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POST-POLLINATION PHENOMENA IN ORCHID FLOWERS

III. EFFECTS AND INTERACTIONS OF AUXIN, KINETIN OR GIBBERELLIN

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Summary

NAA, in concentrations exceeding 0.01 μg/flower, initiates post-pollination phenomena in *Cymbidium* (Orchidaceae) flowers similar to those brought on by pollination. These include stigmatic closure, swelling and loss of curvature of the column, wilting of the perianth, deformation of the calli, and anthocyanin production. Applications of relatively high concentrations of GA_3 induce post-pollination effects which, except for anthocyanin production, are less intense than those brought about by auxin. Kinetin does not induce post-pollination phenomena, but in concentrations of 10 μg and 100 μg/flower causes slight stigmatic closure, and in some combinations with NAA it inhibits wilting. Stigmatic closure, loss of column curvature and changes in calli, all of them NAA induced, cannot be prevented by simultaneous applications of kinetin, GA_3 or ABA. Some combinations of NAA and GA_3 lowered anthocyanin content relative to separate treatments with either hormone. Flowers treated with GA_3 plus kinetin wilted slightly in most cases, but columns did not swell and retained their curvature; calli did not develop colour and anthocyanin content was generally equal to that of flowers given only kinetin. GA_3 and ABA when applied together brought on symtoms which were similar to those caused by ABA only but anthocyanin content was lower than in flowers treated with either hormone alone. This is also true for ABA–kinetin mixtures, but intensities of the effects are different and, with certain concentration ratios, stigmatic closure occurs. The phenomena are discussed relative to fruit-set, seed formation, anthocyanin production, senescence, orchid biology and the possible mode of action of each hormone.

Introduction

Pollination effects on orchid flowers were apparently first noted by Robert Brown during his early studies of nuclei and fertilization (Brown, 1833). Later, descriptions were added by several observers (Anonymous, 1890, 1894, 1899; Fitting, 1909a, b, 1910 and personal communications; Guignard, 1886a, b; Haberlandt, 1902; Hildebrand, 1863; Müller, 1888; Rivière, 1866; Veitch, 1883). These pollination effects were attributed by Fritz Müller to a 'poisonous' influence of the pollen (Darwin, 1904; Hans Fitting, personal communication; Loew, 1897). Later investigations disclosed that post-pollination phenomena can be brought on not only by pollination, but also by dead pollinia and pollen extracts (Fitting, 1909a, b, 1910). This led to the conclusion that the stimulus is chemical in nature, and to the forward-looking suggestion that it is a plant hormone (Fitting, 1909a, b, 1910, personal communications). In calling this factor *pollenhormon*, Fitting

became the first to introduce the term hormone into Botany (Arditti and Knauft, 1969; Bell, 1959; Fitting, 1909a, b, 1910, personal communications; Went and Thimann, 1937). Subsequent work by several investigators demonstrated that Fitting's *pollen-hormon* was either an auxin or acted like one (Arditti and Knauft, 1969; Dolcher, 1959, 1961, 1967; Laibach, 1930, 1932, 1933; Laibach and Maschmann, 1933; Mai, 1934; Maschmann, 1932; Maschmann and Laibach, 1932; Went and Thimann, 1937) and that orchid pollinia are a rich source of IAA (R. Müller, 1953). Eventually it was also shown that IAA, 2,4-D and NAA can bring on post-pollination phenomena (Arditti and Knauft, 1969; Bouriquet, 1954; Burg and Dijkman, 1967; Dolcher, 1959, 1961, 1967; Gessner, 1948; Heslop-Harrison, 1957; Hsiang, 1951a, b; Hubert and Maton, 1939).

A number of hormones are known to participate in fruit-set and development (Addicott and Lyon, 1969; Crane, 1964, 1965, 1969; Letham, 1967, 1969) and can initiate post-pollination symptoms which may vary among orchid genera or species, and with each hormone (Arditti, Flick and Jeffrey, 1971; Arditti and Knauft, 1969; Dolcher, 1959, 1961, 1967; Hayes, 1968; Heslop-Harrison, 1957; Jancke, 1915; Ringstrom, 1968; Roux, 1954) but are definite and easily observable. Orchid flowers are, therefore, not only unique in their structure, beauty and pollination mechanisms (Dodson, 1967; van der Pijl and Dodson, 1966), but also exceedingly interesting in their responses to pollination or hormone treatments (Arditti, 1969; Arditti *et al.*, 1971; Arditti and Knauft, 1969).

Hormones or pollination bring about flower wilting, loss of proteins from sepals and petals (Schumacher, 1931), cessation of scent production and, in some cases, of nectar exudation (Wiefelputz, 1970), swelling and loss of curvature by columns, flow of water and minerals into columns or ovaries (Oertli and Kohl, 1960), stigmatic closure, production of anthocyanins, and other pigments, by columns and labella ('lips'), starch formation in the ovary (Seshagiriah, 1941), as well as loss of turgidity and colour changes in calli (Arditti *et al.*, 1971; Arditti and Knauft, 1969; Burg and Dijkman, 1967; Dodson, 1967; van der Pijl and Dodson, 1966). Auxins (Arditti and Knauft, 1969; Hsiang, 1951a, b; Hubert and Maton, 1939), ethylene (Akamine, 1963; Akamine and Sakamoto, 1951; Burg and Dijkman, 1967; Davidson, 1949; Fischer, 1950; Lindner, 1946) and ABA (Arditti *et al.*, 1971) can all initiate some post-pollination symptoms.

Auxin is known to be present in pollen (R. Müller, 1953) and ethylene is the only hormone currently known to be produced following pollination, auxin treatments or emasculation of orchid flowers (Akamine, 1963; Akamine and Sakamoto, 1951; Burg and Dijkman, 1967; Davidson, 1949; Dijkman and Burg, 1970; Fischer, 1950; Lindner, 1946).

Gibberellins have been applied to *Cymbidium* plants in an effort to initiate flowering under unfavourable conditions (O'Neil, 1958; Zeilmaker, 1958) or at earlier dates (Bivins, 1968). These treatments resulted in earlier as well as bigger flowers (Bivins, 1968), but there are no reports regarding the effect of GA_3 on flower colour or keeping quality (i.e. onset of senescence).

Auxins, gibberellins and cytokinins can induce parthenocarpic fruits in a number of plants (Crane, 1964, 1965, 1969). These hormones have also been implicated in the earliest stages of fruit growth (van Overbeek, 1962) and, therefore, it is not surprising to note that they can initiate some post-pollination (i.e. early fruit-set) symptoms in orchid flowers. *Cymbidium* flowers, because of their sensitivity, are particularly well suited for studies of post-pollination phenomena and the roles played by hormones in anthocyanin synthesis, flower-senescence and fruit-set.

Materials and methods

Flowers of *Cymbidium* cv. Samarkand (Dos Pueblos Orchid Company, Goleta, California) were treated, observed, and analysed as described previously (Arditti *et al.*, 1971; Arditti and Knauft, 1969). Hormones were applied in aqueous solutions, 3% agar (Arditti *et al.*, 1971), or lanolin pastes (Arditti and Knauft, 1969). Increases in width 5 mm below the anther cap (Arditti and Knauft, 1969) were used as a measure of column-swelling (Table 1). Wilting, condition of the calli, stigmatic closure, and hooding of the dorsal sepals (Table 1) are described subjectively (Arditti *et al.*, 1971).

Anthocyanins were extracted by steeping in 1% HCl in methanol and expressed as OD_{525}/g fresh weight (Arditti *et al.*, 1971; Arditti and Knauft, 1969; Furuya and Thimann, 1964; Thimann and Edmondson, 1949).

Results

Swelling of the column

Swelling was induced by NAA (alone or in combinations with all other hormones) at concentrations exceeding 0.01 μg/flower, and slightly by GA_3 in the 1–100 μg/flower range (Table 1).

Loss of curvature by the column

Applications of 0.01–250 μg NAA per flower, and, with one exception (that of 25 μg/flower NAA plus 1 μg kinetin/flower), all combinations of NAA with other hormones brought about straightening of the column (Table 1). None of the other hormones could bring about loss of curvature.

Stigmatic closure

NAA, at concentrations of 0.05–250 μg/flower, alone or in combination with all other hormones, induced stigmatic closure. Slight stigmatic closure was also brought about by 10 and 100 μg GA_3, 100 μg kinetin, or 1 μg GA_3 plus 10 μg kinetin per flower (Table 1). With GA_3 the closure amounted to little more than a limited aperture reduction caused by a low degree of swelling of the sides. Surprisingly, stigmas also closed when flowers were treated with 0.01, 0.05 or 0.1 μg ABA in the presence of 10 μg kinetin (Table 1). Neither kinetin nor ABA, when applied alone, could cause stigmatic closure.

Wilting of sepals and petals

As might be expected, wilting was retarded by kinetin, alone or in several, but not all, combinations with other hormones. Some GA_3 treatments did not initiate wilting and three NAA-ABA combinations (25 μg NAA plus 0.1 or 0.05 μg ABA, or 2.5 μg NAA and 0.05 μg ABA) retarded it (Table 1). The latter was also true for 100 μg GA_3 plus 0.05 μg ABA.

Calli

Loss of turgidity and colour development by the calli, usually together, occurred following all NAA treatments. All GA_3 applications brought about a decrease in firmness, some loss of form, and the development of orange or red coloration. Kinetin had no effect on the calli and could not reverse changes induced by other hormones, except

when 1–100 μg were combined with 10 μg GA_3. Kinetin–ABA combinations also induced changes in the calli (Table 1).

Table 1. *Effects of abscisic acid (ABA), gibberellin (GA_3), kinetin and naphthaleneacetic acid (NAA) singly and in combinations on swelling of columns, stigmatic closure, column curvature, condition of calli and wilting of sepals and petals of* Cymbidium *cv. Samarkand**

Treatment		Swelling: width of column (mm)	Stigma	Condition of column‡	Calli§	Sepals/petals**
None		10.0	Open	C	Y	NW
Water agar		10.0	Open	C	RY	NW
Emasculated		10.6	Open	C	O	SW
Cross pollinated		16.0	Closed	St	O	W
Self pollinated		15.6	Closed	St	O	W
ABA, 0.001–1 μg/flower††		10.0	Open	C	Y-O	NW
NAA (μg/flower)	0	10.0	Open	C	Y	SW
	0.001	10.3	Open	SC	O-R	SW
	0.01	11.3	Open	St	O-R	W
	0.05	11.7	Closed	St	O-R	W
	0.1	15.3	Closed	St	O-R	W
	0.25	15.0	Closed	St	O-R	W
	2.5	15.0	Closed	St	O-R	W
	25	16.0	Closed	St	O-R	W
	250	16.0	Closed	St	O-R	W
GA_3 (μg/flower)	0	10.0	Open	C	Y	NW
	0.001	10.0	Open	C	Y-O	NW
	0.01	9.5	Open	C	Y-O	NW
	0.1	10.5	Open	C	Y-O	SW
	1	11.0	Open	C	Y-O	SW
	10	11.0	Slightly closed	C	Y-O	SW
	100	12.0	Slightly closed	C	Y-O	SW
Kinetin (μg/flower)	0	10.5	Open	C	Y	NW
	0.001	10.0	Open	C	Y-O	NW
	0.01	10.0	Open	C	Y-O	NW
	0.1	10.5	Open	C	Y	NW
	1	10.0	Open	C	Y	NW
	10	10.6	Open	C	Y	NW
	100	10.5	Slightly closed	C	Y	NW
NAA (μg/flower)	GA_3 (μg/flower)					
2.5	10	12.6	Slightly closed	St	O	W
25	1	15.6	Closed	St	O	W
25	10	16.1	Closed	St	O	W
25	100	16.6	Closed	St	O	W
250	10	17.1	Closed	St	O	W
NAA (μg/flower)	ABA (μg/flower)					
2.5	00.05	14.0	Closed	St	O	NW
25	0.01	16.0	Closed	SC	O	W
25	0.05	16.0	Closed	SC	O	NW
25	0.10	16.0	Closed	St	O	NW
250	0.05	17.0	Closed	St	O	W
NAA (μg/flower)	Kinetin (μg/flower)					
2.5	10	12.3	Closed	SC	O	NW
25	1	15.0	Closed	C	O	W
25	10	15.0	Closed	St	O	W
25	100	15.0	Closed	St	O	W
250	10	13.0	Closed	St	O	SW
GA_3 (μg/flower)	ABA (μg/flower)					
1	0.05	9.6	Open	C	O	W
10	0.01	11.0	Open	C	O	W
10	0.05	10.3	Open	C	O	W
10	0.10	10.0	Open	C	O	W
100	0.05	11.0	Open	C	O	NW

Table 1 (*continued*)

Treatment		Swelling: width of column (mm)	Stigma	Condition of column‡	Calli§	Sepals/ petals**
GA_3 (μg/flower)	Kinetin (μg/flower)					
1	10	10.0	Closed	C	O	W
10	1	9.0	Open	C	Y	W
10	10	10.0	Open	C	Y	W
10	100	11.0	Open	C	Y	W
100	10	11.0	Open	C	O	NW
Kinetin (μg/flower)	ABA (μg/flower)					
1	0.05	10.0	Open	C	O	
10	0.01	10.0	Closed	C	O	
10	0.05	10.0	Closed	C	O	
10	0.1	10.0	Closed	C	O	
100	0.05	10.0	Open	C	O	

* All data in this table represent three replicas.
† Measured 5 mm below the anther cap (Arditti and Knauft, 1969), average.
‡ C—curved; SC—slightly curved; St—straight.
§ O—orange; R—red; Y—yellow.
** NW—not wilted; SW—slightly wilted; W—wilted.
†† Summary from a previous paper (Arditti *et al.*, 1971).

Anthocyanin production

Columns and labella differed in their responses to treatments insofar as anthocyanin production was concerned (Figs. 1, 2 and 3). Also, the hormones (singly or in combinations) had different effects. NAA brought about anthocyanin formation in labella only at concentrations exceeding 0.01 μg/flower. Higher concentrations increased anthocyanin content of labella in essentially a straight line fashion. Columns were more erratic in their responses and three peaks were evident (Fig. 1).

Gibberellic acid treatments raised anthocyanin content in the labella relative to solvent-controls, untreated, emasculated and water-treated flowers (Fig. 2). In these flowers pigment levels were lower than in cross-pollinated and, with one exception (0.1 μg GA_3), self-pollinated ones (Fig. 2). Applications of 10 and 100 μg caused reduced anthocyanin content in columns to below that in the GA_3-solvent control (Fig. 2). All other treatments raised anthocyanin levels to above those of the controls.

Anthocyanin content in labella of flowers given GA_3–NAA combinations was always lower than in those treated with equal concentrations of GA_3 alone. Combinations of 25 μg NAA with 1 μg GA_3, and 250 μg NAA with 10 μg GA_3, lowered anthocyanin levels relative to flowers treated with the same amounts of NAA only. Columns developed less pigment than in NAA treatments when 25 and 250 μg NAA were combined with most GA_3 concentrations (Figs. 1 and 2). This was not the case when 2.5 μg NAA were combined with 10 μg GA_3 (Figs. 1 and 2). In the presence of a constant NAA concentration (25 μg), increased amounts of GA_3 raised anthocyanin levels in both columns and labella (Figs. 1 and 2). When GA_3 was kept at 10 μg per flower and NAA was increased (2.5, 25 and 250 μg), anthocyanin content of labella dropped and then increased, whereas in columns the decrease was not reversed (Figs. 1 and 2).

Most kinetin applications did not increase anthocyanin content of either columns or labella to levels higher than those of the controls. Exceptions were 0.1 and 1 μg kinetin which raised anthocyanin content of labella and 0.01 μg which increased it in columns (Fig. 3). Labella and columns of flowers treated with 1 or 10 μg kinetin plus 25 μg

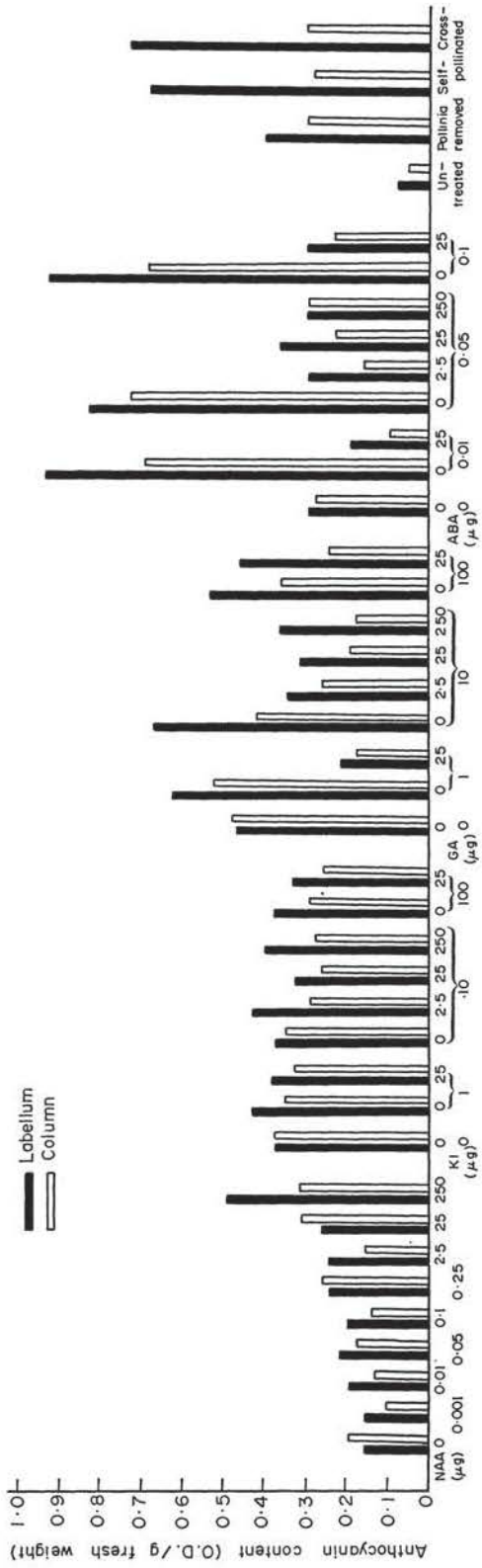

Fig. 1. Anthocyanin content in labella and columns of *Cymbidium* cv. Samarkand 1 week after applications of auxin (NAA), gibberellin (GA_3), cytokinin (KI), and abscisic acid (ABA) singly and in combinations.

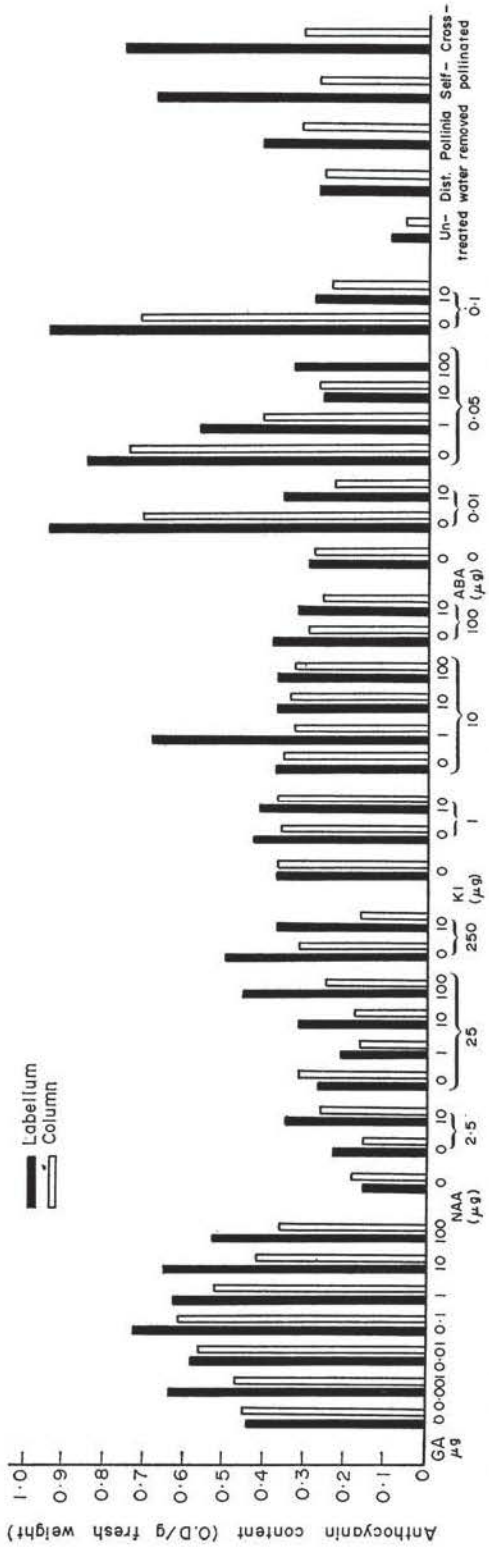

Fig. 2. Anthocyanin content in labella and columns of *Cymbidium* cv. Samarkand 1 week after applications of gibberellin (GA_3), abscisic acid (ABA), kinetin (KI) and auxin (NAA) singly and in combinations.

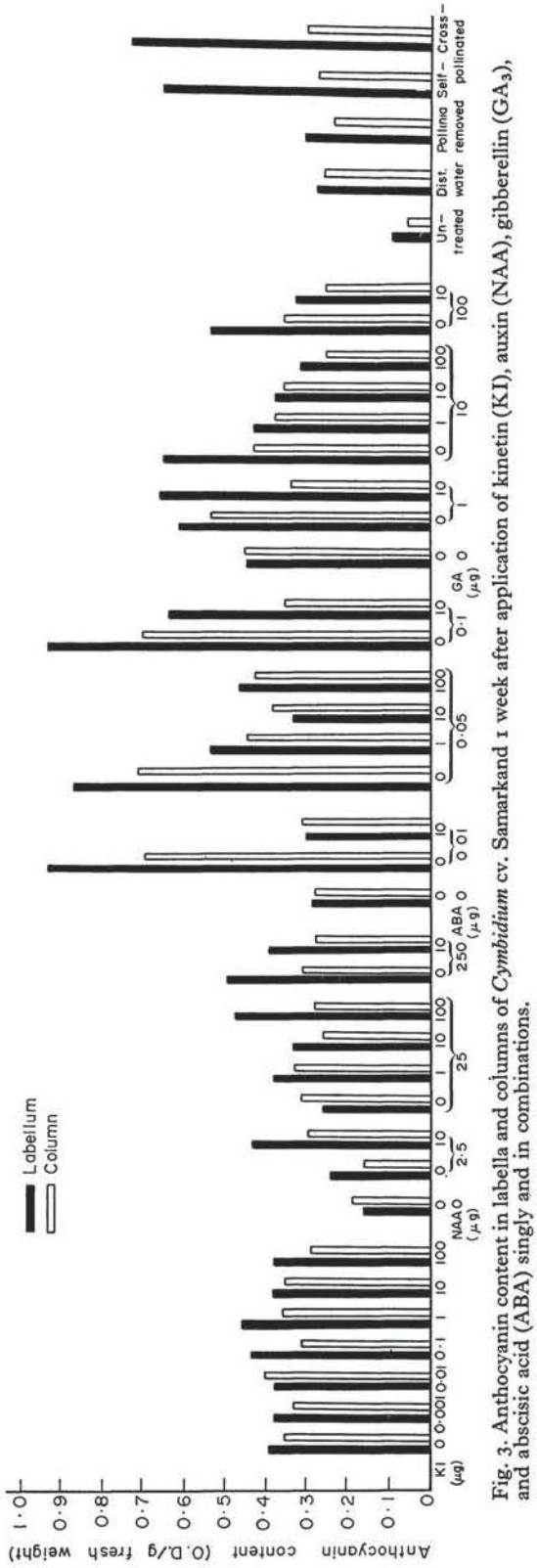

Fig. 3. Anthocyanin content in labella and columns of *Cymbidium* cv. Samarkand 1 week after application of kinetin (KI), auxin (NAA), gibberellin (GA_3), and abscisic acid (ABA) singly and in combinations.

NAA had a lower anthocyanin content than in blooms treated with the cytokinin alone, but higher than in auxin-only treatments (Figs. 1 and 3). With 100 μg kinetin and 25 μg NAA, pigment levels exceeded those produced by each hormone alone. Increased NAA concentrations (2.5, 25 and 250 μg) in the presence of 10 μg kinetin first lowered and then raised anthocyanin content in both columns and labella, but the effect was more pronounced in the latter. The same was true in regard to increased kinetin levels (1, 10 or 100 μg) in combinations with a constant 25 μg NAA (Figs. 1 and 3).

Following treatments with GA_3-kinetin combinations anthocyanin content was generally equal to that of flowers treated with kinetin only, although slight variations did occur (Figs. 2 and 3). In the presence of constant kinetin (10 μg/flower) and increasing amounts of GA_3 (1, 10 and 100 μg/flower) anthocyanin content of both columns and labella remained the same except for a sharp increase, followed by a drop, with 1 μg gibberellin (Fig. 2). When GA_3 was maintained at 10 μg per flower higher concentrations of kinetin (1, 10 or 100 μg/flower) reduced anthocyanin content of both labella and columns (Fig. 3).

ABA, when applied alone, caused anthocyanin formation (Arditti *et al.*, 1971), but in most instances NAA–ABA combinations reduced anthocyanin content relative to either hormone alone (Fig. 1). Exceptions to that were combinations of 25 μg NAA plus either 0.05 or 0.01 μg ABA which increased anthocyanin content in labella, and 2.5 μg NAA together with 0.05 μg ABA per flower which raised it in both the lip and column; both relative to the auxin only (Fig. 1).

Combinations of GA_3 and ABA always reduced pigment levels to below those obtained with either hormone alone (Fig. 2). Increased amounts of GA_3 (1, 10 and 100 μg) in the presence of constant ABA (0.05 μg/flower) first raised and then lowered anthocyanin content of labella and columns (Fig. 2).

Flowers treated with 0.1, 0.05 or 0.01 μg ABA plus 10 μg kinetin contained less anthocyanins than those given ABA only (Fig. 3). Combinations of 0.05 μg ABA with either 100 or 1 μg kinetin increased anthocyanin content relative to the same concentrations of the cytokinin when applied alone. With 0.05 μg ABA plus 10 μg kinetin, anthocyanin content in labella was lower, and that of columns higher than in flowers given an equal amount of kinetin only (Fig. 3).

Discussion

Stigmatic closure and column swelling or loss of curvature

Auxin- or pollination-induced swelling and straightening of columns, as well as closure (Table 1), are for the most part due to cell enlargement (Hsiang, 1951a; Hubert and Maton, 1939; Oertli and Kohl, 1960). Cell division is not involved and the swelling mechanism is not sensitive to actinomycin D, ethionine and puromycin (Arditti and Knauft, 1969).

Gibberellins do not, for the most part, induce marked swelling and straightening of the column or stigmatic closure (Table 1). Because of several reports that gibberellins lead to increased auxin levels, there have been attempts to explain their influence on growth and differentiation processes on this basis (Andersen and Muir, 1969; Kuraishi and Muir, 1962; Lantican and Muir, 1969; Nitsch, 1957; Roberts and Fosket, 1966; Valdovinos, Ernest and Henry, 1967; Valdovinos, Ernest and Perley, 1967). Some of the effects of GA_3 on *Cymbidium* flowers are similar enough to those induced by NAA (Table 1) to suggest that, in this instance, gibberellins may act by increasing auxin levels. On the

other hand, columns retained their curvature, anthocyanin levels were higher than those induced by NAA, but column swelling, stigmatic closure or wilting were not pronounced enough. Therefore, we must conclude that in *Cymbidium* flowers GA_3-effects on the columns and stigmas are in the main not auxin mediated.

Gibberellins can stimulate ethylene production (Pratt and Goeschl, 1969) and the latter has been shown to initiate some post-pollination symptoms in orchid flowers (Akamine, 1963; Akamine and Sakamoto, 1951; Burg and Dijkman, 1967; Davidson, 1949; Dijkman and Burg, 1970; Fischer, 1950; Lindner, 1946). Therefore, it is possible to speculate that GA_3 acts by initiating ethylene production at levels which, although sufficiently high to increase anthocyanin content, are insufficient to cause marked column swelling or loss of curvature, and stigmatic closure. Orchid flowers whose anthers have been dislodged during shipment do produce ethylene (Akamine, 1963; Akamine and Sakamoto, 1951; Fischer, 1950; Lindner, 1946), exhibit post-pollination symptoms such as anthocyanin production, fading and wilting, but not stigmatic closure and column swelling or straightening.

It is not surprising that kinetin cannot induce swelling of the column and brings about only limited stigmatic closure since these are probably caused by IAA or NAA via auxin-specific mechanisms. The limited stigmatic closure (Table 1) initiated by 100 μg kinetin per flower may be due to kinetin-mediated increased IAA levels since it is equal to that which could be brought on by very low auxin concentrations. Surprising, and unexplained, is the slight stigmatic closure caused by three kinetin-ABA combinations (Table 1).

Stigmatic closure as well as column straightening and swelling—all of them NAA-induced phenomena—cannot be reversed by ABA (Arditti *et al.*, 1971), GA_3 or kinetin. This is not surprising in light of recent views regarding the mode of action of these hormones (Addicott and Lyon, 1969; Chrispeels and Varner, 1967; Fox, 1969; Letham, 1967, 1969), and the nature of the swelling (Arditti and Knauft, 1969; Hsiang, 1951a, Hubert and Maton, 1939; Oertli and Kohl, 1960).

Wilting of sepals and petals

Of the several pollination- or hormone-induced post-pollination symptoms in orchid flowers, the wilting sepals, petals and calli are unequivocably signs of senescence. They are induced by ABA, GA_3 and NAA singly or in combinations, but not by kinetin (Table 1), a hormone which generally retards senescence (Letham, 1967, 1969).

Protein and starch are lost from the sepals of *Cattleya labiata*, *Dendrobium nobile*, *Phalaenopsis amabilis* and *Rhynchostylis retusa* (all Orchidaceae) following pollination (Fitting, 1909a, b; Schumacher, 1931). At the same time nitrogenous substances and carbohydrates are transported to the column (Gessner, 1948; Oertli and Kohl, 1960; Schumacher, 1931). This implies hydrolysis and suggests that GA may act by activating the synthesis of hydrolytic enzymes.

How auxins may act in causing the wilting of *Cymbidium* sepals and petals is not entirely clear at present. What emerges clearly is that wilting (i.e. senescence) of sepals and petals is an actively induced process. The implication of this on the problem of senescence and the use of orchid flowers to investigate it are worthy of note.

Calli

Pollination or hormone treatments bring about colour formation and loss of turgidity by the calli. Colour changes are from yellow through orange to red or purple and depend,

at least to a certain extent, on the concentration of the hormone being applied. Although calli have not been analysed separately, the coloration is almost certainly due to anthocyanins. Quite possibly, pigment synthesis in the calli and the labellum are activated simultaneously.

Loss of turgidity generally accompanies colour development and leads to deformation of the calli. Thus, the process (probably loss of water) appears to be the reverse of that occurring in columns (influx of water).

Anthocyanin production

The response of anthocyanin content to NAA concentrations is essentially quantitative (Fig. 1) and agrees with previous reports regarding orchids (Arditti *et al.*, 1971; Arditti and Knauft, 1969; Hsiang 1951a; Hubert and Maton, 1939).

A slight enhancement of anthocyanin content by the medium-range kinetin concentrations (Fig. 3) can be correlated with some previous reports, but not with others. Cytokinins stimulate anthocyanin synthesis in some plants (Crane, 1964; Klein and Hagen, 1961; Thimann and Radner, 1962) and inhibit it in others (Crane, 1965; Crane and van Overbeek, 1965; Hirai, 1966).

Gibberellins enhance flavonoid and anthocyanin contents in several instances (Arnold and Albert, 1964; Brian, Petty and Richmond, 1959; Furuya and Thimann, 1964; McClure, 1970; Norman, 1968) and inhibit it in some cases (Bachelard, 1965; Crane, 1965; Furuya and Thimann, 1964; Hirai, 1966; McClure, 1970; Russell and Galston, 1969). Thus, as with kinetin, our results do not confirm all previous reports. The mode of action of GA remains unclear. Gibberellin-induced auxin production must be excluded since, generally GA_3 does not bring about most typical NAA effects.

NAA inhibition of GA_3-induced anthocyanin synthesis can be reversed by higher concentrations of the gibberellin. And, conversely, increased levels of the auxin tend to reverse GA_3-promotion of anthocyanin content. Parthenocarpic 'bing' cherries induced by auxin plus gibberellin are also anthocyanin-poor (Crane and Hicks, 1968). If NAA stimulates the production of an unstable RNA which is required for anthocyanin synthesis (Arditti and Knauft, 1969; Mohr, 1969; Thimann and Radner, 1962) or at least stabilizes it, and if GA_3 enhances the production of ribonucleases (Chrispeels and Varner, 1967) the antagonism between the two hormones relative to anthocyanin synthesis in *Cymbidium* flowers seems logical.

Both synergism and antagonism have been reported to exist between cytokinins and auxins (Letham, 1967, 1969; van Overbeek, 1962). In orchid flowers, most NAA effects cannot be reversed by kinetin, although wilting and changes in the calli seem to be slowed down. Both are aspects of senescence, a process inhibited by cytokinins (Letham, 1967, 1969). Changes in anthocyanin levels brought on by combinations of NAA and kinetin are limited and it simply appears that if interactions between the two hormones occur in this respect they are minimal.

Exogenous cytokinins bring on few, if any, post-pollination symptoms in *Cymbidium* flowers (Fig. 3; Table 1). When combined with GA_3, kinetin reverses most of the gibberellin effects including enhancement of anthocyanin synthesis. Since some of the post-pollination symptoms in *Cymbidium* flowers are aspects of senescence, these effects of kinetin are in agreement with its well-known ability to delay ageing (Fox, 1969).

Gibberellins and ABA are antagonistic in a variety of systems (Addicott and Lyon, 1969; Khan, 1969) as well as in orchid flowers (Arditti *et al.*, 1971). Possibly, ABA enhances pigment content by inhibiting production of ribonucleases which destroy an

unstable RNA required for anthocyanin synthesis (Radner and Thimann, 1963; Thimann and Radner, 1962). Gibberellins may reverse this inhibition by stimulating ribonuclease synthesis (Chrispeels and Varner, 1967). Decreasing anthocyanin levels in the presence of constant ABA (0.005 μg/flower) and increasing GA_3 concentrations (1, 10 and 100 μg/flower) argue in favour of this hypothesis. In so far as anthocyanin synthesis is concerned, NAA or kinetin are antagonistic to ABA (Arditti *et al.*, 1971) as they are in other systems.

Fruit set v. *anthocyanin synthesis*

All major plant hormones (and different chemical forms of each) have been implicated in fruit-set, growth, development and ripening although plants may vary in their responses or requirements (Addicott and Lyon, 1969; Crane, 1964, 1969; Letham, 1967, 1969; van Overbeek, 1962; Pratt and Goeschl, 1969). Post-pollination phenomena in orchids, including ovule formation are initiated by auxin (Fitting, 1910; Heslop-Harrison, 1957; Hsiang, 1951a; Hubert and Maton, 1939; Morita, 1918; Poddubnaya-Arnoldi, 1964) and possibly also other substances which may be contained in the pollen. Further development may be controlled and supported by hormones produced by the ovary, pollen tubes, placentae, column or remnants of the perianth (Burg and Dijkman, 1967; Dijkman and Burg, 1970; Dolcher, 1967).

Correlations between the capacity of a hormone to initiate parthenocarpic fruit and to regulate anthocyanin synthesis are difficult. Auxins, gibberellins and kinetin can all induce parthenocarpy (Crane, 1965), yet parthenocarpic 'bing' cherries, are deficient in anthocyanins (Crane and Hicks, 1968).

Hormonal interactions

The hormonal balance in a complex system such as *Cymbidium* flowers during post-pollination is of considerable interest. It affects and controls, or results from, senescence or redifferentiation of the perianth (Hayes, 1968; Ringstron, 1968), ovule development (Heslop-Harrison, 1957), anthocyanin formation in columns and labella (Arditti *et al.*, 1971; Arditti and Knauft, 1969; Hsiang, 1951a); pollen germination and tube growth as well as redifferentiation of the column, and hydrolysis of proteins (Schumacher, 1931). Remarkably, all of these occur in close proximity and simultaneously, or in a rapid and ordered succession. Pollinia-auxin is IAA (Maschmann, 1932; R. Müller, 1953), yet when applied exogenously this hormone does not sustain development of parthenocarpic fruits, or parthenogenetic seeds although it accelerates meiosis and embryosac formation in *Dactylorchis* (Heslop-Harrison, 1957). The same is true for *Cymbidium* where auxin stimulates ovule development which soon fails (Dolcher, 1967), other orchids (Huber and Maton, 1939) and several plants (Nitsch, 1952, 1953). Auxins can induce sufficient ethylene formation in *Vanda* to bring about fading (Burg and Dijkman, 1967; Dijkman and Burg, 1970) and 2,4-D or β-naphthoxyacetic acid can cause formation of parthenocarpic fruits in *Vanilla* which develop faster than pollinated ones but produce inferior perfume (Bouriquet, 1954). Spontaneous parthenogenetic haploid embryo formation has been observed in orchids (Hagerup, 1947) but, like in *Datura stramonium* (Solanaceae), it cannot be induced by NAA (Heslop-Harrison, 1957; van Overbeek, Conklin and Blakeslee, 1941).

It appears reasonable to assume that more than only ethylene and auxin are required for normal ovary and ovule development. While orchid pollen has been reported to con-

tain as much as 100 μg auxin/g (R. Müller, 1953), orchid embryos are poor in IAA (Poddubnaya-Arnoldi, 1964; Zinger and Poddubnaya-Arnoldi, 1959). Therefore, pollen or pollen preparations (Fitting, 1910; Morita, 1918) may contain more than just IAA. Fitting himself has suggested (personal communication) that equating his *pollenhormon* with auxin may not be correct. On the whole, the hormonal balance required for ovule development (Heslop-Harrison, 1957) and fruit-set in orchids is unclear at present. Multiple factor requirements for the formation of parthenocarpic fruits are not uncommon in other plants (Crane, 1964, 1969; Crane and Hicks, 1968; Letham, 1967, 1969; van Overbeek, 1962). The same may eventually prove to be the case regarding the multitude of symptoms brought on by pollination or hormone treatments in orchids (Arditti *et al.*, 1971; Arditti and Knauft, 1969; Bouriquet, 1954; Hayes, 1968; Holttum, 1957; Ringstrom, 1968; Roux, 1954; Swamy, 1947).

In considering the effects of exogenously supplied hormones (including NAA and kinetin which do not occur naturally) it is important to remember that they may be interacting with regulators already present in tissues or organs. This may indeed be the case with orchid flowers, but responses to the several concentrations and combinations of the hormones used by us (Arditii, 1969; Arditti *et al.*, 1971; Arditti and Knauft, 1969; Knauft, Arditti and Flick, 1970) indicate that, at the time of application, endogenous levels are not necessarily at optimal physiological ranges. We therefore conclude that the observed effects are due to the treatments but may represent effective concentrations and combinations other than those applied.

Life cycle and pollination ecology of orchids

Orchid flowers are generally pollinated by very specific vectors. Elaborate mechanisms such as deception, mimicry, explosive anthers, food and/or scent production are employed to attract pollinators and insure pollination (Dodson, 1967; Dodson, *et al.*, 1969; van der Pijl and Dodson, 1966). Moreover, in several instances exudates are produced both before and after pollination or fruit-set and act as attractants for ants which serve to protect the plant, flowers, or developing fruits from grazers (Dodson, 1967; Jeffrey, Arditti and Koopowitz, 1970; van der Pijl and Dodson, 1966). All these require considerable expenditures of energy and, indeed, respiration rate of orchid inflorescences are highest when the flowers are fully open and usually actively attracting pollinators (Rosenstock, 1956).

From the evolutionary standpoint it appears reasonable to assume that survival of a species would demand conservation of energy as well as re-utilization of storage or structural compounds following pollination or during fruit development and seed production. These can be achieved by hydrolysis of flower parts which have served their main function, and utilization of their structural and reserve substances. Closing of the stigma and swelling of the column undoubtedly serve to protect germinating pollen and to supply nutrients during the long periods which elapse in many orchids between pollination and fertilization (up to 130 days).

In a very specific plant species–pollinator relationship where the number of pollinators or pollinated flowers are limited, as in some orchids, survival of a species would require that 'unsuccessful' visits to flowers (i.e. those which do not pollinate or do not carry away pollen) be kept to a minimum. It is not surprising, therefore, to find that pollination usually brings about changes which may render orchid flowers no longer attractive to pollinators. These could be interpreted as conserving 'pollinator power'. For example, the calli in *Cymbidium* may either act as insect guides or mimic food (Gellert, 1923) and

therefore attract pollinators. Changes in appearance due to either colour development or loss of turgidity would no doubt reduce or eliminate their usefulness.

Pollination-induced anthocyanin formation alters patterns seen both under visible and ultra violet light on labella and columns (L. B. Thien, unpublished). Such changes in patterns and morphology probably play an important part in the attraction of pollinators. The evidence for this is at present circumstantial, but convincing.

Longevity in orchid flowers increases in direct proportion to the complexity of the pollination mechanism (Decker, 1941). Also, since the first visit by a pollinator may remove pollen without pollinating an orchid flower, successful adaptation would require that an emasculated blossom remain functional and attractive long enough for a second pollinating visit. And, this is indeed the case. Wilting and ethylene production in emasculated flowers are not as fast as in pollinated ones (Burg and Dijkman, 1967; Dijkman, and Burg, 1970). Such an intricate and sensitive system would require the type of delicate control mechanisms which can be provided by interacting hormones. Our findings and those by others confirm this assumption.

General comments

Post-pollination phenomena in orchid flowers include aspects of fruit-set, senescence organogenesis, redifferentiation and newly-expressed biochemical or metabolic capabilities, all in the same organ and in close physical proximity. It is not surprising, therefore, to find interactions between hormones. Possibly, anthocyanin production in orchid flowers, being initiated as it is by NAA, GA and ABA, may be activated by a 'multi-target' trigger, or at several starting points which, once 'turned on', result in the formation of all other necessary factors. Since, under normal (i.e. pollination) conditions, post-pollination phenomena in *Cymbidium* would only be a prelude to fruit-set, it is reasonable to expect that a system (Crane, 1964) involving sequential events as in other plants (Crane, 1964; van Overbeek, 1962) is operative in these, as well as other orchids. Because of their special character, longevity, pollination ecology and mode of fruit set or senescence, it would appear that orchid flowers represent a particularly attractive, yet largely unused, experimental system for studies in these areas.

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