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POSTPOLLINATION PHENOMENA IN ORCHID FLOWERS. X. TRANSPORT AND FATE OF AUXIN[1]

MICHAEL S. STRAUSS AND JOSEPH ARDITTI

Department of Biology, Northeastern University, Boston, Massachusetts 02115; and Department of Developmental and Cell Biology, University of California, Irvine, California 92717

The major portion of indoleacetic acid (IAA-2-^{14}C), applied to stigmas of whole flowers of *Angraecum* cv Veitchii and *Cattleya* cv Porcia 'Cannizaro,' as well as to excised gynostemia of the latter, is immobilized at the point of application. A limited amount of nonpolar transport occurs. Some of the auxin is conjugated into IAA-aspartate in a process which is inhibited by cycloheximide, suggesting a requirement for *de novo* protein synthesis. Because the effects of pollination and auxin application spread quickly to floral segments, we suggest that additional substances, from pollen or produced by pollinated flowers, may participate in the regulation of postpollination phenomena in orchid flowers.

Introduction

Orchid pollen is a rich source of IAA, and auxin applications (IAA, NAA) to stigmas induce postpollination phenomena in orchid flowers, including the production of ethylene (for review, see ARDITTI [1979]). Consequently, it is not clear if postpollination phenomena in floral segments are initiated by (1) auxin transport from the stigma, (2) ethylene which diffuses through the air or tissues, (3) interaction of both, or (4) other substances released by pollen or produced at the stigma. Previous studies of postpollination phenomena in orchid flowers and the effect of ethylene and auxin have provided some answers and raised new questions (ARDITTI 1979), one of which pertains to the nature of IAA transport in orchid flowers.

Material and methods

Whole, fully open, excised flowers or excised gynostemia and ovaries of *Angraecum* cv Veitchii (SANDERS 1947) and *Cattleya* cv Porcia 'Cannizaro' were employed (table 1). Treatments were initiated within 1 h of removal of flowers from plants.

Intact excised flowers were treated with IAA-2-^{14}C (ICN, Irvine, Calif.), 50 μg (sp act, 0.877 mCi/mmol) or 0.78 μg (sp act, 56.2 mCi/mmol), both in 0.2% agar (Bacto-Difco). Agar disks containing 50 μg IAA-2-^{14}C (sp act, 0.877 mCi/mmol) were applied to excised segments of *Cattleya* (table 1). These disks were prepared by pouring the warm agar into Tygon tubing and allowing the solution to solidify before extrusion and then cutting the resulting cylinder with a template of razor blades mounted on an aluminum block.

Whole *Angraecum* and *Cattleya* flowers were treated with auxin, inhibitors, and pollen, applied to the stigma simultaneously and at 2, 4, 6, and 8 h after pollination. Controls for *Angraecum* were NP and AUX.

Donor/receiver experiments were carried out with inverted and upright gynostemia and entire ovaries (fig. 1). In half of the experiments, donor disks were applied to tips and receiver disks to the base. The positions were reversed in the other half of the experiments (gynostemia are connected to ovaries in orchids). We define "tip" as the stigmatic end and "base" as the portion attached to the ovary; for ovaries, "upper" is the section adjoining the gynostemium, and "base" is the end near the pedicel. In one experiment, 5 μl drops containing auxin were applied to stigmas, and receiver blocks were attached to the bases of the gynostemia. The auxin concentration in donor blocks and drops was 50 μg IAA/5 μl (sp act, 0.877 mCi/mmol).

Act. D (10 μg/flower in 5 μl 0.2% agar) or CHI (25 μg/flower in 5 μl 0.2% agar) was applied to *Cattleya* flowers simultaneously with auxin (50 μg IAA, sp act, 0.877 mCi/mmol). All experiments were replicated three times.

Whole excised flowers were maintained in modified Knudson C medium (ARDITTI and KNAUFT 1969). Excised segments were placed in covered vials. Photoperiods, provided by Gro-Lux lamps, were 10 h at 430 lx; temperature was 21 ± 2 C.

Flower segments were separated (table 1) and extracted by steeping, for three successive 24-h

[1] Abbreviations: Act. D = actinomycin D; AUX = treatment with IAA of low specific activity and high concentration (sp act, 0.877 mCi/mmol), unpollinated flowers; CHI = cycloheximide; FW = fresh weight; IAA = indoleacetic acid; IAA-Asp = indoleacetic acid-aspartate; NAA = naphthaleneacetic acid; NP = treatment with IAA of high specific activity and low concentration (sp act, 56.2 mCi/mmol), unpollinated flowers; Pol = IAA treatment with high specific activity and low concentration (sp act, 56.2 mCi/mmol), pollinated flowers.

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Address for correspondence and reprints: MICHAEL S. STRAUSS, Department of Biology, Northeastern University, 360 Huntington Avenue, Boston, Massachusetts 02115.

TABLE 1

TREATMENTS, HARVESTS, AND ASSAYS OF ANGRAECUM AND CATTLEYA FLOWERS OR THEIR PARTS

ORCHID AND TREATMENT	AUXIN[a]		INHIBITOR, μg[b]	HARVEST, TIME AFTER TREATMENT (h)	SEGMENTS ASSAYED[c]	ASSAY[d]
	^{14}C, μCi	μg				
Angraecum CV						
Veitchii: stigmatic application, 5 μl	.25	.78	...	24, 48, 72, 96	Dorsal sepal(1); petals(2); labellum(1); ovary(1)	LSC for all segments
	.25	50.0				
Cattleya CV						
Porcia 'Cannizaro': stigmatic application, 5 μl (whole flowers) or agar blocks on cut surfaces of gynostemia and ovaries	.25	50.0	Act. D, 10 CHI, 25	.8, 1, 2, 2, 3, 4, 6, 8, 12, 24, 48, 72, 120, 168	Gynostemium(1); labellum(1); ovary(1); petals(2); sepals(2); and stigma(1); whole flowers or gynostemium(1); ovary(1); and stigma(1)	LSC and ARG for all segments

[a] In experiments with whole *Angraecum* and *Cattleya* flowers, auxin and pollen were applied simultaneously.
[b] No. in parentheses indicates segments per flower; the labellum is a modified petal.
[c] For *Cattleya*, only whole flowers were treated with CHI or Act. D.
[d] LSC = liquid scintillation counting, ARG = autoradiography.

periods, in 80% aqueous methanol (vol/vol) at room temperature. The extracts were combined in a scintillation vial, reduced to dryness in a 50 C oven, and resuspended in 1-ml absolute methanol before addition of 10-ml scintillation liquid (BRAY 1960).

Excised segments were cut into three sections—stigma (average FW, 600 mg), midsection (467 mg), and base (333 mg)—and extracted by steeping at room temperature for 2 days in 80% aqueous methanol (vol/vol) and for 2 additional days with diethyl ether. The extracts were then treated like those from floral segments.

Extract samples, together with authentic IAA and IAA-Asp, were applied to Whatman no. 1 chromatography paper and developed with isopropanol:ammonia:water (10:1:1, vol/vol/vol) or isopropanol:water (8:2, vol/vol). Visualization was accomplished with ninhydrin or Salkowski's reagent, as modified by SEN and LEOPOLD (ARDITTI and DUNN 1969). Sections of chromatograms were assayed for radioactivity with a liquid scintillation counter, and substances were localized on the intact chromatograms by autoradiography.

Results

The level of ^{14}C in labella 24 h after applying 0.78 μg IAA-2-^{14}C (sp act, 56.2 mCi/mmol) to stigmas of NP *Angraecum* flowers was high. When the concentration of IAA in the solution applied to stigmas was 50 μg (sp act, 0.877 mCi/mmol), radioactivity recovered from labella did not decrease (table 2). By 2 days after pollination, levels of ^{14}C in AUX or NP flowers did not show marked increases. Labella of pollinated flowers had the highest level of radioactivity on day 3 after IAA application. Radioactivity then declined markedly, but this was not evident in AUX controls (table 2).

At 24 h, petals of pollinated flowers had a low level of radioactivity, which increased to a peak on day 3 (table 2). Neither AUX- nor NP-treated flowers displayed this peak, although both had initially higher levels of ^{14}C than pollinated flowers (table 2). Increasing the concentration of IAA in the auxin solution did not reduce counts in labella, even when the solution used (50 μg) had a lower specific activity (0.877 mCi/mmol; tables 1, 2). Distribution of radioactivity in dorsal sepals was similar to that in petals (table 2).

Ovaries of Pol- or NP-treated flowers had similar levels of ^{14}C at 24 and 72 h following treatment (table 2). Application of higher concentrations of auxin resulted in lower levels of radioactivity in ovaries of AUX-treated flowers (table 2). At almost all harvest times the ovary contained more radioactivity than any other single floral segment, except for the site of application (table 2). Less than 10% of the total radioactivity applied was recovered out-

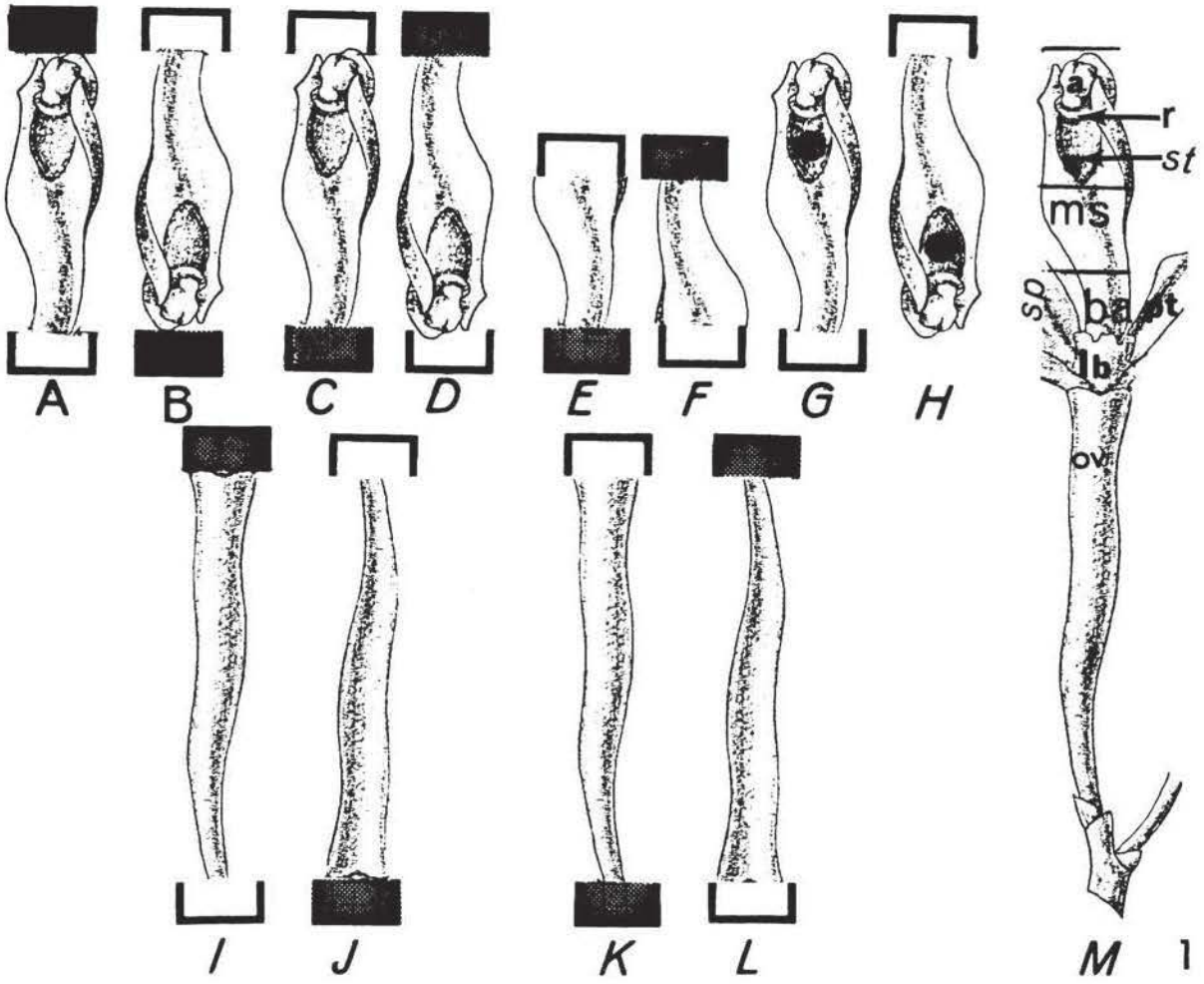

FIG. 1.—Sites of ^{14}C-IAA applications to gynostemia (columns) and ovaries of *Cattleya* cv Porcia 'Cannizaro.' Explanations of symbols: *A–H*, Gynostemia. *I–L*, Ovaries. *M*, Gynostemium, ovary, and stubs of perianth segments showing method of cutting columns for radioactivity determinations. *a*, Anther cap; *ba*, base; *lb*, stub of labellum (lip, median petal); *ms*, midsection; *ov*, ovary; *pt*, stub of petal; *sp*, stub of sepal; *r*, rostellum; *st*, stigma; shaded blocks, ^{14}C-IAA donors; clear blocks, receivers; dark spot, droplet containing ^{14}C-IAA (drawings of gynostemia and ovaries taken from fig. 228, p. 767 of SCHLECHTER [1915]).

TABLE 2

LEVEL OF RADIOACTIVITY IN FLORAL PARTS OF ANGRAECUM CV VEITCHII FROM IAA-2-^{14}C STIGMATICALLY APPLIED TO EXCISED FLOWERS AND THE EFFECT OF POLLINATION OR IAA ON ITS DISTRIBUTION

TREATMENT AND HARVEST TIME (h)	RADIOACTIVITY[a]			
	Labellum	Petals	Dorsal sepal	Ovary
Pol (pollinated), 0.78 μg, sp act, 56.2 mCi/mmol:				
24	129± 27	354± 49	268± 40	21,655±3,543
48	794± 232	1,315±295	732±250	17,645±3,351
72	1,626± 57	4,159±381	3,086± 50	19,325±2,314
96	580± 192	1,037±364	514±160	12,400±1,842
AUX (IAA control), 50 μg, sp act, 0.877 mCi/mmol:				
24	7,537±3,641	1,317±556	872±314	6,944± 944
48	793± 216	2,016±355	2,003±211	2,075±1,333
72	1,068± 219	2,503±460	1,037±132	2,656± 436
96	1,519± 331	3,520±325	2,909±651	1,320± 292
NP (unpollinated control), 0.78 μg, sp act, 56.2 mCi/mmol:				
24	4,125± 128	1,615± 82	1,136± 30	22,247±3,878
48	...	968±134	846±221	5,802± 609
72	959± 174	1,091±259	842±160	13,600±5,600
96	1,076± 221	1,237± 65	883±237	4,672± 960

[a] dpm ± SE.

side the stigma. No loss to the culture medium was detected, and no detectable amounts of ^{14}C could be found in a saturated barium hydroxide trap.

Following IAA treatments, chromatograms of *Cattleya* stigmatic extracts had three radioactive zones: (1) free IAA, (2) IAA-Asp, and (3) probably degradation products (table 3). These spots were also present in extracts of all other floral segments. Application of CHI together with IAA sharply reduced the amount of IAA-Asp formed in stigmas and increased the amounts of high Rf metabolites (table 3). Treatments with Act. D resulted in a slight reduction of conjugate formation but caused no change in the levels of the other radioactive components. In both Act. D and CHI-treated flowers, the levels of free IAA were almost 75% higher than in the controls (table 3).

When ^{14}C-IAA-containing blocks were applied to the tips of upright or inverted *Cattleya* gynostemia (fig. 1*A*, *B*), most of the radioactivity was concentrated in the stigma (fig. 1*M*, *st;* fig. 2). Within 6 days, some of the radioactivity moved to the midsection (fig. 1*M*, *ms;* fig. 2), but very little was transported to the base (fig. 1*M*, *ba*) or the receiver (fig. 2).

In experiments where the donor blocks were placed on the bases of upright (fig. 1*C*) or inverted (fig. 1*D*) whole gynostemia or those without tips and stigmata (fig. 1*E*, *F*), radioactivity moved into the gynostemia but concentrated at the base. Relatively little movement occurred into the midsection. Transport into tips and receivers from basally applied donors was even more limited (figs. 3, 4). Levels of radioactivity in the receiver dropped after 50 min (figs. 3, 4). Movement to the midsection, base, and receiver increased when a droplet of auxin was placed on the stigma (fig. 1*G*, *H;* fig. 5).

Translocation from donors into ovaries was the same regardless of site of application or orientation with respect to gravity (fig. 1*J–L;* fig. 6).

TABLE 3

DISTRIBUTION OF RADIOACTIVITY IN EXTRACTS OF CATTLEYA CV PORTIA 'CANNIZARO' STIGMAS 24 h AFTER TREATMENT WITH IAA-2-^{14}C AND Act. D OR CHI

TREATMENT	TOTAL RADIOACTIVITY APPLIED TO CHROMATOGRAM (%), Rf			TOTAL RECOVERED
	.0–.15[a]	.31–.40[b]	.7–1.0[c]	
IAA + Act. D	27.5	34.7	20.5	82.7
	25.7	34.7	27.1	87.5
IAA + CHI...	12.5	34.5	41.5	88.5
	9.6	33.8	44.5	87.9
IAA alone.....	36.0	20.1	29.7	85.8
	36.3	19.7	28.9	85.9

[a] Corresponds to published Rf values for IAA-Asp (ANDRAE and VAN YSSELSTEIN 1960; ROBINSON, FORMAN, and ADDICOTT 1968; HALLIDAY and WANGERMAN 1972*a*, 1972*b*).

[b] Free IAA (identified by co-chromatography with authentic standard).

[c] Other metabolites (IVERSEN and AASHEIM 1970).

Discussion

Protein and RNA synthesis are implicated in the pollination-induced phenomena in orchid flowers (ARDITTI and KNAUFT 1969; ARDITTI 1979). Auxins induce most of these phenomena and are present in high levels in orchid pollinia (MÜLLER 1953; KLASS 1964; ARDITTI 1979). Both enzymatic destruction and immobilization of IAA, by conjugation, occur in orchid flowers (table 3). There is no indication of polarity of auxin transport in either gyno-

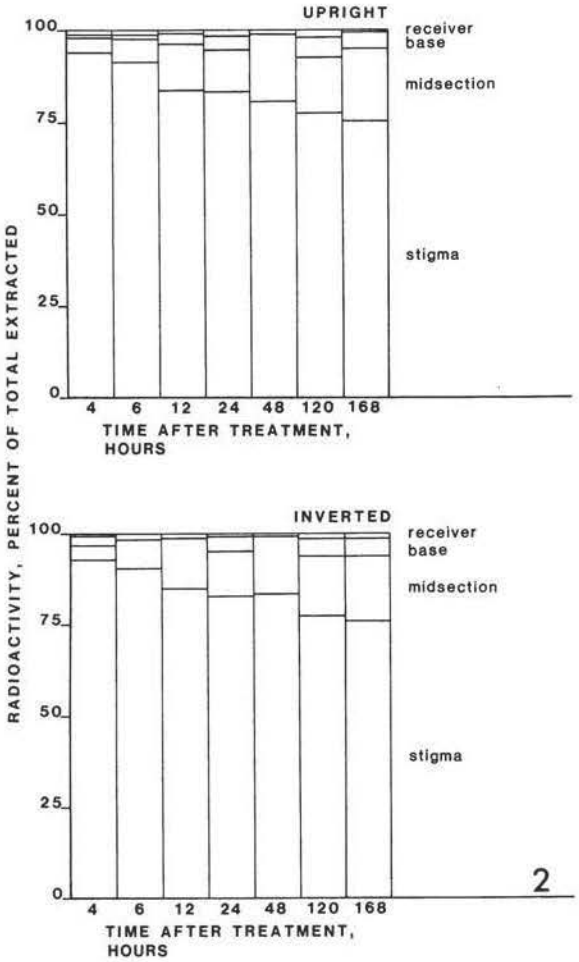

FIG. 2.—Distribution of label from donor blocks containing ^{14}C-IAA on tips of whole gynostemia of *Cattleya* cv Porcia 'Cannizaro' in upright and inverted positions. Data are expressed as percentage of total radioactivity removed from tissue and are divided, beginning at 0, into stigma, midsection, base, and receiver. Radioactivity from receivers at 48 h for upright gynostemia and at 6, 12, and 49 h for inverted gynostemia was less than 1% and is not visible on graphs.

stemia or ovaries under the conditions of this study. Apparently, only relatively small amounts of auxin are translocated over long distances because of immobilization. This may be the reason why auxin, when present in the maintenance medium, does not seem to affect excised gynostemia (ARDITTI and FLICK 1976).

Movement into receiver blocks from gynostemia is limited, but uptake by ovaries from donors is relatively high. In addition, translocation from stigmas is more pronounced than that from donors. Therefore, in intact flowers, stigmas may exert a regulatory influence on auxin movement, and initial downward movement from the gynostemium into the ovary may result, in part, from a sink effect. Exogenous auxin applied to a stigma, which under natural conditions presumably comes from pollen, initiated ovule development in orchids (HESLOP-HARRISON 1957; ARDITTI 1979). However, as the ovules, seeds, and fruits develop, the ovaries become self-sufficient with respect to auxin production (PODDUBNAYA-ARNOLDI 1964).

The distribution of label in *Angraecum* flowers differs from that reported for *Vanda* (BURG and DIJKMAN 1967; DIJKMAN and BURG 1970). Orchid pollinia are a rich source of auxin (MÜLLER 1953; KLASS 1964), but when IAA is applied, it does not fully mimic the effect of pollen on transport of this hormone. Application of IAA to unpollinated (AUX or NP) flowers resulted in a preferential movement of label to labella and other perianth segments similar to that reported for *Vanda* (BURG and DIJKMAN 1967; DIJKMAN and BURG 1970). This pattern of movement was not seen in pollinated flowers treated with low concentrations of IAA (Pol). Since there was no less label in perianth segments after 24 h following treatments with the low specific activity auxin solution than with the high specific activity solution, we conclude that increased auxin concentrations enhance the movement of this hormone to the perianth. The data clearly indicate that auxin alone is not responsible for the postpollination phenomena of orchid flowers. Enzymes, RNA, and DNA diffuse from or are

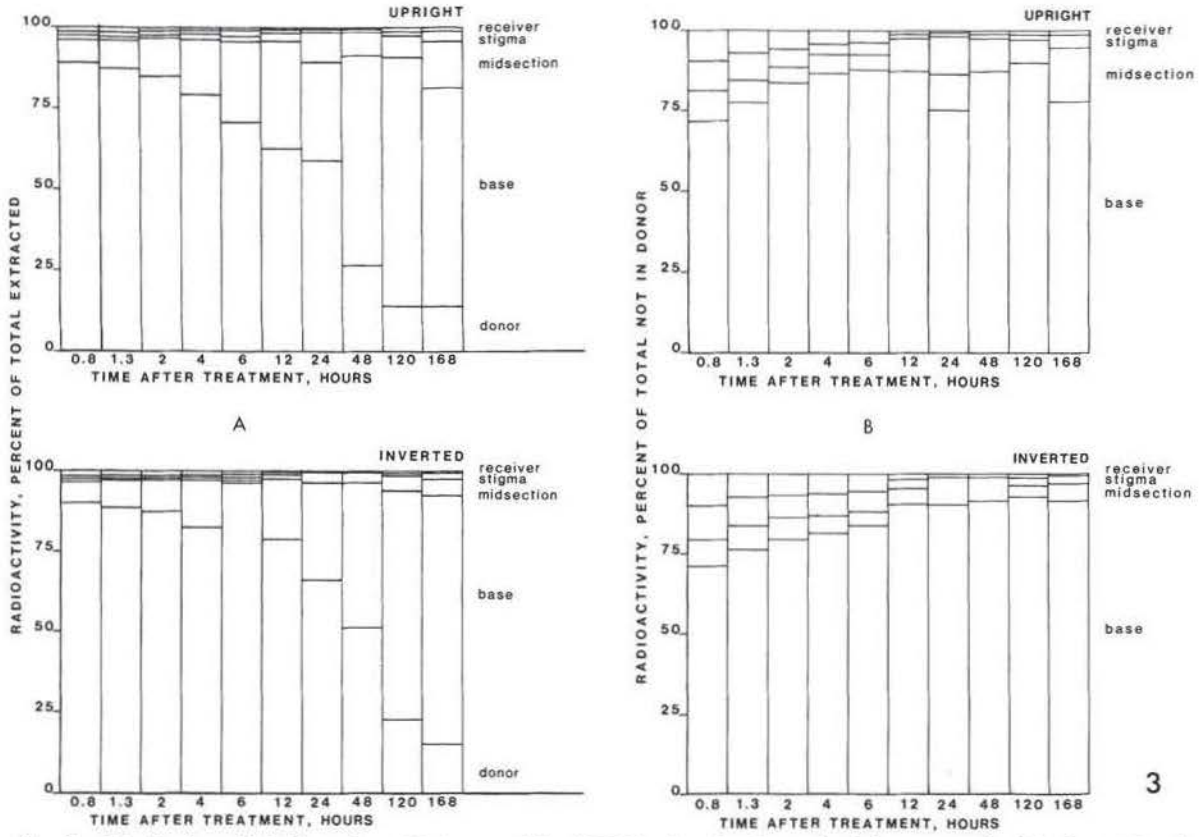

FIG. 3.—Distribution of label from donor blocks containing ^{14}C-IAA, placed on bases of whole gynostemia of *Cattleya* cv Porcia 'Cannizaro' in upright and inverted positions. *A*, Percentage of total radioactivity extracted from tissue and donor, expressed as portion in donor, base, midsection, stigma, and receiver; radioactivity from receivers at 12, 24, and 48 h for upright gynostemia and inverted gynostemia was less than 1.5% and is not readily visible on graphs. *B*, Percentage of total radioactivity extracted from tissue only, expressed as portion in base, midsection, stigma, and receiver; radioactivity from receivers at 24 and 48 h for inverted gynostemia was less than 1% and is not visible on graphs.

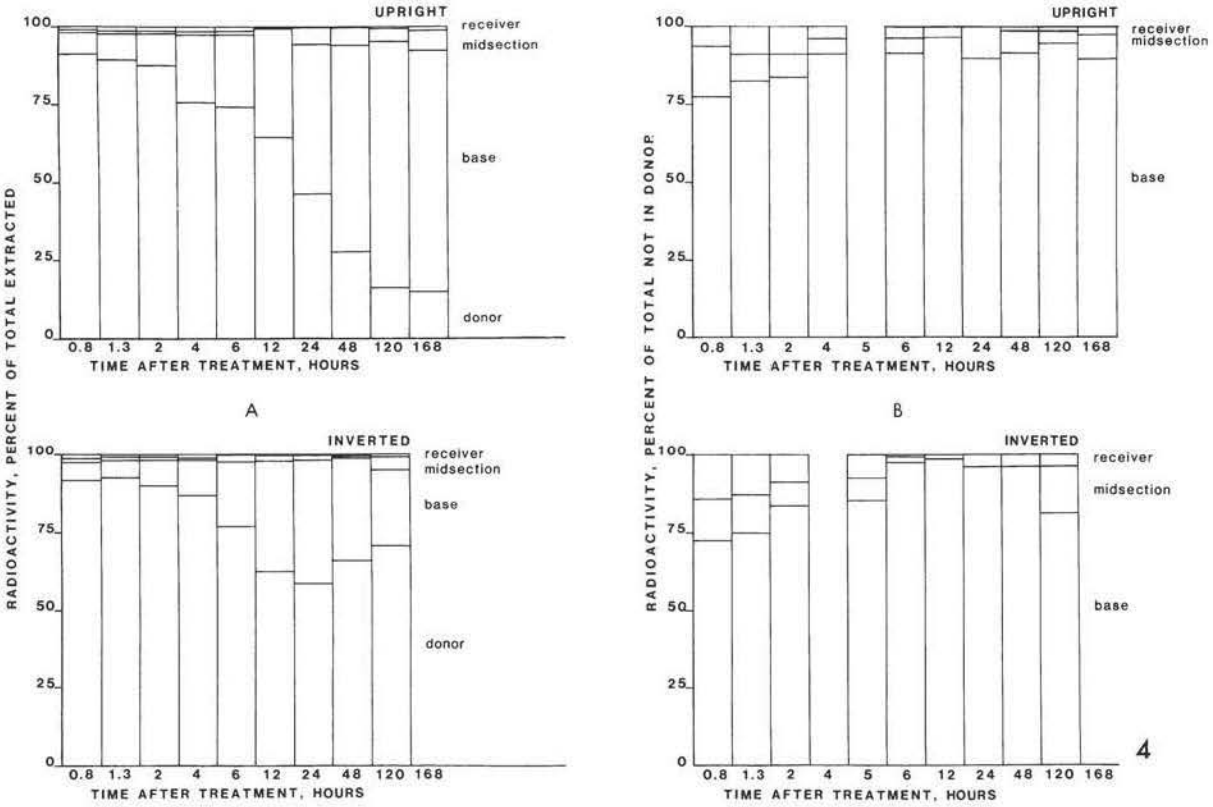

FIG. 4.—Distribution of label from donor blocks containing ^{14}C-IAA, placed on bases of gynostemia of *Cattleya* cv Porcia 'Cannizaro' from which stigmas and tips were removed. *A*, Percentage of total radioactivity extracted from tissue and donor, expressed as portion in donor, base, midsection, and receiver; radioactivity from receivers at 12, 24, and 48 h for upright gynostemia and 6, 12, and 24 h for inverted gynostemia was less than 1% and is not visible on graphs. *B*, Percentage of total radioactivity extracted from tissue only, expressed as portion in base, midsection and receiver; radioactivity from receivers at 12 and 24 h for upright gynostemia and at 12, 24, and 48 h for inverted gynostemia was less than 1% and is not visible on graphs.

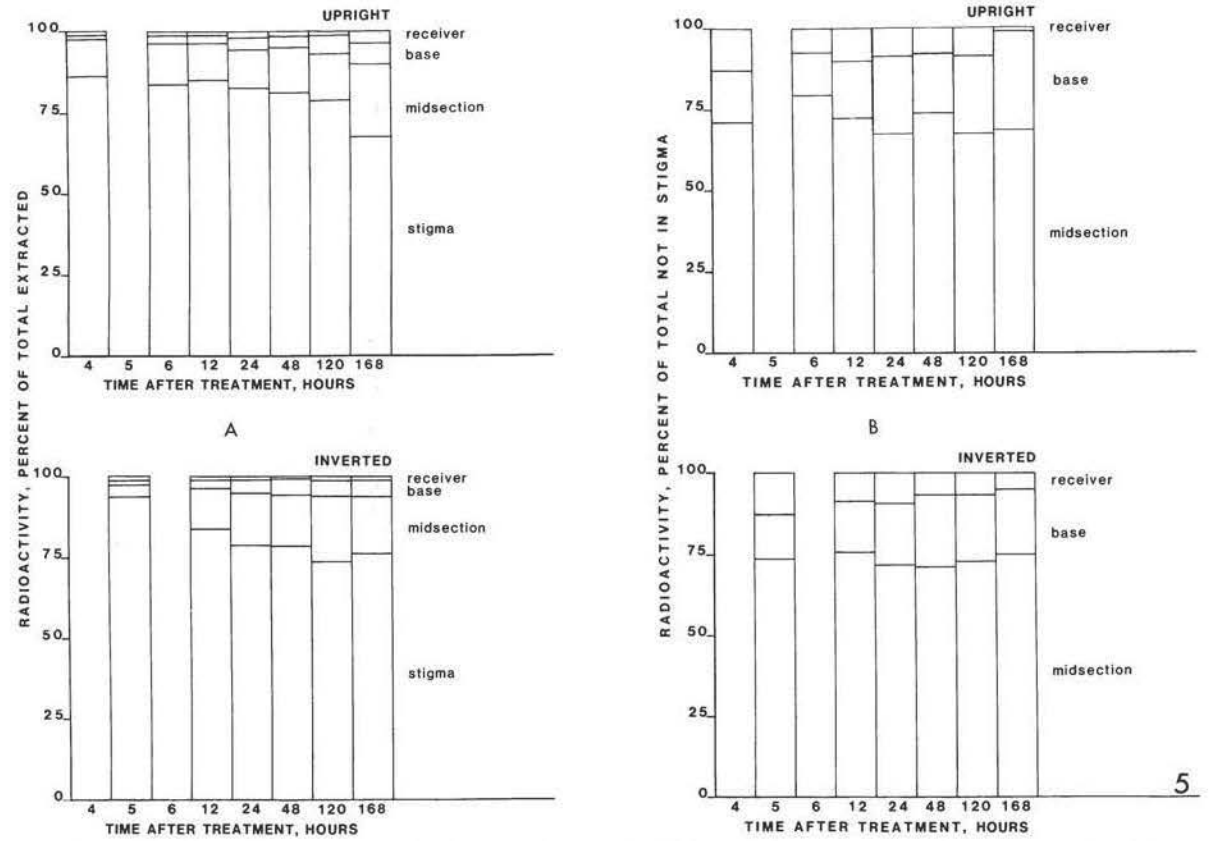

FIG. 5.—Distribution of label from ^{14}C-IAA placed in stigmata of *Cattleya* cv Porcia 'Cannizaro.' *A*, Percentage of total radioactivity extracted from tissue and donor, expressed as portion in stigma, midsection, base, and receiver. *B*, Percentage of total radioactivity from tissue only, expressed as portion in midsection, base, and receiver.

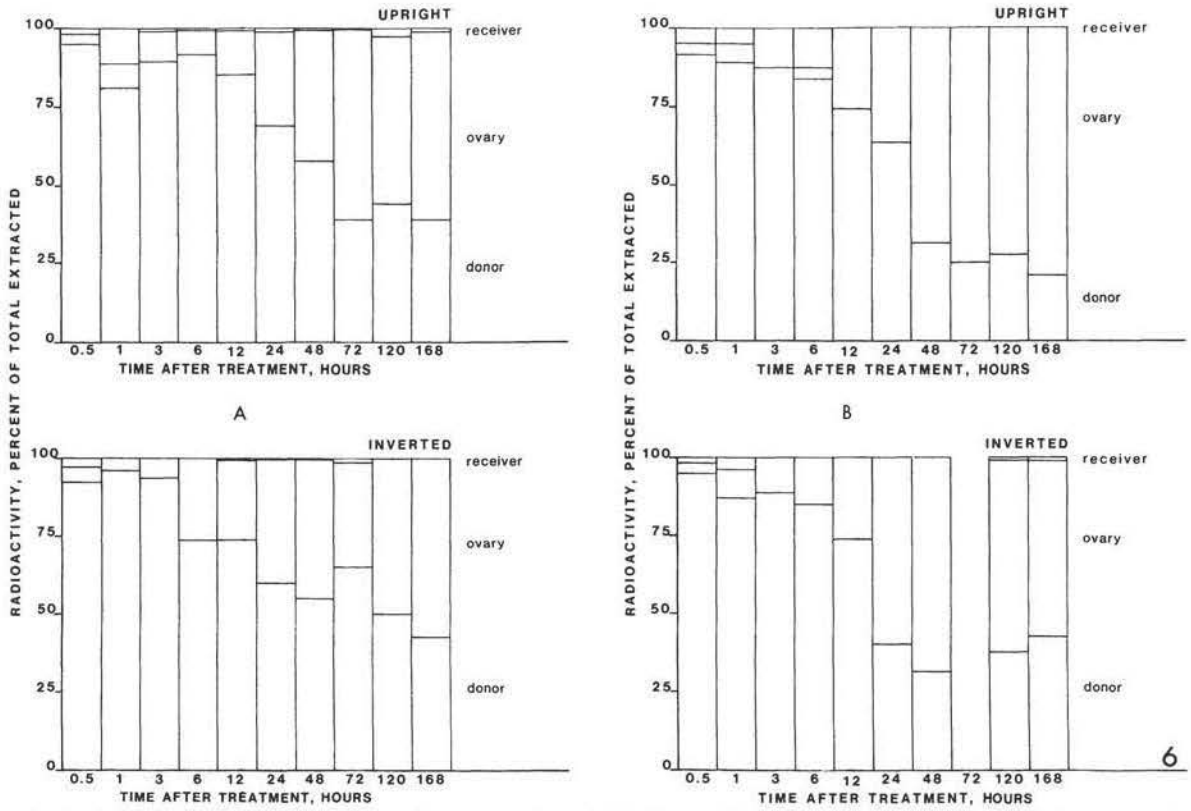

FIG. 6.—Distribution of label from donor blocks containing ^{14}C-IAA, applied to ends of excised ovaries of *Cattleya* cv Porcia 'Cannizaro.' Data expressed as percentage of total radioactivity extracted from donor, ovary, and receiver. *A*, Donor on distal (flower) end of ovary; radioactivity from receivers at 72 h for upright ovaries and at 1, 3, 6, 120, and 168 h for inverted ovaries was less than 1% and is not visible on graphs. *B*, Donor on proximal end of ovary; radioactivity from receivers at 3, 12, 24, 48, 72, 120, and 168 h for upright ovaries and at 3, 6, 12, 24, and 48 h for inverted ovaries was less than 1% and is not visible on graphs.

affected by germinating pollen (MÄKINEN and BREWBAKER 1967; TUPY and RANGASWAMY 1973; NEWBURY, SEDGLEY, and POSSINGHAM 1978; ARDITTI 1979). Consequently, auxin transport may be regulated by substances produced by the pollen and/or stigmas. Hence, orchid pollinia and/or stigmas produce effects resulting from synthesis or release of substances in addition to auxin.

Act. D and CHI can reduce and somewhat delay the onset of ethylene evolution by orchid flowers (CHADWICK, HOGAN, and ARDITTI 1980). Since ethylene can influence the transport and effects of auxin (ABELES 1966, 1973; OSBORNE 1976), the action of these compounds may be direct or mediated by the gas.

Our results support the suggestion that auxin plays an important role in the induction and regulation of postpollination phenomena in orchid flowers. However, it is also clear that other factors, possibly from pollinia, are important and may interact with auxin. Care must be exercised in interpreting exogenous auxin application to be analogous to pollination.

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