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THE ROLES OF AUXIN, ETHYLENE, AND ACID GROWTH IN FILAMENT ELONGATION IN GAILLARDIA GRANDIFLORA (ASTERACEAE)[1]

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ABSTRACT

Filament elongation and the role of auxin in this process in *Gaillardia grandiflora* was investigated. Filament elongation in vivo occurred just prior to anthesis and was accompanied by cell elongation and fresh weight increase. Filaments isolated and exposed to auxin in vitro grew more rapidly than controls and their growth was comparable to that of filaments in vivo. Furthermore, the natural auxin content of disc flowers (determined by double-standard isotope dilution analyses) increased just prior to anthesis and filament elongation. These results imply that auxin controls filament elongation. Applied ethylene slightly promoted filament elongation in vitro, and ethylene production of the flowers (determined by gas chromatography) slightly increased prior to filament growth. Fusicoccin and acidic buffers also stimulated elongation of isolated filaments. Thus, the role of auxin in controlling filament elongation in *Gaillardia* may involve stimulation of ethylene biosynthesis and acid growth.

THE REGULATION of the flowering process of plants has been studied in several phases of flower development. The induction of flowering (Tompsett and Schwabe, 1974; Zeevaart, 1976; Chailakhyan, 1979) and the development of flower primordia (Heslop-Harrison, 1959; Bose and Nitsch, 1970; Bilderback, 1972; Berghoef and Bruinsma, 1979) seem to be environmentally and hormonally influenced. After the differentiation of the primordia, the flower buds develop slowly until the time when the flower opens. During flower opening, several rapid growth responses occur in an organized sequence. The corolla opens by means of rapid elongation and expansion of the petals and subsequently the filaments and style rapidly elongate.

The role of auxin in filament elongation has been studied in several plants (Lang, 1961). The results of recent studies show that filaments of some plants respond to cytokinins (Pool, 1975; Raman and Greyson, 1978), others to gibberellins (Schaeverbeke, 1960; Greyson and Tepfer, 1967; Adolphe, 1971; Murakami, 1973, 1975; Sawhney and Greyson, 1973), and yet others to auxin (Koevenig, 1973; Goldschmidt and Huberman, 1974; Hess and Morré, 1978). The hormonal control of filament elongation in the Asteraceae is unstudied. The purpose of the present work was to measure natural filament elongation, to determine which plant hormone(s) control this process, and to determine whether acid growth is part of the filament elongation mechanism in a member of the Asteraceae.

Gaillardia grandiflora cv 'Goblin' was chosen for this study because the plants are small enough to culture in growth chambers, the inflorescences yield 15 flowers at the appropriate developmental stage, the flowers are large enough to handle easily, there are five genetically and developmentally identical filaments in each flower, and the filaments can be excised without being extensively damaged.

MATERIALS AND METHODS—*Plant culture*—*Gaillardia grandiflora* Van Houtte cv 'Goblin' seeds were obtained from George W. Park Seed Co., Greenwood, South Carolina. Seedlings were grown to a stage with eight leaves under greenhouse conditions, and were transplanted to a field plot of sandy loam at the Matthaei Botanical Garden, Ann Arbor, Michigan. The plants flowered under long-day conditions of summer.

Filament length measurements—The filaments were excised with fine forceps and a razor blade. The length of the filaments was measured to within 0.1 mm using a Finescale

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Comparator (Finescale Tools, Orange, California).

Cell length and cell number determinations—Wet mounts of filaments were made, and the length of each epidermal cell of a longitudinal row of cells was measured with an Olympus Model EH microscope fitted with a calibrated optical micrometer. The cells in several rows were measured separately and compared as described by Greyson and Tepfer (1966). The mean cell length was compared for the filaments at various developmental stages. To determine the uniformity of elongation, the length of cells was plotted as a function of position along the length of the filament. The number of epidermal cells in each row was also counted to show the contribution of cell division to filament elongation.

Fresh and dry weight determinations—Immediately after removal from the inflorescence, groups of filaments from 25 flowers of each developmental stage were weighed on a Sartorius Model 2603 analytical balance. These fresh weight data were compared with dry weight data. The latter were determined by reweighing the filaments after drying them overnight in an oven at 80 C.

In vitro filament elongation—Filaments were excised from 15 Stage II disc flowers using fine forceps to open the flowers and a razor blade to excise the filaments. The five filaments from each flower were placed in separate 6-cm petri dishes containing half a microslide, a disc of 4.5-cm-diam filter paper, and 2 ml of a test solution. All test solutions contained 0.05 M sucrose as a substrate for growth. The dishes were incubated in the dark at 30 C in a Thelco Model 2 incubator. The filaments were measured with the Finescale Comparator at 2-hr intervals. The mean filament length was calculated for each treatment in this randomized-block experiment.

Test solutions[3]—ABA, BA, CCC, $CoCl_2$, IAA, IPA, PCIB, TIBA, and Z were obtained from Sigma Chemical Co., St. Louis, Missouri. AMO 1618 was obtained from Polysciences, Inc., Warrington, Pennsylvania; AVG was a gift from Maag Agrochemicals, Vero Beach, Florida; BS was a gift from Dr. W. Meudt, Beltsville, Maryland, to P. B. Kaufman. Fusicoccin was a gift from Montedison S.P.A., Milano, Italy. The GA_3 and GA_{4+7} were a gift from Imperial Chemical Industries Ltd., England, to P. B. Kaufman. Serial dilutions of 1 mM stock solutions of these compounds were used as test solutions. Na_2HPO_4-citrate buffer (0.01 M) was prepared at pH 3, 4, 5, 6, and 7. One buffer at each pH was also 10^{-5} M IAA. These pH buffers were also used as test solutions. Ethylene (1,000 μl/l) was obtained from Applied Sciences, State College, Pennsylvania, and was applied to filaments in rubber-sealed petri dishes using a syringe for gas introduction. The atmosphere was changed at 2-hr intervals in an exhaust hood.

Auxin analyses—The amount of endogenous auxin was determined for each developmental stage of disc flower opening by using the double-standard isotope dilution assay designed by Cohen and Schulze (1981). A weighed sample of flowers (10–20 g) was ground in 100 ml of 80% aqueous acetone (v/v) in a Waring Blender and extracted overnight at 4 C. The extract was filtered through weighed Whatman #1 filter paper, and the residue was washed with additional 80% acetone, dried in an oven, and weighed. Twenty-three nanocuries of [2-^{14}C]-indole-3-acetic acid (54.5 mCi/mmole), a gift from Dr. Robert Bandurski, East Lansing, Michigan, were added to the combined filtrates. The filtrate was reduced to aqueous in a Buchler rotary evaporator, was acidified with 1 N H_3PO_4 to pH 2.5, and was partitioned against 3 × 1 volumes of ether. The organic phases were pooled, evaporated to the original volume, and partitioned against 1 N sodium bicarbonate. The aqueous phase was washed with 3 × 1 volumes of ether, was slowly acidified with H_3PO_4 to pH 2.5, and was partitioned against 3 × 1 volumes of ether. The ether phases were pooled, washed with 1 volume of distilled water, and evaporated to the water that had remained dissolved in the ether. To this, an equal volume of absolute ethanol was added. The sample was loaded onto a 0.5 × 10-cm column of DEAE-Sephadex A-25 (Sigma) equilibrated with 50% aqueous ethanol (v/v). After washing the loaded column with 20 ml of equilibrating solvent, the IAA was eluted with a linear gradient of 0–5% acetic acid in 50% aqueous ethanol (v/v). The IAA eluted between 20 and 30 ml after starting the gradient. The IAA-containing fractions were pooled, evaporated to about 20 μl in the rotary

[3] Abbreviations: AMO 1618: 4-hydroxy-5-isopropyl-2-methylphenyl trimethyl ammonium chloride-1-piperidine carboxylate; AVG: L-2-amino-4-(2-aminoethoxy)-trans-3-butenoic acid hydrochloride (or aminoethoxyvinylglycine); BS: brassinolide; CCC: 2-chloroethyltrimethyl ammonium chloride; FC: fusicoccin; GA_{4+7}: gibberellin A_4 and A_7 mixture; IPA: isopentenyl adenine; PCIB: 4-chlorophenoxy-isobutyric acid; TIBA: 2,3,5-triiodobenzoic acid; Z: zeatin.

evaporator, mixed with about 80 μl of spectrograde methanol, and subjected to HPLC (high performance liquid chromatography). The 100 μl sample was loaded onto a 25 × 0.46 cm Whatman Partisil 10 ODS-3 C_{18} reverse-phase column (Anspec Co., Ann Arbor, Michigan) and eluted sequentially with 15 ml of 5%, 15 ml of 30%, and 30 ml of 100% aqueous spectrograde methanol (v/v). The elution rate was adjusted to 1 ml/min with a Milton-Roy Mini-pump FR (Anspec Co.). The IAA eluted between 2 and 6 ml of 30% methanol. The IAA-containing fractions were pooled, evaporated to about 20 μl in the rotary evaporator, transferred to a tapered vial, evaporated to dryness in a stream of nitrogen, and taken up in 200 μl of spectrograde methanol. Five μl of this solution were counted in a Beckman LS 230 scintillation counter, and percent recovery was determined. The recoveries ranged from 25 to 55 percent, with 35 percent being a typical value. To the recovered IAA, 1.5 nanocuries of[2-ring-^{14}C]-indole-3-butyric acid (508 μCi/mmole), a gift from Dr. Jerry Cohen, East Lansing, Michigan, were added. The mixture was derivatized using ethereal diazomethane (Schlenk and Gellerman, 1960) to form the methyl esters of IAA and IBA. The esters were taken to dryness under a stream of nitrogen, taken up in 50 μl of freshly distilled tetrahydrofuran, and subjected to gas chromatography. The Varian 2740 gas chromatograph was equipped with a Varian Thermionic Specific Detector, a flame ionization detector, and matched 1.8-m × 2-mm-ID glass columns. The columns were packed with 3% OV-17 on 100/120 Gas Chrom Q (Applied Sciences). The oven temperature was 170 C, and the carrier gas flow rate was 30 ml/min. The eluents of replicate injections of 1 μl of the derivatized sample were detected using the Thermionic Specific Detector connected to a chart recorder. There were two major peaks corresponding to retention times, 3′22″ and 6′50″, for authentic IAA and IBA (Sigma) methyl esters, respectively. The detector responds to organic nitrogen, so the peak areas of equal amounts of IAA and IBA were equal. The amount of IAA (in μg) in the vial at the time of derivatization was determined by the relationship:

$$\frac{\text{peak area (IAA)}}{\text{peak area (IBA)}} \times \mu\text{g IBA added to sample.}$$

Replicate injections of 10 μl of the derivatized sample were made into the flame ionization detector channel. The flame was extinguished, a 20-cm Pyrex tube fitted with a loosely-fitting plug of silanized glass wool was lowered over the jet, and the eluates allowed to condense in the tube during the elution interval determined for authentic IAA methyl ester. A second tube was used to collect eluates during the elution interval determined for authentic IBA methyl ester. The collection tubes were rinsed into scintillation vials with ACS scintillation fluid, and the vials were counted as before. The radioactivity of the collected IAA and IBA was used to determine the radioactivity (in μCi) of the IAA in the vial at the time of derivatization by the relationship:

$$\frac{\mu\text{Ci IAA collected}}{\mu\text{Ci IBA collected}} \times \mu\text{Ci IBA added to sample.}$$

The specific activity of the IAA in the vial at the time of derivatization was determined as the ratio of microCuries to micrograms of IAA in the sample at the time of derivatization. The relationship of Rittenberg and Foster (1940) was used to determine the amount of IAA in the original sample of flowers:

$$\left[\frac{\text{specific activity of IAA added to extract}}{\text{specific activity of IAA in vial at derivatization}} - 1\right] \times \mu\text{g IAA added to extract.}$$

The amount of IAA (in μg) in the flowers is presented as a function of the stage of flower development and is expressed as μg per g fresh and dry wt.

Ethylene analyses—A weighed sample (approximately 0.35 g) of fresh flowers at a single developmental stage was packed into a 5-ml screwcap vial and sealed with a neoprene sleeve septum (Pierce Chemical Co., Rockford, Illinois). The vials were placed under negative pressure (to extract the ethylene) by inserting the needles of three 30-ml syringes, retracting their plungers, and holding them in place with dowels for 4 min. The rods were then removed, and the negative pressure pulled the plungers back to zero barrel volume. The syringes were removed and the flowers allowed to equilibrate for 1 hr. Two 2.5-ml aliquots of the atmosphere of the vial were injected separately into a Varian 1400 gas chromatograph. The chromatograph was fitted with a 1.8-m × 2-mm-ID stainless steel column packed with Porapak Q 80/100. A flame ionization detector connected to a chart recorder was used to detect ethylene. With the oven temperature at 55 C and the carrier gas flow rate at 30 ml per min, ethylene had a retention time of 90 sec. The peak areas of flower-produced ethylene at each stage were summed and converted to moles of ethylene against a calibration curve of peak areas of various amounts of authentic ethylene (Ap-

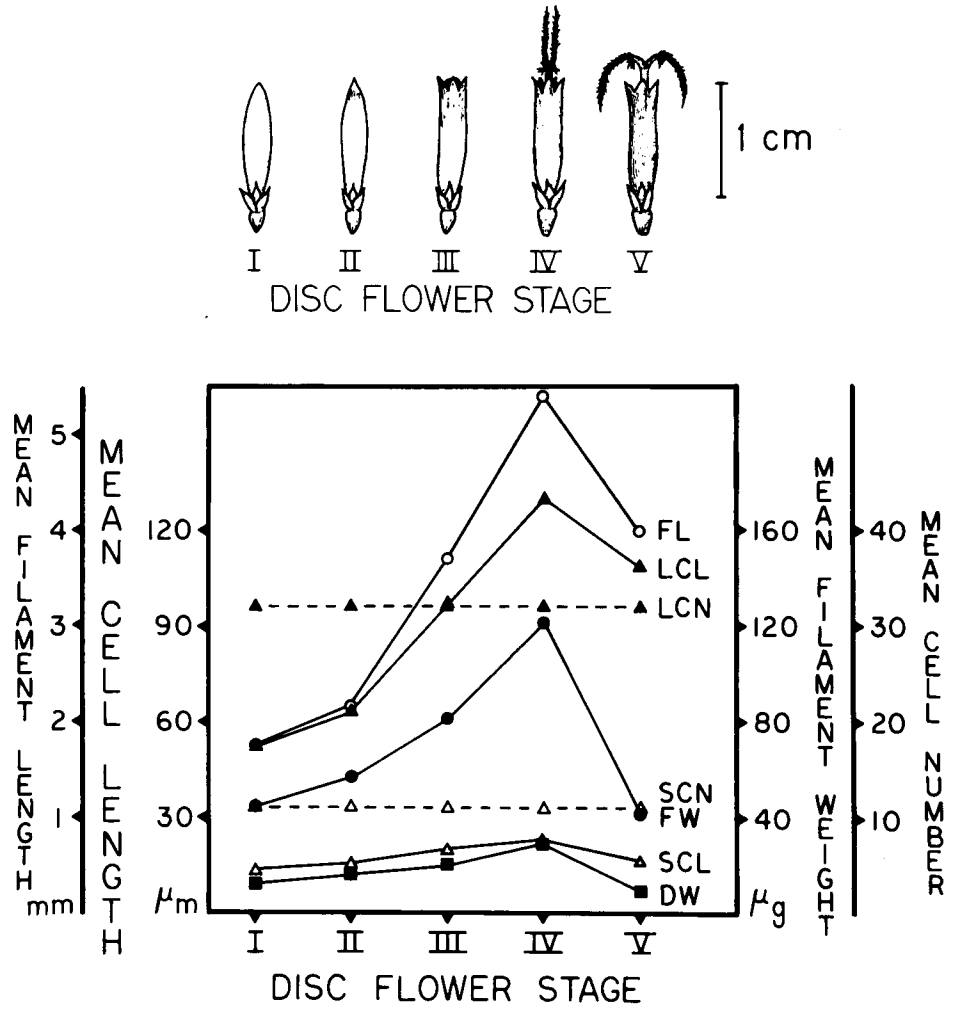

Fig. 1. Parameters of in vivo filament elongation through five developmental stages of opening *Gaillardia* disc flowers. Stages III and IV represent the morning and afternoon of the day of anthesis, respectively. The parameters are: filament length (—○—FL), cell length (long cells —▲— LCL, short cells —△— SCL), cell number (long cells – –▲– – LCN, short cells – –△– – SCN), filament wt (fresh —●— FW, dry —■— DW).

plied Sciences). The total amount of ethylene detected in the flowers is presented as a function of the stage of flower development.

RESULTS AND DISCUSSION—*Disc flower development*—The 80 to 100 disc flowers differentiate in rows that spiral around each other and converge at the apex of the meristem in the inflorescence. The disc flowers develop centripetally in the rows so that about 15 flowers of each of five easily-distinguished stages (Fig. 1) are present at one time in the inflorescence. In Stage I, the disc flower is relatively unpigmented and tightly closed. This stage lasts for about 15 days. By Stage II, which lasts 3 days, the tip of the corolla is pigmented (red). In Stage III, the day of anthesis, the growth processes of the flower opening phase begin. The filaments elongate, lift the tube of fused anthers, and push open the corolla. The pollen is shed in late morning and falls upon the stigma hairs. In the afternoon, the style and stigma elongate, and the stigma is pushed through the anther tube. Like a bottle brush, the two hairy stigma branches effectively remove the pollen from the anther tube as they emerge from the top of the flower (Stage IV). During the day after anthesis, pollinators remove the pollen from the emerging stigma while the stigma branches reflex away from each other. The stigmatic surface between the branches is exposed, and the flower is pollinated by Stage V. As the

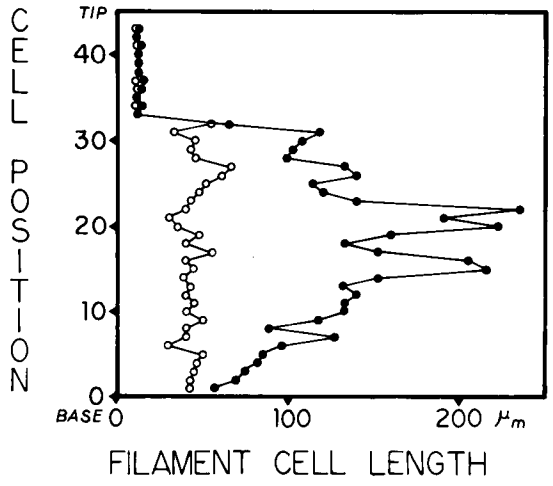

Fig. 2. The length of epidermal cells as a function of their position in a row of cells along the length of a *Gaillardia* filament before (○) and after (●) filament elongation. The 43 cells were counted from the base to the tip of the filament to designate cell position. Note the 11 apical short cells.

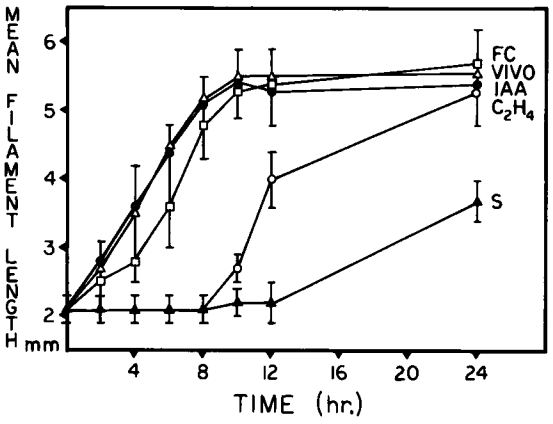

Fig. 3. The time course of *Gaillardia* filament elongation in vivo (△), and in response to 10^{-7} M fusicoccin (□), 10^{-5} M auxin (●), 10 μml/l ethylene (○) and 0.05 M sucrose (▲) in vitro. Vertical lines indicate ±S.D.

fruit develops, the flower senesces and abscises at a point just above the insertion of the calyx to the inferior ovary.

Filament elongation parameters—Filament elongation in disc flower opening on the morning of anthesis was reflected in epidermal cell elongation (Fig. 1). The cell layer beneath the epidermis could not be observed because of the small diameter of the filament and the presence of the central vascular bundle. This inner layer was only one cell thick, as verified by free-hand sections, and in any case may be torn during filament elongation (Cheignon, 1972). Epidermal cell elongation was not uniform along the length of the filament (Fig. 2). The filaments were composed of two cell types: the basal long cells, which elongated to lift the anther tube, and the apical short cells, which did not elongate. Furthermore, the elongation of the long cells was greatest in cells near the middle of the filament. There was no increase in the width of the epidermal cells during elongation. The number of long and short cells remained constant (32 and 11, respectively) in each longitudinal row of epidermal cells (Fig. 1). Filament elongation was accompanied by cell elongation, fresh and dry weight increases but not by cell division in the epidermis. Parallel weight losses were observed during filament shrinkage in senescence (Stage V). Filament elongation in some monocots (Cheignon and Schaeverbeke, 1965; Schaeverbeke, 1965, 1966; Cheignon, 1972) and several dicots (Greyson and Tepfer, 1966; Ganapathy, 1969; Murakami, 1973, 1975; Sawhney and Greyson, 1973) is accompanied by fresh weight increases and cell elongation (greatest near the base of the filament). In contrast, the filaments of *Cleome* elongate mostly near the tip (Koevenig, 1973), but the other parameters of their growth are similar to those above. *Gaillardia* filaments also elongate in the usual way, but show greatest elongation near the *middle* of the filament and have an apical group of cells which neither elongate nor divide.

In vitro filament elongation—Excised *Zea* filaments elongate reliably in vitro if placed in an incubator at 30 C in the dark and supplied with sucrose (Schaeverbeke, 1967). *Gaillardia* filaments elongated more rapidly with the addition of IAA than in plain 0.05 M sucrose medium (Fig. 3), and the response of filaments to auxin was dose-dependent (Fig. 4). The rate of growth and final size of the filaments responding to 10^{-5} M IAA in vitro were comparable to normal growth of filaments in vivo (Fig. 3). These findings, like those for *Cleome* (Koevenig, 1973), *Citrus* (Goldschmidt and Huberman, 1974), and *Lilium* (Hess and Morré, 1978), indicate that auxin induces filament growth in vivo.

As previously cited, filaments of other plants respond to other plant growth regulators. *Gaillardia* filaments were not affected by applications of auxin antagonists, polar and less-polar gibberellins, anti-gibberellins, synthetic and natural cytokinins, brassinolide, and abscisic acid (Table 1). These other growth regulators could interact with natural auxin to modulate rapid filament growth as in *Zea* (Cheignon and Schaeverbeke, 1965; Schaeverbeke, 1966), but

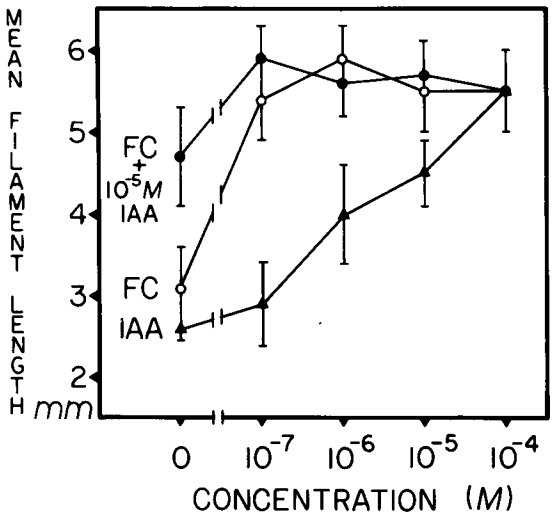

Fig. 4. The effects of auxin (▲) and fusicoccin (in the presence (●) and absence (○) of 10^{-5} M auxin) on *Gaillardia* filament length after 6 hr in vitro. Vertical lines indicate ±S.D.

simultaneous and sequential hormone applications showed no significant synergistic, additive, or antagonistic relationships between any hormone pairs in *Gaillardia* (Koning, 1981).

Of other growth regulators, ethylene had a significant effect upon filament elongation in *Gaillardia,* but this effect was much less than that for auxin (Fig. 3). Aminoethoxyvinylglycine (AVG) and cobalt ions are inhibitors of ethylene biosynthesis in some plants (Mohan Ram and Sett, 1979; Yu and Yang, 1979). In the presence and absence of IAA, Co^{2+} did not promote or significantly inhibit *Gaillardia* filament elongation in vitro; AVG only slightly inhibited the growth of *Gaillardia* filaments when applied simultaneously with auxin (Koning, 1981). Taken together, these results indicate minor importance for ethylene biosynthesis in the growth response induced with auxin.

In most auxin-sensitive systems, low pH can mimic the early auxin response ("acid growth"). The auxin apparently induces the pumping of protons across the cell membrane, lowering the pH of the cell wall milieu, optimizing the activity of hydrolytic enzymes, weakening the wall polysaccharides, and turgor drives the subsequent elongation of the cell (Evans, 1974). Acidic growing media provide acid conditions without the need for the auxin induction. Acid growth can be induced by low pH in *Gaillardia* filaments; they elongated more in a pH-4 buffer without auxin than in a pH-7 buffer with auxin (Fig. 5). This initial acid induced growth is only part of the auxin response, since the filaments stopped growing after six hours in the low-pH buffers while those in unbuffered auxin and higher-pH buffers continued to grow.

Another compound that induces proton pumping in auxin-sensitive systems is fusicoccin (Marré, 1979). Since pH changes induced by fusicoccin are due to increased proton efflux at the plasma membrane, the effects mimic the auxin-induced acid growth more meaningfully than the externally applied acid conditions of pH buffers. In *Gaillardia* filaments, fusicoccin caused extremely rapid elongation (Fig. 3), which was dose dependent (Fig. 4). The filaments were 1,000 times more sensitive to this compound than to auxin; the growth kinetics with 10^{-7} M FC were virtually identical to those with 10^{-5} M IAA. Thus auxin may promote growth through stimulating acid-growth.

TABLE 1. *Treatments having no significant dose response in* Gaillardia *filament length after 12 hr in vitro*

Test solution	Mean filament length (mm) ± S.D. (15)				
	0 M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
TIBA	2.9 ± 0.2	2.9 ± 0.3	2.7 ± 0.3	2.9 ± 0.3	3.1 ± 0.3
PCIB	3.0 ± 0.3	3.0 ± 0.3	3.1 ± 0.3	3.1± 0.4	3.1 ± 0.4
GA_3	2.7 ± 0.6	2.7 ± 0.9	2.7 ± 0.9	2.6 ± 0.8	2.4 ± 0.5
GA_{4+7}	3.1 ± 1.2	2.9 ± 1.2	2.8 ± 1.0	2.8 ± 1.0	2.7 ± 0.8
AMO_{1618}	2.6 ± 0.5	2.8 ± 0.5	2.8 ± 0.4	2.7 ± 0.4	2.8 ± 0.5
CCC	2.6 ± 0.4	2.6 ± 0.3	2.6 ± 0.4	2.6 ± 0.4	2.6 ± 0.4
BA	2.8 ± 0.4	2.8 ± 0.4	3.0 ± 0.3	3.3 ± 0.4	3.6 ± 0.7
IPA	2.6 ± 0.4	2.7 ± 0.5	2.7 ± 0.4	2.9 ± 0.7	2.6 ± 0.3
Z	2.8 ± 0.6	2.8 ± 0.5	2.8 ± 0.6	2.8 ± 0.6	2.8 ± 0.6
BS	3.0 ± 0.2	3.0 ± 0.2	2.9 ± 0.2	3.0 ± 0.2	3.0 ± 0.1
ABA	2.6 ± 0.1	2.7 ± 0.1	2.6 ± 0.1	2.6 ± 0.1	2.6 ± 0.1
Co^{2+}	2.5 ± 0.2	2.4 ± 0.3	2.5 ± 0.2	2.5 ± 0.2	2.4 ± 0.3
AVG	2.6 ± 0.2	2.4 ± 0.3	2.4 ± 0.2	2.3 ± 0.6	2.3 ± 0.3

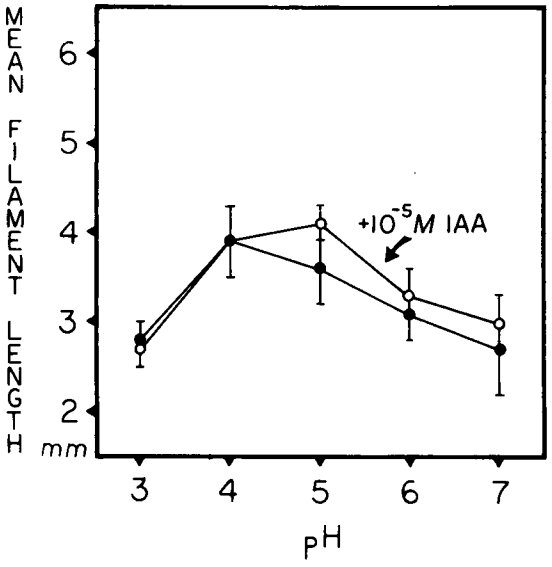

Fig. 5. The effects of pH buffers, in the presence (O) and absence (●) of auxin, on *Gaillardia* filament length after 6 hr in vitro. Vertical lines indicate ±S.D.

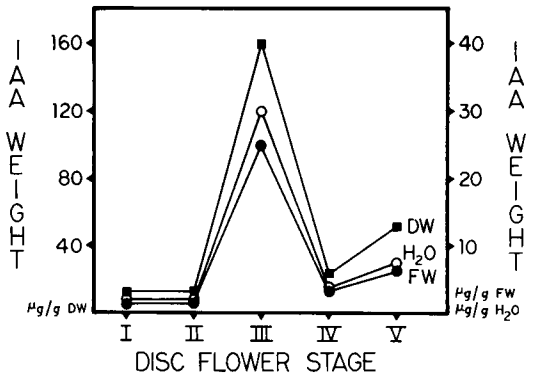

Fig. 6. The auxin content of *Gaillardia* disc flowers at the five stages of flower opening. The content is expressed as μg/g fresh (●), dry (■), and water (FW − DW = H_2O) wt (O) of the flowers.

Natural hormone levels—Although auxin appears to be necessary for the rapid growth of *Gaillardia* filaments, the involvement of this hormone is uncertain until parallel variation of auxin levels and filament elongation is demonstrated (Jacobs, 1959). The level of free auxin in *Gaillardia* disc flowers increased ten-fold just prior to anthesis and filament elongation (Fig. 6). The IAA produced at Stage III is metabolized or transported from the flower by Stage IV in the field; a collection of Stage IV flowers that was made when no pollinators were active (during unusually overcast, cool weather) was found to contain 574 μg IAA/g FW. It is likely that the auxin is contained in pollen (Fitting, 1909; Hatcher, 1945; Sheldrake, 1973) that is usually removed as the style elongates prior to Stage IV. Natural auxin has been shown to vary in a parallel fashion with flower opening in several species (Hatcher, 1945; Jeffcoat and Cockshull, 1972; Sladký, 1972; Hänischtencate et al., 1975; Gopal and Venkataramanan, 1976; Janardhan, Raju and Gopal, 1977; Ilahi, 1979; Zieslin et al., 1979). In these plants, the natural auxin level increases just prior to flower opening and anthesis, and then it drops to a very low level. Furthermore, the high level of auxin found in *Gaillardia* disc flowers at stage III is consistent with high levels found in *Coffea* (Janardhan et al., 1977) and *Cucumis* (Galun, Izhar and Atsmon, 1965). This amount is sufficient to induce the auxin-mediated responses shown by auxin applications (Fig. 3, 4); the amount of measured free-auxin dissolved in the water content of Stage III flowers would result in a concentration of 7×10^{-4} M (providing the auxin is distributed evenly in all aqueous compartments). If certain cell and tissue compartments contain less than this concentration of auxin, then the other compartments must contain an even greater concentration of auxin. Thus parallel variation of auxin levels and filament elongation is demonstrated, and the peak auxin level is sufficient to induce rapid elongation of the filaments.

A small (1½-fold) increase in ethylene production was measured prior to anthesis and filament elongation, but was followed by greater production during senescence at Stage V (Fig. 7). The first increase accompanied the free auxin increase and preceded filament elongation. Since applied ethylene slightly promoted

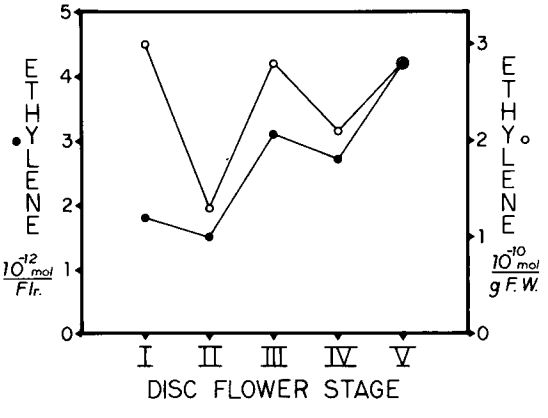

Fig. 7. The ethylene content of *Gaillardia* disc flowers at the five stages of flower opening expressed in moles per flower.

filament elongation and biosynthesis inhibitors inhibited filament elongation, this auxin-induced natural ethylene production increase may play a minor role in filament elongation in vivo. In studies of post-pollination development, pollination and auxin applications both increase the ethylene production of the female organs of flowers and advance development (Hall and Forsyth, 1967). In addition, the reproductive parts of the flower produce much more ethylene than the petals and sepals (Lipe and Morgan, 1973; Nichols, 1977; Veen, 1979). The greater production of ethylene in later stages (V) may be more important in later flower opening processes such as stigma unfolding than in the earlier processes such as filament elongation in *Gaillardia*.

Taken together, the production of free auxin and ethylene coincides with the natural timing of filament elongation. Isolated filaments respond to applied auxin (and to a lesser degree to ethylene) in amounts that compare with the concentrations observed in vivo. The rate and amount of growth observed in filaments responding to this amount of auxin is commensurate with that observed in vivo. These results indicate that auxin is indeed the hormone controlling filament elongation in *Gaillardia*. Moreover, auxin probably regulates growth in part by stimulating proton efflux and its associated "acid growth" and in part by stimulating ethylene biosynthesis.

LITERATURE CITED

ADOLPHE, A. 1971. Action des facteurs physiques et de l'acide gibbérellique sur le rythme de l'anthèse du *Lolium italicum* Br. Bull. Soc. Bot. Fr. 118: 463–470.

BERGHOEF, J., AND J. BRUINSMA. 1979. Flower development of *Begonia franconis* Liebm. III. Effects of growth regulating substances on organ initiation in flower buds in vitro. Z. Pflanzenphysiol. 93: 377–386.

BILDERBACK, D. E. 1972. The effects of hormones upon the development of excised floral buds of *Aquilegia*. Amer. J. Bot. 59: 525–529.

BOSE, T. K., AND J. P. NITSCH. 1970. Chemical alteration of sex expression in *Luffa acutangula*. Physiol. Plant. 23: 1206–1211.

CHAILAKHYAN, M. K. 1979. Genetic and hormonal regulation of growth, flowering, and sex expression in plants. Amer. J. Bot. 66: 717–736.

CHEIGNON, M. 1972. Modifications structurales des parois cellulaires au cours de l'allongement du filet staminal de *Zea mays*. C. R. Acad. Sci. Sér. D 275: 549–551.

———, AND J. SCHAEVERBEKE. 1965. L'ultrastructure des filets staminaux du *Zea mays* L. et ses modifications sous l'action de l'acide gibbérellique et de l'acide β-indolyl-acétique. C. R. Acad. Sci. Sér. D 260: 5085–5088.

COHEN, J. D., AND A. SCHULZE. 1981. Double standard isotope dilution assay. I. Quantitative assay of indole-3-acetic acid. Anal. Biochem. 112: 249–257.

EVANS, M. L. 1974. Rapid responses to plant hormones. Annu. Rev. Plant Physiol. 25: 195–223.

FITTING, H. 1909. Die Beeinflussung der Orchideenblüten durch die Bestäubung und durch andere Umstände. Z. Bot. 1: 1–86.

GALUN, E., S. IZHAR, AND D. ATSMON. 1965. Determination of relative auxin content in hermaphrodite and andromonoecious *Cucumis sativus* L. Plant Physiol. 40: 321–326.

GANAPATHY, P. S. 1969. Floral morphogenesis and flowering in aseptic cultures of *Browallia demissa* L. Biol. Plant. 11: 165–174.

GOLDSCHMIDT, E. E., AND M. HUBERMAN. 1974. The coordination of organ growth in developing *Citrus* flowers: a possibility for sink-type regulation. J. Exp. Bot. 25: 534–541.

GOPAL, N. H., AND D. VENKATARAMANAN. 1976. Physiological studies on flowering in coffee under South Indian conditions. V. Growth-substance content during flower bud enlargement and anthesis. Turrialba 26: 74–79.

GREYSON, R. I., AND S. S. TEPFER. 1966. An analysis of stamen filament growth of *Nigella hispanica*. Amer. J. Bot. 53: 485–490.

———, AND ———. 1967. Emasculation effects on the stamen filament of *Nigella hispanica* and their partial reversal by gibberellic acid. Amer. J. Bot. 54: 971–976.

HALL, I. V., AND F. R. FORSYTH. 1967. Production of ethylene by flowers following pollination and treatments with water and auxin. Can. J. Bot. 45: 1163–1166.

HÄNISCHTENCATE, C. H., J. BERGHOEF, A. M. H. VANDERHOORN, AND J. BRUINSMA. 1975. Hormonal regulation of pedicel abscission in *Begonia* flower buds. Physiol. Plant. 33: 280–284.

HATCHER, E. S. J. 1945. Studies on the vernalization of cereals. IX. Auxin production during development and ripening of the anther and carpel of spring and winter rye. Ann. Bot. 9: 235–266.

HESLOP-HARRISON, J. 1959. Growth substances and flower morphogenesis. J. Linn. Soc. Bot. 56: 269–281.

HESS, E., AND D. J. MORRÉ. 1978. Fine structural analysis of the elongation zone of Easter lily (*Lilium longiflorum*) staminal filaments. Bot. Gaz. 139: 312–321.

ILAHI I. 1979. Nature and balance of growth regulators in the reproductive organs of *Ceratonia siliqua* L. Phyton 37: 29–34.

JACOBS, W. P. 1959. What substance normally controls a given biological process? I. Formulation of some rules. Dev. Biol. 1: 527–533.

JANARDHAN, K. V., K. I. RAJU, AND N. H. GOPAL. 1977. Physiological studies on flowering in coffee under South Indian conditions. VI. Changes in growth rate, indoleacetic acid, and carbohydrate metabolism during flower bud development and anthesis. Turrialba 27: 29–36.

JEFFCOAT, B., AND K. E. COCKSHULL. 1972. Changes in the levels of endogenous growth regulators during development of the flowers of *Chrysanthemum morifolium*. J. Exp. Bot. 23: 722–732.

KOEVENIG, J. L. 1973. Floral development and stamen filament elongation in *Cleome hassleriana*. Amer. J. Bot. 60: 122–129.

KONING, R. E. 1981. Control of flower opening by plant hormones in *Gaillardia grandiflora*. Ph.D. thesis, University of Michigan, Ann Arbor.

LANG, A. 1961. Auxins in flowering. Encycl. Plant Physiol. 14: 909–950.

LIPE, J. A., AND P. W. MORGAN. 1973. Location of ethylene production in cotton flowers and dehiscing fruits. Planta 115: 93–96.

MARRÉ, E. 1979. Fusicoccin: a tool in plant physiology. Annu. Rev. Plant Physiol. 30: 273–312.

MOHAN RAM, H. Y., AND R. SETT. 1979. Sex reversal in the female plants of *Cannabis sativa* by cobalt ion. Proc. Indian Acad. Sci. Ser. B II 88: 303–308.

MURAKAMI, Y. 1973. The role of gibberellins in the growth of floral organs of *Pharbitis nil.* Plant Cell Physiol. 14: 91–102.

———. 1975. The role of gibberellins in the growth of floral organs of *Mirabilis jalapa.* Plant Cell Physiol. 16: 337–345.

NICHOLS, R. 1977. Sites of ethylene production in the pollinated and unpollinated senescing carnation (*Dianthus caryophyllus*) inflorescence. Planta 135: 155–159.

POOL, R. M. 1975. Effect of cytokinin on in vitro development of 'Concord' flowers. Amer. J. Enol. Viticult. 26: 43–46.

RAMAN, K., AND R. I. GREYSON. 1978. Further observations on the differential sensitivities to plant growth regulators by cultured "single" and "double" flower buds of *Nigella damascena* L. (Ranunculaceae). Amer. J. Bot. 65: 180–191.

RITTENBERG, D., AND G. L. FOSTER. 1940. A new procedure for quantitative analysis by isotope dilution, with application to the determination of amino acids and fatty acids. J. Biol. Chem. 133: 737–744.

SAWHNEY, V. K., AND R. I. GREYSON. 1973. Morphogenesis of the stamenless-2 mutant in tomato. II. Modifications of sex organs in the mutant and normal flowers by plant hormones. Can. J. Bot. 51: 2473–2479.

SCHAEVERBEKE, J. 1960. Action de la gibberélline sur l'allongement des filets staminaux chez les graminées. C. R. Acad. Sci. Sér. D 251: 1176–1178.

———. 1965. Actions compareés de l'acide gibbérellique et de l'acide indolyl-β-acétique sur l'allongement des filets staminaux isolés du *Zea mays* L. C. R. Acad. Sci. Sér. D 260: 4580–4582.

———. 1966. Contribution à l'étude de l'anthèse des graminées: intervention de la gibbérelline dans le métabolisme. Ph.D. thesis, University of Paris.

———. 1967. Gibbérellines et synthese de saccharase dans les filets staminaux de *Zea mays* L. Proposition d'un test specifique des gibbérellines. C. R. Acad. Sci. Sér. D 264: 326–329.

SCHLENK, H., AND J. L. GELLERMAN. 1960. Esterification of fatty acids with diazomethane on a small scale. Anal. Chem. 32: 1412–1414.

SHELDRAKE, A. R. 1973. The production of hormones in higher plants. Biol. Rev. 48: 509–559.

SLADKÝ, Z. 1972. The role of endogenous growth regulators in the differentiation processes of walnut (*Juglans regia* L.). Biol. Plant. 14: 273–278.

TOMPSETT, P. B., AND W. W. SCHWABE. 1974. Growth hormone changes in *Chrysanthemum morifolium.* Effects of environmental factors controlling flowering. Ann. Bot. 38: 269–285.

VEEN, H. 1979. Effects of silver on ethylene synthesis and action in cut carnations. Planta 145: 467–470.

YU, B., AND S. F. YANG. 1979. Auxin-induced ethylene production and its inhibition by aminoethoxyvinylglycine and cobalt ion. Plant Physiol. 64: 1074–1077.

ZEEVAART, J. A. D. 1976. Physiology of flower formation. Annu. Rev. Plant Physiol. 27: 321–348.

ZIESLIN, N., G. MADORI, AND A. H. HALEVY. 1979. Involvement of hormonal balance in the control of the 'bullhead' malformation in Baccara rose flowers. J. Exp. Bot. 30: 15–25.