0	139	0.9
Complete extract	51	3.2	59	3.1	51	7.0	119	2.8
Sugar fraction	68	0.9	57	0.9	102	1.1	115	0.8
Amino acid fraction	128	0.9	99	1.6	81	2.5	138	0.8
Organic acid fraction	121	1.0	113	3.3	118	4.1	136	1.0

Table 2 - Effect of IAA applied onto stigmas (2 µl) on ethylene synthesis of excised styles (2-7h) and flower longevity in situ. Data are averages of 2 experiments.

Amount of IAA applied (nmol)	Ethylene production (nl/h/style)	W_{50} (h)
0	0.0	134
0.2	0.0	134
2.0	0.0	129
10.0	0.1	124

Table 4 - Compositional analysis (HPLC) of the purified sugar fractions in eluates of 4 different pollen species.

Pollen species	Components in sugar fraction (weight %)				
	Fructose	Glucose	Sucrose	Unknown	% Recovery
Typha	2.8	4.4	92.8	0	94.8
Cosmos (old)	5.2	4.9	31.8	58.0	63.8
Nicotiana alata	5.3	3.5	91.2	0	97.8
Petunia	5.0	3.7	91.3	0	95.3

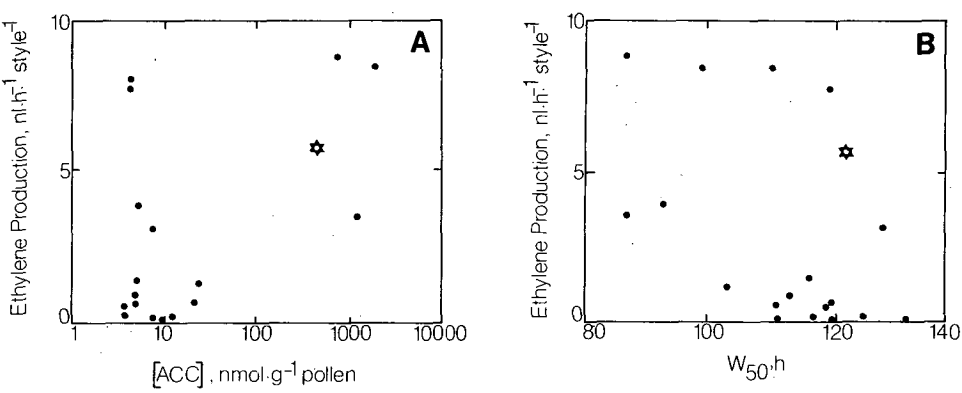

Figure 2 A-B - Correlation between ACC contents of the different pollen species and early ethylene productions by the pollinated pistils (A), and between pistillate ethylene productions and the corolla longevities (B). The data are from table 1. The asterisk stands for the γ-irradiation-killed petunia pollen.