THE INFLUENCE OF N-(DIMETHYLAMINO)SUCCINAMIC ACID ON INFLORESCENCE INITIATION AND ENDOGENOUS GIBBERELLIN CONTENT OF RED WING AZALEA (*RHODODENDRON* CV.)

by

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ABSTRACT

Some anatomical and physiological aspects of inflorescence initiation in the Red Wing azalea (*Rhododendron* cv.) induced by N-(dimethylamino)-succinamic acid (B-9) treatment was studied. The anatomy of inflorescence initiation and development is described.

B-9 induces inflorescence initiation. Gibberellic acid (GA_3) completely reverses the B-9 effect, and prevents natural initiation.

The dwarf pea epicotyl bioassay of eluates from thin layer chromatograms of ethyl acetate extractions from immature leaves revealed one acidic and two basic gibberellin-like substances. A modified cucumber hypocotyl bioassay detected a third basic substance. Two of the basic gibberellin-like substances may be involved with inflorescence development.

B-9 does not act by decreasing the endogenous gibberellin levels. In fact, two basic gibberellin-like substances appear after B-9 treatment.

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INTRODUCTION

The growth retardant N-(dimethylamino)succinamic acid (B-9) has been reported to retard growth in many plants (9). It has been reported to induce floral initiation in azaleas (*Rhododendron* cv.) and some rhododendrons (9, 41, 78). The azalea Red Wing is one of the responsive cultivars. The effects of B-9 on floral initiation and the anatomy of this azalea have not been described. Naskalli (55) described the normal floral ontogeny of the azalea Coral Bells, a Kurume cultivar, Since Red Wing is a Pericat cultivar, the inflorescence initiation and ontogeny may be different.

The mechanism through which B-9 induces floral initiation is unknown. In other systems it has been hypothesized that growth retardants act by inhibiting the biosynthesis of endogenous gibberellins (1, 61). It is possible that B-9 may act in this way on azaleas since continued application of gibberellic acid (GA_3) has been found to prevent flowering (2, 15). The presence of endogenous gibberellins in azaleas, and their involvement with flowering, has not been investigated.

Accordingly, experiments were undertaken to study some of the anatomical and physiological aspects of inflorescence initiation and development in the Red Wing azalea.

REVIEW OF LITERATURE

The horticultural azaleas belong to the genus *Rhododendron*, in the family Ericaceae (20). The Belgian Indicas are a group of hydrids developed in Belgium from *Rhododendron simsii*, a native of central China and Yunnan. The Kurume Hybrids are a group of small evergreen azaleas developed on Kyushu Island from species involving *Rhododendron obtusum*, *Rhododendron obtusum* var. *kaempferi*, and *Rhododendron obtusum* var. *kiusianum*. The Pericat hybrids are a more recent group, derived from crossing a Belgian Indica and a Kurume azalea. The cultivar Red Wing is in this group. Red Wing is a rapidly growing evergreen azalea, with large, open, cerise-red flowers with ruffled petals and a petaloid calyx.

In 1962 a new plant growth retardant, N-dimethylaminosuccinamic acid (B995), was reported (70). This chemical has since appeared in the literature under a variety of names: N,N-dimethylaminosuccinamic acid, N-dimethyl succinamic acid, 1,1-dimethylhydrazide of succinic acid, B-995, B-9, B-Nine, and Alar. The correct chemical name, according to the Merck Index (43), is succinic acid 2,2-dimethylhydrazide. The next most acceptable name is N-(dimethylamino)succinamic acid. For the sake of simplicity, it will be referred to as B-9 hereafter. The main effect of B-9 has been growth retardation without toxicity over a wide range of plants. Inhibition of flower formation, which is reversed by gibberellic acid treatment, has been reported for the short day plant *Pharbitis nil* (84). When applied to vernalized *Oenothera biennis* L, a biennial with an absolute cold requirmeent for floral initiation, B-9 has retarded or prevented floral initiation (67). However, in azaleas, B-9 induces floral initiation (78), and is now used commercially to regulate azalea flowering (83).

A number of hypotheses have been advanced to explain the mechanism of B-9 growth retardation. These are: inhibitions which are not directly related to auxin or gibberellin metabolism (52); a blocking of gibberellin biosynthesis (17, 61); an interference with auxin metabolism (14, 21, 69); an uncoupling of oxidative phosphorylation (26); some mechanism which is GA_3 reversible, but does not involve gibberellin biosynthesis inhibition (46); or an inhibition of some non-hormonal aspect of growth (14).

A new group of plant growth hormones was established in 1935 when the Japanese chemist Yabuta crystallized the causative agent of the "bakanae" disease of rice, and named it gibberellin after the fungus *Gibberella fujikuroi* which produced it (77). It was not until the 1950's that western scientists began working on the gibberellins. By 1956 (68) and 1957 (65) the first isolations of gibberellin-like substances from higher plants were reported. Thereafter, gibberellins were isolated and chemically characterized in ever increasing numbers, until at the present time there have been 23 gibberellins isolated from higher plants or cultures of *Gibberella fujikuroi* and chemically characterized (36).

Until recently, only acidic plant extracts were tested for gibberellin activity, since the extraction procedures were based on that used to isolate the acidic fungal gibberellins (66). Water soluble (30, 47), neutral (23, 24), and most recently, basic gibberellin-like substances (27, 62) have been extracted from higher plants.

Immature seeds have been found to be a rich source of gibberellin-like substances, and it has been suggested that they synthesize these substances (16). Other evidence indicates that immature leaves (31, 39) and root tips (11, 31, 51) are sites of endogenous gibberellin production. Chloroplasts may also be sites of synthesis (76).

Gibberellin-like substances have been separated from solvent extracts by column chromatography (35), paper chromatography (58), and by thin layer chromatography (32). The most recent development is the combination of thin layer electrophoresis and chromatography employed by Jackson (27).

A number of the known gibberellins can be detected on chromatograms by ultraviolet fluorescence after spraying and heating with sulphuric acid (42). However, reports differ as to which gibberellins will respond to sulphuric acid (66).

Perhaps the most reliable means of detection is by means of a bioassay. Many bioassays have been developed, including rice seedling (59), barley endosperm (56), dwarf pea (64), lettuce hypocotyl (19), and cucumber hypocotyl (6). Most of these are very specific to growth induction by gibberellins. Most bioassay plants are more sensitive to some gibberellins than to others (7), and are sensitive in varying degrees to the specific gibberellins.

Gibberellin has been found to replace cold and long-day requirements in the flowering response of many plants, but usually can not substitute for short day requirements (37). Gibberellin has also induced flowering in a gymnosperm (63), and has prevented flowering in lilac (18). It has reduced the number of lateral inflorescences in Indianapolis Yellow chrysanthemums (10), and reduced the number of flowers when applied to Yellow Shasta chrysanthemums (3).

It has a profound effect on enzyme levels (25), and induces synthesis of at least one enzyme, α-amylase (82). Gibberellin may affect the sulfhydryl state of enzymes (44). It is involved in starch hydrolysis (54, 81) and transport of carbohydrates, and appears to be transported with the carbohydrates (13). Ion absorption and transport have also been influenced (40). The influence of gibberellin on the specific systems mentioned above may result in the more gross effects of gibberellin on cell division and elongation, stem growth, bolting and flowering, reversal of dwarfism and of red light stem growth inhibition (66), and changes in endogenous auxin levels (60).

MATERIALS AND METHODS

Plant Material

The Red Wing azaleas used in this study were grown from the time of propagation as cuttings in a 1:1 peat and sand potting mixture in an unshaded greenhouse. The plants were fertilized with a weak solution of 3 parts ammonium sulphate and one part 20-20-20 every 3 weeks during the summer, and every 4 weeks after September 1. The plants received natural day lengths. The minimum night temperature was maintained at 20°C, while the maximum day and night temperatures were uncontrolled. A similar regime was followed during the experimental peridd, which extended from May to August, 1968.

Time of B-9 Application

In an experiment designed to determine the effect of time of application of B-9 after shoot tip removal on inflorescence initiation, sixty-six plants, about one year old, were arranged randomly along a greenhouse bench, and the shoot tips were uniformly removed. A preliminary experiment had indicated that a large proportion of Red Wing plants initiate inflorescences naturally before the recommended time of spraying with B-9, at 5 weeks after shoot tip removal (83). Three groups of 6 plants were sprayed to run off with B-9 at 2500 ppm (0.25%) with 0.1% Tween 20 (sorbitan polyoxyethylene monolaurate) at 2, 3 and 4 weeks after shoot tip removal. Three similar groups of 6 plants were sprayed with the check solution, distilled water with 0.1% Tween 20. All treatments were applied once a week for 2 consecutive weeks.

Interactions of B-9 and GA_3

At 4 weeks after removal of shoot tips, the remaining plants were treated in groups of 6 with 2 different concentrations of B-9 and GA_3, and various combinations of B-9 with GA_3 (Table III). This experiment was performed to find the effects of B-9 at a higher concentration, and if GA_3 could reverse the effects of B-9 on flowering.

Before spraying, and each week until 7 weeks after shoot tip removal and again at 10 weeks, one bud was collected from each plant. The buds were dissected under a dissecting microscope, and ranked according to inflorescence stage (Table I).

The data were transformed using the Normal Score Transformation (38). Data concerning the time of treatment after shoot tip removal were analyzed for significance at the 5% level with the F-test. Data from experiments of B-9 and GA_3 treatments applied at 4 weeks after removal of shoot tips were analyzed at the 5% level of significance with the New Multiple Range Test (38).

Four Year Old Azaleas

An additional experiment was undertaken to study the levels of endogenous gibberellin-like substances during floral initiation, and whether or not these levels were influenced by B-9 treatment. A parallel study of the anatomy was carried out to determine if there were any correlations between endogenous gibberellin levels and inflorescence initiation. Twenty large Red Wing azaleas, about 4 years old, had shoot tips uniformly removed on each of July 8, 9, 10 and 11, 1968, to establish 4 replicate experiments. The plants were randomly arranged along a greenhouse bench in blocks of 10 control plants and

10 plants treated with B-9 at 2500 ppm Treatment was started at 4 weeks after removal of shoot tips, and spraying was carried out as before.

Ten buds were taken from the new growth on each plant at weeks 4, 5, 6, and 7 after shoot tip removal. Thus, a sample of 100 buds was obtained from each experiment block at each sampling date. One bud from each plant was immediately dissected and fixed in formalin-propionic acid-alcohol (FPA) (29). Air was evacuated from the tissue with a water aspirator for 10 minutes. After about one week, the FPA fixed material was dehydrated according to the tertiary butyl alcohol series (29) and embedded in Paraplast.

Serial sections 8µ thick were cut on a rotary microtome and stained with Heidenhain's haematoxylin-Orange G (28). The sectioned apices were ranked as to stage of inflorescence initiation and development (Table I).

A second bud from each plant was dissected and photographed through a dissecting microscope with Plus-X, 35 mm film, and ranked as to stage of inflorescence initiation and development (Table I). These data were combined with those obtained from the sectioned apices to get a mean of 20 stages for each treatment block. Statistical analysis of the means was carried out using the Sign Test (38).

All the leaves under 1 cm. in length which were removed from this bud were combined with the leaves dissected from the other 80 shoot tips collected within one treatment block, to provide a sample consisting of leaves from 90 buds for each block. The leaves were packaged in a folded #3 Whatman filter paper disc, frozen on dry ice for 30 minutes, and lyophilized for 24 hours on a Virtis freeze drier. The lyophilized tissue was stored at -11°C in a tightly capped jar.

Gibberellin-like Substance Extractions

The lyophilized tissue was weighed to the nearest 0.1 of a milligram, and ground through a #40 mesh screen in a Wiley mill. The dry powder was immediately extracted for gibberellin-like substances by a procedure modified from Pegg (62), as illustrated in Figure 1.

The tissue was shaken in a cork stoppered flask with 50 ml of 75% ethanol for 24 hours at room temperature. The ethanol was filtered off and stored in a refrigerator, and the residue extracted as before. The ethanol extracts were combined, and evaporated to the water phase under a moving air stream at room temperature. The water phase (25 ml) was adjusted to pH 2.5 with 1 N HCl and extracted in a separatory funnel with 3 equal volumes of ethyl acetate. The 3 aliquots of ethyl acetate were then each extracted individually once with equal volumes of 5% $NaHCO_3$, and the ethyl acetate combined to form the neutral fraction.

The water phase (25 ml) from the ethanol extract was adjusted to pH 8.2 using a saturated $NaHCO_3$ solution, and extracted with 3 equal volumes of ethyl acetate. The ethyl acetate was combined to make the basic fraction.

This water phase from the basic fraction was added to the 5% $NaHCO_3$ phase from the neutral fraction, and the whole acidified to pH 2.5 with 12 N HCl. This was extracted with 3 equal volumes (100 ml) of ethyl acetate. The combined ethyl acetate phase constituted the acidic fraction.

The water phase was discarded.

The ethyl acetate extracts were frozen in a deep freeze (-11°C) for at least 24 hours. Water containing impurities, such as pigments, froze to the bottom of the flask. The ethyl acetate was decanted into an

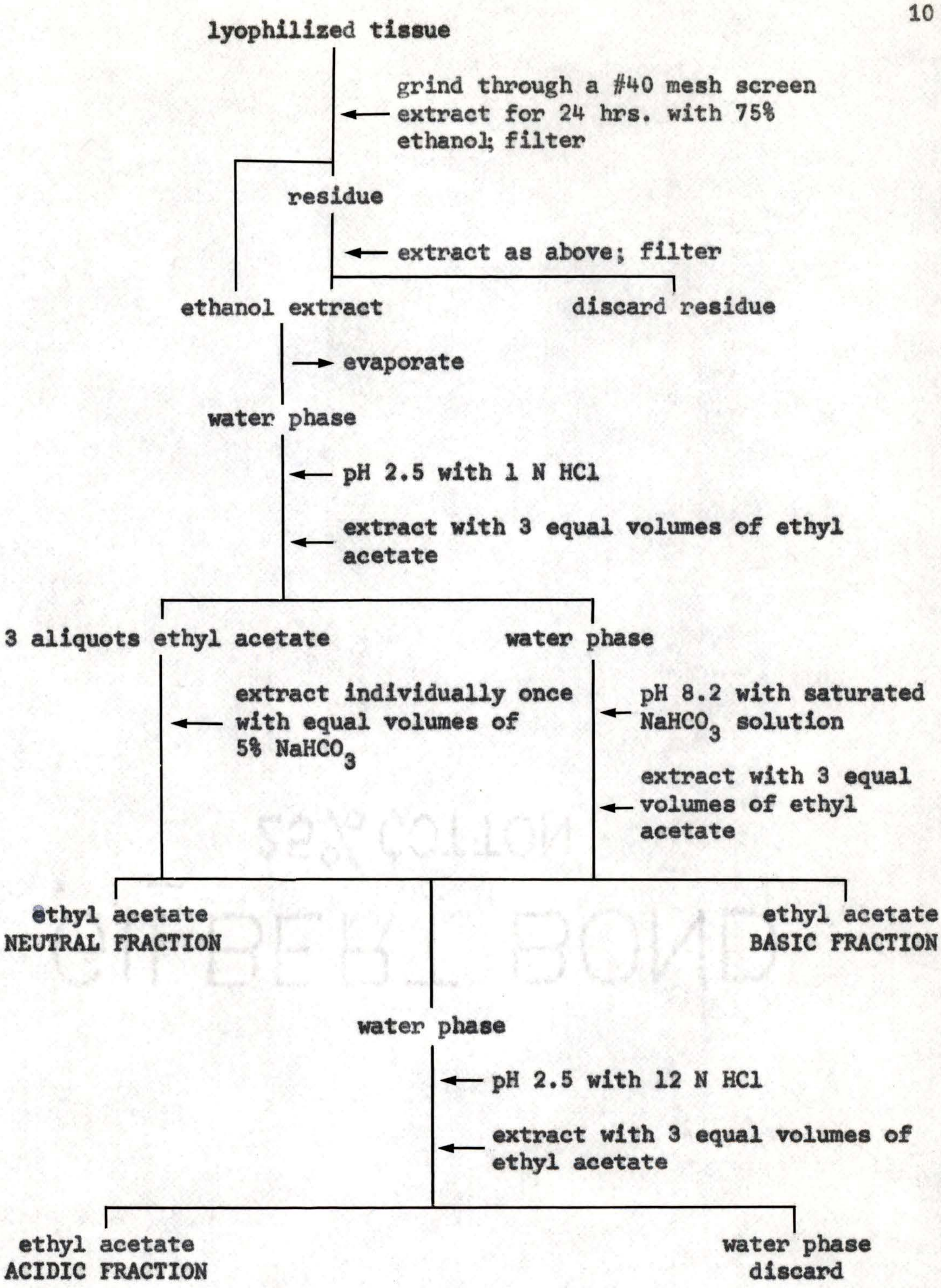


Fig. 1. Procedure for the extraction of gibberellin-like substances from the young leaves of Red Wing azaleas.

evaporating dish, and taken to dryness at room temperature under a moving air stream. The soluble residue was taken up in 2 ml of ethyl acetate, and stored in cork stoppered vials in the deep freeze.

For each block, 3 extractions were made: one from the control plants (Ck), one from the B-9 treated plants, and one from 75% ethanol. The ethanol extraction was necessary because it has been found that ethyl acetate contains growth promoting substances which are active in gibberellin bioassays (8).

Chromatography

Gibberellin-like substances in the extracts were separated by thin layer chromatography, using Silica Gel G, with benzene/n-butanol/acetic acid (80:15:5) (v:v:v) as the solvent (32). A Camag Chromatocharger was used to apply a uniform streak of 0.25 ml of extract to the plates. The applied extract was dried immediately in a forced air stream. The plates were developed to 10 cm , and dried in a moving air stream for 3 hours prior to dwarf pea bioassay. When the cucumber bioassay was used, the chromatoplates were dried for 24 hours, because the cucumbers were very sensitive to the developing solvent residues. The developed gel was divided into 10 equal R_f sections, which were scraped off the plates into 15 ml Nalgene centrifuge tubes and shaken vigorously with 2 ml of distilled water for 15 seconds. The suspension was centrifuged for 15 - 20 minutes, and the clear supernatant decanted into shell vials for bioassay.

Bioassays

Gibberellin-like substances in the extracts were detected with the dwarf pea epicotyl and cucumber hypocotyl bioassays.

Dwarf Pea Bioassay

The bioassay used was modified from that described by Phillips and Jones (64). Dwarf peas, variety Meteor, were surface sterilized for 20 minutes with 0.25% commercial bleach, and washed 3 times with distilled water. The sterilized peas were soaked for 4 - 6 hours at room temperature in distilled water. They were washed once more, and sown in aluminum trays filled with damp sterile vermiculite. After growth in darkness at 23°C for 6 days, the tops were severed from the roots and cotyledons, and thoroughly washed in distilled water. The epicotyls were cut to a standard length of 3.0 cm , measured from the base of the blade of the leaf subtending the apical bud. Ten epicotyls were put in each vial of chromatogram eluate. After 3 days of growth under continuous fluorescent light at about 20°C, the increase in length was measured to the nearest millimeter with a ruler.

Cucumber Bioassay

The cucumber bioassay used was based on the methods of Katsumi, Phinney and Purves (34). Cucumber seeds of the variety National Pickling, which had been powdered with Captan and Pyrethrum, were soaked for 2 - 3 hours in distilled water at room temperature. The imbibed seeds were washed 3 times with distilled water, and spread out on several layers of damp cheesecloth in plastic trays. The trays were covered with aluminum foil to prevent moisture loss. The seeds were incubated in darkness at 23°C for 4 days, at which time most of the hypocotyls were 2 cm. or more in length. The tops were snipped free of the roots with scissors, and washed in distilled water. The hypocotyls were cut to a standard length of 2.0 cm. measured from the base of the cotyledonary node. Ten hypocotyls were stood in each vial of chromatogram eluate. After 3 days growth at 27°C under a bank of combined fluorescent and incandescent lights, the increase in length was measured to the nearest millimeter with a ruler.

The growth responses of both the dwarf pea epicotyl and cucumber hypocotyl sections to GA_3 were determined by growing the sections in distilled water, and a series of GA_3 concentrations from 10^{-10} M to 10^{-3} M (Table V). The results were analyzed with the New Multiple Range Test (38).

The dwarf pea and cucumber hypocotyl bioassays were used because of their ease of application, and because the two plants differ in sensitivity to specific gibberellins. Dwarf peas are most sensitive to gibberellins A_3, A_1, and A_5. Cucumber hypocotyls are most sensitive to gibberellins A_7, A_4, and A_9 (7).

The 4 replicate means for each R_f value of the ethanol, check, and B-9 extracts were compared at the 5% level of significance using the New Multiple Range Test for the randomized block design (38).

In an attempt to detect the phytoactive substances by ultraviolet fluorescence, extracts showing biological activity were re-chromatographed, and the plates treated with 70% aqueous sulphuric acid, and 5% ethanolic sulphuric acid (60). Four replicate chromatoplates of standard GA_3 dissolved in ethyl acetate were similarly treated, and the average R_f of GA_3 was calculated.

In case B-9 might have been extracted from the treated plants, and might interfere with the bioassays, an aqueous solution of B-9 was chromatographed. The compound was made visible as brown spots by placing the chromatoplates in a closed chamber with subliming iodine (71).

RESULTS

Anatomy

The anatomy of the Red Wing azalea is very similar to that described for the azalea Coral Bells by Naskalli (55). His nomenclature of apical zonation and inflorescence structures will be followed here.

The Vegetative Apex

The vegetative apex (Stage 1, Table I), is a relatively small structure comprising the apical meristem and the young leaf primordia (Figs. 2, 3, and 13). The apical meristem, that portion of the apex above the youngest primordium, varies from dome shaped to almost flat, depending on the plastochron stage. The maximum height and width occur just before leaf initiation, and the minimum height and width just after leaf initiation.

The zonation appears to be the same as that for Coral Bells. Zone 1, the mantle, is usually composed of 2 layers of somewhat longitudinally elongated cells (Fig. 2). The mantle layers correspond to the tunica. Anticlinal divisions occur in the inner mantle layer. Zone 2, the sub-apical initials, is a lenticular group of isodiametric cells between the mantle and the pith. This zone corresponds to the corpus. Zone 3, the central meristem, consists of one or 2 layers of longitudinally flattened cells just below the sub-apical initials. They give rise to the rectangular pith cells. Zone 4, the peripheral meristem, consists of a cylinder of cells inside the protoderm and enclosing the central meristem. The cortex and procambium arise from cells in this zone.

Leaf primordia are formed from cells in the mantle layers and sub-apical initial zone. The primordia are triangular in cross section

Table I. The stages of inflorescence initiation and development used for ranking Red Wing azalea shoot tips.

Stage	Characteristics
1	Vegetative apex, relatively small and flat. (Figs. 2, 3, and 13)
2	Inflorescence apex, relatively tall and domed. From the earliest clearly recognizable stage to just before floral apex formation. May have 1-6(7) bud scales. (Figs. 4 and 14)
3	1-3(4) floral apices present, with or without floral bracts. (Figs. 5-8 and 15)
4	Flower primordium with sepal primordia. From this stage on, an average of the stages of the flower primordia in the bud is taken as the inflorescence stage. (Fig. 9)
5	Flower primordium with petal primordia. (Fig. 16)
6	Flower primordium with stamen and carpel primordia, prior to elongation of the floral parts. Petal primordia folded over the inner parts. (Figs. 11 and 17)
7	Floral parts elongating; petals erect and open at the mouth of the flower. (Fig. 18)

and spirally arranged (Fig. 2). A shell meristem forms in the axil of each leaf primordium (Fig. 4). Continued cell divisions and enlargement in the shell meristem give rise to an axillary shoot apex.

The Inflorescence Apex

After floral induction, the vegetative apex undergoes transition to an inflorescence apex (Stage 2, Table I). The pith cells change from rectangular to isodiametric, to vertically elongate. The inflorescence apex is highly domed, and is as tall or taller than its width (Figs. 4, 5, 14, and 15). Most of the zonation present in the vegetative apex becomes disrupted, except for the mantle layers. This disruption is probably caused by the rapid growth and primordium formation of the inflorescence apex. Zonation of the vegetative apex is also rarely discernible when the apex is in a state of rapid growth.

The inflorescence apex forms bud scale primordia in the same manner as leaf primordia are formed. They are thinner than leaf primordia and tend to wrap around the apex and overlap laterally (Fig. 6). As each bud scale forms, a large shell meristem initiates in its axil (Fig. 4). Each of these is a potential floral apex. Unlike Coral Bells, in which the inflorescence apex may cease activity after the first bud scale and shell meristem are formed, the inforescence apex of Red Wing continues to form bud scales and shell meristems. The usual number of bud scales formed is 6 or sometimes 7, and more rarely 8. Only the shell meristems in the axils of the last 2 or 3 bud scales formed will develop into floral apices. The first formed shell meristems remain undeveloped, except for the formation of small bracts.

The Floral Apex

The shell meristems in the axils of the last 2 or 3 bud scales enlarge rapidly and form floral apices (Stage 3, Table I). They occasion-

Figures 2-7

Figure 2. Median longitudinal section through an actively growing vegetative apex (Stage 1), showing zonation. X200

Figure 3. Cross section of a vegetative bud (Stage 1) showing the spiral leaf arrangement. X200.

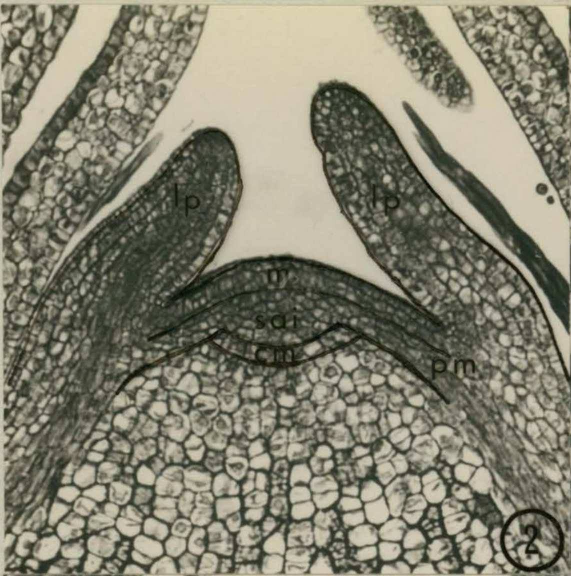
Figure 4. Longitudinal section of a reproductive bud (Stage 2), showing the inflorescence apex, bud scales, and a shell meristem in the axil of a bud scale. This shell meristem is a potential floral apex. The shell meristem in the axil of the leaf is a potential axillary vegetative bud. X200.

Figure 5. Longitudinal section of an inflorescence apex with a floral apex developing from a shell meristem (early Stage 3). X200.

Figure 6. Transverse section of an inflorescence apex initiating floral apices (Stage 3). X135.

Figure 7. Longitudinal section of a floral apex with a bract primordium (late Stage 3). X135.

brp = bract primordium
bsp = bud scale primordium
cm = central meristem
fa = floral apex
ia = inflorescence apex
l = leaf
lp = leaf primordium
m = mantle
pm = peripheral meristem
sai = sub-apical initials
sm = shell meristem
va = vegetative apex



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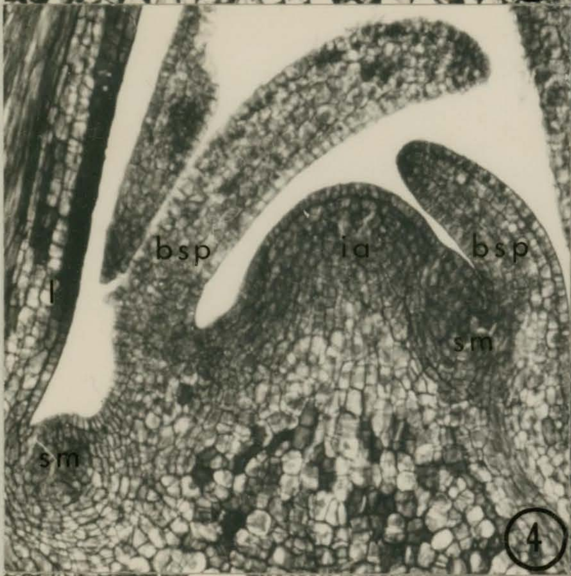

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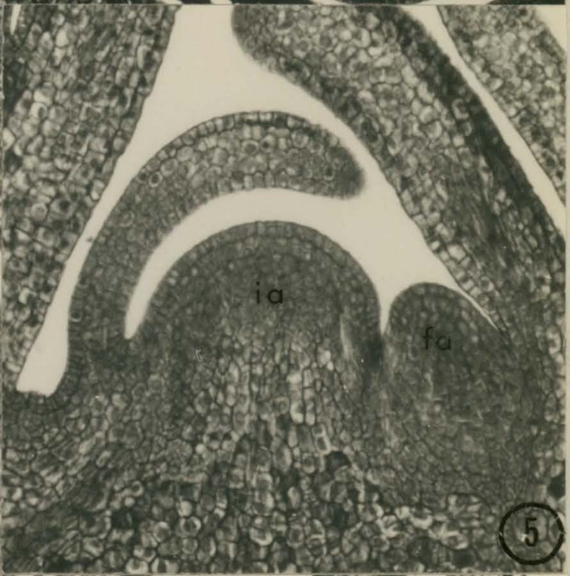

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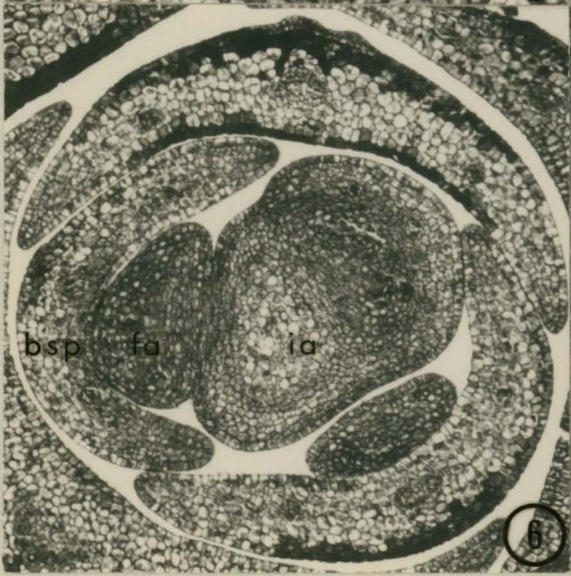

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ally exhibit zonation like that of the vegetative apex, (Figs. 5 , 6 , and 9). As soon as a floral apex has formed, it initiates 2 bracts. These are different in structure from the broadbased bud scales, being tall, spathulate, and hispid. They are always formed from the sides of the floral apex adjacent to the side which is adaxial to the inflorescence apex (Figs. 8 and 15). These structures do not occur in Coral Bells (55).

The floral apex and its bracts grow rapidly, so that the floral apex becomes much taller and broader than the inflorescence apex. The 2 bracts may become twice the height of the floral apex. The upper parts of the bracts arch over the developing floral apex. At first the floral apex has a broad, domed apical meristem but this flattens out to a broad plate prior to floral organ initiation.

The sepals (Fig. 9) are initiated separately in a compressed helix in the same manner as leaf primordia, high on the flanks of the floral apex (Stage 4, Table I). Very shortly after initiation, the furrows separating the sepals from the floral apex join laterally to form an undulating calyx ridge with 5 rounded points (Fig. 16). Shortly after calyx formation, the sepals almost cease developing, and remain small and undifferentiated until well after all the other floral organs have formed.

Once the calyx has formed, a total of 5 petal primordia are initiated in a helix alternating with the sepal helix, (Stage 5, Table I) (Fig. 16). They grow rapidly as broad, but separate, primordia.

Once the petals have enlarged enough to arch over one another, a helix composed of 5 stamen primordia is initiated opposite the sepals. The 5 separate carpel primordia form almost immediately in a helix

Figures 8-13

Figure 8. Transverse section of an inflorescence apex and 3 floral apices (Stage 3) showing the orientation of the bract primordia. X135.

Figure 9. Longitudinal section of a remnant apex and a floral apex initiating sepals (Stage 4). X135.

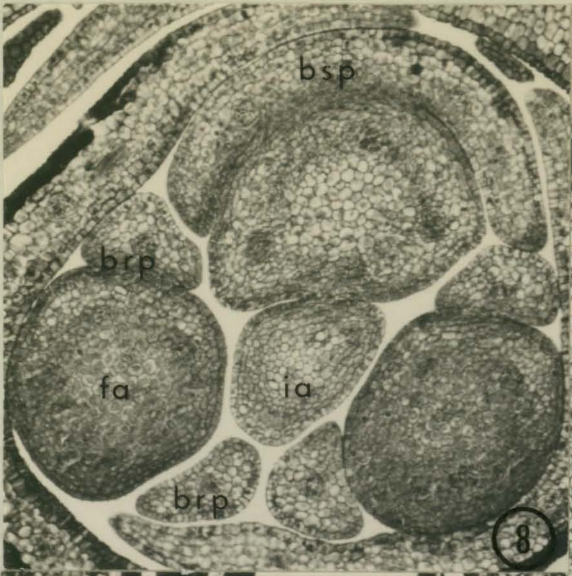
Figure 10. Longitudinal section of a remnant apex showing elongate, vacuolate cells extending almost to the summit. X200.

Figure 11. Longitudinal section of an early Stage 6 flower primordium with the petal primordia curving over the stamen and carpel primordia. Note the meristematic plate remaining between the carpel primordia. X135.

Figure 12. Longitudinal section of a young Stage 7 flower showing the elongating style and the space remaining between the carpel primordia. X75.

Figure 13. Lateral view of an intact vegetative apex (Stage 1). X73.

br = bract
brp = bract primordium
bsp = bud scale primordium
cp = carpel primordium
fa = floral apex
ia = inflorescence apex
pep = petal primordium
ra = remnant apex
sep = sepal primordium
stp = stamen primordium
sy = style



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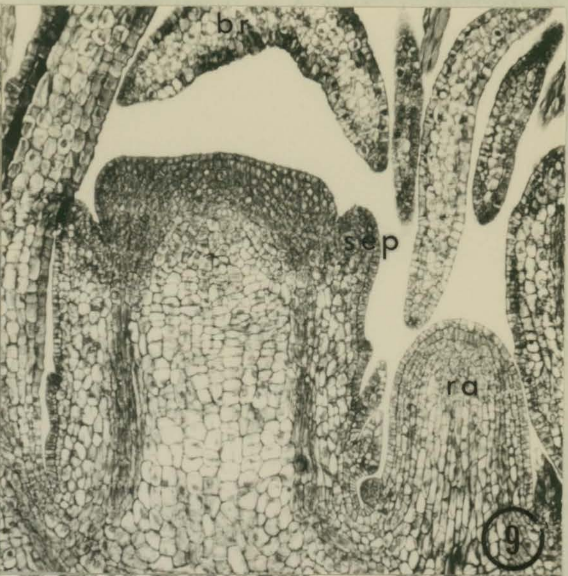

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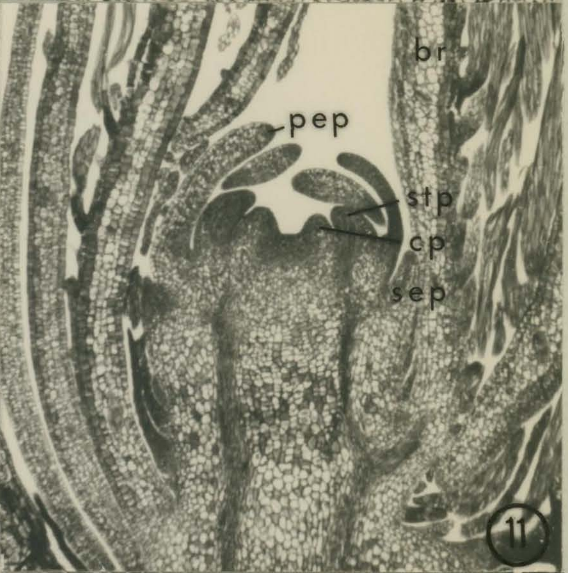

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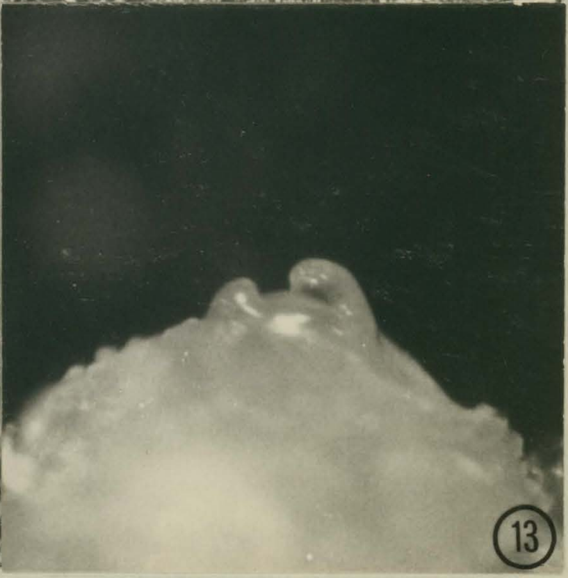
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alternating with the stamens (Stage 6, Table I, Fig. 11).

After carpel initiation, a small flat portion of the floral apex remains, indicating that all the floral organs are lateral appendages, as are the flowers themselves.

The stamens and carpels develop rapidly under the enlarging, overarched petals. Once the styles form, they elongate swiftly, grow together, and appear to push the petals apart, making them stand upright (Stage 7, Table I, Figs. 12 and 18). The space between the carpels is still present at this stage. The calyx does not develop until later. The tissue at the base of the petals eventually develops into a corolla tube.

The Remnant Apex

At any time after the first floral apex is initiated the inflorescence apex may lose its vigour and begin transition to a dormant remnant apex. The inflorescence apex may cease development without forming any primordia, but often it forms a small abnormal bud scale or a hair-like process. If the inflorescence apex is quite large after the floral apices which will develop have initiated it may produce a small bud scale with a tiny floral apex in the axil. Rarely, this floral apex forms bracts, or even sepal primordia before becoming quiescent. This type of development seems to occur more often when the plant is treated with B-9. The inflorescence apex never develops into a rudimentary flower bud or a perfect terminal flower as with Coral Bells (55).

The remnant apex is characteristically a tall, narrowly cylindrical dome with vertically elongated, vacuolate cells extending almost to the summit (Figs. 9 and 10). The remnant apex remains alive after all the floral apices are initiated, and is discernible with a hand lens in the

Figures 14-19

Figures 14-19.	Lateral views of dissected buds showing stage of flower bud development.
Figure 14.	An inflorescence apex with second and third bud scales (Stage 3). X73.
Figure 15.	Inflorescence apex and 2 floral apices forming bracts (Stage 3). X73.
Figure 16.	A flower primordium showing the calyx and the initiation of petal primordia (early Stage 5). X73.
Figure 17	Two Stage 6 flower primordia with one bract each removed. X27.
Figure 18.	A partially dissected Stage 7 flower (right) and an older flower. X16.
Figure 19.	Typical Red Wing inflorescence with 2 flowers. Note the petaloid calyx. X½.

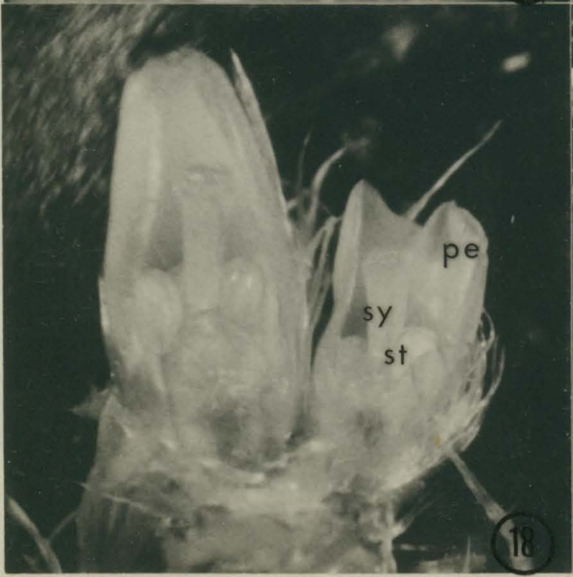
br = bract
fa = floral apex
ia = inflorescence apex
pe = petal
st = stamen
sy = style


14


fa
ia
15


16


br
br


pe
sy
st
18


19

newly opened flower bud between the pedicels of the flowers.

The typical Red Wing inflorescence consists of 2 flowers (Fig. 19). Buds with 1 or 3 flowers are less common. Because they are initiated sequentially in a spiral the flowers often mature at different times, particularly in buds with 3 flowers.

The stages described above were selected because they could be easily distinguished under a dissecting microscope. They do not necessarily represent developmental phases equally spaced in time, or of equal importance.

Time of B-9 Application

When one year old Red Wing azaleas were treated with 2 consecutive weekly sprays of B-9, beginning at 2 or 4 weeks after shoot tip removal, inflorescence initiation occurred at 2 weeks after the last B-9 spray (Table II). Inflorescence initiation occurred 1 week after the last spray when B-9 treatment was applied at 3 weeks after shoot tip removal. The most rapid rate of inflorescence development occurred when plants were treated at 4 weeks after removal of shoot tips. The control plants remained vegetative until 10 weeks or more after shoot tip removal.

Interactions of B-9 and GA_3

In this experiment, B-9 at 2500 ppm and 7500 ppm applied for 2 consecutive weekly sprays beginning at 4 weeks after shoot tip removal induced inflorescence initiation within 1 week after the last spray (Table III). There was no statistically significant difference between the 2 concentrations of B-9.

Treatment with GA_3 alone or in combination with B-9 seemed to prevent inflorescence initiation. Plants treated with the combinations of GA_3 at 200 ppm with B-9 at 7500 ppm were morphologically almost

Table II. The effect of treatment with N-(dimenthylamino) succinamic acid (B-9)[1] at 2500 ppm beginning at 2, 3, and 4 weeks after shoot tip removal on the inflorescence initiation and development of one year old Red Wing azaleas.

WEEKS AFTER SHOOT TIP REMOVAL	AVERAGE STAGE OF INFLORESCENCE					
	2 Weeks		3 Weeks		4 Weeks	
	Ck	B-9	Ck	B-9	Ck	B-9
2	1.0[2]	1.0				
3	1.0	1.0	1.0	1.0		
4	1.0	1.2	1.0	1.0	1.0	1.0
5	1.0	2.3*	1.0	1.8*	1.0	1.1
6	1.0	2.5*	1.0	2.0*	1.0	1.6
7	1.0	3.6*	1.0	3.2*	1.0	2.1*
10	1.2	5.5*	2.3	5.8*	1.8	5.1*

[1] *Succinic acid 2,2-dimethylhydrazide.*

[2] *Each value is the mean of 6 observations. Treatment means followed by an asterisk are significantly different from their respective controls at the 5% level by the F-test.*

Table III. The influence of different concentrations of N-(dimethylamino) succinamic acid (B-9)[1] and gibberellic acid (GA_3), and their combinations, on inflorescence initiation and development of one year old Red Wing azaleas treated at 4 weeks after shoot tip removal.

TREATMENT	WEEKS AFTER SHOOT TIP REMOVAL 4	5	6	7	10
Ck	1.0 a[2]	1.0 a	1.0 a	1.0 a	1.8 b
B-9 (2500 ppm)	1.0 a	1.1 a	1.6 b	2.1 b	5.1 c
B-9 (7500 ppm)	1.0 a	1.3 a	2.3 b	2.7 b	5.6 c
GA_3 (100 ppm)	1.0 a	1.0 a	1.0 a	1.0 a	1.0 a
GA_3 (100 ppm) +B-9 (2500 ppm)	1.0 a	1.0 a	1.0 a	1.0 a	1.0 a
GA_3 (100 ppm) +B-9 (7500 ppm)	1.0 a	1.0 a	1.0 a	1.0 a	1.3 ab
GA_3 (200 ppm.) +B-9 (7500 ppm)	1.0 a	1.0 a	1.0 a	1.0 a	1.0 a

---

[1] *Succinic acid 2,2-dimethylhydrazide.*

[2] *Each value is the mean of 6 observations. Values followed by a different letter or letters are significantly different within the same week at the 5% level.*

indistinguishable from those treated with GA_3 alone. None of these plants showed any indication of flowering within the period of the experiment. Plants treated with GA_3 at 100 ppm and B-9 at 7500 ppm were almost indistinguishable from the controls, and began to initiate inflorescences at about the same time as the control plants.

Four Year Old Azaleas

The 4 year old azaleas treated 4 and 5 weeks after shoot tip removal for growth substance extraction initiated inflorescences one week after the second spray with B-9 at 2500 ppm (Table IV).

Gibberellin-like Substance Extractions

Table V shows the growth responses of Meteor peas and National Pickling cucumbers to a series of GA_3 concentrations.

Acidic Fraction

The Meteor pea bioassay revealed the presence of a gibberellin-like substance in the acidic extract. These substances appeared at R_f 0.3-0.4 at 5 weeks after shoot tip removal; R_f 0.2-0.3 at 6 weeks after shoot tip removal and spread from R_f 0.0-0.5 in the B-9 extracts, and 0.1-0.5 in the control extracts at 7 weeks after shoot tip removal (Table VI). There were no significant differences between extracts from control plants and those treated with B-9, except in R_f 0.0-0.1 at 7 weeks after removal of shoot tips. A phytoactive substance was present in the B-9 treated plants, but not in the control plants.

None of the phytoactivity of this fraction extracted from plants at 7 weeks after shoot tip removal was detected by the National Pickling cucumber bioassay.

Table IV. The effect of treatment with N-(dimethylamino) succinamic acid (B-9)[1] (2500 ppm) at 4 weeks after shoot tip removal on inflorescence initiation and development of 4 year old Red Wing azaleas.

WEEKS AFTER SHOOT TIP REMOVAL	AVERAGE STAGE OF INFLORESCENCE	
	Ck	B-9
4	1.01[2]	1.00
5	1.05	1.13
6	1.36	2.04*
7	1.74	3.04*

---

[1] *Succinic acid 2,2-dimethylhydrazide.*

[2] *Each value is the mean of 4 replicates of 20 apices. Values followed by an asterisk are significantly different from their respective controls at the 5% level by the Sign Test.*

Table V. Linear growth response in centimetres of Meteor pea epicotyl and National Pickling cucumber hypocotyl sections to gibberellic acid (GA_3) concentrations over 3 days in the light. Temperature during the growth period was 20°C for the pea sections, and 27°C for the cucumber sections.

GA_3 Concentration *(M)*	Meteor pea	National Pickling cucumber
H_2O	0.93 a[1]	1.34 a
10^{-10}	0.85 a	1.38 ab
10^{-9}	0.86 a	1.31 a
10^{-8}	1.13 b	1.13 a
10^{-7}	1.36 c	1.10 a
10^{-6}	1.53 d	1.15 a
10^{-5}	1.57 d	1.70 b
10^{-4}	1.51 d	2.38 c
10^{-3}	1.56 d	2.45 c

[1] *Each value is the mean of 4 replicates of 10 sections. Means followed by a different letter or letters are significantly different at the 5% level.*

Table VI. Linear growth in centimetres of Meteor pea epicotyl sections exposed to thin layer chromatogram region (R_f) eluates of the acidic extract from immature leaves of Red Wing azalea treated with N-(dimethylamino)succinamic acid (B-9).[1]

WEEKS AFTER SHOOT TIP REMOVAL

	WEEK 4			WEEK 5		
R_f	EtOH	Ck	B-9	EtOH	Ck	B-9
0.0-0.1	0.85 a[2]	0.85 a	0.85 a	0.70 a	0.83 a	0.72 a
0.1-0.2	0.93 a	0.89 a	0.84 a	0.70 a	0.94 a	0.83 a
0.2-0.3	0.84 a	0.85 a	0.87 a	0.88 a	1.15 a	1.18 a
0.3-0.4	0.89 a	0.87 a	0.87 a	0.91 a	1.45 b	1.17 ab
0.4-0.5	0.84 a	0.97 a	0.95 a	0.79 a	1.13 a	0.99 a
0.5-0.6	0.92 a	0.91 a	0.92 a	0.75 a	0.94 a	0.90 a
0.6-0.7	0.95 a	0.92 a	0.83 a	0.83 a	0.98 a	0.97 a
0.7-0.8	0.92 a	0.90 a	0.86 a	0.78 a	0.74 a	0.90 a
0.8-0.9	0.94 a	0.84 a	0.82 a	0.75 a	0.88 a	0.91 a
0.9-1.0	0.87 a	0.92 a	0.86 a	0.74 a	0.80 a	0.87 a

	WEEK 6			WEEK 7		
R_f	EtOH	Ck	B-9	EtOH	Ck	B-9
0.0-0.1	0.77 a	0.86 a	0.83 a	0.69 a	0.72 a	0.83 b
0.1-0.2	0.76 a	0.93 a	0.86 a	0.75 a	0.83 ab	0.96 b
0.2-0.3	0.76 a	1.11 b	1.17 b	0.88 a	1.13 b	1.30 b
0.3-0.4	0.93 a	1.15 a	0.99 a	0.70 a	1.05 b	1.04 b
0.4-0.5	0.84 a	0.87 a	0.83 a	0.74 a	0.89 b	0.83 ab
0.5-0.6	0.77 a	0.80 a	0.80 a	0.70 a	0.73 a	0.73 a
0.6-0.7	0.76 a	0.81 a	0.76 a	0.76 a	0.78 a	0.83 a
0.7-0.8	0.71 a	0.78 a	0.76 a	0.72 a	0.77 a	0.79 a
0.8-0.9	0.77 a	0.85 a	0.77 a	0.72 a	0.78 a	0.87 a
0.9-1.0	0.74 a	0.77 a	0.82 a	0.76 a	0.87 a	0.82 a

[1] *Succinic acid 2,2-dimethylhydrazide.*

[2] *Each value is a mean of 4 replicates of 10 epicotyls. Means followed by a different letter or letters are significantly different at the 5% level within each* R_f *region and week.*

Basic Fraction

The Meteor pea bioassay detected significant amounts of phytoactivity in the extracts from B-9 treated plants at 7 weeks after shoot tip removal (Table VII). These gibberellin-like substances occurred at R_f 0.4-0.5 and R_f 0.8-0.9. They were absent in the extract from control plants.

The cucumber bioassay revealed an additional basic gibberellin-like substance at 7 weeks after shoot tip removal at R_f 0.1-0.2, but the cucumbers did not respond to the substances detected by the dwarf peas (Table VIII). There were no significant differences in bioassays of extracts from control and B-9 treated plants.

Neutral Fraction

The neutral fraction of extracts from 7 weeks after shoot tip removal failed to show any phytoactivity with either bioassay.

Attempts to observe the growth promoting substances on the chromatoplates with ultraviolet fluorescence induced by sulphuric acid sprays were unsuccessful.

The R_f of GA_3 dissolved in ethyl acetate was 0.37. The published R_f in the solvent used is 0.19 (32).

B-9 remained in the region of R_f 0.0-0.1.

Table VII. Linear growth in centimetres of Meteor pea epicotyl sections exposed to thin layer chromatogram region (R_f) eluates of the basic extract from immature leaves of Red Wing azalea treated with N-(dimethylamino) succinamic acid (B-9)[1] at 7 weeks after shoot tip removal.

R_f	EtOH	Ck	B-9
0.0-0.1	0.75 a[2]	0.82 a	0.79 a
0.1-0.2	0.79 a	0.77 a	0.84 a
0.2-0.3	0.82 a	0.81 a	0.83 a
0.3-0.4	0.74 a	0.82 a	0.86 a
0.4-0.5	0.74 a	0.76 a	0.88 b
0.5-0.6	0.82 a	0.81 a	0.80 a
0.6-0.7	0.81 a	0.83 a	0.85 a
0.7-0.8	0.80 a	0.77 a	0.85 a
0.8-0.9	0.72 a	0.78 a	0.85 b
0.9-1.0	0.71 a	0.78 a	0.79 a

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[1] *Succinic acid 2,2-dimethylhydrazide.*

[2] *Each value is a mean of 4 replicates of 10 epicotyls. Means followed by a different letter or letter are significantly different at the 5% level within each R_f region.*

Table VIII. Linear growth in centimetres of National Pickling cucumber hypocotyl sections exposed to thin layer chromatogram region (R_f) eluates of the basic extract from immature leaves of Red Wing azalea treated with N-(dimethylamino) succinamic acid (B-9)[1] at 7 weeks after shoot tip removal.

R_f	EtOH	Ck	B-9
0.0-0.1	0.90 a[2]	0.94 a	0.94 a
0.1-0.2	0.87 a	0.98 b	1.07 b
0.2-0.3	0.90 a	0.99 a	0.93 a
0.3-0.4	0.92 a	1.00 a	0.98 a
0.4-0.5	0.91 a	1.00 a	0.87 a
0.5-0.6	0.93 a	1.00 a	0.95 a
0.6-0.7	0.98 a	1.03 a	0.98 a
0.7-0.8	0.91 a	1.04 a	0.97 a
0.8-0.9	0.97 a	0.98 a	0.93 a
0.9-1.0	0.92 a	0.98 a	0.91 a

---

[1] *Succinic acid 2,2-dimethylhydrazide.*

[2] *Each value is a mean of 4 replicates of 10 hypocotyls. Means followed by a different letter or letters are significantly different at the 5% level within each R_f region.*

DISCUSSION

The data indicate that B-9 will induce inflorescence initiation in Red Wing azaleas that would otherwise have remained vegetative (Tables II and III). It is effective on the new growth when applied as early as 2 weeks after shoot tip removal. It is possible that growers could safely treat plants immediately after shoot tip removal to get earlier flower initiation and more compact plants.

When treated with either 2500 ppm or 7500 ppm B-9, the one-year-old plants initiated inflorescences one or 2 weeks after the last spray. The variation in time of initiation did not seem to be related to the time of B-9 treatment after shoot tip removal and may have been due to sampling discrepancies, or the small sample size.

The 4 year old plants were treated later in the summer when they were beginning to form inflorescences naturally (Table IV). These plants produced inflorescences one week after the last B-9 application. Red Wing azaleas appear to remain vegetative in the spring and early summer, and begin forming flowers in late July and early August. Plants treated while in vigorous vegetative growth, as were the one year old azaleas, appear to require more B-9 to induce initiation. It may be advisable for commercial azalea growers to adjust the concentration of B-9 and the number of applications to the time of year. Age of the plants may also be important.

Gibberellic acid prevents both natural and B-9 induced floral initiation (Table III). Considering the morphological observations, it seems probable that B-9 could counter the GA_3 effect, and induce flowering if the concentrations of the two chemicals were in the correct balance.

Inhibition of flowering by gibberellin has been reported in other plants; for example, lilac (18), citrus (45), and fuchia (72). The inhibitory effects of GA_3 on flowering are probably linked to its multiple effects on plant metabolism; in particular, the inhibition of starch accumulation may play a part. An accumulation of starch (74,75), and sucrose availability (57) appear to be necessary for floral initiation in some plants. Gibberellin has been reported to enhance the hydrolytic activity in internodes and decrease starch content, presumably by mobilizing the reserve foods (53,54). In tobacco callus tissue, gibberellin has prevented starch accumulation and subsequent shoot initiation (81).

Gibberellin may also prevent floral initiation by stimulating mitoses in areas of the apex which enhance vegetative growth. In *Rudbeckia,* GA_3 stimulated mitoses most in the central zone of the apex, resulting in flowering (5). But for *Perilla,* the activation included the central and peripheral zones, and resulted in vegetative elongation. Similarly, elongation was caused in *Samolus* and *Chrysanthemum* by stimulation of the sub-apical meristem. Amo-1618, a growth retardant, inhibited mitoses in this region, and the effect was reversible by GA_3 (73). Perhaps GA_3 and B-9 interact on the Red Wing apex in a similar fashion.

Immature leaves of the Red Wing azalea were found to contain a number of gibberellin-like substances (Tables VI, VII, VIII). Little can be concluded about the number or identity of the acidic gibberellin-like substances, (Table VI), especially since ultraviolet fluorescence in sulphuric acid was not detected. Lack of fluorescence may have been caused by masking substances on the plates, or impurity of the phytoactive substance. Also, precursors of gibberellins may be active in bioassays (49,50), but may not fluoresce.

The spread of phytoactivity to R_f's 0.0-0.5 at 7 weeks after shoot tip removal may have resulted from the "tailing" of a substance present in high concentrations, since the earlier 2 weeks showed only one band of activity (Table VI). The shift in R_f of the substance from R_f 0.3-0.4 at 5 weeks after shoot tip removal to R_f 0.2-0.3 at 6 weeks after shoot tip removal was probably caused by some variant in the chromatography method affecting the reproducibility of R_f regions. Alternately, there may have been conversion of one substance to another, or a halt in production of one substance and the commencement of synthesis of another. If the concentration of the acidic gibberellin-like substance had increased at 7 weeks after shoot tip removal a corresponding increase in phytoactivity would not necessarily have occurred. Not all gibberellins show an increase in phytoactivity with an increase in concentration similar to GA_3. For example, the activity of GA_{18} levels off at a relatively low concentration (33). Any change in amount or occurrence of the phytoactive substance was unlikely to be caused by a variation in the amount of material that was extracted because the dry weights of the samples varied little throughout the experiment.

There are definitely 3 separate basic gibberellin-like substances present in Red Wing azaleas. None of the recently discovered basic gibberellin-like substances have yet been chemically characterized. Comparison with the basic substances detected by other workers is not possible because they used different chromatography methods.

The involvement of gibberellins with flowering in the Red Wing azalea seems definite from the interaction of GA_3 and B-9. Since GA_3 prevents inflorescence initiation, it seems paradoxical that an acidic gibberellin-like substance appeared in the immature leaves prior to initiation and persisted while initiation occurred (Tables IV and VI). However, there was no significant difference in the amount or occurrence of the active material between the control and B-9 treatment indicating that this gibberellin-like substance is not involved in the flowering process. It has been shown that for a given plant, one gibberellin will influence only vegetative growth, while another will influence flowering (12). If GA_3 is not one of the naturally occurring endogenous gibberellins of Red Wing, it may act on the plant in a different manner than the native gibberellin-like substances.

It is impossible to ascertain the role of the basic gibberellin-like substances in inflorescence initiation, because lack of time prevented chromatography and bioassay of extracts from weeks 4,5, and 6 after removal of shoot tips. The 2 active substances detected by the Meteor pea bioassay (Table VII) occur only in the extract from B-9 treated plants. This indicates that they may be important in inflorescence development, and particularly in the initiation of the floral apices.

The basic gibberellin-like substance active in the cucumber bioassay was present in approximately equal amounts in both the control and B-9 treatment extracts (Table VIII). This material then is probably not involved in floral initiation, but like the acidic gibberellin-like substance, (Table VI), appears as a part of the normal sequence of events concerned with the growth and development of a shoot from an axillary bud released from dormancy.

It is quite possible that the Red Wing azalea contains other gibberellin-like substances, which may be more important in flowering than these, but which were not extracted by the procedure followed. It has been found that gibberellin-like substances occur in bound forms, from which they are only released by chemical or enzyme hydrolysis (48,62). There are also water soluble gibberellin-like substances which are not soluble in ethyl acetate (47,79).

It is apparent that B-9 does not interfere with the biosynthesis, or cause the destruction of ethyl acetate soluble gibberellin-like substances in the azalea. Indeed, B-9 treatment leads to the production of 2 basic gibberellin-like substances (Table VII). Moore (46) has reported that while B-9 inhibition may be reversed by GA_3, B-9 does not appear to inhibit gibberellin biosynthesis.

It has been demonstrated that B-9 blocks the synthesis of indole-3-acetic acid (IAA) in pea stems (69), resulting in the suggestion that B-9 retards growth by decreasing the auxin content of plants. Applying this to the azalea, a drop in endogenous gibberellin levels would not be expected; instead, auxin levels would decrease with B-9 treatment and flowering. High auxin levels would prevent inflorescence initiation. This hypothesis is in agreement with the inhibitory action of GA_3 on initiation, as gibberellin has been shown to act through increasing the levels of endogenous auxin in numerous cases (60). If this applies to the azalea, it would be expected that detectable amounts of auxin-like substances would be found in the vegetative buds, and that application of an anti-auxin would promote the flowering response in the same manner as does B-9. However, Ballantyne and Link (4) failed to detect a wheat coleoptile growth promoter in the vegetative buds of Triumph azaleas. When Hexe azaleas were sprayed with 2,3, 5 triiodobenzoic acid (TIBA, an anti-auxin) at about the time of inflorescence initiation, multiple flower bud formation was inhibited. Multiple flower bud formation is promoted by B-9 treatment, and should also be promoted by TIBA treatment if a decrease in auxin level is necessary for flower initiation. Zeevaart (84) has reported that in *Pharbitis*, GA_3 reversed B-9 inhibition of growth and flower initiation, while IAA and naphthalene acetic acid (NAA, a synthetic auxin) did not. Thus, it appears that auxins are not singularly important for inflorescence initiation of the azalea, and that B-9 inhibition of auxin biosynthesis may not be a universal phenomenon among plants.

Both auxins and gibberellins may be involved in floral induction and initiation. A number of endogenous auxins and gibberellins have been detected in *Nicotiana* (58), and in *Rudbeckia* and *Chrysanthemum* (22). The levels of these substances changed markedly during flower induction and initiation. Some auxin and gibberellin-like materials increased, while others decreased.

Cytokinins may also be involved in the flowering response. Cytokinins and nucleic acid metabolites have been found to promote flowering in various plants (12). Kinetin has been reported to substitute for GA_3 in reversing inhibition of flowering in *Chenopodium* by (2-chloroethyl)-trimethylammonium chloride (CCC, a growth retardant) (80), although it reduces endogenous gibberellin levels similar to CCC. Nitsch and Nitsch (57) have found auxins and gibberellins to inhibit inflorescence production in *Plumbago* cultures, whereas kinetin, adenine, and relatively high sucrose concentrations may promote flowering.

Apparently inflorescence initiation is a very complex process, involving much more than the presence, absence, or change in level of a certain hormone. It is probably controlled by the relative abundance of and the balance between a large number of factors.

LITERATURE CITED

1. Baldev, B. and A. Lang. 1965. Control of flower formation by growth retardants and gibberellin in *Samolus parviflorus*, a long-day plant. Am. J. Botany 52: 408-417.

2. Ballantyne, D.J. 1959. Unpublished data.

3. ______________. 1962. A growth inhibitor from the roots of *Chrysanthemum morifolium* Ramat. and its influence upon the gibberellic acid response. Can.J. Botany 40: 1229-1235.

4. ______________., and C.B. Link. 1961. Growth regulators and the flowering of evergreen azaleas (*Rhododendron* cv.). Proc. Am. Soc. Hort. Sci. 78: 521-531.

5. Bernier, G., R. Bronchart, and A. Jacqmard. 1964. Action of gibberellic acid on the mitotic activity of the different zones of the shoot apex of *Rudbeckia bicolor* and *Perilla nankinensis*. Planta 61: 236-244.

6. Brian, P.W. and H.G. Hemming. 1961. Promotion of cucumber hypocotyl growth by two new gibberellins. Nature 189: 74.

7. __________, ______________, and D. Lowe. 1964. Comparative potency of nine gibberellins. Ann. Bot. 28: 369-389.

8. Briggs, D.E. 1966. Residues from organic solvents showing gibberellin-like biological activity. Nature 210: 419-421.

9. Cathey, H.M. 1964. Physiology of growth retarding chemicals. Ann. Rev. Plant Physiol. 15: 217-302.

10. _____, and N.W. Stuart. 1958. Growth and flowering of *Chrysanthemum morifolium* Ramat. as affected by time of application of gibberellic acid. Proc. Am. Soc. Hort. Sci. 71: 547-554.

11. Carr, D.J., D.M. Reid, and K.G.M. Skene. 1964. The supply of gibberellins from the root to the shoot. Planta 63: 382-392.

12. Chailakhyan, M.K. 1968. Internal factors of plant flowering. Ann. Rev. Plant Physiol. 19: 1-37.

13. Chin, T.Y. and J.A. Lockhart. 1965. Translocation of applied gibberellin in bean seedlings. Am. J. Botany 52: 828-833.

14. Cleland, R. 1965. Evidence on the site of action of growth retardants. Plant and Cell Physiol. 6: 7-15.

15. Criley, R.A. 1967. Flower initiation delayed in azalea by gibberellic acid. Report from the 1967 meetings of the Am.Soc. Hort. Sci., Aug. 28-30. 1967. in Maryland Florist 138: 1. Oct. 1967.

16. Corcoran, Mary R. and B.O. Phinney. 1962. Changes in amounts of gibberellin-like substances in developing seed of *Echinocystis*, *Lupinus* and *Phaseolus*. Physiol. Plant. 15: 252-262.

17. Dennis, D.T., C.D. Upper, and C.A. West. 1965. An enzymatic site of inhibition of gibberellin biosynthesis by Amo-1618 and other plant growth retardants. Plant Physiol. 40: 948-952.

18. Dostál, R. 1964. Effect of gibberellic acid on the initiation of buds in lilac. Nature 201: 843-844.

19. Frankland, B. and P.F. Wareing. 1960. Effect of gibberellic acid on hypocotyl growth of lettuce seedlings. Nature 185: 255-256.

20. Graf, A.B. 1963. Exotica 3. Pictoral Cyclopedia of Exotic Plants. Roehrs Co., Rutherford, N.J. pp. 1541-1542.

21. Halevy. A.H. 1963. Interaction of growth-retarding compounds and gibberellin on indoleacetic acid oxidase and peroxidase of cucumber seedlings. Plant Physiol. 38: 731-737.

22. Harada, H. and J.P. Nitsch. 1959. Changes in endogenous growth substances during flower development. Plant Physiol. 34: 409-415.

23. Hashimoto, T. and L. Rappaport. 1966. Variations in endogenous gibberellins in developing bean seeds. I. Occurrence of neutral and acidic substances. Plant.Physiol. 41: 623-628.

24. Hayashi, F., S. Blumenthal-Goldschmidt, and L. Rappaport. 1962. Acid and neutral gibberellin-like substances in potato tubers. Plant Physiol. 37: 774-780.

25. _______, T., Y. Murakami, and S. Matsunaka. 1956. Biochemical studies on "bakanae" fungus. Part XXXVI. The physiological action of gibberellin. VIII. Changes in the activities of various enzymes in leaf-sheaths of rice plants treated with gibberellin. Bull. Agr. Chem. Soc. Japan 20: 159-164.

26. Heatherbell, D.A., B.H. Howard, and A.J. Wicken. 1966. The effects of growth retardants on the respiration and coupled phosphorylation of preparations from etiolated pea seedlings. Phytochem. 5: 635-642.

27. Jackson, D.J. 1967. Gibberellin-like substances in peach tissue: Separation by thin-layer electrophoresis and chromatography. Planta 74: 324-329.

28. Jensen, W.A. 1962. Botanical Histochemistry. W.W. Freeman & Co., San Francisco.

29. Johansen, D.A. 1940. Plant Microtechnique. McGraw-Hill Book Co., Inc., New York.

30. Jones, R.L. 1968. Aqueous extraction of gibberellins from pea. Planta 81: 97-105.

31. Jones, R.L. and I.D.J. Phillips. 1966. Organs of gibberellin synthesis in light-grown sunflower plants. Plant Physiol. 41: 1381-1386.

32. Kagawa, T., T. Fukinbara, and Y. Sumiki. 1963. Thin layer chromatography of gibberellins. Agr. Biol. Chem. 27: 598-599.

33. Katsumi, M., Y. Ogawa, and K. Koshimizu. 1968. Biological properties of gibberellin A_{18} in comparison with those of gibberellin A_3. Bot. Mag. Tokyo 81: 491-497.

34. __________, B.O. Phinney, and W.M. Purves. 1965. The roles of gibberellin and auxin in cucumber hypocotyl growth. Physiol. Plant. 8: 462-473.

35. Khalifah, R.A., L.N. Lewis, and C.W. Coggins, Jr. 1965. Gradient elution column chromatography systems for the separation and identification of gibberellins. Anal. Biochem. 12: 113-118.

36. Koshimizu, K., H. Fukui, M. Inui, Y. Ogawa, and T. Mitsui. 1968. Gibberellin A_{23} in immature seeds of *Lupinus luteus*. Tetrahedron Lett. No. 9, pp. 1143-1147.

37. Lang, A. 1957. The effect of gibberellin upon flower formation. Proc. Natl. Acad. Sci. 43: 709-717.

38. Li, J.C.R. 1965. Statistical Inference I. Edward Brothers, Inc., Mich.

39. Lockhart, J.A. 1957. Studies on the organ of production of the natural gibberellin factor in higher plants. Plant Physiol. 32: 204-207.

40. Lüttge, U., K. Bauer, und D. Köhler. 1968. Frühwirkungen von Gibberellinsäure auf Membrantransporte in jungen Erbsenpflanzen. Biochem. Biophys. Acta 150: 452-459.

41. McDowell, T.C. and R.A. Larson. 1966. Effects of (2-chloroethyl) trimethyl ammonium chloride (Cycocel), N-dimethyl succinamic acid (B-9), and photoperiod on flower bud initiation and development in azaleas. Proc. Am. Soc. Hort. Sci. 88: 600-605.

42. MacMillan, J. and P.J. Suter. 1963. Thin layer chromatography of the gibberellins. Nature 197: 179.

43. The Merck Index. An Encyclopedia of Chemicals and Drugs. 8th. Ed. Merck & Co., Inc. Rathway, N.J. 1968.

44. Mertz, D. 1968. Hormonal control of root growth. III. The role of sulfhydryl groups in cell elongation. Advan. Frontiers Plant Sci. 19: 61-71.

45. Monselise, S.P., R. Goren, and A.H. Halevy. 1966. Effects of B Nine, Cycocel, and benzothiazole oxyacetate on flower bud induction of lemon trees. Proc. Am. Soc. Hort. Sci. 89: 195-200.

46. Moore. T.C. 1967. Kinetics of growth retardant and hormone interactions in affecting cucumber hypocotyl elongation. Plant Physiol. 42: 677-684.

47. Murakami, Y. 1962. Occurrence of 'water-soluble' gibberellin in higher plants. Bot. Mag. Tokyo 75: 451-452.

48. _________. 1968. Evidence for the occurrence of an inactive bound gibberellin in the seed of *Pharbitis nil*. Bot. Mag. Tokyo 81: 278-280.

49. _________. 1968. Gibberellin-like activity of (-)-kaurene, (-)-kauren-19-ol and (-)-kauren-19-oic acid in leaf sheath elongation of 'Tan-ginbozu' dwarf of *Oryza sativa*. Bot. Mag. Tokyo 81: 100-102.

50. _________. 1968. Gibberellin-like activity of steviol in leaf elongation of the 'Tan-ginbozu' dwarf of *Oryza sativa*. Bot. Mag. Tokyo 81: 464-466.

51. _________. 1968. Gibberellin-like substances in roots of *Oryza sativa*, *Pharbitis nil*, and *Ipomea batatas*, and the site of their synthesis in the plant. Bot. Mag. Tokyo 81: 334-343.

52. Murashige, T. 1965. Effects of stem-elongation retardants and gibberellin on callus growth and organ formation in tobacco tissue culture. Physiol. Plant. 18: 665-673.

53. Nanda, K.K. and R.S. Dhindsa. 1968. Effect of gibberellic acid on starch content of soybean (*Glycine Max* L.) and its correlation with extension growth. Plant and Cell Physiol. 9: 423-32.

54. _________., and A.N. Purohit. 1965. Effect of gibberellin on mobilization of reserve food and its co-relation with extension growth. Planta 66: 121-125.

55. Naskalli, R.J. 1961. Initiation and early ontogeny of the inflorescence of *Rhododendron obtusum*. Unpublished Master's Thesis. Ohio State Univ., Columbus.

56. Nicholls, P.B. and L.G. Paleg. 1963. A barley endosperm bioassay for gibberellins. Nature 199: 823-824.

57. Nitsch, Colette and J.P. Nitsch. 1967. The induction of flowering in vitro in stem segments of *Plumbago indica* L. II. The production of reproductive buds. Planta 72: 371-384.

58. Nitsch, J.P. and Colette Nitsch. 1965. The induction of flowering in *Nicotiana*. III. Variations in the level of endogenous growth substances. Am. J. Botany 52: 591-598.

59. Ogawa, Y. 1963. Studies on the conditions for gibberellin assay using rice seedling. Plant and Cell Physiol. 4: 227-237.

60. Paleg, L.G. 1965. Physiological effects of gibberellins. Ann. Rev. Plant Physiol. 16: 291-322.

61. __________, H. Kende, H. Ninneman, and A. Lang. 1965. Physiological effects of gibberellic acid. VIII. Growth retardants on barley endosperm. Plant Physiol. 40: 165-169.

62. Pegg, G.F. 1966. Changes in levels of naturally occurring gibberellin-like substances during germination of seed of *Lycopersicon esculentum*. J. Exp. Botany 17: 215-230.

63. Pharis, R.P., M.D.E. Ruddat, C.C. Phillips, and E. Heftmann. 1965. Precocious flowering of Arizona cypress with gibberellin. Can. J. Botany 43: 923-927.

64. Phillips, I.D.J. and R.L. Jones. 1964. Gibberellin-like activity in bleeding-sap of root systems of *Helianthus annus* detected by a new dwarf pea epicotyl assay and other methods. Planta 63: 269-278.

65. Phinney, B.O., C.A. West, Mary Ritzel, and P.M. Neely. 1957. Evidence for "gibberellin-like" substances from flowering plants. Proc. Natl. Acad. Sci. 43: 398-404.

66. Phinney, B.O. and C.A. West. 1961. Gibberellins and plant growth. in Encyclopedia of plant physiology. XIV. Growth and growth substances. ed. Springer-Verlag, Berlin. pp. 1185-1227.

67. Picard, C. 1967. Action du CCC et du B 995 sur la mise à fleur d'une plante bisannuelle, l'*Oenothera biennis*. Planta 74: 302-312.

68. Radley, Margaret. 1956. Occurrence of substances similar to gibberellic acid in higher plants. Nature 178: 1070-1071.

69. Reed, D.J., T.C. Moore, and J.D. Anderson. 1965. Plant growth retardant B-995: A possible mode of action. Science 148B: 1469-1471.

70. Riddell, J.A., H.A. Hageman, C.M. J'Anthony, and W.L. Hubbard. 1962. Retardation of plant growth by a new group of chemicals. Science 136: 319.

71. Robinson, F.P. 1969. Personal cummunication.

72. Sachs, R.M., A.M. Kofranels, and S-Y. Shyr. 1967. Gibberellin-induced inhibition of floral initiation in fuchia. Am. J. Botany 54: 921-929.

73. Sachs, R.M. and A. Lang. 1961. Shoot histogenesis and the subapical meristem: the action of gibberellic acid, Amo-1618, and maleic hydrazide. in Plant Growth Regulation. The Iowa State Univ. Press, Ames, Iowa. pp. 567-578.

74. Sadik, S. and J.L. Ozbun. 1968. The association of carbohydrate changes in the shoot tip of cauliflower with flowering. Plant Physiol. 43: 1696-1698.

75. ____________________. 1967. Histochemical changes in the shoot tip of cauliflower during floral induction. Can. J. Botany 45: 955-959.

76. Stoddart, J.L. 1968. The association of gibberellin-like activity with the chloroplast fraction of leaf homogenates. Planta 81: 106-112.

77. Stowe, B.B., F.H. Stodola, T. Hayashi, and P.W. Brian. 1961. The early history of gibberellin research. in Plant Growth Regulation. The Iowa State Univ. Press, Ames, Iowa. pp. 465-471.

78. Stuart, N.W. 1962. Azalea growth rate regulated by chemicals. Flor. Rev. 130: 35-36.

79. Tamuro, S., N. Takahashi, T. Yokota, and N. Naurofushi. 1968. Isolation of water-soluble gibberellins from immature seeds of *Pharbitis nil*. Planta 78: 208-212.

80. Teltscherová, Lola. 1968. Changes in the levels of endogenous gibberellins and auxins in apical buds of *Chenopodium rubrum* L. after application of growth substances reversing the effect of (2-chloroethyl)-trimethylammonium chloride (CCC) on flowering. Biol. Plant. 10: 305-310.

81. Thorpe, T.A. and T. Murashige. 1968. Starch accumulation in shoot-forming tobacco callus cultures. Science 160: 421-422.

82. Varner, J.E. 1964. Gibberellic acid controlled synthesis of α-amylase in barley endosperm. Plant Physiol. 39: 413-415.

83. Year-Round Azaleas. in Grower Talks. Geo. J. Ball, Inc., West Chicago, Illinois. 1967. pp. 1-14.

84. Zeevaart, J.A.D. 1966. Inhibition of stem growth and flower formation in *Pharbitis nil* with N,N-dimethylaminosuccinamic acid (B 995). Planta 71: 68-80.

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