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HORMONAL CONTROL OF FRUIT DEVELOPMENT IN STRAWBERRY (*FRAGARIA X ANANASSA* DUCH.)

By

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ABSTRACT

HORMONAL CONTROL OF FRUIT DEVELOPMENT IN STRAWBERRY
(FRAGARIA X ANANASSA DUCH.)

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Indoleacetic acid (IAA) was identified by gas chromatography-mass spectrometry in extracts of achene and receptacle tissue of strawberry. Free, ester-conjugated and amide-conjugated IAA present in achene and receptacle tissue from anthesis to maturity were quantified by a double-standard isotope dilution method using ^{14}C-IAA and ^{14}C-indolebutyric acid as internal standards. Whole fruit at anthesis contained high concentrations of free IAA, small amounts of ester-conjugated IAA, and no amide-linked IAA. Maximum concentrations of free IAA in achenes were 4- (1980) and 1.2-fold (1981) as high as those in receptacle tissue; maxima occurred simultaneously in the two tissues 16 (1980) and 14 (1981) days after anthesis. The maximum concentration of ester-conjugated IAA in achene tissue was 3.4- (1980) and 17.5-fold (1981) as high as that in receptacle tissue with the maxima occurring at 6 (1980) and 8 (1981) days after anthesis with a second maximum at 22 days after anthesis in 1980. Amide-conjugated IAA was found in significant quantities in achene tissue at 11 days and again at maturity. Maximum concentrations in achenes were

21- (1980) and 34-fold (1981) as great as those in receptacle tissue. Secondary fruit on the cyme contained higher concentrations of free IAA in the achenes, but not in the receptacle tissue, than did primary fruit.

Abscisic acid was detected in whole fruit at anthesis by electron capture gas chromatography. The concentration in the achenes declined until midway through development, then increased as fruit approached maturity. The total quantities in both achenes and receptacle increased as fruit ripened. The ratio of free IAA to free ABA changed during fruit development but was not well correlated with fruit growth rate.

Treatment of emasculated flowers with aqueous solutions of naphthaleneacetic acid (NAA), gibberellins (GA_3, $GA_{4/7}$), NAA + GA_3, or NAA + $GA_{4/7}$, each at 10^{-3} M in 2% dimethylsulfoxide (DMSO) and 0.1% Tween 80, induced parthenocarpy. All fruits stopped growing within 12 days of treatment, except those induced with NAA or NAA + GA_3. Re-treatment with the same or another growth regulator at 20 days after initial treatment stimulated continued growth as compared to no re-treatment. All induced fruits were considerably smaller than pollinated fruit; sizes ranged from 40 to 70% of pollinated fruit.

Free IAA concentration in NAA-treated fruit was 5-fold as high as that in control flowers and 3-fold as great as that in $GA_{4/7}$-treated fruit 6 days after treatment. By 14 days after treatment levels had declined in all treated fruit. These results are discussed in relation to the role of IAA in strawberry fruit development.

Complete achene removal 12 days after pollination stopped receptacle growth. Removal of achenes from half of the receptacle diminished growth as compared with that of intact fruit. Free IAA concentration in the receptacle tissue of intact fruit at 14 days after pollination was nearly equal to that in achenes. Removal of achenes significantly reduced the IAA content of receptacle tissue, complete removal being more effective than partial removal. The extent of achene removal and growth rates of receptacles were positively correlated with free IAA content.

Achenes were removed from some fruits 16 days after pollination, and the receptacles were treated with aqueous solutions of NAA, GA_3, or $GA_{4/7}$ at 10^{-3}M in 2% DMSO and 0.1% Tween 80. None of the growth regulators were as effective as were achenes in maintaining growth. NAA treatment of receptacles produced fruit 75% the size of controls, while fruit treated with GA_3 and $GA_{4/7}$ did not grow. Removal of achenes 24 days after pollination had no effect on enlargement of receptacles.

For Rhonda

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ABA	abscisic acid
DMSO	dimethylsulfoxide
EC	electron capture
EtOH	ethyl alcohol
EtOAc	ethyl acetate
FID	flame ionization detector
GA	denotes the series of gibberellins--use of a subscript denotes a specific gibberellin, as GA_3
IAA	indoleacetic acid
IBA	indolebutyric acid
MeOH	methyl alcohol
NAA	naphthaleneacetic acid
NAD,NAAm	naphthaleneacetamide
NOA	naphthoxyacetic acid
NSD	nitrogen sensitive detector
THF	tetrahydrofuran

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LITERATURE REVIEW

The tree fruit industry relies heavily on several plant growth regulators to control various aspects of fruit physiology (56), but with the exception of fruit thinning, only limited control of fruit set and early development has been realized (14). Species and cultivar differences in response to applied compounds have contributed to the problem; thus, the goal of regulated cropping has remained elusive since F. G. Gustafson demonstrated that plant hormones induce parthenocarpy (18). A better understanding of the role of endogenous plant hormones in fruit set and development could provide a basis for developing effective means of controlling yield.

This review will be concerned primarily with strawberry fruit set and development and the control of these phenomena by endogenous and exogenous growth regulators. Although many other species have been studied (9,10), strawberry represents a model system.

Role of Seeds in Fruit Development

Association between seeds and fruit development. Though not essential to fruit development, as evidenced by parthenocarpy, seeds play an important role in influencing the growth of surrounding fruit tissues. Seed distribution can affect both fruit shape and volume (32,37,54). Fruit weight is directly correlated with achene or seed number in strawberry and other multi-seeded fruit (1,37,38).

Premature seed removal may result in cessation of both growth and nutrient accumulation in the surrounding tissues (28,38), or hasten ripening of fruit approaching maturity (38,53).

Seeds as sources of hormones. Immature seeds are rich sources of hormones. Because these compounds affect fruit growth when exogenously applied (26,38,58), they may be responsible for the effects of seeds. However, the presence of a multiplicity of hormones makes the determination of their precise roles difficult,for little is known about hormonal interactions.

Strawberry: a model system for studying the role of seeds in fruit development. Strawberry, Fragaria spp., is not a true fruit. The fruit are the individual 'seeds' which lie on the surface of the fleshy receptacle; they are actually ovaries, termed achenes. Horticulturally, the strawberry receptacle is a fruit; it accumulates sugars and ripens as do true fruits. Most fruits bear seeds within the fleshy tissue; seed removal incurs extensive damage to the flesh. Because the achenes are on the surface of the strawberry receptacle, they can be removed with minimal damage to the supporting tissue. Thus, a model system for studying the influence of seeds on fruit development exists. Further discussion will be confined to this system.

Strawberry Fruit Development and Morphology

The strawberry fruit is borne on an inflorescence with one primary, two secondary, four tertiary, and up to eight quaternary

flowers (13). The flower has five sepals and petals, while stamens occur in multiples of five. The number of achenes on the surface of the receptacle ranges from 50 to 500. The petals and stamens senesce within 24 to 48 hrs after pollination and receptacle enlargement begins within 48 hrs, most growth being due to cell enlargement, cell division occurring at a low rate (21). Receptacle enlargement continues for ca. 25 days and exhibits a sigmoidal pattern. Achene enlargement is almost linear until maturity (11). Change from the free nuclear to cellular endosperm occurs at 10 to 14 days after pollination (49). Fruit mature about 30 days after pollination but varietal differences and environmental factors can shorten or prolong this time. Unpollinated flowers remain attached to the plant; an abcission zone is absent from the pedicel.

Primary fruit are the largest, followed by secondary, tertiary, etc. Berry size is linearly proportional to achene number, and the larger the receptacle, the more achenes it bears (60). However, berry weight per achene is relatively constant across classes (34), and achene number and spacing can be used to calculate potential yields (2). Because primary flowers have the largest receptacles and quaternary flowers the smallest, both achene number and berry size decline with class (1°, 2°, etc.).

Role of Hormones in Fruit Development

Hormones may control two distinct yet related phenomena: fruit set and fruit development. Fruit set is considered an inductive phase beginning at pollination or chemical treatment. Growth

of the ovary and/or associated structures begins within a short time, often prior to fertilization. Accompanying morphological changes include petal and stamen wilting and abscission (26). The transition from a flower into a young fruit is termed fruit set. Fruit development begins with the initial swelling of the fruit tissues and ends at maturity. It is characterized by increasing fresh and dry weights and changes in sugar, starch, and organic acid content (8).

Exogenous control--in vivo studies. Due to their external location on the receptacle, achenes could be considered as an exogenous factor in controlling fruit development. However, their continuity with the tissues of the receptacle preclude considering them as exogenous. This term will be reserved for influences arising outside the plant.

Lanolin paste was the carrier of choice in early experiments with applied growth regulators (30,31,36,37,48,50,51,52). Although lanolin provides a continuous supply of the hormone, it does not allow calculation of the precise dose. Aqueous applications have often been ineffective, possibly because of limited uptake by the treated tissues. Mudge et al. (35) found that addition of DMSO doubled the response to auxin and resulted in 100% fruit set. Thus, penetration of the applied compound was rapid and effective, permitting the study of dosage effect without the uncertainty involved with the use of lanolin.

Treatment of whole plants of pistillate varieties with aqueous or ethanolic solutions of growth regulators has had limited effect

in inducing parthenocarpy. Gardner and Marth (16) reported the first success. Although high concentrations of IAA were effective when applied at full bloom, no more than one fruit per inflorescence was obtained. Achenes developed but were devoid of embryos. Vapors of methyl and ethyl esters of NAA (64) and single and repeated applications of IBA, NOA, GA, or combinations thereof were relatively ineffective in inducing fruit set (51). Tafazoli and Vince-Prue (48) reported that GA_3 treatment of perfect-flowered cultivars prior to anthesis inhibited fruit set by inducing sterility. Though NOA applied prior to anthesis can increase fruit set, it cannot overcome the inhibitory effect of GA_3.

Erratic or incomplete pollination, generally due to poor pollen production, although environmental factors cannot be excluded, results in low yields and small or misshapen fruit (5,19,25,29). Applications of aqueous sprays containing IAA, NOA, and NAA to open-pollinated plants at bloom or thereafter have increased yields (6,45, 63,65); however, the investigators have seldom estimated the extent of pollination or distinguished between effects on fruit set and fruit development. Most reports of increased yields cite increased numbers of 'normal' fruit, although Swarbrick (45) states that increased fruit size rather than fruit number was responsible for the higher yield. Thus, spraying whole plants with auxin enhances fruit development, possibly substituting for those achenes not pollinated. Field applications of GA at or shortly after bloom to open-pollinated varieties may increase yields or fruit size (6,48), but reports are

contradictory. The evidence suggests that low concentrations may be promotive while high concentrations have an inhibitory effect.

Treatment of individual flowers has been more effective than applications to whole plants in most studies. Hunter (22) and Wong (62) reported that applications of NAA and IBA in 95% EtOH to blossoms stimulated parthenocarpy, though percent set was not reported. Tukey (55) was unable to induce parthenocarpy in a single fruit in an extensive study utilizing 60 varieties of strawberry and several auxins. On the other hand, Lord and White (31) succeeded by using much higher concentrations of auxin in lanolin paste. IBA application resulted in 90% of the treated flowers setting fruit, NAD in 60%, and NOA, NAA, and IAA slightly less than 50%. Thompson (50,51,52) induced fruit set with GA_3, $GA_{4/7}$, and auxins, but subsequent development varied with the chemical treatment. Cytokinins alone are unable to induce fruit set and do not affect the response to auxins when applied simultaneously with them (50).

Parthenocarpic strawberry fruits induced by auxin applied in lanolin vary in size from 50 to 100% of that of pollinated fruit (51,52), the response depending on the specific auxins applied and their concentrations. Varieties and species differ with respect to response to specific auxins. Generally, IBA, NOA, NAA, and IAA all stimulate parthenocarpic development, but IBA produces small fruit, NOA large fruit, and NAA and IAA fruit of intermediate size.

Parthenocarpic fruit are usually smaller than pollinated controls, although varieties differ in response. 'Freya' flowers treated with NOA produced fruit nearly equal in size to pollinated

controls, while 'Tardive de Leopold' produced fruit only half the size of control fruit. Between 10 and 20 days after treatment, the growth rate of auxin-treated parthenocarpic fruit declines (51,52). This suggests that most of the initially-applied dose has been utilized. Re-application of auxin or GA or both increased final fruit size, which is a function of both initial and final treatment. When flowers were treated with NOA, then retreated after 10 days with either NOA or GA_3, GA_3 induced the greater response (52). Fruit induced with GA_3 responded equally well to retreatment with NOA or GA_3. Mudge et al. (35) reported that parthenocarpic fruit ceased growth about 10 days after treatment with aqueous solutions of auxin containing DMSO and would not resume growth unless retreated. Retreatment was not necessary when lanolin paste was used.

$GA_{4/7}$ is more effective than GA_3 at equivalent concentrations (51). At optimal concentrations, both yield fruit only slightly smaller than auxin-induced fruit. Synergism between auxins and GAs has been noted (51,52). NOA-induced fruit were half the size of pollinated controls in 'Tardive de Leopold'; adding GA_3 increased fruit size to 75% of that of the controls.

All results are complicated by the effect of the applied growth regulator on maturation. NAA- and GA-treated fruit mature early, IBA- and NOA-treated fruit mature late (50,51,52). Retreatment of auxin-induced fruit with GA shortens the time to maturity. The longer the developmental period, the larger the fruit. Thus, NOA produces large fruit but the growth rate is low.

Removal of fertilized achenes before 8 days after anthesis prevents receptacle growth regardless of subsequent treatment (35,55), possibly because of wounding and desiccation. Nitsch (36,37) removed achenes 8 days after pollination and substituted lanolin paste containing IBA or NOA. He obtained fruit nearly as large as intact fruit. A more extensive study of 15 auxin analogs demonstrated that IBA and NAA were more effective than IAA and NOA (35). GA_3 is less effective than auxin (46).

Exogenous control--in vitro studies. Culturing fruits in vitro has provided a useful means of examining more closely the effects of growth regulators on fruit set and development. According to some investigators, inclusion of auxins such as NAA, NOA, and NAAm, and of GAs and kinetin in the supporting media stimulates receptacle growth; other workers have been unsuccessful in similar attempts (3,12,17,23). The reasons for these contradictions are not apparent unless varietal differences affected the results. Intact receptacles in vivo respond to a variety of auxins as described earlier, thus the use of different auxins cannot be the explanation for these discrepancies. Other chemicals such as ABA and maleic hydrazide do not promote receptacle growth (3,17,23). There are no known reports of sub-optimal auxin concentrations promoting set when combined with another growth regulator, such as GA or cytokinin.

Effects of growth regulators on parthenocarpic fruit growth, size, and length of development in vitro have also been noted. NAAm alone promotes receptacle enlargement and synergizes with GA_3 (23).

NAAm tends to delay ripening while GA_3 hastens it. Both the application of cytokinins and the presence of intact carpels delay both growth and ripening.

Endogenous control--significance of achenes and the calyx. The discussion to this point has dealt with the application of growth regulators to intact flowers, i.e., achenes plus receptacles. The response differs following the removal of achenes, which appear to mediate fruit set and initiate fruit growth. Intact achenes are required for the receptacle to begin growing following fertilization or growth regulator application in vivo. Achene removal during the first week after anthesis results in severe wounding of the receptacle and growth of the naked receptacle cannot be induced (35,56). This failure suggests that the achenes supply more than auxins or GAs to the receptacle. However, wounding may prevent response to applied compounds.

Thompson (48) reported that unfertilized achenes inhibit growth of the adjacent receptacle tissue. Growth will not occur in this area even if all the surrounding achenes develop. One might assume that achene-derived hormones would diffuse in all directions to stimulate development, but the evidence indicates that there is a localization of the stimulus. Modification of the environment to stimulate production of receptacles with bracts in place of achenes, or treatment of inflorescences with maleic hydrazide to inhibit achene development, can result in naturally parthenocarpic fruit (48). Again, receptacle tissue adjacent to the few viable but unfertilized

achenes does not develop. The mechanism responsible for this localized effect of the achenes is not known.

Receptacles cultured in vitro following achene removal at anthesis are capable of initiating growth when auxins or GA are supplied (12,17). Apparently, damage to the receptacle is slight, although this was not discussed. Intact achenes, whether viable or not, inhibit receptacle expansion significantly in vitro (17). Calyx removal accompanied flower excision in the cited studies. Although the calyx is not essential to fruit growth in vivo, in vitro work indicates that removal results in smaller fruit (3,57). The calyx may supply an unknown factor required for normal fruit growth which other plant parts supply in vivo. This factor cannot be replaced by auxin, GA, or cytokinin and thus would be absent in culture. Its absence may restrict embryo development, thereby reducing the production of growth promoter(s) and reducing fruit size.

The inhibitor from achenes has been characterized to a limited extent. Crude aqueous extracts of achenes collected prior to bloom inhibit GA activity in the barley endosperm bioassay whereas extracts of fertilized achenes exhibit little or no inhibition (12). However, no attempt was made to separate promoters from inhibitors; therefore, an increase in GA content following fertilization could be responsible for the difference. Extracts of unfertilized and fertilized achenes are equally inhibitory to growth of receptacles in vitro (17) and wheat coleoptile sections (46). Following normal fractionation procedures with ether, the inhibitor remains in the water fraction and is not adsorbed on cation exchange resin.

There is some evidence available that suggests that the fruit set stimulus may arise from locations other than the achene. Hunter (22) treated selected pistillate flowers on some inflorescences with IAA or NAA and noted that the receptacles of nontreated flowers on the same inflorescences grew. Accidental spraying was eliminated as a potential cause of the phenomenon. Thompson (48) observed a similar effect. Following repeated application of 50 µg GA at weekly intervals to a mature leaf, swelling of the neck region of the primary fruit occurred. Thus, the hormone must have moved from treated flowers or leaves to nontreated flowers.

Endogenous control--hormones. Few studies have been made of growth hormones in strawberry fruit tissue. Nitsch (35,39) reported that auxin activity of achenes increased from 3 through 12 days after anthesis and then declined. Maximum levels detected by the Avena curvature bioassay were 0.3 to 0.5 µg IAA-equivalents per 100 mg dry weight. No activity was detected in receptacle tissue despite Nitsch's suggestion that auxin controlled receptacle growth and his demonstration that auxins could replace achenes in stimulating receptacle development.

Lis et al. (29) reported that the peak in auxin activity in receptacle extracts coincided with that in achenes but that the maximum concentration was 10-fold less per kg fresh weight. No activity was detected prior to 2 days after pollination. Although the developmental period was longer in this study, the peak in auxin activity occurred at 7 to 8 days after pollination, earlier than that observed

by Nitsch. Activity in both tissues declined after 8 days but was detectable in achenes through maturation.

The decline in auxin activity in the achenes occurs during berry swelling, and auxin production by the achenes correlates more closely with changes in the achene itself than with changes in receptacle growth. The peak in activity corresponds to the change from free to cellular endosperm about 10 days after pollination (49). The endosperm rather than the embryo may be responsible for auxin production. By periodic application of maleic hydrazide to fruits before and after pollination to inhibit achene development, Thompson (49) showed that a viable embryo was not required for receptacle enlargement to occur. He also noted that the greatest response to applied auxin occurred about 10 days after pollination. Thus, he suggested a 2-phase growth period. The first phase, pollination through 10 days, was characterized by low auxin sensitivity but the control mechanism was unknown. The second phase at 10 days and later was mediated by auxin.

Nitsch (39) tentatively identified IAA as the major auxin present in the achenes, though several unknown compounds with auxin activity were present. He estimated the maximum concentration of IAA in the achenes at 1.5 to 2.0 μg per gram dry weight. This was less than half the total detectable activity. Thus, auxins other than IAA may be involved in controlling receptacle growth.

The history, chemistry, and physiology of conjugated, or bound, auxins have been extensively reviewed (4,43). Though most

reviews deal with conjugates in relation to IAA metabolism and function, a recent review presents evidence for other roles (7). IAA conjugates may participate in systems of (a) IAA transport, (b) storage and reuse of IAA, (c) protection of IAA from enzymatic degradation, and (d) homeostatic control of the concentration of free IAA in the tissues.

Because auxins, particularly IAA, are the most active growth regulators that promote fruit set and development in strawberry, IAA conjugates may play an essential role. Conjugated auxin extracted from maize kernels as well as the synthetic ethyl and methyl esters of IAA induce parthenocarpy in tomato (42,61). The ethyl ester of IAA has been tentatively identified in extracts of immature apple seeds (40,47), though it may be an artifact (33). Application of ^{14}C-IAA to strawberry fruit yields ^{14}C-IAA aspartate *in vitro* and ^{14}C-IAA-glucose *in vivo* (27). The reason for this discrepancy is not apparent, but the mechanisms for conjugation are obviously present in strawberry fruit.

GA-like activity has been quantified in extracts of strawberry achenes and receptacles via the barley endosperm bioassay (29) and the lettuce hypocotyl bioassay (46). At peak levels the receptacle contained higher concentrations of such compounds than did the achenes. Activity in receptacles was highest at 5-6 days after pollination, then declined. Extracts of flowers and receptacles were active at 3 days after pollination. No pattern was apparent in achenes. Floral diffusates exhibited activity in the *Rumex*-leaf

senescence bioassay, but extracts of flowers showed little activity (15).

Abscisic acid has been tentatively identified in extracts of both ripe and unripe fruit (41) and quantified by the wheat coleoptile bioassay (29). No inhibitory activity could be detected two days after pollination. In achenes, levels remained relatively constant until late stages of development, then increased as fruit ripened. Concentration in achenes was 2- to 10-fold higher than in receptacle tissue until maturity, when levels in achenes rose 10-fold. The total quantity per fruit was approximately evenly divided between achenes and receptacles until maturity.

Cytokinin-like activity, as measured by the Amaranthus (29) or soybean callus bioassay (24) was detected in flowers at anthesis and increased in achenes and receptacle through 7 or 15 days, depending on the study, then declined. Activity in receptacle tissue paralleled that in achenes but was several-fold less per unit weight.

Ethylene has been detected in the volatiles emanating from pollinated strawberry flowers (20). An increase in evolution was noted following pollination, perhaps associated with petal or stamen wilting or abscission.

Strawberry fruit set may be governed by a balance of promoters and inhibitors (44). Evidence for the existence of an inhibitor in strawberry fruit has been presented. Auxin and cytokinin levels rise in whole fruit as soon as 2 days after pollination when a small growth increment is evident (29) and GA levels increase in receptacle tissue

within 3 days (46). Total GA activity is higher in the receptacle tissue than in the achenes (29). Levels prior to 2 days after pollination are unknown, though some activity is present at anthesis. The increase in promoter level(s) occurs when berry enlargement is first noted.

Summary

Seeds play a very important role in influencing growth of surrounding fruit tissues, an effect possibly mediated by seed-derived hormones. Strawberry, whose 'seeds' or achenes occur on the surface of the receptacle, provides an excellent model system with which to study the mechanisms of control.

The strawberry fruit exhibits a sigmoidal growth pattern. Most receptacle growth is due to cell enlargement rather than cell division. Fruit mature about 30 days after pollination, though varieties differ. Primary fruit are the largest, and berry size is linearly proportional to achene number.

Applied auxins and GAs elicit fruit set *in vivo*, lanolin paste being more effective than aqueous solutions. Addition of DMSO to the aqueous solution nearly doubles the response as compared to aqueous solutions lacking DMSO. Work *in vitro* has not yielded clear-cut results; some investigators have reported positive effects of auxins, while others have reported no effects.

Parthenocarpic fruit growth in strawberry is most effectively induced by applied auxins; GAs are capable of promoting limited development, while cytokinins have little effect. Synergism occurs

between auxins and GAs. Compounds differ in their effects on the length of the developmental period. Auxins can replace achenes, sustaining growth nearly as well as achenes. Field applications of auxins reportedly increase yields, while GAs are ineffective. Auxins probably increase fruit size rather than fruit number.

Unfertilized achenes inhibit growth of the receptacle in the region of attachment *in vivo*, whereas both fertilized and unfertilized achenes inhibit development *in vitro*. *In vitro* studies are confounded by calyx removal, which reduces fruit growth. Cytokinins, auxins, or GAs cannot substitute for the calyx.

Endogenous levels of auxin-like compounds parallel achene and receptacle development in early phases of growth, then decline. Maximum activity in achenes is 10-fold higher than in receptacle tissue, IAA having been tentatively identified as the major auxin present. GA-like activity is high soon after pollination in receptacles, while no pattern is evident in the achenes. ABA levels increase in both achene and receptacles as maturity approaches. Cytokinin-like activity increases through 1 to 2 weeks after pollination and subsequently declines.

Fruit set in strawberry may be governed by a balance of promoters and inhibitors. Rapid increases in cytokinins and auxins within 3 days and GAs within 5 days after pollination have been observed; these promoters may counteract the effect of an inhibitor present in achenes.

Auxin conjugates may play a role in fruit development, for these compounds are capable of eliciting fruit set and growth and the

mechanisms for conjugation are present in strawberry fruit. These facts, together with the observation that free auxin levels are not well correlated with fruit set or development, warrant an investigation of the levels of such conjugates in the strawberry during its development.

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SECTION I

QUANTIFICATION OF FREE ABSCISIC ACID AND FREE AND CONJUGATED INDOLEACETIC ACID IN STRAWBERRY (*FRAGARIA X ANANASSA* DUCH.) ACHENE AND RECEPTACLE TISSUE DURING FRUIT DEVELOPMENT

Quantification of Free Abscisic Acid and Free and Conjugated Indoleacetic Acid in Strawberry (Fragaria x ananassa Duch.) Achene and Receptacle Tissue During Fruit Development

Abstract

Indoleacetic acid (IAA) was identified by gas chromatography-mass spectrometry in extracts of achene and receptacle tissue of strawberry. Free, ester-conjugated and amide-conjugated IAA present in achene and receptacle tissue from anthesis to maturity were quantified by a double-standard isotope dilution method using ^{14}C-IAA and ^{14}C-indolebutyric acid as internal standards. Whole fruit at anthesis contained high concentrations of free IAA, small amounts of ester-conjugated IAA, and no amide-linked IAA. Maximum concentrations of free IAA in achenes were 4- (1980) and 1.2-fold (1981) as high as those in receptacle tissue; maxima occurred simultaneously in the two tissues 16 (1980) and 14 (1981) days after anthesis. The maximum concentration of ester-conjugated IAA in achene tissue was 3.4- (1980) and 17.5-fold (1981) as high as that in receptacle tissue with the maxima occurring at 6 (1980) and 8 (1981) days after anthesis with a second maximum at 22 days after anthesis in 1980. Amide-conjugated IAA was found in significant quantities in achene tissue at 11 days and again at maturity. Maximum concentrations in achenes were

21- (1980) and 34-fold (1981) as great as those in receptacle tissue. Secondary fruit on the cyme contained higher concentrations of free IAA in the achenes, but not in the receptacle tissue, than did primary fruit.

Abscisic acid (ABA) was detected in whole fruit at anthesis by electron capture gas chromatography. The concentration in the achenes declined until midway through development, then increased as fruit approached maturity. The total quantities in both achenes and receptacle increased as fruit ripened. The ratio of free IAA to free ABA changed during fruit development but was not well-correlated with fruit growth rate.

The strawberry has provided a classic model for studies of seed and hormonal control of fruit development. IAA is believed to exert primary control over strawberry fruit development. Applied auxins can stimulate parthenocarpy (14,15), enhance fruit development of pollinated fruit, and increase yields (1,12,16). Nitsch (8,9) demonstrated that removal of achenes stopped fruit enlargement; subsequent treatment with lanolin containing synthetic auxins permitted continued growth. A variety of auxins are capable of replacing achenes and each differs in its efficacy (7). Indoleacetic acid has been tentatively identified as the major auxin present in strawberry achenes, comprising 1/2 to 2/3 of the auxin activity, as measured by bioassay (10). However, no unequivocal methods have been used for its positive identification. Bioassays have indicated the presence of auxin-like compounds in both achene and receptacle extracts, the former being more active (6,9,10). Auxin-like activity increases in both achene and receptacle tissue through 8 to 12 days after pollination and subsequently declines. However, fruit growth, as measured by fresh or dry weight gain, is not well-correlated with auxin content of achenes or receptacles (6,9,10).

ABA has been tentatively identified in extracts of ripe and unripe strawberry fruit (11) and quantified by bioassay (6). ABA-like activity was not detectable at anthesis and the concentration was greater in achene than receptacle tissue throughout development. At maturity, however, activity in extracts of achenes rose 10-fold.

The poor correlation between fruit growth and auxin content, or content of other hormones, raises questions as to the role of hormones in controlling fruit development. Cohen and Bandurski (3) suggested that conjugates of IAA play crucial roles in many physiological processes; these could include fruit development. Following exogenous application of ^{14}C-IAA to strawberry fruit, ^{14}C-IAA-aspartate can be recovered *in vitro* and ^{14}C-IAA-glucose *in vivo* (6). Thus, mechanisms for conjugation exist in strawberry.

The objectives of this research were to (a) identify IAA in strawberry fruit tissue by GC-MS, and (b) to quantify IAA and its conjugates and ABA in achene and receptacle tissue during fruit development. The method used, the double-standard isotope dilution assay, allows more precise measurement of endogenous IAA, both free and conjugated, than does a bioassay.

Materials and Methods

Plant material. Fruit were collected in May-June 1980 and 1981 at a commercial farm near East Lansing, MI. Prior to bloom two 1 m sections were marked at opposite ends of one row 100 m long in order to monitor flower opening. Daily counts were made of total numbers of open primary and secondary flowers and these data were converted to percentage to estimate the time of 50 to 75% bloom (anthesis) for each class of flower. On each sampling data, all primary fruits were collected from a section of row until 1 kg of fruit had been harvested. Consecutive sections were harvested on

subsequent dates. Secondary fruit were harvested in a similar manner on selected dates only.

In 1980, samples of primary fruit of 'Sparkle' were collected at anthesis (May 27) and at 6, 11, 16, 22, and 27 (ripe) days after anthesis. Fruits from secondary flowers were collected 9, 14, and 19 days after anthesis (May 30). In 1981, samples of primary fruit of 'Midway' were collected at anthesis (May 23) and at 4, 8, 11, 14, 17, 20, and 23 (ripe) days after anthesis. Fruits from secondary flowers were collected 6, 13, and 16 days after anthesis (May 25).

At harvest fruits were weighed, placed on ice, and transported to a freezer (-18°C). When frozen, the fruits were lyophilized and stored at 4°C in the dark with a drying agent.

Tissue preparation. Following lyophilization, achenes were separated from the receptacle by gentle scraping with a spatula. Where this was difficult, as with mature fruit, the tissue was gently ground to a powder with mortar and pestle. Achenes were separated by filtering the tissue through nylon mesh, the achenes being retained. All achenes and all receptacles from each sampling date were pooled, and 0.2-3 g and 5-25 g, respectively, of each tissue were analyzed for IAA content.

IAA analysis. Achene and receptacle tissues were analyzed for free and conjugated IAA by the double-standard isotope dilution assay (4). Solvents used were either re-distilled or MS-grade. Water was double-distilled in glass. Tissue was extracted in 70% acetone at 23-25°C, to which a known quantity of ^{14}C-IAA (Research,

Products International, Mount Prospect, Il; sp. act. 48 mCi/mmol; purity, 99%) had been added, by macerating in a Waring blendor for 305 min. The solvent was filtered off on a Buchner funnel and stored at 0° in the dark. The tissue was re-extracted with 70% acetone overnight in the dark at 23-25°, and the extract added to the first.

The extract was divided into 3 approximately equal parts and processed to yield free, ester-conjugated, and amide-conjugated IAA (Fig. 1). A column of DEAE-Sephadex was equilibrated with 50% 2-propanol. For HPLC a Waters Associates model 6000A solvent delivery system and a model U6K injector equipped with a 3-ml inlet coil were used. The second internal standard, ^{14}C-IBA (courtesy of J. Cohen and R. S. Bandurski) had a sp. act. of 5545 dpm/μg.

Gas chromatography. Samples were analyzed on a Varian 2740 instrument equipped with FID and NSD detectors. Identical columns, 1.83 m x 2-mm (i.d.) packed with 3% OV-17 on 100/120 GasChrom Q, were used. Injection and detector temperatures were 240-260°C, and column temperature was 170-180°; N_2 carrier gas flow rate was adjusted to produce elution times of 4-6 min for IAA and 10-12 min for IBA. ^{14}C-IAA and ^{14}C-IBA were collected from one column (FID) by extinguishing the flame and placing a 100 mm x 3 mm (i.d.) glass tube over the exit port for predetermined intervals. The contents of the tube were rinsed into scintillation vials with ca. 7 ml scintillation fluid (Amersham-Searle aqueous counting scintillant). Each sample was counted for 5 min, and data were corrected for background and efficiency.

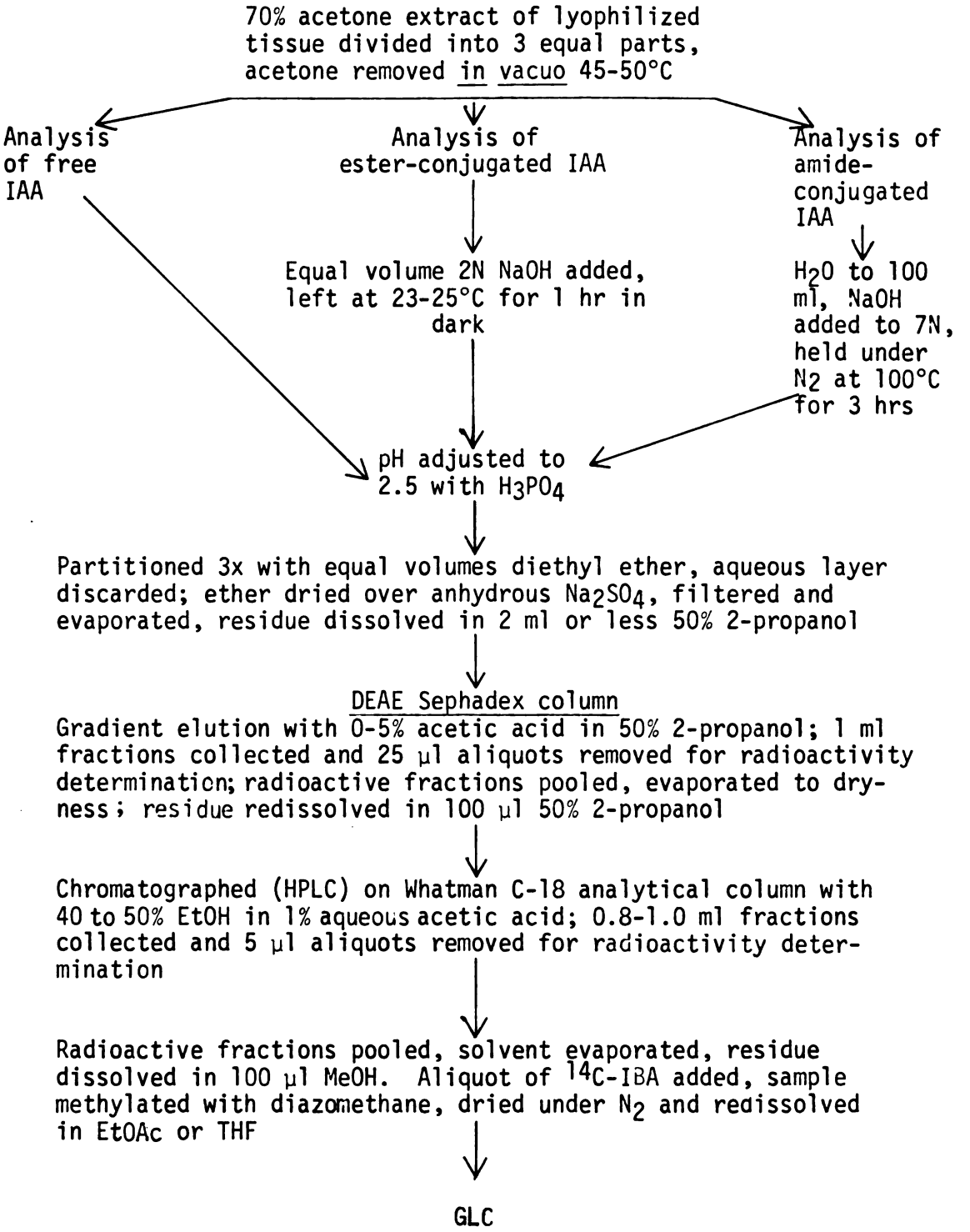


Figure 1. Flow diagram of procedure for purification and preparation of strawberry achene and receptacle extracts for GLC analysis of free and conjugated IAA.

Calculation of endogenous IAA in sample. The quantity of IAA in each sample was calculated as described by Cohen and Schulze (4). The specific activity of the ^{14}C-IAA standard decreases as a result of dilution by endogenous IAA in the tissue being extracted. The final specific activity is calculated and used to determine the quantity of endogenous IAA as shown:

$$\left(\frac{\text{Initial sp. act.}}{\text{Final sp. act.}} - 1 \right) \times \text{Amount of } ^{14}\text{C-IAA initially added} = \text{Amount of IAA in tissue}$$

Because hydrolysis with 2 N NaOH yields free IAA plus ester-linked IAA, and hydrolysis with 7 N NaOH yields free IAA plus ester- and amide-conjugated IAA, data for esterified IAA were corrected by subtraction of values for free IAA and those for amide-linked IAA were corrected by subtraction of values for both free and esterified IAA. Based on these computations, no conjugated IAA was evident in some samples.

Duplicate aliquots of each sample were injected and the data for area and radioactivity ratios were averaged. Actual values varied from 90 to 110% of the average values. Data are presented as µg/g dry weight or ng per organ. The latter totals were based on estimates of 246 (1980) and 275 (1981) achenes per fruit; the total quantity of IAA in the achenes of one fruit was calculated for comparison with the total amount in the receptacle. Each data point represents one extraction of a sub-sample of tissue from each sampling date.

GC-MS identification. Fruit collected 14 days after anthesis in 1981 were used to verify the presence of IAA in achene and receptacle tissue. Samples were analyzed on a Hewlett-Packard 5985a GC/MS/COM instrument containing a 3.05 m x 2 mm (i.d.) glass column packed with 3% SP-2250 on 80/100 Supelcoport, using a repetitive scan at 70 eV. The carrier gas was He, and ion source and inlet temperatures were 200° and 250°C, respectively; column temperature was programmed from 200-260° at 10°/min.

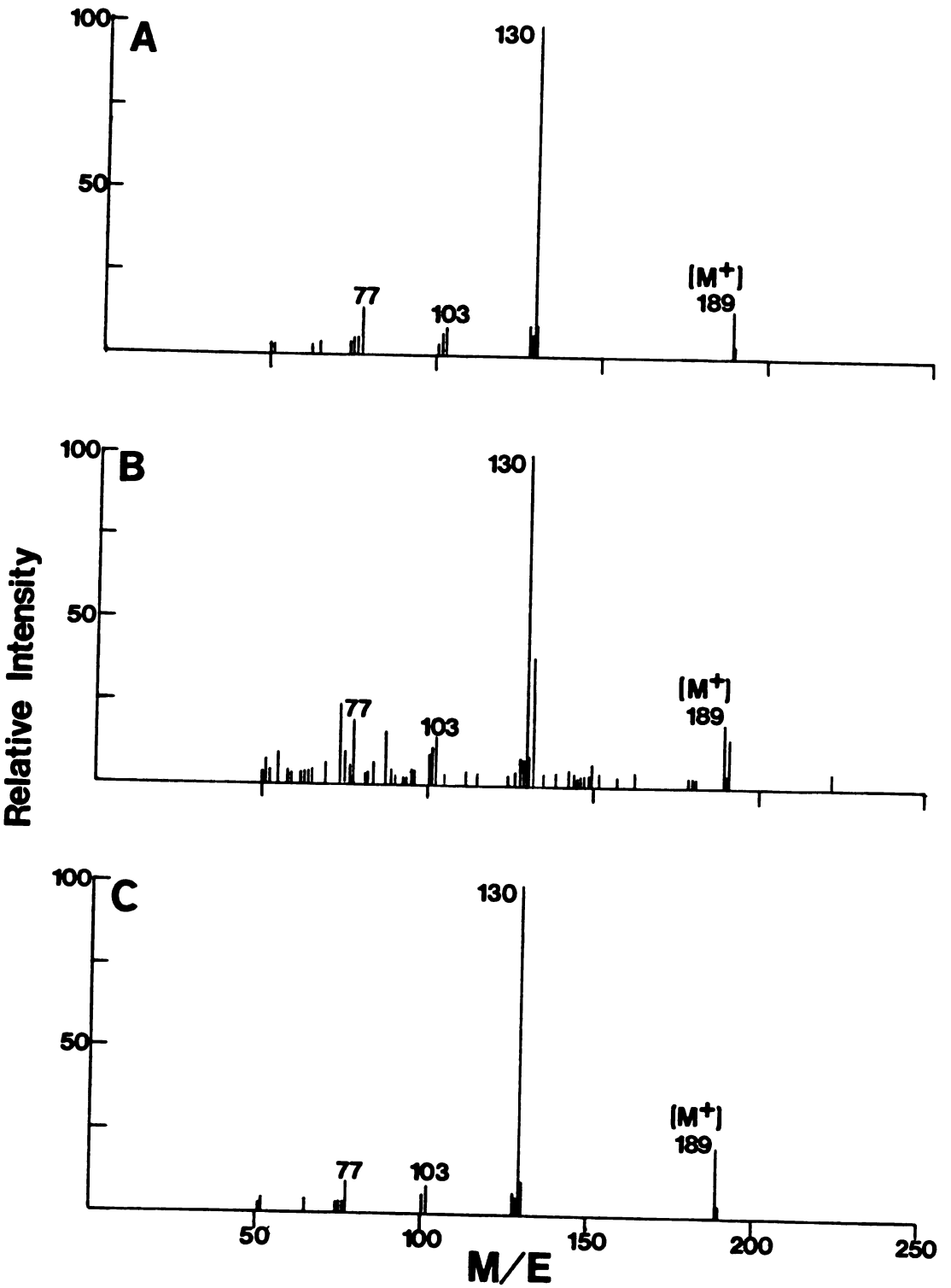
ABA analysis. Tissue samples were extracted with 70% acetone or aliquots were taken from extracts used for analysis of free IAA. The procedure used for partial purification was the same as that used for IAA through partitioning with ether (Fig. 1). The ether residue was redissolved in ca. 100 µl 100% MeOH and methylated with diazomethane. Samples were analyzed on a Packard 7400 GC equipped with an EC detector containing ^{63}Ni foil. The instrument contained a 1.8 m x 2 mm (i.d.) glass column packed with 3% XE-60 on Gas Chrom Q. N_2 was used as the carrier gas, and column, detector, and injector temperatures were 180°, 240°, and 240°C, respectively. Using a ^{14}C-ABA internal standard, recovery was estimated to be 66%. Data are presented as µg/g dry weight or ng per organ and represent one extraction of a sub-sample of tissue from each sampling date.

Identification of IAA. Aliquots of the final preparations of the free IAA fractions of extracts of 'Midway' achene and receptacle tissue sampled 14 days after anthesis, representing ca. 97 and

560 µg dry weight, respectively, were analyzed by GC-MS. The rentention time for both presumed and authentic Me-IAA was ca. 6.7 min. Mass spectrometry of both samples showed a molecular ion (M^+) at 189, a base peak at 130, and fragments at 103 and 77, all identical to synthetic Me-IAA. Slight contamination of the receptacle extract contributed to the background evident in the spectrum, and ^{14}C-Me-IAA may be present, as peaks at 191 and 132 were detected. These data confirm Nitsch's tentative identification of IAA in extracts of achenes.

Effect of lyophilization on free IAA content. To determine if significant losses of free IAA occurred during tissue preparation, the free IAA content of fresh vs. frozen and lyophilized whole fruit was measured. Fruit were from pooled samples of primary, secondary, and tertiary berries, and equal numbers of fruit from each class were divided between treatments. After weighing, one sub-sample of fruit was macerated in 100% acetone containing ^{14}C-IAA and analyzed as described. The volume of acetone was adjusted so that the water contributed by the fruit led to a final concentration of 70% acetone. The other sub-sample was prepared as described above (see Tissue preparation). Estimates of free IAA concentration in a first trial were fresh, 0.157, and lyophilized, 0.100 µg/g dry weight. A second experiment with a different sample of fruit gave values of 1.61 and 1.03 µg/g dry weight for fresh and lyophilized tissue, respectively. Thus, freezing and lyophilization resulted in a loss of 36 to 37% of the free IAA.

Figure 2. Mass spectra of putative Me-IAA in methylated extracts of achene (A) and receptacle (B) tissue from 'Midway' strawberry fruits harvested 14 days after anthesis, and of authentic Me-IAA (C).


A
100
50
130
77
103
[M+]
189
B
100
50
130
77
103
[M+]
189
Relative Intensity
C
100
50
130
77
103
[M+]
189
50
100
150
200
250
M/E

Variability in IAA recovery among replicate samples. Estimates of recovery of ^{14}C-IAA in routine analyses ranged from 30 to 60%. To determine the variability among replicate extractions, three replicate samples of achene and receptacle tissue collected 11 days after anthesis in 1981 were extracted. The concentrations found in 'Midway' achene tissue were 2.22, 2.34, and 2.63 µg/g dry weight, or 2.43 ± 0.21; those in receptacle tissue were 0.23, 0.27, and 0.31 µg/g dry weight, or 0.27 ± 0.04. Thus, the ranges with 3 samples were 18% (achene) and 30% (receptacle) of the means.

Levels of free and conjugated IAA during fruit development. Levels of free IAA in the whole fruit at anthesis were high as compared with subsequent levels in the achenes and receptacles (Tables 1 and 2; Figs. 3-5). The concentration in the achenes rose to a maximum 2 weeks after anthesis, then declined gradually to a very low level at maturity. Concentrations in receptacle tissue paralleled those in achene tissue both years, but levels were generally considerably lower than in achenes. The concentration 2 weeks after anthesis in 1981 was high and approached that found in the achenes. Peak concentrations in achenes were 1.2- to 4-fold as high as those found in receptacles. Sixteen days after anthesis in 1980, achenes contained 4.5 times the total quantity of free IAA in the receptacle; however, 14 days after anthesis in 1981 the maximum amount in the achenes was only 0.72 times that found in the receptacle.

Most of the ester-conjugated IAA was recovered from achenes, though some was recovered from receptacle tissues (Tables 1 and 2;

TABLE 1. Rate of receptacle growth and quantities of free, ester- and amide-conjugated and total IAA in achene and receptacle tissue of primary 'Sparkle' strawberry (Fragaria x ananassa Duch.) fruits from anthesis to maturity, 1980

Days after anthesis	Growth Rate[z] (mg/day)	Free		Ester		Amide		Total	
		A[y]	R[y]	A	R	A	R	A	R
Concentration (µg/g dry wt.)									
0		4.26		0.16		nd[x]		4.32	
6	11.3	0.05	nd	3.16	nd	nd	0.53	3.21	0.53
11	18.5	1.36	0.03	2.18	0.40	8.24	nd	11.78	0.43
16	90.5	3.98	0.96	nd	0.62	5.99	nd	9.93	1.58
22	28.2	2.00	0.02	1.93	0.92	10.92	nd	14.85	0.94
27 (Ripe)		nd	nd	nd	nd	10.06	nd	10.06	nd
Total (ng) per fruit[w]									
0		36.64		1.36		nd		38.00	
6	11.3	2.46	nd	777.36	nd	nd	41.38	779.82	41.38
11	18.5	334.56	4.04	536.28	53.90	2027.04	nd	2397.88	57.94
16	90.5	979.08	218.11	nd	140.86	1473.54	nd	2452.62	162.67
22	28.2	492.00	11.55	474.78	708.60	2686.32	nd	2653.10	720.15
27 (Ripe)		nd	nd	nd	nd	2474.76	nd	2474.76	nd

[z]Rate of receptacle growth between sampling dates, mg dry wt./day.

[y]A = achene, R = receptacle

[x]No detectable IAA when value adjusted for quantities present in other forms

[w]Total quantity in 246 achenes or 1 receptacle

TABLE 2. Rate of receptacle growth and quantities of free, ester- and amide-conjugated and total IAA in achene and receptacle tissue of primary 'Midway' strawberry (Fragaria x ananassa Duch.) fruits from anthesis to maturity, 1981

Days after anthesis	Growth Rate[z] (mg/day)	Free		Ester		Amide		Total	
		A[y]	R[y]	A	R	A	R	A	R
		Concentration (µg/g dry wt.)							
0		6.21		2.01		nd[x]		8.22	
4	15.4	2.06	nd	4.15	0.26	3.67	0.68	9.88	1.66
8	31.3	1.35	nd	10.67	nd	2.24	nd	14.26	nd
11	76.8	2.22	0.23	0.64	nd	14.13	0.78	16.99	1.01
14	31.3	3.12	2.72	nd	nd	7.04	0.06	10.16	2.78
17	82.9	1.77	0.15	nd	0.61	2.90	nd	4.67	0.76
20	23.8	1.26	0.49	nd	nd	6.56	nd	7.82	0.49
23 (Ripe)		0.26	0.79	nd	nd	26.93	nd	27.19	0.79
		Total (ng) per fruit[w]							
0		49.05		15.88		nd		64.93	
4	15.4	57.75	nd	1141.25	12.95	1009.25	34.00	2208.25	46.95
8	31.4	371.25	nd	2934.25	nd	616.00	nd	3921.50	nd
11	76.8	610.50	47.15	176.00	nd	3885.75	159.90	4672.25	207.05
14	31.3	858.00	1189.65	nd	nd	1936.00	26.22	2794.00	1215.87
17	82.9	486.75	79.50	nd	323.18	797.50	nd	1284.25	402.68
20	23.8	346.50	381.46	nd	nd	1804.00	nd	2150.50	381.46
23 (Ripe)		71.50	671.50	nd	nd	7405.75	nd	7477.25	671.50

[z]Rate of receptacle growth between sampling dates, mg dry wt./day

[y]A = achene, R = receptacle

[x]No detectable IAA when value adjusted for quantities in other forms

[w]Total quantity in 275 achenes or 1 receptacle.

Figure 3. Receptacle and achene weights and concentrations of free, ester-conjugated and amide-conjugated IAA in primary 'Sparkle' strawberry fruit from anthesis to maturity, 1980.

A. Fresh and dry weights of whole fruit and individual achenes and receptacles. Averages of 5 fruits or 25 achenes.

B-D. Concentrations (μg/g dry weight) of free (B), ester-conjugated (C), and amide-conjugated (D) IAA in achene and receptacle tissue.

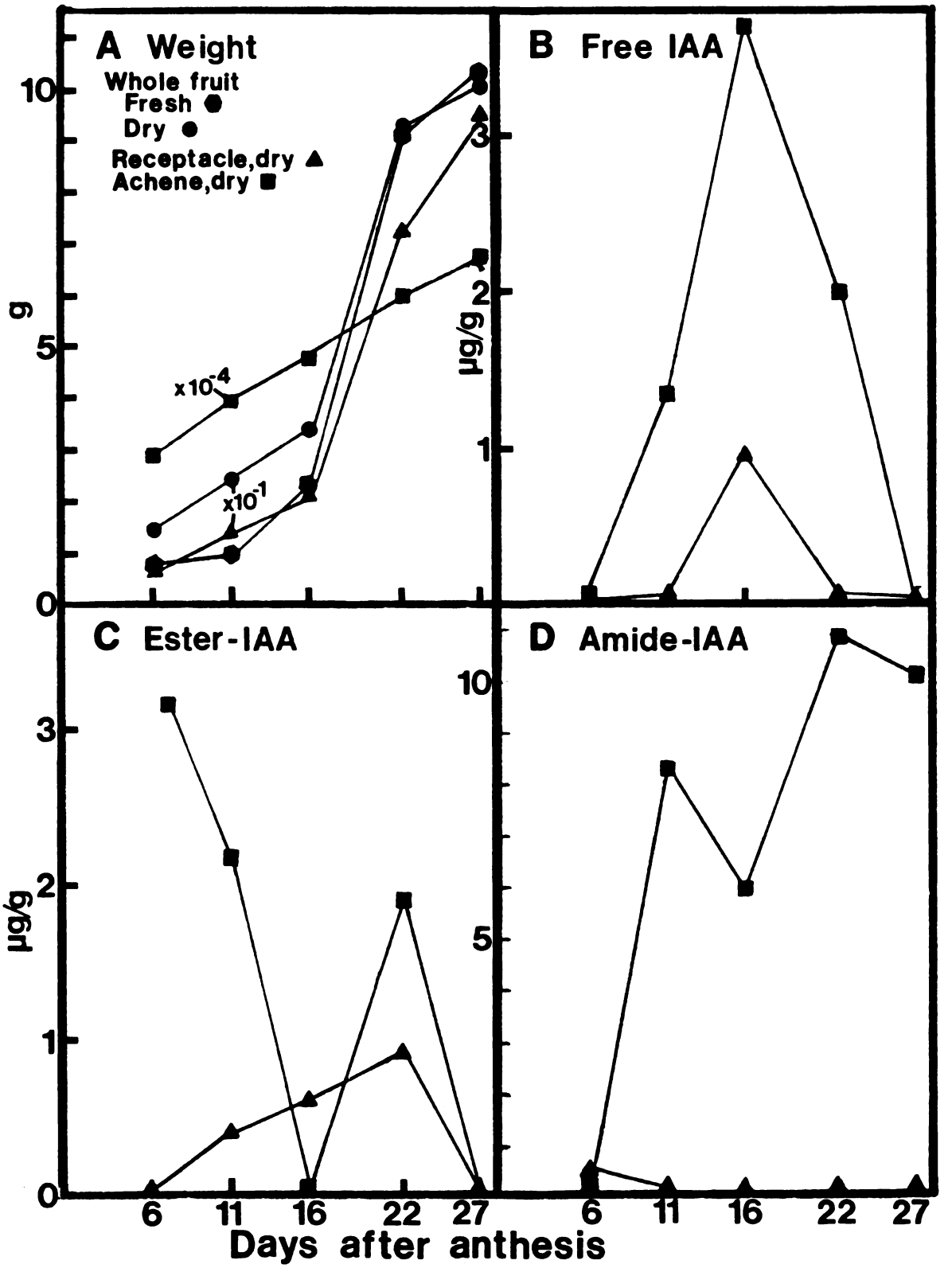

A Weight
Whole fruit
Fresh
Dry
Receptacle,dry
Achene,dry
x10-4
x10-1
g
B Free IAA
µg/g
C Ester-IAA
µg/g
D Amide-IAA
6
11
16
22
27
Days after anthesis

Figure 4. Receptacle and achene weights and concentrations of free, ester-conjugated and amide-conjugated IAA in primary 'Midway' strawberry fruit from anthesis to maturity, 1981.

A. Fresh and dry weights of whole fruit and individual achenes and receptacles. Averages of 5 fruits or 25 achenes.

B-D. Concentrations (µg/g dry weight) of free (B), ester-conjugated (C), and amide-conjugated (D) IAA in achene and receptacle tissue.

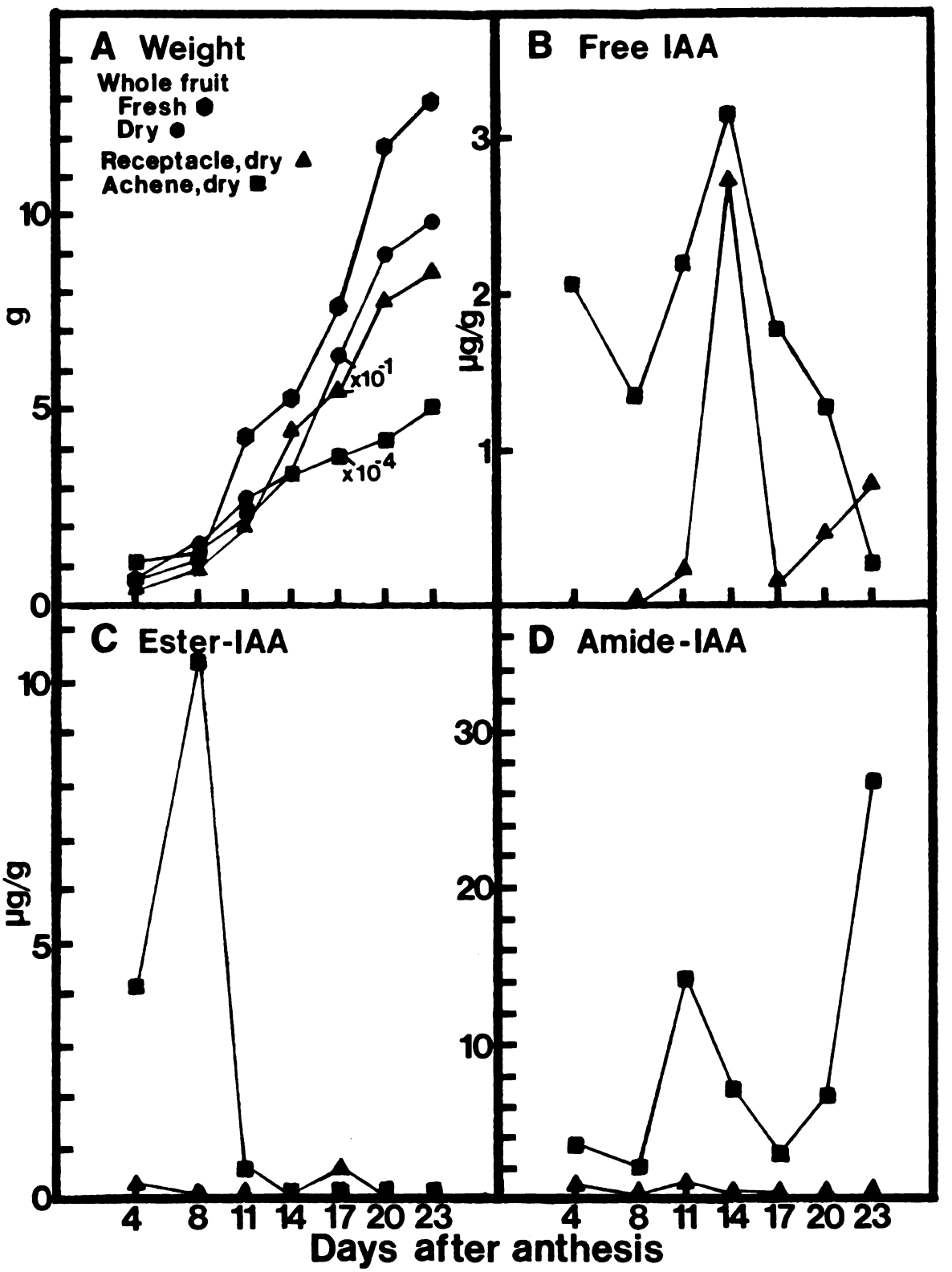

A Weight
Whole fruit
Fresh
Dry
Receptacle, dry
Achene, dry
x10-1
x10-4
g
B Free IAA
µg/g
C Ester-IAA
µg/g
D Amide-IAA
4 8 11 14 17 20 23
Days after anthesis

Figure 5. Total free, ester-conjugated and amide-conjugated IAA per primary strawberry fruit in achenes (A,C) and receptacle (B,D). A,B -- 'Sparkle', 1980; C,D -- 'Midway', 1981.

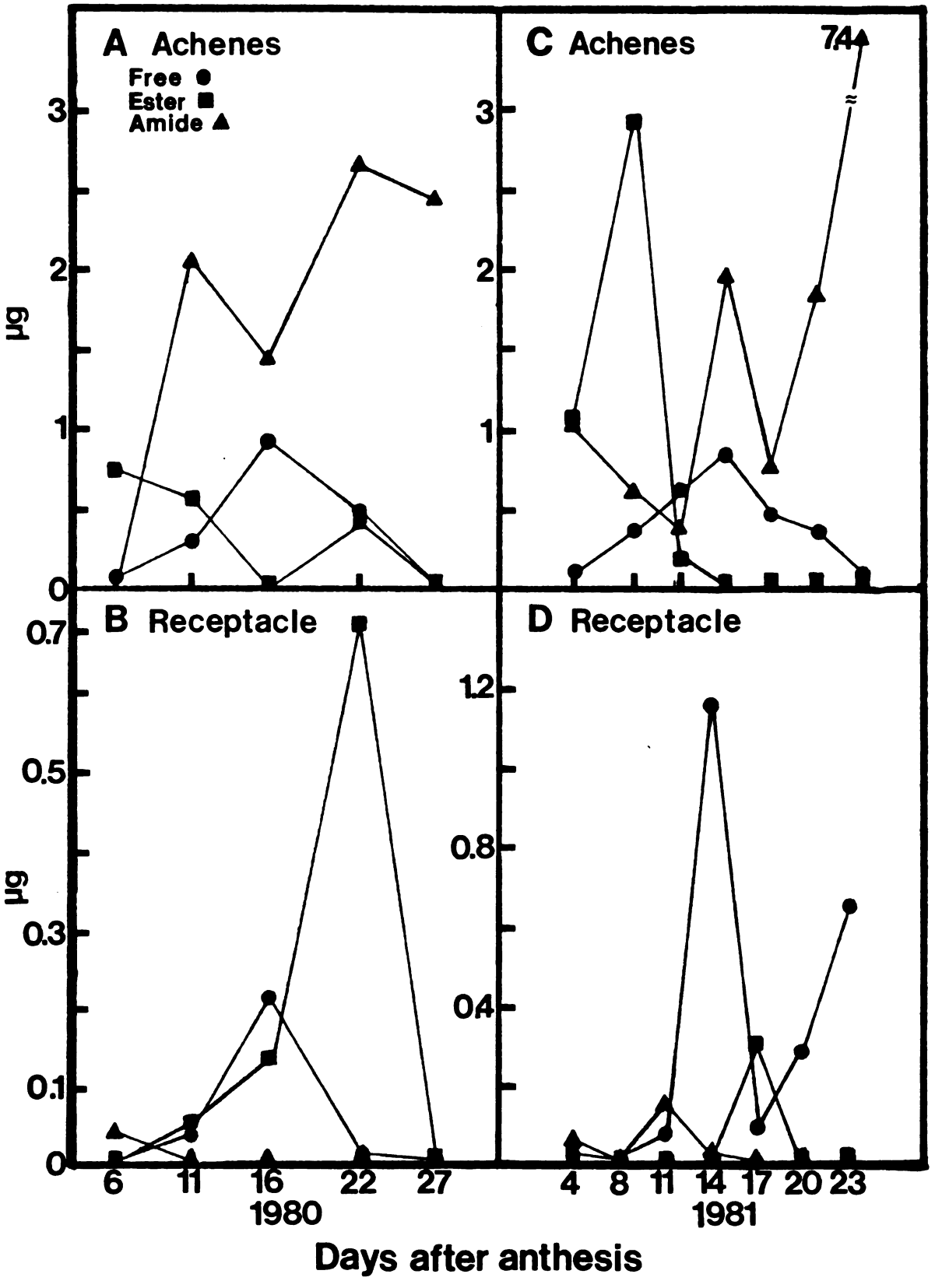

A Achenes
Free ●
Ester ■
Amide ▲
C Achenes
74
B Receptacle
D Receptacle
µg
µg
3
2
1
0
0.7
0.5
0.3
0.1
0
1.2
0.8
0.4
6 11 16 22 27
1980
4 8 11 14 17 20 23
1981
Days after anthesis

Fig. 3-5). Maximum concentrations in the achenes were 3.4- (1980) and 17.5-fold (1981) as high as those in receptacle tissue and roughly equal to (1980) or 3-fold as great as (1981) the levels of free IAA in the achenes. Maximum concentrations of ester-linked IAA in the achenes occurred at 6 (1980) and 8 (1981) days after anthesis vs. 22 and 17 days after anthesis in receptacles. The concentration increased in the receptacle between 6 and 22 days after anthesis in 1980, but no pattern was found in 1981. The maximum concentrations in achene tissue were higher in 1981 than in 1980. The consistent features from both years were that some ester-conjugated IAA was evident in whole fruit at anthesis and a significant quantity was found in achenes 6 to 11 days later. The maximum amount in the achenes was 1.1- (1980) to 9.1-fold (1981) as great as that in the receptacle. An increase in total ester-conjugated IAA in the receptacle follows the maximum total free IAA in the receptacle both years.

No amide-conjugated IAA was found in whole fruit at anthesis in either year (Tables 1 and 2; Figs. 3-5). The maximum concentrations were 20.6- (1980) and 34.5-fold (1981) higher in achene than in receptacle tissue, and the total amount in achenes was 64.9- (1980) and 46.3-fold (1981) as high as those found in the receptacle. Peak concentrations of the amide form in achene tissue were 2.7- (1980) and 8.3-fold (1981) as great as the levels of free IAA, and 3.4- (1980) and 2.5-fold (1981) the levels of ester-conjugated IAA. Peak concentrations of ester-conjugated IAA in receptacle tissue were

1.7-fold (1980) and 0.78 (1981) as great as the levels of amide-conjugated IAA. The maximum amount in the achenes of one fruit was 65- (1980) and 46-fold (1981) that in receptacle tissue. No particular pattern of change was evident in the receptacle, but a bimodal distribution was evident in achenes with peaks at 11 days after anthesis and at or near maturity.

Free IAA in secondary fruit. More free IAA was recovered from achenes of secondary fruit than from those of primary fruit, but levels in the receptacles were similar (Table 3). The maximum concentrations in the achenes were 9- (1980) and 2.9-fold (1981) as high as those found in the receptacle tissue with peaks at 14 and 13 days after anthesis, respectively.

Free ABA in primary fruit. Free ABA was detected in extracts of whole fruit at anthesis (Table 4; Fig. 6). The concentrations in achene tissue were high 4 to 6 days after anthesis, dropped to somewhat lower levels during the time of rapid growth, then increased as fruit approached maturity. No clear pattern of concentration was evident in receptacle tissue either year, though total ABA increased as fruit approached maturity, then declined at maturity. Maximum concentrations in achene tissue were 4- to 6-fold as high as those found in receptacle tissue. The achenes contained more total ABA than did the receptacle at all sampling dates both years, except at 20 days in 1981.

The ratio of free IAA to free ABA changed in both achene and receptacle tissue during fruit development. In whole flowers at

TABLE 3. Concentration of free IAA in achene and receptacle tissue of secondary strawberry (Fragaria x ananassa Duch.) fruits during their development

Days after anthesis	IAA (µg/g)	
	Achene	Receptacle
	1980, cv 'Sparkle'	
9	1.15	nd*
14	8.13	0.90
19	4.31	0.17
	1981, cv 'Midway'	
6	5.06	2.30
13	6.72	2.10
16	5.64	nd*

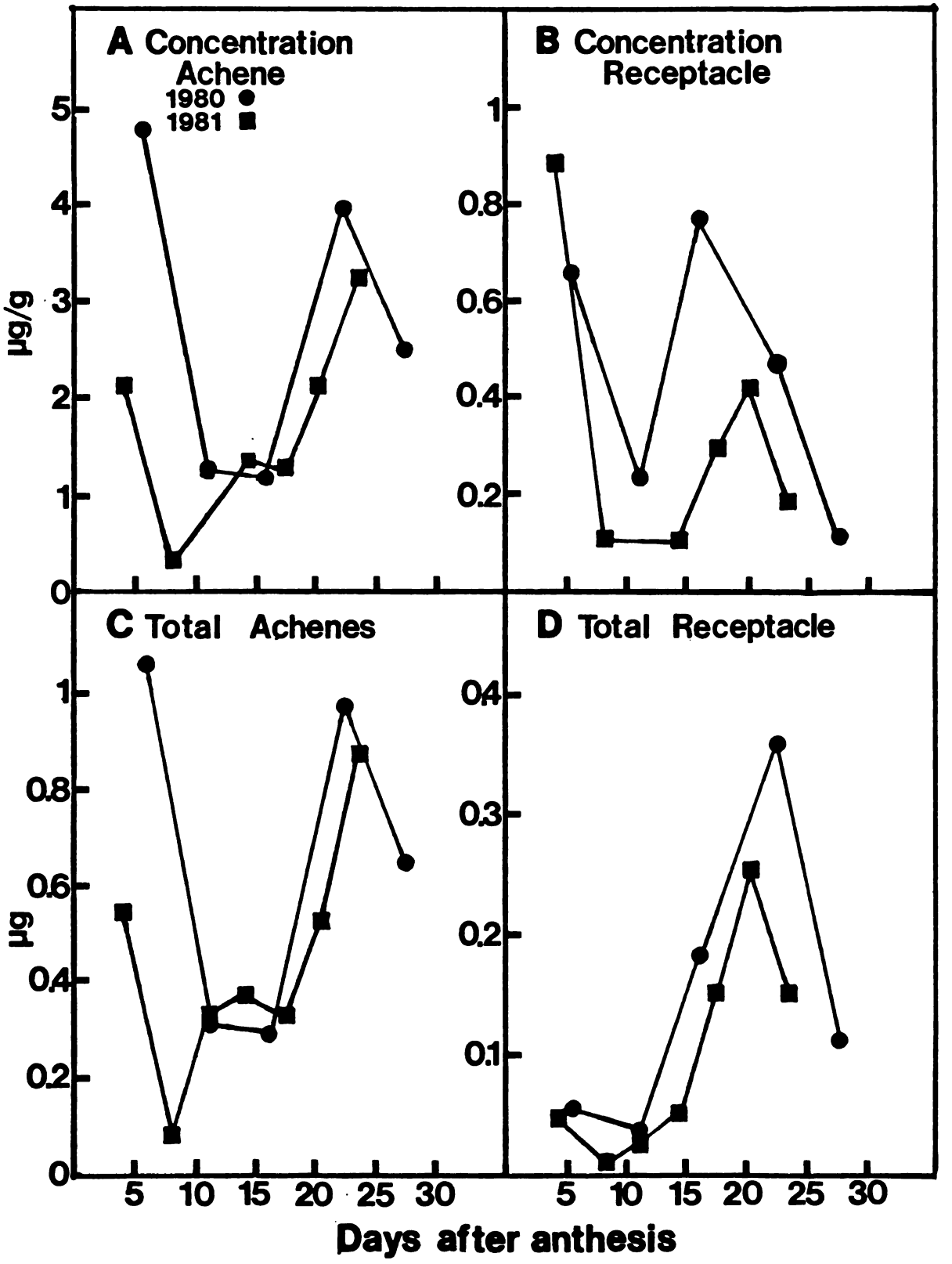
*No detectable IAA.

TABLE 4. Quantity of free ABA in achene and receptacle tissue of primary strawberry (Fragaria x ananassa Duch.) fruits from anthesis to maturity

Days after Anthesis	Concentration (µg/g dry wt.)		Total (ng) per fruit*	
	Achene	Receptacle	Achene	Receptacle
	1980, cv 'Sparkle'			
0	3.66		31.48	
6	4.40	0.65	1082.40	50.70
11	1.31	0.22	322.26	29.70
16	1.26	0.78	309.96	177.22
22	3.99	0.45	981.54	346.59
27 (Ripe)	2.53	0.12	622.38	109.34
	1981, cv 'Midway'			
0	4.63		31.02	
4	2.10	0.89	577.50	44.32
8	0.34	0.11	93.50	11.88
11	1.25	0.22	343.75	24.42
14	1.41	0.11	387.75	48.07
17	1.31	0.31	360.25	164.03
20	2.06	0.41	566.50	266.50
23 (Ripe)	3.22	0.19	885.50	161.50

*Total quantity in 246 (1980) or 275 (1981) achenes or 1 receptacle.

Figure 6. Concentration (A,B) and total amount of ABA per primary fruit (C,D) in achenes (A,C) or receptacle (B,D) of 'Sparkle' and 'Midway' strawberry from anthesis to maturity in 1980 or 1981.


A Concentration
Achene
1980 ●
1981 ■
B Concentration
Receptacle
C Total Achenes
D Total Receptacle
µg/g
µg
Days after anthesis
0
1
2
3
4
5
0.2
0.4
0.6
0.8
0.1
0.3
5
10
15
20
25
30

anthesis, the ratio was 1.16 (1980) and 1.34 (1981), and in both achene and receptacle tissue the balance shifted to less than 1 by 4 or 6 days after anthesis. As ABA levels declined in achene tissue, IAA levels increased and the ratio was greater than 1 through 16 or 17 days after anthesis with maxima of 3.2 (1980) and 4.07 (1981) at 16 and 8 days after anthesis, respectively. The correlation between growth rate and IAA/ABA ratio was low and non-significant both years. However, a decrease in the ratio was generally associated with lower growth rate.

Discussion

The patterns of change in free IAA concentrations in achene tissue are quite similar to those reported by Nitsch (9,10) and Lis et al. (6). The concentration of free IAA in achene and receptacle tissue did not parallel fruit growth rate. The maximum in previous studies occurred at 12 (9,10) or 8 (6) days after pollination, but was evident immediately prior to the greatest increase in fruit size and declined as growth rate increased. The maximum concentrations in achenes in this study, 16 or 14 days after anthesis, roughly correspond to those found by Lis et al. and to the time of change of free nuclear to cellular endosperm (13), though maximum receptacle levels in 1981 were surprisingly high in comparison with published data.

Though Nitsch was unable to recover auxin-like activity from receptacle tissue, Lis et al. observed levels similar to those found

here. Maximum concentrations were 3-fold greater in achenes than in receptacles, within the 1.2- to 4-fold range which I observed.

Nitsch (10) estimated that 1/2 to 2/3 of the auxin in the achenes was present as IAA, or 1.5 to 2.0 μg/g dry weight at the maximum. Converting the data of Lis et al. to dry weight, one obtains estimates of 2 to 5 μg/g dry weight, in the same range as my values. More free IAA was recovered from 'Midway' than from 'Sparkle' fruit in the present study. Cultivars probably vary in auxin content, and the general vigor of the plant and the environmental conditions under which it is grown may affect the levels also. Nitsch, using one cultivar, obtained 1.6 times as much auxin activity per unit weight in one study as in another (9, 10), while Lis et al., using a third cultivar, obtained an intermediate value (6). Methodology could have been responsible for some of the differences also. Thus, Nitsch's inability to recover auxin-like activity in receptacle extracts may have resulted from either cultivar differences, technique, or environmental effects.

The interrelationships among the various conjugates and free IAA remain obscure and the paucity of data on the role of conjugates in fruit development leaves much to speculation. Ester-conjugated IAA in the achene may be a source of free IAA in the early stages of development. It was present in sufficient quantity both years to provide the quantity of free IAA found in the achene. This does not preclude de novo synthesis of free IAA as proposed by Nitsch (10); hydrolysis of the ester may augment the supply of newly-synthesized

IAA or be a temporary storage form. The ester form found in the receptacle immediately following the date of the maximum total amount of free IAA may be either a temporary storage form or an end-product. Thus, the metabolism of IAA may differ in achene vs. receptacle. The bimodal distribution of amide-conjugated IAA in the achenes makes interpretation of its role difficult. Growth rate was also bimodal in 1981 though out of phase with the amide-conjugated IAA levels. The amide form appeared to accumulate in the achene as fruit approached maturity. High concentrations of amide-conjugated IAA have been found in mature seed; IAA-aspartate accounts for an estimated 1/2 of the total IAA in mature soybean seed (2). In the strawberry achene amide-conjugated IAA comprised nearly all the IAA in the tissue.

The reasons for the higher estimated concentration of free IAA in achenes of secondary fruit as compared to primary fruit are unknown. In contrast, maximum concentrations in the receptacle were roughly equal both years. Secondary fruit are smaller than primary fruit and fewer achenes occur on the receptacle. The higher concentration of IAA in the achenes may raise the total auxin content per fruit to or above that found in the primary fruit. One may speculate that a fruit placed at a competitive disadvantage survives only if it produces more hormone(s), thereby creating a stronger sink.

Fruit growth may be governed by a balance between promoters and inhibitors, but my data suggest that a low ratio favors, rather than restricts, growth. However, the correlation between the change

in the ratio and the growth rate is low, and a more accurate assessment of the situation must include levels of all hormones, free and bound.

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SECTION II

EFFECTS OF EXOGENOUS APPLICATION OF AUXIN AND/OR GIBBERELLIN ON STRAWBERRY FRUIT SET, GROWTH AND ENDOGENOUS INDOLEACETIC ACID CONTENT

Effects of Exogenous Application of Auxin and/or Gibberellin on Strawberry Fruit Set, Growth and Endogenous Indoleacetic Acid Content

Abstract

Treatment of emasculated strawberry (Fragaria x ananassa Duch.) flowers with aqueous solutions of naphthaleneacetic acid (NAA), gibberellins (GA_3, $GA_{4/7}$), NAA + GA_3, or NAA + $GA_{4/7}$, each at 10^{-3} M in 2% dimethylsulfoxide (DMSO) and 0.1% Tween 80 induced parthenocarpy. All fruits stopped growing within 12 days of treatment, except those induced with NAA or NAA + GA_3. Re-treatment with the same or another growth regulator at 20 days after initial treatment stimulated continued growth as compared to no re-treatment. All induced fruits were considerably smaller than pollinated fruit; sizes ranged from 40 to 70% of pollinated fruit.

Free IAA concentration in NAA-treated fruit was 5-fold greater than that in control flowers and 3-fold as great as that in $GA_{4/7}$-treated fruit 6 days after treatment. By 14 days after treatment levels had declined in all treated fruit. These results are discussed in relation to the role of IAA in strawberry fruit development.

Exogenously-applied auxin and GA are capable of inducing fruit set in many species, including strawberry (3,7,8), auxin-induced fruits being larger than GA-induced fruits. When aqueous solutions are used, growth of parthenocarpic fruit parallels that of pollinated fruit during the first 10 days after treatment, then ceases (5), and ripening does not occur. When lanolin is used as the carrier, growth parallels that of pollinated fruit through 10 days after treatment, then slows but ripening eventually occurs (8). Both situations suggest that a continuous supply of hormone is required to maintain growth. Both vegetatively parthenocarpic (3) and hormone-induced fruit (2,86) of other species contain endogenous hormones. The purpose of the following work was to determine if the IAA content of parthenocarpic strawberry fruit parallels their rates of fruit growth.

Materials and Methods

Strawberry, 'Our Own', plants were obtained from Ahrens Nursery, Huntingburg, IN, in June 1980, and grown in 22 cm diameter pots in a glasshouse. Plants were overwintered outdoors in a cold frame and brought into the glasshouse in April 1981. The plants were maintained on a daily watering and bi-weekly fertilizer regime (20:20:20, Peters SoilTest). Experiment 1 was performed during June-October, 1980, and Experiment 2 was performed May-September, 1981.

Flowers were emasculated and tagged one day prior to anthesis, using primary through quaternary flowers on a cyme. The following treatments were applied in Experiment 1: Hand-pollination; control (dist. H_2O); NAA; GA_3; $GA_{4/7}$; NAA+GA_3; and NAA+$GA_{4/7}$. In Experiment 2, only water, NAA, and $GA_{4/7}$ were used. Each growth regulator was applied at 10^{-3}M; and contained 2% DMSO and 0.1% Tween 80; all solutions were adjusted to pH 7.0 with phosphate buffer. Twenty microliters of each solution was spread evenly over the surface of the receptacle, treatments being assigned at random. In some cases receptacles were re-treated with the same or a different (NAA or $GA_{4/7}$) chemical 20 days later, using twice the volume of solution at the same concentration.

In Experiment 1, receptacle diameters (minimum width) were measured on alternate days beginning on the day of treatment and continuing until maturity, using 12 flowers per treatment. In Experiment 2, diameters were measured 6, 8, 10, and 12 days after treatment, using a minimum of 30 fruit per treatment.

Free IAA content of the fruits was determined at both 6 and 12 days after treatment, using half the fruits in each treatment on each date. Harvested fruit were frozen at -18°C, lyophilized, and stored in plastic bags at 4°C in the dark with a drying agent. Whole fruit were analyzed for free IAA content as described in Section 1.

Results

Effects of hormones on fruit development. Applications of NAA, GA_3, and $GA_{4/7}$, alone and in combination, induced parthenocarpy

(Fig. 1), the receptacle diameter 6 days after treatment being comparable to that of pollinated fruit regardless of chemical used. NAA-treated fruit grew through 30 days after treatment, whereas GA-induced fruits had ceased growing 12 days after treatment. All fruits were significantly smaller than pollinated fruits. Addition of GA_3 to NAA had no appreciable effect on response to auxin, whereas addition of $GA_{4/7}$ prevented the response to NAA, the final fruit size being no greater than that of fruits induced by $GA_{4/7}$ alone (data not shown). Re-treatment with the same or a different growth regulator increased receptacle size by 30 days after initial treatment as compared with no re-treatment (Fig. 2). Receptacle size of GA_3- and $GA_{4/7}$-induced fruit declined if not re-treated. The effect of re-treatment was significant by LSD at 0.15 and/or 0.10, but not at 0.05.

In Experiment 2, the receptacles had enlarged significantly in comparison with controls 6 days after treatment, regardless of chemical used (Fig. 3). NAA-treated fruit grew more rapidly during the subsequent 4 days than did GA-induced fruits, but growth rates were similar thereafter. The fruit were harvested for determination of free IAA content at 14 days, precluding further measurement.

Effects of hormones on free IAA content. Free IAA was detected in all treatments in Experiment 2 (Fig. 3). Six days after treatment, control, NAA-treated, and $GA_{4/7}$-treated receptacles contained 0.64, 3.23, and 0.96 µg IAA/g dry weight. After 14 days, the values were 0.15, 1.13, and 0.91 µg IAA/g dry weight. The

Figure 1. Effect of application of aqueous solutions of NAA, $GA_{4/7}$, or GA_3 at 10^{-3}M in 2% DMSO and 0.1% Tween 80 to emasculated strawberry flowers on fruit growth from anthesis to maturity. Values are means for 12 fruits per treatment.

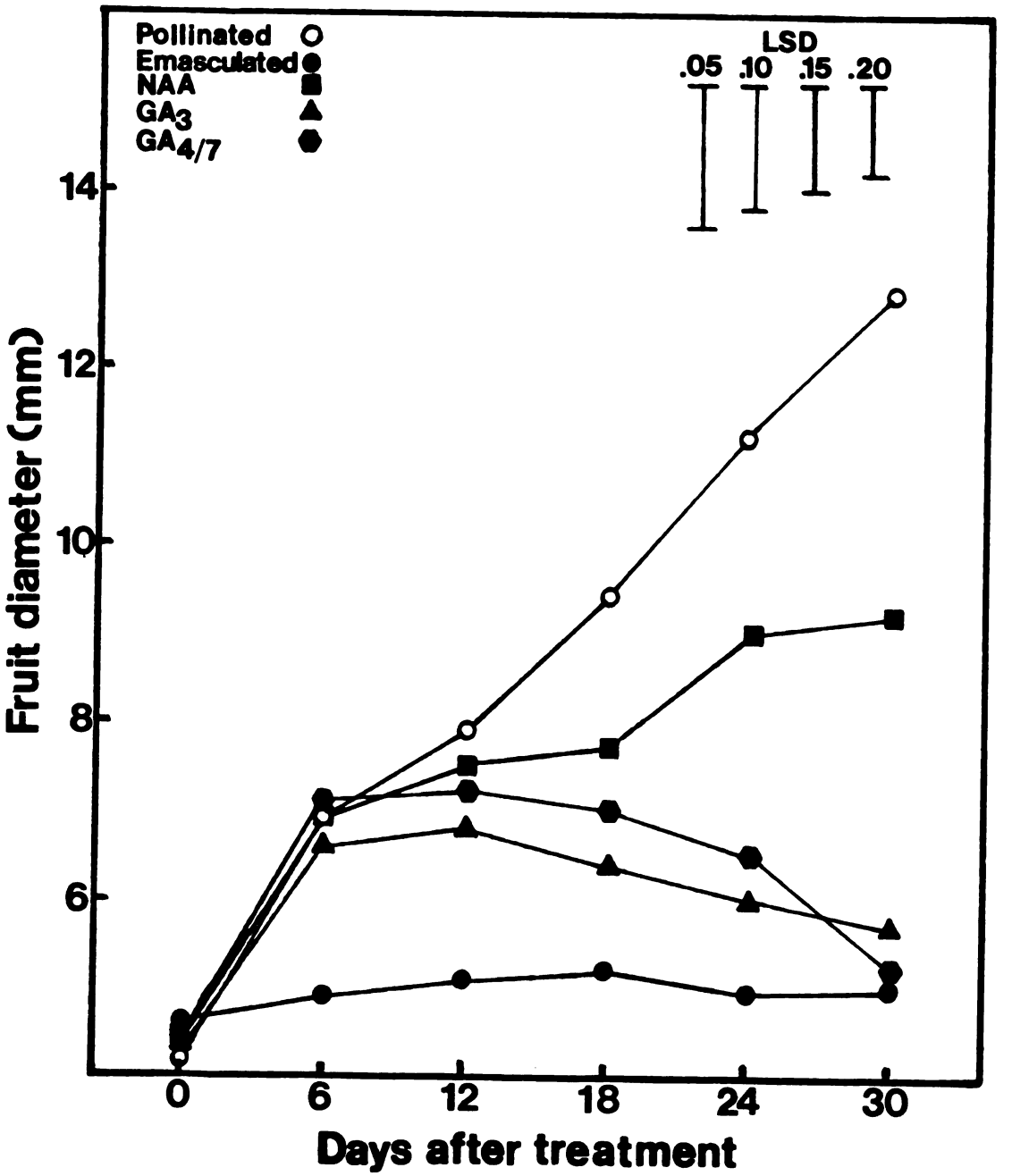

Pollinated
Emasculated
NAA
GA3
GA4/7
LSD
.05
.10
.15
.20
Fruit diameter (mm)
14
12
10
8
6
0
6
12
18
24
30
Days after treatment

Figure 2. Effect of re-treatment of parthenocarpic strawberry fruit with NAA or $GA_{4/7}$ at 20 days after initial treatment on subsequent growth. Receptacles were initially treated with NAA (A), GA_3 (B), or $GA_{4/7}$ (C) at 10^{-3}M in 2% DMSO and 0.1% Tween 80. Values are means for 4 fruit per treatment.

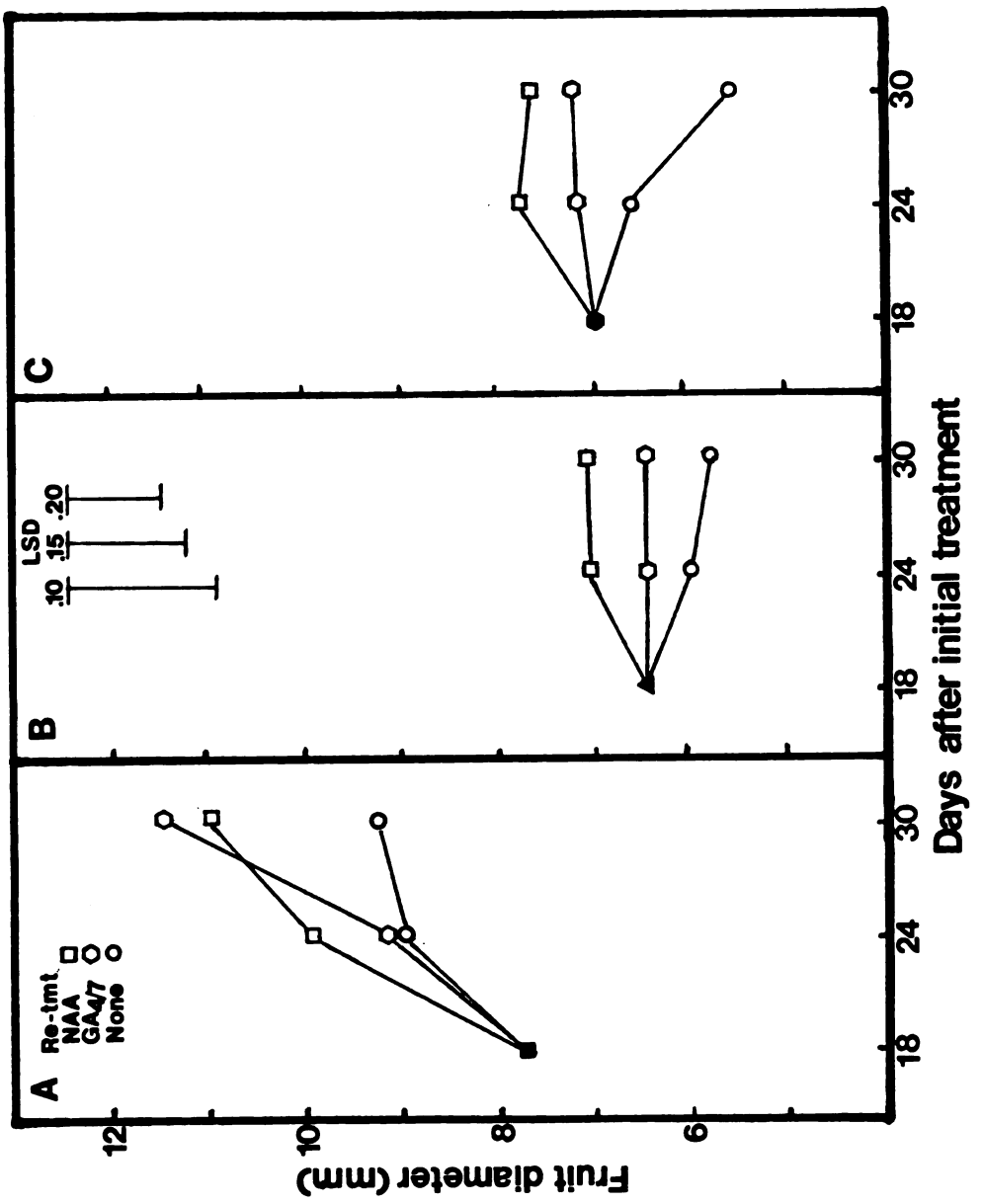
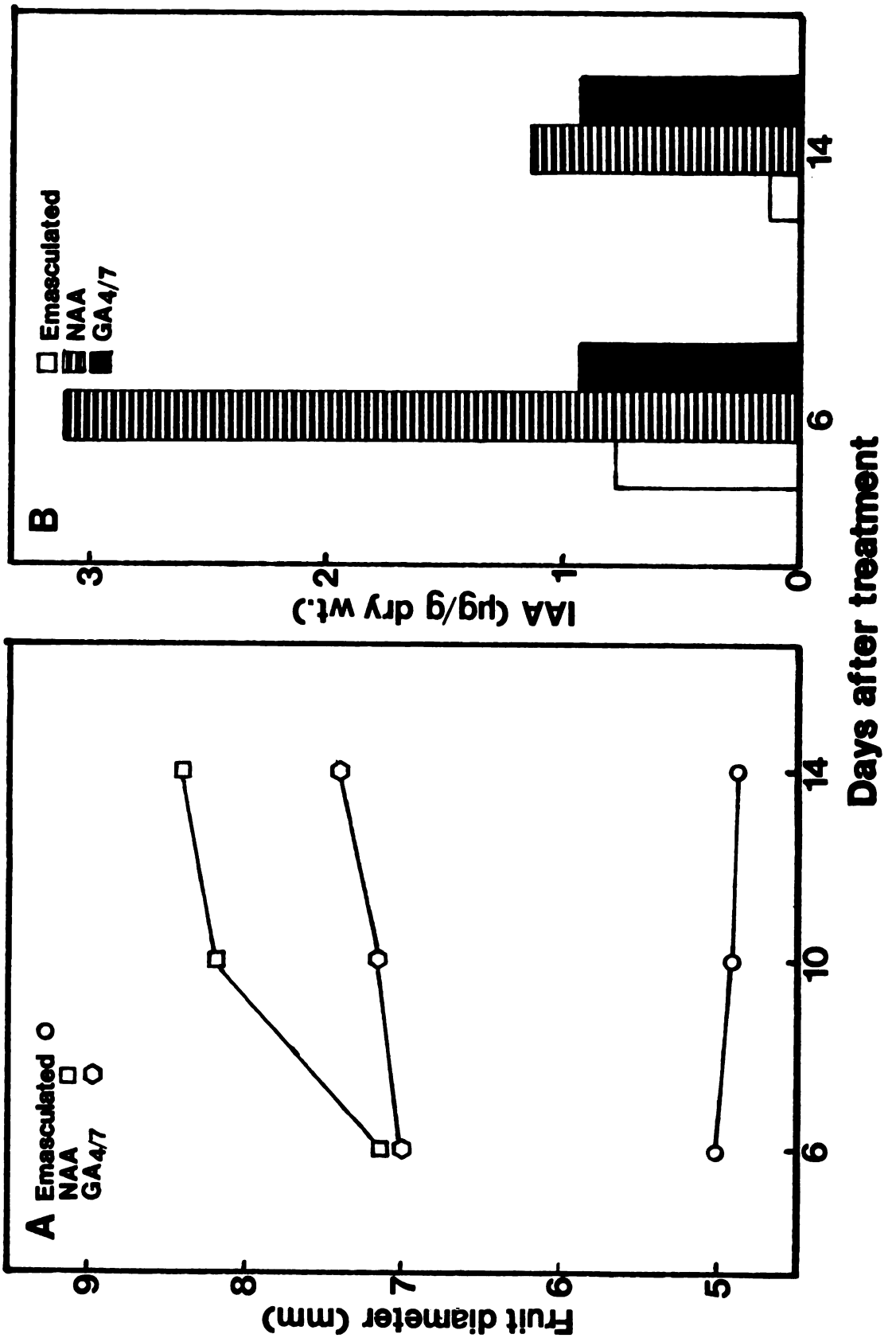

A
B
C
Re-tmt.
NAA
GA4/7
None
LSD
.10
.15
.20
Fruit diameter (mm)
12
10
8
6
18
24
30
Days after initial treatment

Figure 3. A. Effect of application of aqueous solutions of NAA or $GA_{4/7}$ at 10^{-3}M in 2% DMSO and 0.1% Tween 80 to emasculated strawberry flowers on fruit growth between 6 and 14 days after treatment. Values are means for a minimum of 30 fruits per treatment.

B. Free IAA concentration in induced fruits (receptacles plus achenes) 6 and 14 days after treatment.


A
Emasculated
NAA
GA4/7
Fruit diameter (mm)
9
8
7
6
5
6
10
14
B
Emasculated
NAA
GA4/7
IAA (µg/g dry wt.)
3
2
1
0
6
14
Days after treatment

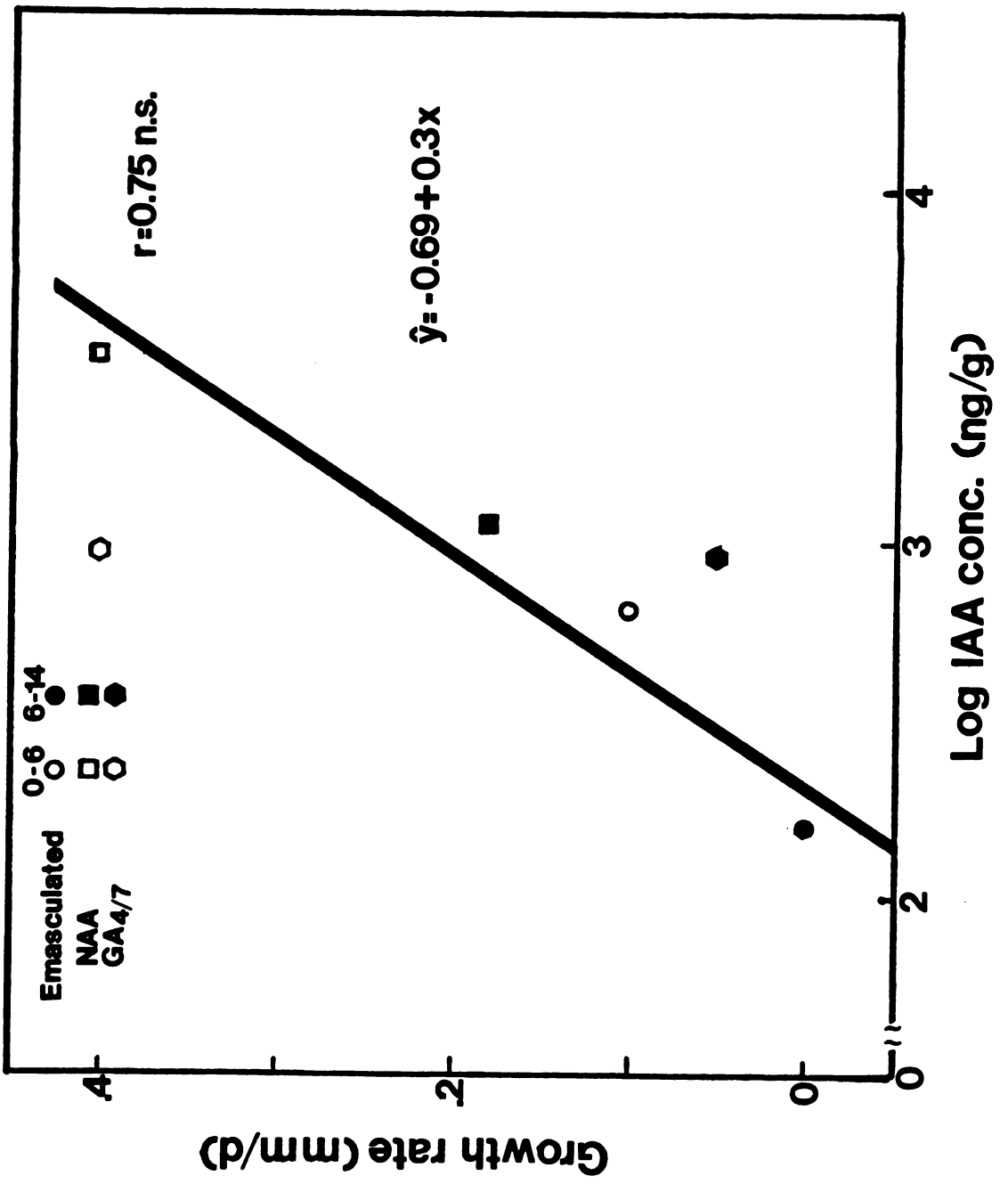
concentration of IAA declined considerably in both control and NAA-treated fruit between 6 and 14 days after treatment, but the decline observed in $GA_{4/7}$-treated fruit was negligible. When growth rates were calculated for both 0-6 and 6-14 days and plotted against log IAA concentration at 6 and 14 days, a reasonably good correlation was obtained ($r = 0.75$, n.s. at 0.05), although the value for $GA_{4/7}$-induced fruit at 0-6 days was higher than would be expected (Fig. 4).

Discussion

The results from studies on the effects on fruit development support earlier work on initial and repeat applications of growth regulators to strawberry receptacles, except that no difference between hormone used for re-treatment was noted in the earlier research (5,7,8). Growth of GA_3-and $GA_{4/7}$-induced fruit had stopped 12 days after treatment; their apparent decline in size through 30 days was probably a result of water loss (1). NAA-induced fruit continued growing through 30 days without re-treatment, contradicting an earlier report (5).

NAA and $GA_{4/7}$ stimulated IAA production, NAA being more effective than $GA_{4/7}$ at the concentration used. GA-induced parthenocarpic tomato fruit also produce significantly less IAA than auxin-induced fruit (6). The concentration of IAA had fallen in NAA-induced strawberry fruit harvested 12 days after treatment, and this decline was associated with a reduction in growth rate (Fig. 4). However, the results with $GA_{4/7}$ do not show a similar correlation.

Figure 4. Log free IAA concentration vs growth rate of parthenocarpic strawberry fruit induced with NAA or $GA_{4/7}$ at 10^{-3}M in 2% DMSO and 0.1% Tween 80 at 6 and 14 days after treatment. Auxin extracted from fruits collected 6 and 14 days after treatment with growth regulators; growth rates calculated for 0 to 6 and 6 to 14 days after treatment.


Growth rate (mm/d)
Log IAA conc. (ng/g)
r=0.75 n.s.
ŷ= -0.69+0.3x
Emasculated
NAA
GA4/7
0-6
6-14
0
2
4
0
2
3
4

Endogenous GA levels are higher in auxin-induced tomato fruit than GA-induced fruit (6), and fruit size is more closely associated with extractable IAA than GA (2). Tomato fruit induced with aqueous solutions of growth regulators do not stop growing, whereas strawberry fruits do, suggesting different systems of control in the two species.

GA levels were not measured in strawberry. NAA application obviously increased IAA levels in the treated tissue, but may also induce the production of GAs or cytokinins which supplement or synergize with NAA and/or IAA in promoting growth. $GA_{4/7}$, on the other hand, appears to be less effective than NAA in stimulating both IAA production and receptacle growth. The direct effects of the applied hormones on fruit development are difficult to separate from their indirect effects via stimulation of the synthesis of other hormones. In addition, NAA treatment may have affected IAA conjugate levels (4), as the enzymes responsible for NAA and, presumably, IAA conjugation are inducible by NAA treatment. Therefore, the mechanisms of action of both exogenous and endogenous hormones must be better understood before we can determine their roles in fruit development.

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SECTION III

EFFECTS OF STRAWBERRY ACHENE REMOVAL ON RECEPTACLE GROWTH AND FREE INDOLEACETIC ACID CONCENTRATION AND OF AUXINS AND GIBBERELLINS IN REPLACING ACHENES

Effects of Strawberry Achene Removal on Receptacle Growth and Free Indoleacetic Acid Concentration and of Auxins and Gibberellins in Replacing Achenes

Abstract

Complete achene removal 12 days after pollination stopped strawberry (Fragaria x ananassa Duch.) receptacle growth. Removal of achenes from half of the receptacle diminished growth as compared with that of intact fruit. Free IAA concentration in the receptacle tissue of intact fruit at 14 days after pollination was nearly equal to that in achenes. Removal of achenes significantly reduced the IAA content of receptacle tissue, complete removal being more effective than partial removal. The extent of achene removal and growth rates of receptacles were positively correlated with free IAA content.

Achenes were removed from some fruits 16 days after pollination, and the receptacles were treated with aqueous solutions of naphthaleneacetic acid (NAA) or gibberellins (GA_3, $GA_{4/7}$) at 10^{-3}M in 2% dimethylsulfoxide and 0.1% Tween 80. None of the growth regulators were as effective as were achenes in maintaining growth. NAA treatment of receptacles produced fruit 75% the size of controls, while fruit treated with GA_3 and $GA_{4/7}$ did not grow. Removal of achenes

24 days after pollination had no effect on enlargement of receptacles.

Removal of achenes early in fruit development results in cessation of strawberry receptacle growth (4,5,6); removal at later times stops growth and hastens fruit ripening (6). Replacing achenes with lanolin containing auxin or GA will maintain receptacle growth, GA being less effective than auxin (4,5,6,8). These observations suggest that growth is mediated by achene-derived hormones. Achenes are rich sources of hormones (3,5,7), but receptacle growth is not well correlated with endogenous hormone content of achene or receptacle tissue.

The purpose of this study was to determine if the free IAA concentration in the receptacle was affected by removal of achenes.

Materials and Methods

Plant material. Everbearing starwberry plants, 'Our Own', were obtained from Ahrens Nursery, Huntingburg, IN., in June 1980, potted in 22 cm diameter pots and placed in a glasshouse. They overwintered outdoors in a cold frame until April 1981 when they were brought into the glasshouse. Two- and three-year-old 'Midway' strawberry plants were dug from the Sodus Experiment Station, Sodus, MI, in early April 1981 and potted as were 'Our Own' plants.

The plants were maintained on a daily watering and bi-weekly fertilizer regime (20:20:20, Peters SoilTest). Flowers from both sets of plants produced between May and September, 1981, were used.

Pollination and removal of achenes. Primary, secondary, tertiary, and quaternary flowers were tagged at anthesis and hand-pollinated with pollen collected from plants used in the achene substitution study. Diameters of fruits were measured at 8 and 10 days after pollination. At 12 days after pollination, the following treatments were randomly assigned within position classes to individual fruit: (a) complete achene removal, (b) achenes removed from a radial half of the receptacle, (c) achenes intact. Following treatment, diameters of fruits were again measured. In treatment (b) the diameter measured was perpendicular to the line of demarcation between halves of the fruit with and without achenes. Two days after treatment (14 days after pollination), all fruits were measured, harvested, and frozen at -18°C. Following lyophilization, achenes were separated from receptacle tissue and all receptacles from a single treatment were pooled.

IAA analysis. Achene and receptacle tissue were analyzed for free IAA content as described in Section I.

Achene substitution. At 16 or 24 days after pollination of 'Our Own' flowers, achenes were gently scraped off the receptacle with a spatula. One of the following treatments was randomly assigned to each receptacle: control (dist. H_2O), and NAA, GA_3, and $GA_{4/7}$, each at 10^{-3} M. All solutions were adjusted to pH 7.0 with phosphate buffer and contained 2% DMSO and 0.1% Tween 80. Each fruit was dipped into the proper solution for ca. 10 s. The basal diameter of each fruit was measured across its narrowest basal width at 18, 24,

and 30 days after pollination. The few replications, 4 per treatment, resulted in large standard deviations; therefore, only ratios of final diameter to initial diameter are presented.

Results

Complete achene removal stopped receptacle growth (Fig. 1); removing half the achenes reduced growth in comparison with intact fruit. A slight decline in receptacle diameter occurred following complete achene removal of 'Our Own' fruit. The correlation between growth rate and the extent of achene removal was high ($r = 0.73$), but not significant. Two days after the treatments were applied, free IAA concentration in the receptacle tissue of intact fruit was high, similar to that of achenes (Fig. 1). Removal of achenes significantly reduced ($p < 0.05$) the IAA level in receptacle tissue, complete achene removal being more effective than partial removal. The free IAA content in the receptacle was significantly correlated ($r = 0.85$, $p < 0.05$), with the extent of achene removal. The growth rate of the receptacle between 12 and 14 days after pollination was highly correlated ($r = 0.91$; $p < 0.05$) with the free IAA content.

In the second experiment, complete achene removal 16 days after pollination stopped receptacle growth, and none of the hormones used to replace the achenes maintained growth equivalent to that of pollinated controls, although NAA-treated receptacles grew to a diameter 75% that of pollinated fruit (Fig. 2).

Neither achene removal 24 days after pollination nor substitution of hormones affected growth (data not shown).

Figure 1. Effect of achene removal on growth of (A,C) and free IAA concentration in (B,D) 'Midway' (A,B) and 'Our Own' (C,D) strawberry receptacles. Tissue extracted 14 days after pollination following treatment at 12 days for (a) intact fruit; (b) 1/2 achenes removed; (c) all achenes removed. Decline in diameters of fruit following removal of achenes at 12 days shown by dotted line. IAA not detectable (nd) in 'Our Own' receptacle tissue following complete achene removal.

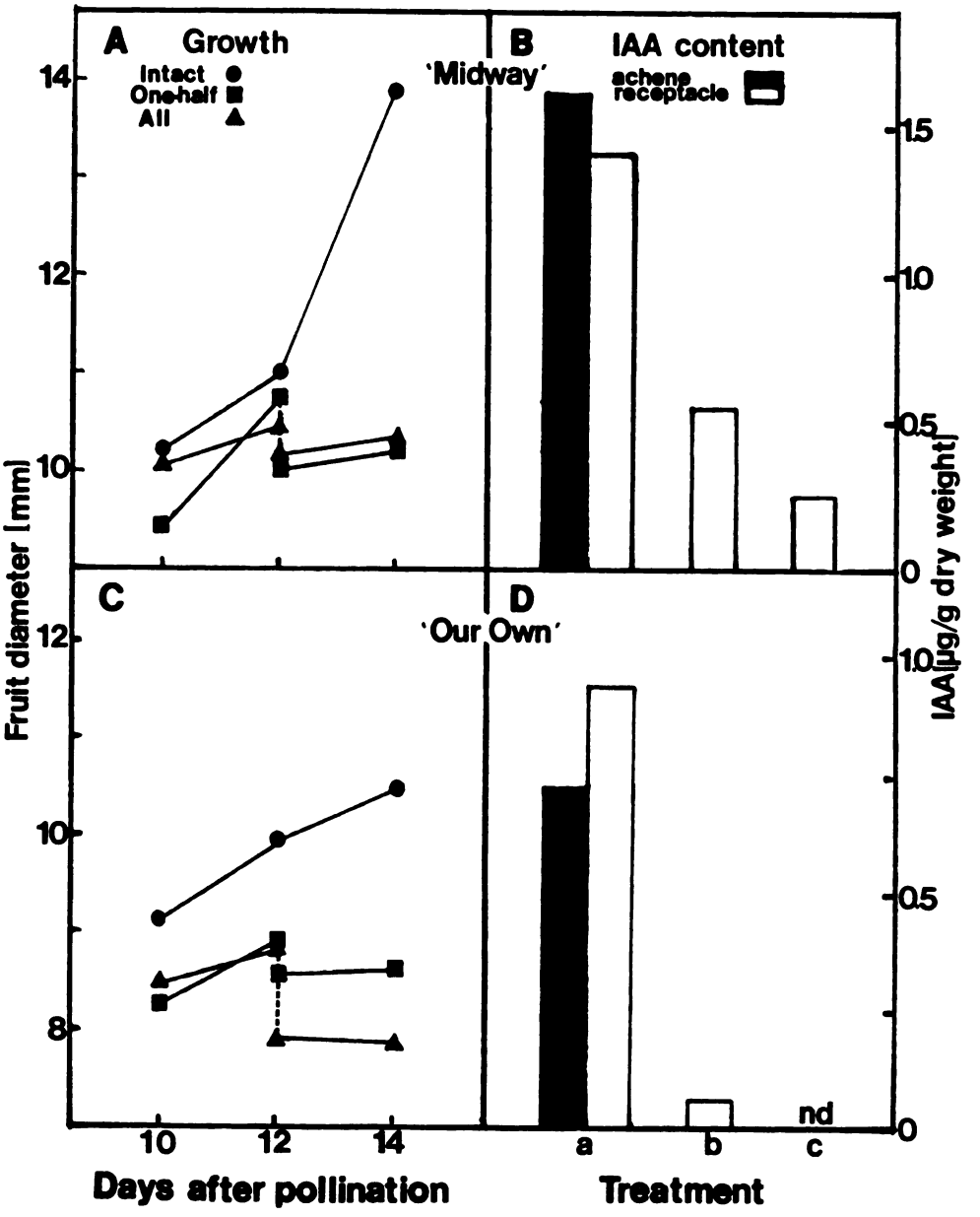
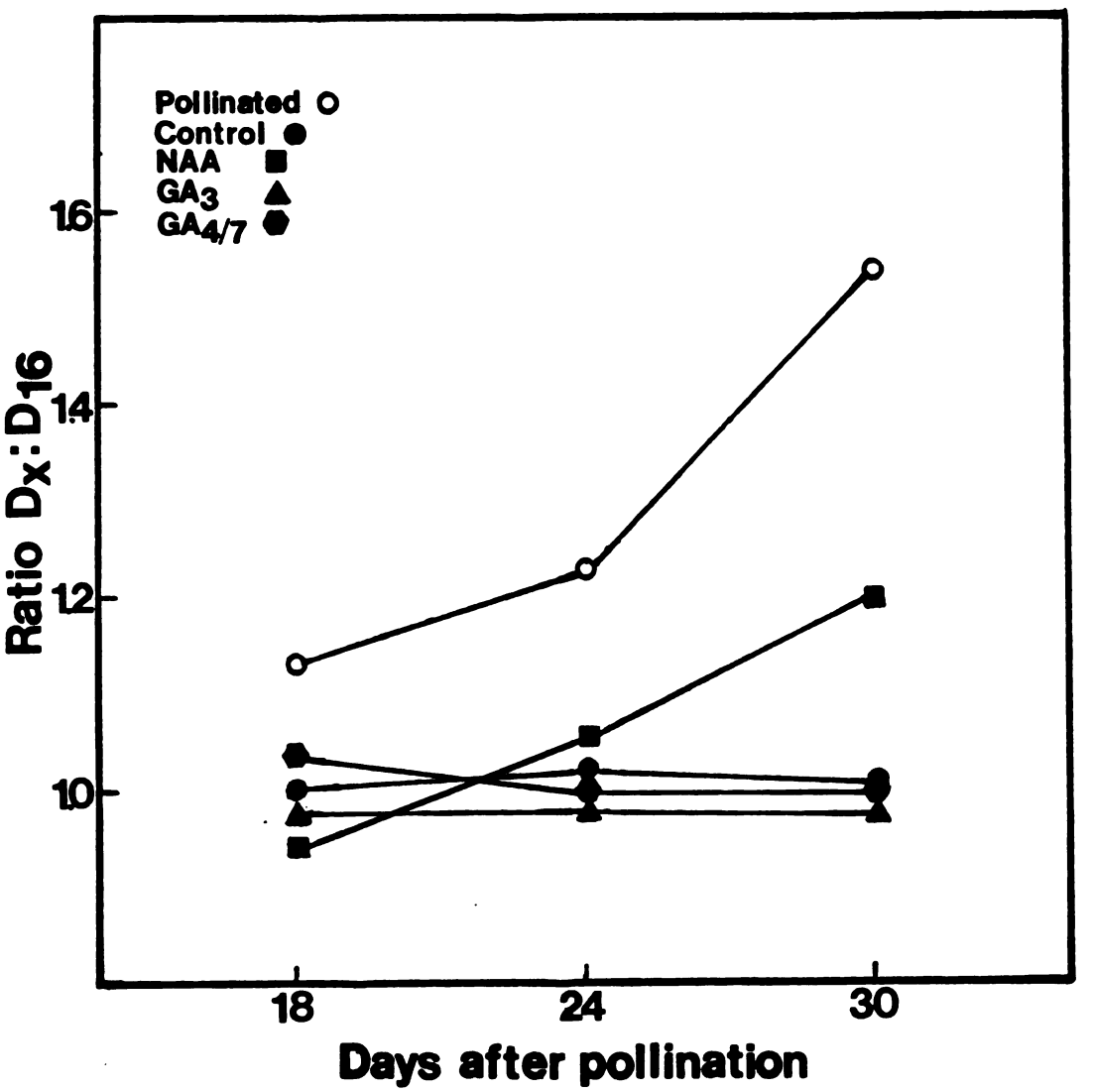

A Growth
Intact
One-half
All
B IAA content
'Midway'
achene
receptacle
C
D
'Our Own'
Fruit diameter [mm]
IAA [µg/g dry weight]
14
12
10
8
1.5
1.0
0.5
0
nd
10
12
14
a
b
c
Days after pollination
Treatment

Figure 2. Effect of removal of achene 16 days after pollination of 'Our Own' flowers and treatment of receptacles with aqueous solutions of growth regulators at 10^{-3}M in 2% DMSO and 0.1% Tween 80 upon the ratio of diameter (D_x) at 18, 24, or 30 days after pollination to diameter (D_{16}) at 16 days.


Pollinated
Control
NAA
GA3
GA4/7
1.6
1.4
1.2
1.0
Ratio Dx:D16
18
24
30
Days after pollination

Discussion

The data support the work of many others who have found that achene removal leads to cessation of fruit growth, and growth resumes if the proper growth regulators are substituted for the achene (4,5,6). Contrary to a previous report (8), neither GA_3 nor $GA_{4/7}$ were capable of replacing achenes 16 days after pollination in this study. The later date of removal here (16 vs 9 days) may have affected the response. Lis and Antoszewski (2) found that GA_3 treatment had no effect on movement of labelled assimilate into receptacles within 6 hrs following achene removal at 14 or 15 days after pollination. Endogenous GA levels are high in achene and receptacle tissue early in development and decline within 14 days of pollination (3); thus, the receptacle may only be responsive to GA treatment early in its development.

My data concerning IAA content of receptacle tissue following achene removal support the theory that the achenes supply IAA to the receptacle. The free IAA content of the receptacle at 14 days after pollination was highly correlated both with the extent of achene removal and with growth rate between 12 and 14 days after pollination. The receptacle undoubtedly contained IAA 12 days after pollination, and IAA metabolism, degradation, and/or conjugation in the receptacle probably continued through 14 days, as levels were very low in the absence of achenes. Because enlargement stopped immediately, growth may be a function of IAA pool size (declining following achene removal) or rate of IAA diffusion (stopped by achene removal) within

the receptacle (1), rather than of IAA metabolism, which should have continued for some time after achene removal.

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CONCLUSION

The hypothesis upon which these studies were based is that the growth of the strawberry receptacle is primarily controlled by auxin (IAA) produced by the achene. Because the precise roles of hormones in physiological processes are not known, there are many difficulties in interpreting results. Dennis (3,4) reviews the assumptions inherent in the accepted methods of analysis and hypotheses concerning the roles of hormones. No single approach can satisfactorily answer all the questions. Most physiological processes are probably mediated by hormonal interactions. Thus, study of a single hormone will never resolve all the pertinent issues. Jacobs (6) proposed a useful set of rules to determine whether a specific hormone controls a specific physiological process. Keeping the difficulties inherent in this approach in mind, these rules can be utilized to study the roles of hormones in natural systems. The data presented in this dissertation bring us closer to fulfilling some of these rules.

Parallel variation. This rule states that the hormone normally be present in the tissues in question and that the amount of the structure (fresh or dry weight, diameter, length) vary in a parallel manner with the amount of the hormone. It is based on the assumption that the pool size controls growth, which may or may not

be true (3,4). Rates of utilization, degradation, and/or conjugation by the target tissue may be more important than pool size. In addition,compartmentation may occur, leading to several pools of endogenous hormone each having a different function. Thus, measurement of the total amount of endogenous hormone would not yield information on the physiologically relevant pool.

The presence of IAA in both achene and receptacle tissue was verified by GC-MS in the present study. Growth of the receptacle was not well correlated with free IAA content, as has been shown by others (8,11,12). However, IAA concentration declined as the growth rate of NAA-induced fruit fell. Following complete achene removal, receptacle growth stopped and free IAA levels plummeted within 2 days. The decline in IAA levels in the receptacle paralleled the extent of achene removal. Thus, while studies of normal fruit development show that the correlation between changes in IAA level and growth rate is low, short-term studies indicate that growth rate parallels IAA level.

Source excision. Simply stated, removal of the presumed source of the hormone should result in the absence of the normal developmental phenomena, i.e., receptacle growth. This assumes that the 'source' is not a 'sink' for the hormone produced in a non-reproductive plant organ. Nitsch (10,11) was the first to demonstrate that the receptacle stopped growing following achene removal. Because IAA levels in the receptacle are well correlated with levels

in the achenes and decline after achene removal, the achene is probably the source of the IAA found in the receptacle.

Substitution. This rule states that if the pure chemical is substituted for the normal source of the hormone, the developmental event should occur. It implicitly means that the effective concentration of applied hormone is equivalent to its endogenous levels (3,4). A difficulty with this is determining the extent of uptake of the applied compound; although high concentrations may be applied, low levels may be absorbed. Also target tissue sensitivity may change with time such that the response to a given dose varies (4).

Following achene removal, auxins (10,11) and GAs (18) are capable of maintaining receptacle growth. Mudge et al. (7) determined that NAA and IBA were the most effective auxins in substituting for achenes; IAA was also effective, producing receptacles 75% the size of intact fruit. Data from the present study suggest that the time of replacement may influence the response. Substituting auxin for achenes at 24 days after pollination had no effect on receptacle size; replacement at 16 days was effective when auxins were used but not GAs. In an earlier report (13) a response occurred when GAs were applied 9 days after pollination. Thus, auxins are the most effective hormones in substituting for achenes, but they may not be the only active compounds.

Isolation. The ability to culture fruits allows one to isolate the system under study and demonstrate that the hormone has the

same effect *in vitro* as *in vivo*, thereby assuring that the event studied is not a secondary effect of another process in the intact plant.

In vitro work has demonstrated that NAA and NAAm in the supporting media will stimulate receptacle growth of unpollinated flowers (2,7) and overcome the inhibitory effect of unfertilized achenes. GAs are capable of supporting receptacle growth of unpollinated flowers *in vitro*, but growth of intact fruits is confined to the basal part of the fruit, which is devoid of achenes. Contradictory results (1,5,13) may be due to cultivar differences or methodology. Though the evidence is not conclusive, auxins appear to be the only hormone stimulating growth of the entire receptacle *in vitro*.

Generality. This rule assumes that many species have similar mechanisms of control, which may or may not be true. Extending the observed effects and role of auxins and IAA in controlling strawberry receptacle development to other species is difficult, though some such as tomato do respond to applied auxins.

Specificity. Another rule states that no naturally-occurring hormones other than IAA have the same effect on the observed event. This is not the case in strawberry or most other species. GA-like activity has been quantified in achene and receptacle extracts (8), and GAs stimulate receptacle growth under some conditions (13,14). Several auxin-like compounds other than IAA have been found in achene

extracts (12). Thus, hormones other than IAA probably play roles in controlling strawberry receptacle development.

Greater knowledge as to the levels of free and conjugated forms of hormones may indicate the importance of IAA in fruit growth. With the exception of the final two rules, a good case can be made for an auxin as the controlling factor in strawberry fruit development. A more detailed knowledge of the pathways of IAA utilization by the receptacle might clarify the remaining problems.

The presence of IAA conjugates in strawberry fruit suggests that they may provide a tool, when exogenously applied, with which to control fruit development in strawberry. The fact that levels of each form of conjugate change during development implies that these changes are related to the developmental process. Further studies of IAA utilization, metabolism, and conjugation in strawberry fruit tissue are necessary to determine the validity of this concept. In addition, studies on the levels of other hormones, free and bound, in fruit development and their effects on IAA physiology and biochemistry would lead to a better understanding of hormonal control of fruit development.

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