GROWTH AND FLOWERING OF *CLERODENDRUM THOMSONAE* BALF.

BY

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ABSTRACT

GROWTH AND FLOWERING OF CLERODENDRUM THOMSONAE BALF.

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These investigations were conducted to determine the effect of growth regulators and environmental factors on the growth and flowering of Clerodendrum thomsonae Balf. "Wisconsin" clone. Whether terminal or stem-bud cuttings were used, there was little difference in growth and flowering of the plants. Plants propagated from cuttings taken from the uppermost portion of the stem of young stock plants (four months) developed about five times as many flowers as those from old stock plants (one and one-half years). Plants defoliated and water stressed for one week produced axillary shoots at 79% of the nodes compared to axillary shoots at 5.3% of the nodes of non-defoliated and non-water stressed plants.

Of eight different media used, a peat-vermiculite-sand medium was most satisfactory for growth and flowering. Plants grown in a medium at pH (6.9) and treated with ancymidol (30 ppm used as a drench at the rate of 30 ml/four-inch pot) produced more flowers than plants grown in media with a pH of 5.0 or lower.

Ancymidol treated plants had twice the concentration

of N, P, and K present in untreated plants. Plants receiving as low as 80 ppm N and as high as 320 ppm N showed little difference in growth and flower development. The dry wt of leaves, stems, and roots decreased with increased ancymidol concentrations.

Plants grown from stem-tips given heat therapy were virus free and produced the same number of flowers as plants that did not receive heat therapy.

Elongation of untreated plants was considerably greater than ancymidol treated ones regardless of photoperiod; whereas, there was little effect of light intensity on the elongation of treated and untreated plants. Ancymidol treated plants produced as much as a 50-fold increase in flowers compared to untreated ones. In controlled environment chambers, fewer flowers were produced under low light intensity (under 1200 ft-c) than under high light intensity (6500 ft-c). Plants exposed to 1 hour of red light before the dark period in both short and long days flowered profusely; whereas, those exposed to far-red remained vegetative.

Of the numerous growth regulators tested, encapsulated cycocel and ancymidol stimulated flowering and inhibited excessive elongation. Studies on the former were discontinued because of severe chlorosis and leaf distortion. Morphactin stimulated axillary shoot development, but also caused extensive apical and foliar injury.

Ancymidol retarded growth of plants whereas gibberellic acid (GA_3) only and GA_3 plus ancymidol promoted stem

elongation. Untreated and ancymidol treated plants grown under a 15-hour photoperiod remained vegetative, but flowered under a 12-hour photoperiod. Plants treated with GA_3 and GA_3 plus ancymidol remained vegetative in both 12 and 15-hour photoperiods.

Anatomical examinations of the stem tips revealed that ancymidol enhanced the early development of floral parts; however, untreated plants flowered at nearly the same time as treated ones. The reason for this may be that the greater number of flowers produced on ancymidol treated plants require a greater amount of photosynthate. There was a striking increase in gibberellic acid in flowering compared to vegetative plants. The effectiveness of ancymidol to increase the GA concentration which may induce plants to flower strengthens the hypothesis that ancymidol does not interfere with inhibition of GA_3 biosynthesis.

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INTRODUCTION

The interest and demand for flowering potted plants by consumers in this country has been phenomenal the past few years. The production and use of flowering potted plants in the United States is at a record high of approximately four hundred and fifty million annually with a prediction of seven hundred million annually in 1980. The public is always enthusiastic about a plant that is unique and requires a minimum of care. Therefore, plants other than the currently most popular should be evaluated for possible market introduction.

A plant relatively unknown to the public is Clerodendrum thomsonae Balf., or Glory Bower, which may have considerable potential as an attractive flowering potted plant. The genus Clerodendrum includes more than one hundred species of shrubs, trees and vines, most of which are native to the Eastern Hemisphere. Clerodendrum has been grown in Europe for many years. Approximately seventy-five thousand plants have been grown in Norway for Mother's Day. The flowers consist of a velvety crimson red corolla which is in vivid contrast to the white persistent calyxes (Fig. 1). Other appealing characteristics of this plant are the production of flowers only eight to ten weeks after cuttings are rooted and the capacity to flower for two to three months.

The two clones commonly known are the "European" and the "Wisconsin." Because of its floriferousness, the "European" clone has been studied extensively and has subse-

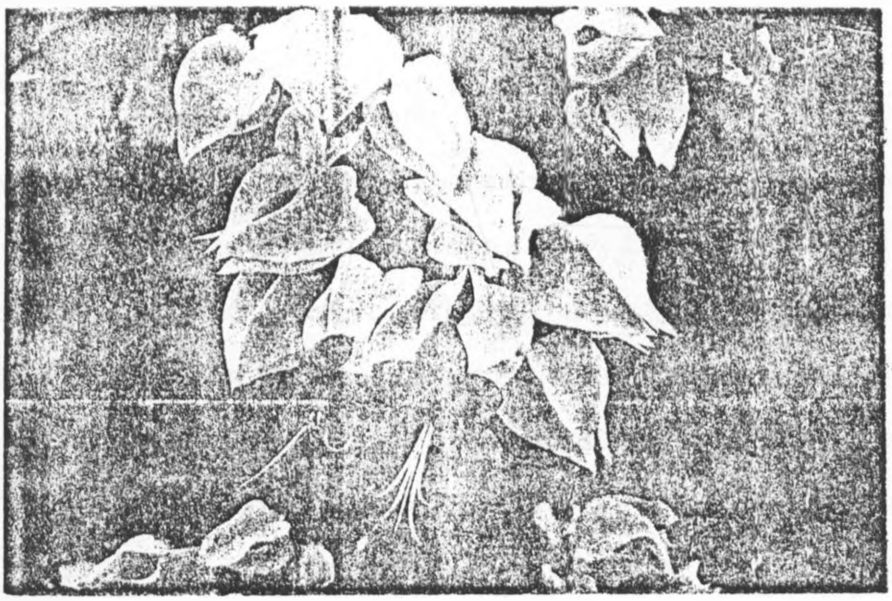


Fig. 1. Close-up view of unopened and opened Clerodendrum flowers treated with 30 ppm ancymidol and grown in short days and full light intensity.

quently become a popular pot plant in Europe (Fig. 2). Cuttings of the "Wisconsin" clone were obtained from the Milwaukee County Park System, Mitchell Park Horticultural Conservatory. The "Wisconsin" clone was selected for detailed investigation to determine its feasibility for the commercial market, as it does not display the severe flower abscission characteristic of the "European" clone. The abscission problem in the "European" clone has reduced its commercial value.

The "Wisconsin" clone is a tall viny plant which produces very few flowers. Control of elongation and increase in number of flowers are necessary for this plant to become a popular potted plant. These investigations were conducted to determine the cultural procedures and requirements, such as, propagation, nutrition, environmental responses, and the effect of growth regulators, to obtain optimum growth and flowering. Experiments were conducted in both greenhouses and controlled environment chambers.

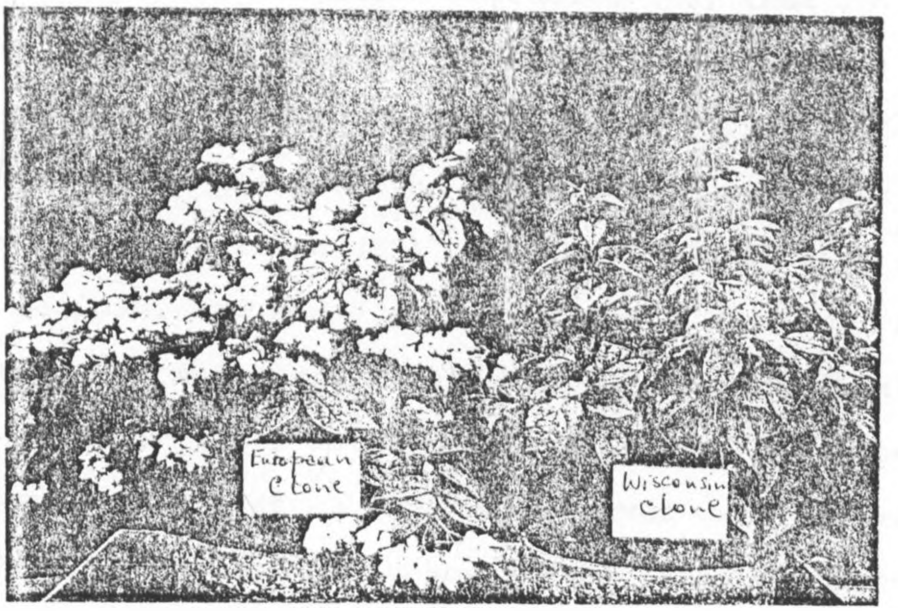


Fig. 2. Untreated plants of "European" and "Wisconsin" clone showing the profuse flowering of the "European" clone and the few incompletely differentiated flowers in the "Wisconsin" clone.

LITERATURE REVIEW

Available information concerning the propagation and maintenance of the "European" clone, Clerodendrum thomsonae Balf., is confined to Hildrum's studies (23) that revealed node cuttings may be the best method of propagation. The feasibility of using stem-bud cuttings in asexual propagation of blueberries to increase the number of cuttings per plant was reported by Parliman (36). The stem-bud cutting included a leaf, petiole, bud and a short section of a stem. Although the cuttings formed roots, less than 10% developed axillary shoots. Granger (20), working with Hibiscus, described the development of axillary shoots when leaves were removed from the cuttings. Holley (24) reported that heel cuttings of carnation should have 14 to 15 pair of leaves when removed from the rooting medium. The lower 6 to 8 pair each differentiated one vegetative lateral, while the upper ones produced flower buds with graduations in reproductiveness. Later Holley (25) discovered that all the unpinched laterals flowered quickly, however, the basal ones required a much longer time to flower. To avoid flowering of the upper shoots the terminal meristem was removed by pinching so that all cuttings would remain vegetative (25).

Blackman (4), in his classic paper, demonstrated that light intensity limited the rate of photosynthesis. Blackman and Wilson (5) showed that species differed in their light requirement for maximum growth. Some species grew best at

about 80 to 85% full summer daylight; some shade plants had an optimal intensity below 80% and for others, theoretically, light intensities greater than full sunlight were optimal.

The influence of light intensity on the growth and yield of greenhouse roses was studied by Chandler and Watson (9) using light reductions of 0, 42 and 65% of solar radiation. The level of 42% of full sunlight reduced growth only slightly; reduction below this level resulted in a decrease in total growth.

Tuskamoto (46), using French marigold, found that light intensity below 50% of full sunlight, prevented anthesis in long days. Rünger (41) reported similar results with Kalanchoe. Much of the research on the effect of light energy on plant growth and reproduction has been oriented toward morphological characteristics including height, weight and flower production.

Walker and Craig (51) found that cumulative solar energy was a major factor controlling flowering in Pelargonium hortorum. They observed that geranium seedlings flowered in about 90 days in summer but required twice as long in winter.

Early work regarding the influence of light quality on plant growth and flowering did not include accurate information concerning the control of temperature and light intensity under the various wave-lengths. Popp (38) was the first to consider these factors. He showed sufficient evidence to indicate that the red portion of the spectrum stimulated and the blue-violet portion inhibited stem

elongation. Leaf growth of pea seedlings increased, and internodes were shorter under red and orange light than those exposed to other regions of the spectrum (56). An intensive study of plants grown in colored light was undertaken by van der Veen (48). Although tomato plants were shortest when grown in blue light, they elongated rapidly when a trace of infra-red was added. Tomato plants grown in red light were of medium height; greater intensities of infra-red than red were required to increase stem elongation. When red and blue light were combined, plants were as short, or shorter than those grown in blue light alone.

Daylength effect on growth and flower development in Clerodendrum depends on the source of supplementary light (23). It has been demonstrated in many species that incandescent light promotes greater stem elongation than fluorescent light (32, 49). Piringer and Cathey (37) reported that petunias flowered under high ratios of red light and remained vegetative under ratios of far-red light. Roodenburg (40) suggested that "incandescent light had a much more daylengthening effect than daylight itself due to the excess of the short-wave infra-red rays (far-red) of the tungsten filament lamp." Wassink and Stolwijk (53, 54) showed that flowering resulting from the use of the incandescent light was probably due to its high emission of red radiant energy and that near infra-red (far-red) is more effective than red for the promotion of flowering of Brassica rapa.

Meijer (32) studied the effect of specific light

qualities on petunias grown under 16-hour photoperiods. Under red and green lights the plants rosetted and remained vegetative, whereas, in blue light, or in a combination of green and far-red light, the stem immediately elongated and plants flowered early. van der Veen and Meijer (48) also reported that the inhibition of stem elongation of petunia by red light was reversed by subsequent far-red irridation. This red--far-red--reversible pigment system controlling photomorphogenic growth was demonstrated in petunia by Ogawara and Ono (35), who showed that germination of petunia seeds was enhanced by a short period of red light and, like that of lettuce seed, (6), was inhibited by far-red.

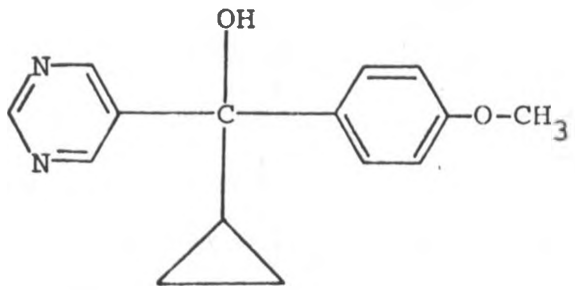
Downs et al. (15) found that plants such as dill, millet and barley, where stem elongation is an integral part of the flowering process, flowered sooner under supplemental incandescent than under supplemental fluorescent light. Radish plants given a 5-minute exposure to far-red radiation at the end of each day remained vegetative (39).

Vince-Prue and Guttridge (50) showed that floral initiation in strawberry was inhibited only with red light during the second half of a 16-hour night, and by far-red light only in the first half.

According to Hackett and Sachs (21) Bougainvillea plants flowered under short days with natural light in a greenhouse, but not with artificial light in controlled environmental chambers. They indicated that the lack of flowering in controlled environmental chambers was related to the high ratio

of red to far-red radiation from fluorescent light sources.

The growth retarding effects of EL-531, a-Cyclopropyl-a-(4-methoxyphenyl)-5-pyrimidinemethanol (ancymidol) was first reported by Tschabold and Meredith (44). The compound was renamed A-Rest or Ancymidol. The structure of this compound is:



Ancymidol affects many plants (16, 17, 19, 29). It is active not only on most of the species that respond to growth retardants, but also those unaffected by most other growth regulators.

The major use of growth retardants has been to produce shorter compact plants. Ancymidol is so active on most chrysanthemum cultivars that the stage of growth must be considered when application is made (8). Pinching and treating with ancymidol spray at the beginning of short days resulted in greater growth responses than applications made 2 wk later (8).

Ancymidol is the preferred growth retardant for poinsettias and is extremely active on both single-stem, and on pinched plants (3). Ancymidol causes almost no visible injury to the foliage; all cultivars respond similarly (12,

31).

Lilies generally grew too tall without a growth retardant, therefore ancymidol has been used to provide the best height control (22). It effectively controlled stem elongation when incorporated into the growing medium before planting (26), when applied as a drench from the time of shoot emergence until the shoot was 25 cm tall or when several foliar applications were made during the growth period (29).

Growth retardants are applied to the foliage or as a drench to the root medium. Ancymidol is unique because it is 10 to 100 times more active on a wt/vol basis than other growth retardants. A slight change in the concn of the solution can greatly modify the percentage of stem growth according to Cathey (8). He also found that when ancymidol was applied to a limited region of the growing medium, some plants were affected more than others resulting in variable plant growth. Weisser and Sachs (55) showed that chrysanthemum root systems absorbed ancymidol more rapidly than the leaves.

Drenching the growing medium with ancymidol is associated with the state of development of the root systems (42) Because ancymidol is extremely active, the time of drenching must be carefully considered to avoid drastic arrest of root growth (3).

Tschabold et al. (45) found that the treatment of Chrysanthemum morifolium Ram. with ancymidol as a drench was ineffective when the potting medium contained

high percentages of ground pine bark. Movement of ancymidol was restricted by pine bark and sand in leaching tests (27). Cathey (3) has reported that ancymidol promoted flowering in rhododendron and Bouvardia.

Information on the physiological effects of growth substances on flowering has passed through an empirical period which showed that under some conditions each of the known hormones can regulate flowering in some species. With the establishment of the gibberellins as natural growth regulators in plants, they were invoked as endogenous controls of flowering based on substantial experimental evidence (28). The discovery that gibberellins caused rosette plants to elongate in a manner similar to bolting quickly led to further investigations (57). Carr (7) reported that while most gibberellin stimulation of flowering has been reported for long-day plants and vernalizable species, some important stimulative effect also has been observed for short-day plants. Carr (7) applied gibberellic acid to Pharabitis and induced flowering. Evans (18) concluded that the presence of gibberellins was a probable prerequisite for the induction of flowering in all plants and that the gibberellin level was often limiting in long-day plants. Chouard (10) demonstrated that many long-day plants have been induced to flower with gibberellins, although it may be more accurate to say that gibberellin may cause the tendency of long-day plants to flower instead of entirely replacing the long-day requirement. Stoddart (43) suggested that gibberellin is

metabolized more rapidly and to different end products under short than long-days; Wareing and El-Antably (52) suggested that the lowering of gibberellin content in several species under short photoperiods may regulate induction through a combined action with increasing levels of abscisic acid.

Nitsch (34) found that in many short-day plants, gibberellin inhibited flowering. Baldev and Lang (2) suggested that the growth retardant effects of synthetic compounds that can inhibit growth and flowering may be a consequence of decreased gibberellin synthesis. Although there are some exceptions (11) gibberellin application ordinarily restores flowering (58).

The mode of action of ancymidol has been investigated the past few years. This chemical provides new possibilities for controlling and analyzing the endogenous plant growth.

Weisser and Sachs (55) showed that in chrysanthemum the main site of action of ancymidol occurred in the shoot; the retardation was independent of ancymidol-induced effects upon the root.

Shoub and De Hertogh (42), working with tulips found that GA_4, GA_7 and ancymidol applied simultaneously completely reversed the growth inhibition of the ancymidol, but GA_3 was relatively ineffective alone. This agrees with the finding of Van Bragt and Zijldyts (47) that injected GA_4-GA_7 was more effective than GA_3. Aung *et al.* (1) observed that GA_3 was not among the GA's tentatively isolated from the tulip shoot. In addition, De Hertogh and Blakely (13) found that

Lilium longiflorum was more responsive to applied GA_4 and GA_7 than GA_3 alone.

Dicks et al. (14) reported that the number of flowers produced on lily plants treated with ancymidol were unaffected, but anthesis was delayed 3 to 6 days and basal leaves senescenced earlier. They also showed that ancymidol delayed anthesis by 5 days by increasing levels of GA_3 which in the absence of ancymidol did not influence anthesis. Ancymidol and GA_3 synergistically promoted pedicel elongation.

According to Leopold (30) gibberellin-regulated growth of lettuce hypocotyls was inhibited by ancymidol. In the same studies he showed that auxin-regulated growth of coleoptile sections was inhibited only slightly by ancymidol. Ancymidol shows little or no antagonism of gibberellin actions which do not involve growth, e.g., the barley endosperm test and the Rumex leaf senescence test.

Cycocel and alar caused earlier flower bud initiation and more rapid development than occurred in the untreated plants (33).

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CHAPTER I

EFFECTS OF DIFFERENT CULTURAL PRACTICES ON GROWTH AND FLOWERING OF *CLERODENDRUM THOMSONAE* BALF.

ABSTRACT

Investigations on the "Wisconsin" clone of Clerodendrum thomsonae Balf. revealed that elongation and number of flowers were approximately the same for both terminal and stem-bud cuttings; however, the time to flowering was less with terminal cuttings. Defoliated axillary shoot cuttings differentiated faster than non-defoliated ones. In both young and old stock plants, there was no flowering at the basal nodes, however, the number of flowers per node increased greatly acropetally, and at the uppermost nodes fewer flowers were produced. The plants produced from young stock plants developed about 5 times more flowers than did plants produced from the old stock plants. Plants defoliated and water stressed for 1 wk produced axillary shoots at almost every node compared to the non-defoliated and non-water stressed plants which produced very few axillary shoots.

Of the 8 different media used, all except the peat-vermiculite-sand medium were satisfactory for normal growth and flowering. Plants produced in media with the highest pH (6.9) and treated with ancymidol had the greatest number of flowers. Ancymidol treated plants had twice the dry wt of N, P, and K compared to the untreated ones. High and medium concn of N, P, and K produced optimum growth; at very low concn growth was stunted. The average number of flowers for unpinched, ancymidol treated plants was about 30 percent

less than for pinched, treated plants. Pinching of the young plants had little influence on number of days to flowering.

Of 16 3 mm length stem tips of the "Wisconsin" clone given heat therapy, serological methods indicated 14 to be virus free. Stem tips that did not receive heat therapy showed tobacco ring spot virus (TRSV) symptoms. Plants from heat treated stem tips grew more rapidly but flowering was similar.

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INTRODUCTION

Little information is available concerning the propagation and optimal environment for the commercial production of Clerodendrum thomsonae Balf. The common name for this plant is "Glory Bower," however, since it is not commonly used it will, in this chapter, be referred to as Clerodendrum. The "Wisconsin" clone only was used in this investigation. Hildrum (8) studied certain of the cultural requirements for the "European" clone and reported that flower abscission was a serious problem; for this reason the "Wisconsin" clone showing little, if any, of the abscission problem was chosen for this investigation.

Since the plant produces virtually no seed, asexual reproduction is vital. Node cuttings have been used; however, this method requires considerable time for the production of mature plants. Therefore, it is important to obtain basic information on rapid methods of propagation to facilitate commercial production. Hildrum (8) used Clerodendrum node cuttings in all of his experiments. Parliman (15) reported that non-defoliated stem-bud cuttings from half-high blueberries rooted but axillary shoots failed to develop. Granger (5) has shown that defoliated stem-bud cuttings from Hibiscus plants produced both roots and axillary shoots.

Because of limited and inconsistent information on propagation, it was necessary to ascertain what effect stock plant age and the position on the plant where the cutting was

taken had on growth and flowering. Holley (10) reported that shoots from axillary buds at the top of carnation plants become reproductive, whereas, those at the base remained vegetative. According to Trippi and Brulfert (19), the capacity for flowering and rooting in cuttings may decrease with age in a given clone.

There is insufficient information on the media for satisfactory growth of Clerodendrum. Many crops require a specific medium for the most favorable growth, thus, it is essential to determine the nutritional requirement for a crop. The amount of P and K required for optimum growth should be determined so efficient amounts will be used and excesses or deficiencies will be avoided.

Pinching is a practice used to increase the number of axillary shoots, however, this practice may result in considerable delay of flowering. Therefore, it is essential to determine the effect of pinching on elongation, number of days to flowering, and number of flowers.

The "Wisconsin" clone was found to be infected with the tobacco ring spot virus (TRSV) and displayed delayed flower abscission compared to the uninfected "European" clone. "Wisconsin" clone plants were subjected to heat therapy to eliminate the virus. Symptoms including reduced plant vigor, chlorosis, and less flowering have been observed by numerous workers (1, 7, 11, 16, 17) in virus-infected plants. The pertinent questions then was, do virus-free plants of the "Wisconsin" clone display abscission and floriferousness as

does the "European" clone, or are these responses unchanged by heat therapy? This would solve the dilemma as to whether the virus has an effect on abscission and flowering.

Studies on the "Wisconsin" clone were designed to assess the effects of: type of cuttings, differences in stock plant age, position of node on the stock plant at which cuttings are taken, different nutritional regimes, virus infection, pinching, defoliation and water stress on vegetative plant growth and flowering.

MATERIALS AND METHODS

Type of cuttings: Terminal, stem-bud, and active eye cuttings were taken from the same 25 stock plants. Stem bud cuttings consisted of a single node, 1 pair of leaves, and 1 inch of the stem below the node; other stem buds were defoliated. Active eye cuttings had leaves and stem, but the axillary buds had elongated to 3 cm before they were removed from the stock plants. Cuttings were rooted in a medium of peat-perlite (1:1 v.v) (standard medium) under intermittent mist with a medium temp of 74°F and an air temp of 72°F. When the roots reached 2 inches in length, plants were potted in 4-inch plastic pots in the same type of medium as above and placed in greenhouses with 76°F day and 72°F night temp. One-half strength Hoagland's solution was used every 3rd irrigation.

The number of days required for non-defoliated cuttings from axillary shoots to grow 3 cm, final elongation, time to flowering and total number of flowers was recorded for each plant. The plant was considered to be flowering when the red petals emerged from the white calyxes. Days to flowering were counted in this manner in subsequent experiments.

Temperature and rooting: Terminal cuttings were placed in the medium at temp of 68, 74, and 78°F. One-half of each group was treated with 0.4% 1-napthalene acetamide. The root length was measured after 2 wk.

Nodal position on stem: Ten uniform young plants (4 months old) were selected and leaves removed from stem-bud cuttings taken starting at the base and progressing acropetally. Cuttings were placed in the rooting medium under intermittent mist. After 3 ½ wk, rooted cuttings were potted in 4-inch pots with standard medium and placed in the greenhouse with 78°F day and 72°F night temp. Final elongation, time to flowering and total number of flowers were recorded for each plant. Ten older plants (18 months old) of equal height also were selected and treated similarly.

Defoliation and stress: To determine the possibility of increasing the number of axillary shoots which develop adequately for cutting production, the following treatments were used: removal of the terminal apex, defoliation, hard pinch, and water stress. Stock plants 8 months old were subjected

to different conditions as follows: control, treatment with a 5 ppm solution ancymidol (30 ml/pot), defoliation and removal of terminal apices, and pinching of 1/2 its original height. Defoliated plants were grown 1 wk without watering after which normal irrigation was resumed. At the termination of the experiment, elongation, number of nodes, axillary shoots and flowers were recorded. Greenhouse temp in the summer varied considerably during this experiment ranging from a high of 120°F to a mean temp of approximately 88°F.

Different media: Cuttings rooted for 10 days were potted into 4-inch pots containing 7 different media and placed in a greenhouse at above mentioned temp. The media used were: soil, peat and perlite at ratios of 1:1:1, 3:1:1, and 1:2:1; vermiculite, peat and sand at 1:1:1; soil, peat, perlite, and bark at 1:1:1:1; soil, peat (Heco), perlite at 1:1:1 and 1:2:1 and peat and perlite at 1:1. One-half of the plants in each medium were drenched with 30 ppm ancymidol (30 ml/pot). Elongation, average number of days to flowering, average number of flowers and dry wt were recorded at harvest. The harvested plants were washed with double distilled water and dried. Tissue of both untreated and treated plants was analyzed for N, P, and K. The Kjeldahl method was used for N, a colormeteric procedure for P and the flame photometer for K (22).

Different concn of P and K: Cuttings rooted for 16 days were potted in the standard medium with 6 different concn of P and K. The available P and K treatments were: P 188 ppm and K 105 ppm; P 150 ppm and K 182 ppm; P 200 ppm and K 138 ppm; P 175 ppm and K 75 ppm; P 125 ppm and K 193 ppm; and P 34 ppm and K 184 ppm. The available P and K was determined by using the #1 Bray test (a standard test using HCl and NH_4NO_3) (2). Elongation, average number of days to flowering, average number of flowers and dry wt were recorded before washing plants with double distilled water and drying. Concn of N, P, and K were determined and recorded for untreated and treated plants.

Unpinched, pinched, untreated and treated plants: Experiments were designed to determine if pinching terminal cuttings affected number of days to flowering, and number of flowers.

Terminal cuttings were taken and rooted in 14 days in the standard medium. The rooted cuttings were pinched and potted in 4-inch pots with standard medium. One-half of the plants were pinched, the other half were unpinched and transferred to a greenhouse with 78°F day and 72°F night temp. Two wk after transplanting, elongation was recorded and ancymidol was applied to both unpinched and pinched plants at concn of 7, 15, and 30 ppm, using 30 ml of solution per pot. Elongation from time of treatment to termination was recorded in all experiments.

Virus studies: Initially, callus derived from the apical meristem was used in an attempt to secure TRSV-free plants of the "Wisconsin" clone. Callus tissue was produced only on agar media that contained 2,4-D and coconut milk. Several concn of indoleacetic acid (IAA) and kinetin were used to induce callus tissue to differentiate roots and shoots but all attempts failed.

Infected virus stock plants were grown in a heat chamber to eliminate the virus. Uniform temp control at 95°F with <1.5° variation was accomplished with electric heating coils controlled by a sensitive thermostat and a blower system which maintained continuous air circulation. The plants were pinched twice and 2 to 6 mm of the stems that developed from 3 cm axillary shoots of the 2nd pinch were removed and placed for 30-60 sec in 1% tri-sodium phosphate solution and then surface-sterilized for 15 min in a 10% sodium hydrochloride solution. They were washed 6 times in sterile glass-distilled water and aseptically cultured for 6 wk in 6 oz prescription bottles on 40 ml of the following sterile media solidified with DIFCO BACTO AGAR: C-, D-, White's (21), Muroshige and Skoog's medium (14), and 0.1 Hoagland's solution (9), containing 1 mg IAA.

The plantlets with roots were transferred from the prescription bottles to 2 ½-inch pots containing peat and perlite (1:1) and grown in insect-free controlled environmental chambers at approximately 75% relative humidity. After

plants were established, they were transferred to 4-inch pots and grown in insect-free cages in the greenhouse to prevent virus infection.

Plants were indexed for tobacco ring spot virus (TRSV) by sap inoculations on cowpea, Vigna sinensis 'Black Eye'. Inoculum was prepared by grinding the infected tissues in 0.03 M potassium phosphate buffer, pH 7.0 and rubbed on carborundum dusted primary leaves of cowpea, which were maintained in a greenhouse 75 $\pm$ 5°F. The heat treated plants were scored for local and systematic symptoms 1 wk after inoculation. Inocula prepared from Clerodendrum thomsonae "European" clone leaves and TRSV-infected Gomphrena globosa served as controls.

TRSV was detected serologically in naturally infected samples by agar-gel double diffusion tests with clarified sap. Four samples of heat treated leaves were homogenized in 20 ml of 0.1 M potassium phosphate buffer containing 0.01 M sodium diethyldithiocarbamate (DIECA) and 0.02 M 2-mercaptoethanol, pH 7.0, and then emulsified with 10 ml of n-butanol:chloroform mixture (1:2, v/v). The emulsion was broken by centrifugation (10,000 x g for 10 min).

Polyethylene glycol (PEG, MW 6000) and NaCl were dissolved in the aqueous phase to final concn of 10% and 0.3 M, respectively. The mixture was incubated for 1 hr at 39°F centrifuged at 10,000 x g for 10 min and the resulting pellet was resuspended in 1.0 ml of 0.03 M potassium phosphate buffer

with 0.01 M ethylenediaminetetraacetate (EDTA), and a pH of 7.0 to concentrate the viral protein.

The virus used for producing antisera was purified by (a) homogenizing infected tobacco leaves in 0.5 M sodium borate buffer, pH 7.0, containing 0.01 M DIECA and 0.02 M 2-mercaptoethanol; (b) clarifying the sap with n-butanol:chloroform; (c) incubating the clarified sap for 8-10 hr at 72°F and centrifuging at 10,000 x g for 15 min and (d) sedimenting the virus from supernatant fluid by differential centrifugations (12, 13).

The antiserum to TRSV was produced by injecting a rabbit, absorbed with healthy host protein preparations and then concentrated by ammonium sulfate $(NH_4)SO_4$ precipitation. Agar gel double diffusion plates were prepared with 0.9% ion agar dissolved in tris(hydroxymethyl)-aminomethane HCl-saline buffer (18).

Ten microliters of antiserum solution were placed at the center of an agar-gel double diffusion plate. Two drops of the heat treated extract solution were placed around the antiserum and the diffusion plate was observed for a halo effect around the extract drops which would indicate the presence of virus.

RESULTS AND DISCUSSION

Effect of type of cutting on growth and flowering: Whether a terminal or stem-bud cutting was taken, elongation and number of flowers produced were essentially the same (Table 1).

Table 1. The effect of type of cutting on rooting, elongation, days to flowering and number of flowers on *Clerodendrum*.

	LSD 0.05	Terminal	Stem bud		Active eye[z]
			with leaves	defoliated	
Days to reach 2" root clump	4.3	10[y]	23	19	8
Days for axillary shoots to reach 3 cm[w]	6.7	--	43	31	21[x]
Days to potting	5.6	10	43	31	29
Final height (cm)	4.8	24	19	19	22
Days to flower	6.3	64	95	89	82
No. flowers per plant	5.3	14	19	19	18

[z]Stem-bud cuttings with axillary shoots 3 cm.

[y]Mean (7 plants per treatment).

[x]Days required for axillary shoots to reach 3 cm after pinching.

[w]3 cm when cuttings were taken.

The non-defoliated and defoliated stem-bud cuttings were taken from plants of the same age and rooted at approximately the same time; however, axillary shoots developed sooner in the latter.

In 1 group of cuttings stock plants were pinched and when the axillary shoots were 3 cm in length, active eye cuttings were taken.

The active eye cuttings flowered 1 wk earlier than the defoliated stem-bud cuttings because the axillaries on the active eye cuttings enhanced rooting.

The time required for rooting of the unpinched terminal and active eye cuttings was very similar. Approximately 3 wk are needed for axillary shoots to develop from the active eye cuttings and they rooted and flowered as quickly as the terminals. The longer period required for rooting of the stem-bud cuttings without axillary shoot development could be related to the small amount of leaf area and the resultant low photosynthate production level. The defoliated, pinched cuttings rooted more rapidly, possibly because leaf removal may have resulted in a reduced level of the IAA allowing rapid development of axillary shoots which then increased the level of photosynthate production. Shoot apices have the highest level of IAA; however, it has been shown by Granger (5) that there is a sufficiently high level of IAA in the leaves near the apex to reduce axillary shoot growth. Both unpinched terminal and active eye cuttings possibly had higher levels of photosynthate production facilitating faster rooting. Slower development of axillary shoots from non-defoliated cuttings

may also be attributed to IAA concn in the leaves.

Effect of temp on rooting: Root growth in both untreated and ancymidol treated cuttings was 2 to 3 times greater at 78°F than at 68°F 2 wk after the cuttings were stuck (Table 2) (Fig. 1). Similar results have been reported by numerous workers (3, 4, 6) for different plants.

Effect of nodal position on growth and flowering: The elongation of axillary shoots from nodes 1 through 9 from old plants taken acropetally (Table 3) was greatest at approximately node 5. Flowers were not initiated on plants produced from nodes 1 through 3; a small, but gradually increasing number developed on plants from nodes 4 through 7; and beyond that the number decreased. Number of days to flowering was greatest for plants produced from node 4 and gradually decreased thereafter.

Axillary shoots from young plants also were grown from nodes 1 through 11 taken acropetally (Table 3). Final elongation of the shoots from node 4 was the greatest. Plants from successive nodes, decreased and then increased to a 2nd maximum at node 10. Plants from nodes 1 through 4 did not flower; flowering began on plants from node 5, increased dramatically to a maximum on those from node 8 and declined sharply thereafter. Days to flowering decreased from the maximum for plants from 5 to the least for plants from node 11.

Table 2. The effect of soil temp on development of Clerodendrum roots.

Temp (°F)	Root length (cm) after 2 weeks	
	Treated[z]	Untreated
68	1.9[y]	1.9
74	2.4	2.4
78	5.1	4.8

[z]0.4% 1-napethalene acetamide.

[y]Mean (10 plants per treatment).

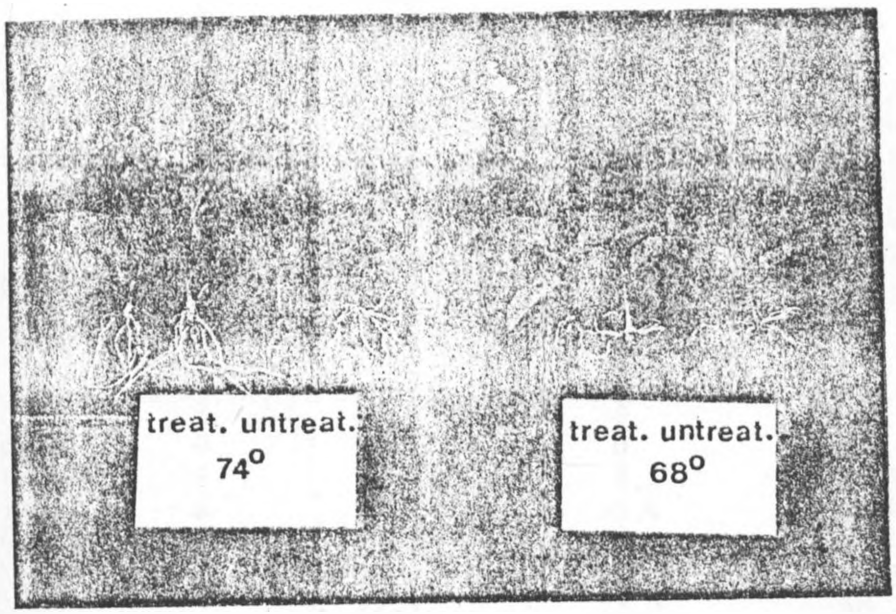

Fig. 1. Rooted terminal Clerodendrum cuttings of "Wisconsin" clone grown at 74°F, treated with 0.4% NAA and untreated. The roots were longer and more numerous in treated than untreated plants. At 74°F the degree of rooting was similar for untreated and treated cuttings and more than cuttings rooted at 68°F.

Table 3. The effect of axillary shoots differentiated at consecutive nodes on elongation and flowering of Clerodendrum.

Acropetal nodes from base		Elongation (cm)[z]	Flowers (number)	Days to flowering (number)
Stock plants ($1\frac{1}{2}$ years)	1	8.3[y]	0	---
	2	12.3	0	---
	3	18.6	0	---
	4	24.2	2	84.3
	5	28.6	3.4	82.4
	6	24.2	4.0	79.3
	7	20.3	8.0	72.3
	8	23.2	4.6	71.4
	9	26.3	3.2	70.2
LSD 0.05		3.1	3.7	5.2
Young stock plants (4 months)	1	6.1	0	---
	2	9.3	0	---
	3	27.6	0	---
	4	28.4	0	---
	5	21.3	8.2	81.3
	6	14.3	26.4	74.4
	7	14.2	32.5	74.4
	8	15.6	46.3	64.3
	9	22.3	23.0	68.2
	10	24.2	15.0	68.3
	11	23.4	8.0	63.5
LSD 0.05		3.1	6.2	6.5

[z]Time of potting to termination.

[y]Mean (10 plants per treatment).

Progeny from old and young plants grew to similar heights; progeny of young plants flowered profusely, and from old plants few flowers were produced. Plants from nodes 6 through 9 produced the most flowers in all experiments. Elongation and number of flowers on plants from nodes 6 through 9 varied inversely. Young plants flowered sooner than the old ones. Ancymidol treatment increased the level of gibberellic acid (GA) in induced plants (Chapter IV). Possibly the GA concn at the nodes which initiate the most flowers is also increased.

Effects of defoliation and stress: In mature plants, percent of nodes producing axillary shoots was lowest in untreated plants. Ancymidol treated plants, conversely, showed a high percentage of axillary shoot development (Table 4).

The highest percentage of axillary shoot development occurred when leaves were removed and the plants were water stressed; without stress, the plants developed about 1/2 as many axillary shoots.

When leaves and shoot apices were removed, 70 percent of the nodes developed axillary shoots; however, plants given a hard pinch developed 17% fewer axillary shoots.

In some cases, where a high percentage of axillary shoots developed, different mechanisms may be operative. In the ancymidol treated plants and in plants with leaves and apices removed, auxin concn may have decreased. With leaves removed and plants stressed, one might speculate that auxin

Table 4. The effects of defoliation and water stress on untreated and 8 ppm ancymidol treated *Clerodendrum*.

Treatment	Elongation (cm)[z]	Flowers (number)	Axillary shoots (number)	Nodes (number)	% of nodes producing axillary shoots
Intact plants, untreated	51.3[y]	0	2	37.5	5.3
Intact plants, treated	10.6	15.4	7.9	11.8	66.9
Leaves and apex removed	20.0	0	19.6	27.9	70.0
Leaves removed	25.5	0	15.3	36.8	42.0
Leaves removed (hard pinch)	23.9	0	4.3	8.3	53.0
Leaves removed (plants under stress)	12.3	0	22.4	28.3	79.0
LSD 0.05	11.1	NS	3.8	5.5	4.9

[z]The increase from treatment to termination.

[y]Mean (7 plants per treatment).

and other inhibitor concn may decrease.

Under greenhouse conditions, leaves abscised under stress and the plants developed not only axillary shoots, but numerous flowers.

Effects of different media on growth and flowering: There was greater elongation of untreated plants compared to those treated with ancymidol (Table 5). Untreated plants grew the least with vermiculite-peat-sand and the most with peat-perlite.

Flowering occurred in 55% of the plants grown in the untreated and in all of the plants produced in the ancymidol treated media. The number of days to flowering were not statistically significant for plants in the untreated media. Plants in soil-peat-perlite-bark required more than 20 percent more time to flower than did plants in the other media.

Untreated plants produced markedly fewer flowers. There was considerable variation in flower production on plants produced in different ancymidol treated media. Ancymidol treated plants (Table 5) in media with pH below 5 produced the least flowers.

The dry wt of untreated plants was approximately double the dry wt of plants grown in the treated media.

Plants, when treated with ancymidol, and grown in vermiculite-peat-sand produced the fewest number of

Table 5. The effect of 8 different media on growth and flowering of untreated and 30 ppm ancymidol treated Clerodendrum.

Treatment	pH[z]	Elongation (cm)[y]		Days to flowering (number)		Flowers (number)		Dry wt.	
		Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated
Soil-peat-perlite (1:1:1)	6.7	46.2[x]	3.5	69.0	77.7	12.1	96.3	5.3	2.4
Soil-peat-perlite (3:1:1)	6.9	44.2	2.5	---	63.7	---	129.6	5.8	3.2
Soil-peat-perlite (1:2:1)	5.6	45.6	4.0	---	65.0	---	77.6	6.3	3.5
Vermiculite-peat-sand (1:1:1)	4.2	35.3	2.1	---	69.7	---	10.5	4.3	1.1
Soil-peat-perlite-bark (1:1:1:1)	5.1	51.4	4.3	71.0	93.3	12.3	64.3	4.7	1.9
Soil-peat(Heco)-perlite (1:1:1)	5.6	49.2	4.6	68.5	59.5	10.1	89.7	5.3	2.2
Soil-peat(Heco)-perlite (1:2:1)	5.8	45.0	4.0	67.0	65.2	14.2	78.6	6.1	4.2
Peat-perlite (1:1)	4.9	57.0	4.8	---	66.4	---	54.3	4.0	2.8
LSD 0.05	.64	8.1	1.5	4.7	8.4	3.7	8.3	.8	.9

[z]At time of potting.
[y]Time of treatment to termination.
[x]Mean (7 plants per treatment).

flowers. The lowest dry wt for treated plants occurred in vermiculite-peat-sand while plants grown in peat-perlite showed the least dry wt of untreated plants.

The effect of N, P, and K on growth and flowering: Ancymidol treated plants showed a substantial increase in concn of N, P, and K, regardless of the media compared to untreated plants (Table 6). The increased concn of N, P, and K may be the result of ancymidol treated plants being smaller in size but absorbing quantities of nutrients similar to those absorbed by untreated plants.

Different concn of P and K: The untreated plants elongated as much as 13.5 times that of treated plants (Table 7). In ancymidol treated plants, the fewest flowers developed with the lowest concn of either P or K. The least dry wt for untreated plants occurred when the media contained the smallest concn of P or K. By visual observations and from the data in Table 7, it is apparent that we can grow saleable plants at many different concn of P or K; but this information is important in that it will enable us to use a concn of these 2 elements to produce crops without excesses of P or K or amounts that might limit growth.

Concn of N, P, and K determined by tissue analysis (Table 8) were similar to those found in other ornamental plants (20). The treated plants

Table 6. The effect of 7 different media on the concn of N, P, and K in the tissue of untreated and 30 ppm ancymidol treated Clerodendrum.

Media	Untreated (%)			Treated (%)		
	N	P	K	N	P	K
Soil-peat-perlite (1:1:1)	1.50[z]	.35	.83	3.00	.92	1.22
Soil-peat-perlite (3:1:1)	2.10	.38	.90	3.20	.80	1.35
Soil-peat-perlite (1:2:1)	1.90	.48	.81	3.23	1.08	1.39
Vermiculite-peat-sand (1:1:1)	2.18	.40	.88	3.23	1.10	1.09
Soil-peat-perlite-bark (1:1:1:1)	1.90	.48	.86	3.10	.99	1.50
Soil-peat(Heco)-perlite (1:1:1)	1.68	.44	.72	3.07	.98	1.56
Soil-peat(Heco)-perlite (1:2:1)	1.90	.64	1.10	2.78	.95	1.20
Peat-perlite (1:1)	1.70	.51	.88	2.60	.77	1.04
LSD 0.05	.55	.09	.15	.42	.16	.39

[z]Mean (7 plants per treatment).

Table 7. The effect of 6 concn of P and K on growth and flowering of untreated and 30 ppm ancymidol treated Clerodendrum.

Treatment	Elongation (cm)[z]		Days to flowering (number)		Flowers (number)		Dry wt.	
	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated
P-200 ppm[y] and K-138 ppm	64.6	4.8	0	66.2	0	98.6	5.3	3.9
P-188 ppm and K-105 ppm	54.5[x]	4.2	77.5	70.0	5.3	96.4	6.6	3.1
P-175 ppm and K-85 ppm	42.0	3.1	84.0	67.4	3.2	43.6	2.6	2.5
P-150 ppm and K-182 ppm	54.0	8.6	0	74.0	0	101.2	4.6	3.1
P-125 ppm and K-193 ppm	65.3	9.4	0	68.0	0	110.5	6.1	2.6
P-34 ppm and K-184 ppm	45.6	3.3	68.3	71.8	6.3	28.6	3.2	2.8
LSD 0.05	12.8	2.8	9.2	6.3	2.6	8.8	1.0	1.0

[z]Time of potting to termination.

[y]The concn of the element in the medium as determined by the Bray method.

[x]Mean (7 plants per treatment).

Table 8. The effect of 6 concn of P and K on the N, P, and K in tops of untreated and 30 ppm ancymidol treated Clerodendrum.

Treatment	Untreated (% dry wt)			Treated (% dry wt)		
	N	P	K	N	P	K
P-200 ppm[z] and K-138 ppm	2.04	.35	.43	3.04	.57	.92
P-188 ppm and K-105 ppm	2.13[y]	.41	.61	3.27	.81	.95
P-175 ppm and K-75 ppm	2.26	.44	.49	3.34	.73	.69
P-150 ppm and K-182 ppm	1.30	.31	.50	2.90	.63	.97
P-125 ppm and K-193 ppm	2.10	.44	.69	3.44	.70	.52
P-34 ppm and K-184 ppm	2.40	.21	1.29	2.49	.34	.90
LSD 0.05	.53	.11	.20	.31	.36	.22

[z]The concn of the element in the medium as determined by the Bray method.

[y]Mean (7 plants per treatment).

had less vegetative growth, however, similar amounts of these elements were absorbed by untreated and treated plants which could account for the higher % in the latter.

Difference between unpinched and pinched, untreated and treated plants: The elongation of unpinched, untreated plants was more than 11 times greater than pinched, treated plants. The pinched plants without ancymidol produced few flowers; and unpinched ones produced none (Table 9).

Unpinched and pinched treated plants were shorter than untreated ones. The elongation of unpinched plants was less than 2 times that of pinched ones treated with 30, 15 and 7 ppm ancymidol. Both unpinched and pinched ancymidol treated plants produced more flowers than untreated ones. Although the number of flowers increased with increasing concn of ancymidol, the increases are not significant for unpinched plants.

The differences in days to flowering for unpinched and pinched ancymidol treated plants was not significant; the number of flowers produced by unpinched vs. pinched treated plants were significantly different at each of the 3 ancymidol concn.

The limited vegetative growth in pinched treated plants may be the result of a higher concn of ancymidol in leaves and shoots which may induce more flowering.

Elongation increases under these conditions are predictable on the basis of previous experiments.

Table 9. The effect of ancymidol on elongation and flowering of unpinched and pinched *Clerodendrum*[z].

Treatment	Height at treatment (cm)	Elongation (cm)[y]	Days to flowering (number)	Flowers (number)
Untreated				
Unpinched	28.5[x]	82.5	0.0	0.0
Pinched	7.0	20.4	57.9	17.1
Ancymidol				
Unpinched				
7 (ppm)	17.7	12.1	75.8	47.2
15	23.6	10.2	74.0	50.8
30	26.0	7.0	74.6	54.6
Pinched				
7	8.4	7.2	72.2	59.4
15	7.6	5.1	65.9	62.7
30	7.7	4.9	65.8	75.8
LSD 0.05	7.3	4.6	13.4	8.2

[z]Plants pinched 92 days and treated for 78 days when data collected.

[y]Increase in height from time of treatment to flowering.

[x]Mean (10 plants per treatment).

"Wisconsin" clone on the indicator host cowpea, was unreliable and unsatisfactory. During the summer months, the virus could easily be transmitted to indicator hosts by mechanical inoculation; however, in the winter, only occasional transmission was obtained. Higher light intensity and temp are associated with the increased activity of the virus, therefore, it was more easily transmitted in the summer (Fig. 2).

Serology using agar-gel double diffusion tests and crude sap from infected plants and several concn of antiserum against TRSV were not always successful. When sap from infected plants was clarified and TRSV was concentrated by ultracentrifugation, a specific precipitin line of the virus and antibody was always observed. The failures in identifying the virus by indexing or serology probably resulted from lower concn of TRSV in the plants. It was necessary to concentrate the virus from Clerodendrum by either the PEG-NaCl precipitation or by ultracentrifugation before using it as an antigen. The PEG-NaCl procedure for serology was easier and more reliable than indexing on cowpea.

Stem tips not subjected to heat therapy and heat treated stem tips formed callus and roots only on a Hoagland's 0.1 mg IAA medium. Fourteen of 16 plants subjected to heat treatment and tested by serological techniques were found to be virus free. The 8 plants not heat treated contained the virus.

The heat treatment eliminated the virus from the plants.

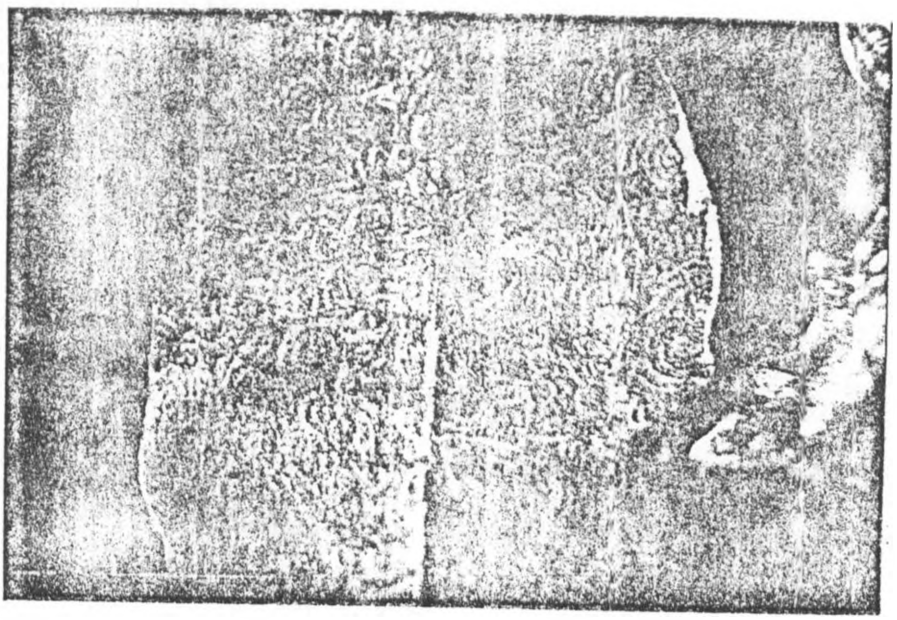

Fig. 2. Close-up view of a leaf from the "Wisconsin" clone showing symptoms of tobacco ring spot virus.

This has seldom been accomplished for other plants exposed to this treatment. There have been arguments concerning the validity of this technique, however, it definitely was successful with Clerodendrum thomsonae. Stem-tips taken from the first pinch were not virus free. However, small stem-tips taken from the axillaries developed from the 2nd pinch still receiving the heat treatment were virus free. Possibly the greater amount of growth, and the length of exposure of the virus at a temp where it becomes inactive were factors in the success of this technique.

The growth and flowering of virus-free plants of the "Wisconsin" clone was similar to infected plants except growth rate was slightly slower in the latter. These results probably indicate flowering is independent of the presence of virus.

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CHAPTER II

EFFECTS OF SOME ENVIRONMENTAL FACTORS ON THE GROWTH AND FLOWERING OF *CLERODENDRUM THOMSONAE* BALF.

ABSTRACT

Response of untreated and ancymidol treated "Wisconsin" and "European" clones of Clerodendrum to environmental factors, such as light intensity, photoperiod, temp and light quality was investigated.

In the "Wisconsin" clone elongation of untreated plants was considerably greater than for ancymidol treated ones for both terminal and nodal cuttings grown in short, long, and interrupted night photoperiods. However, there was little variation in elongation within the untreated and treated plants with different photoperiods and light intensities. The number of flowers produced by treated plants was as much as 50-fold greater than untreated plants.

With low light intensity (20% of the normal), plants of the "Wisconsin" clone had fewer flowers and the number of days to flowering was greater in short and long days compared to higher light intensities (60% and 100%). Unpinched, treated plants of the "European" clone produced more flowers sooner than pinched ones under the low light intensity. Regardless of treatment, the least number of flowers were differentiated at the low light intensity.

Temp of 68, 75, and 84^{o}F resulted in more flowers on treated than untreated plants for the "European" clone compared to the very few flowers produced on the "Wisconsin" clone.

Treated plants had less vegetative growth under both

incandescent and natural daylength than untreated ones. Young and old stock plants had fewer flowers as the time of exposure to incandescent light increased. Plants of the "Wisconsin" clone exposed to 1 hr of red light before the dark period in both short and long days flowered profusely; whereas, those exposed to far-red remained vegetative.

The elongation of terminal and nodal cuttings treated with 30 ppm of the growth retardant ancymidol (a-cyclopropyl-a-(4-methoxyphenyl)-5-pyrimidine methanol) applied as a drench at 30 ml per 4-inch pot was considerably less than untreated cuttings. The total number of flowers was strikingly greater for treated compared to untreated plants grown under 3 different light intensities and 3 photoperiods.

INTRODUCTION

There is meager information regarding the effect of light intensity and conflicting reports on the photoperiod responses of Clerodendrum. Hildrum (7) earlier reported that daylength does not influence flower induction, but does affect flower bud development. In his later findings he observed that short days did influence flower induction (8). The effects of daylength on flower bud development have been reported for Caryopteris clandonensis by Piringer et al. (13) and for Bougainvillea by Hackett and Sachs (5).

Because of the sparse information on light intensity, it is important to determine the optimum light intensity so that Clerodendrum can be grown at the appropriate season of the year. It also is imperative to understand the photoperiod requirements as related to flower induction, so that growers can manipulate the suitable daylength for flowering.

Hildrum (8) stated that a temp above 68°F was required for good growth and flowering; whereas, temp of 90°F might retard flowering in the "European" clone. Preliminary studies on the "Wisconsin" clone revealed that temp does not affect flowering, therefore, in depth investigations were necessary to determine more accurately its relationship to growth and flowering. This information would be useful in screening different clones that respond satisfactorily to low temp, which is presently critical to fuel conservation.

Another aspect of this investigation was how to keep

plants vegetative so that when flower induction is desired, uniform flowering can be obtained. Hildrum (8) stated that daylength effect on flower bud development is a function of the source of supplementary light. Since it is not clear if photoperiod causes induction, possibly flowering is related to light quality. It has been demonstrated in many species that incandescent light promotes greater stem elongation than fluorescent light (2, 12). Stem elongation is especially promoted by far-red (FR) light (1, 11, 16). Proctor (14) exposed radish plants to FR radiation 5 min before the dark period to maintain vegetative plants. Vince-Prue and Guttridge (20) showed that floral initiation in strawberry was inhibited when the daylength was extended with red (R) light but not with FR light. Combining R and FR light was inhibitory to flowering. Hackett and Sachs (6) showed that lack of flowering in Bougainvillea was related to the high ratio of R to FR radiation with fluorescent light sources.

Studies on R and FR radiation were made to determine if light quality was associated with flowering in Clerodendrum. When we discover what triggers flowering, this information may be related to and utilized by the grower. Without this information, it is doubtful if the plant can be grown profitably.

Although the "European" clone was included in these investigations, emphasis was placed on the "Wisconsin" clone because of the severe abscission problem that occurs in the former.

The purpose of this investigation was to determine the effect of environmental factors, i.e. light intensity, photoperiod, temp, light quality, and treatment with ancymidol on growth and flowering of the "Wisconsin" and "European" clones.

MATERIALS AND METHODS

Cross gradient, "Wisconsin" clone: Terminal and node cuttings were taken from the "Wisconsin" clone on May 25 and June 28. The cuttings were placed in peat and perlite at 76°F (1:1 v/v) under mist. After 2 wk, the rooted cuttings were transplanted into 4-inch pots containing the same medium. Two wk after potting the plants were pinched. Four wk after transplanting, the soil was drenched with 30 ml of 30 ppm ancymidol solution per pot. One-half strength Hoagland's solution (10) was applied every 3rd irrigation; distilled water was used for the other irrigations. Five single plant replicates were included in each treatment. The 3 daylength treatments were a) short days with a 9-hr photoperiod, b) long days with a 16-hr photoperiod, and c) short days (same as a) with a 2-hr incandescent light interruption in the middle of the dark period was obtained by using a 60 watt bulb spaced 4 ft apart, 60 inches from the top of the plant.

Three light intensities in both short and long days were a) full light intensity under greenhouse conditions (6300 ft-c, average at mid-day), b) 60% of full light intensity as

defined in a., and c) 20% of full light intensity. Light intensity was reduced by surrounding the plants with layers of cheesecloth. Light intensity levels, which varied less than 5 ft-c in b and c were determined with a Weston (Model 758) illumination meter.

Average ambient temp was 85°F during the day and 75°F at night in the greenhouse. The area under the shadecloth (black cloth) used for the daylength treatments and the cheesecloth used to reduce light intensity did not vary more than 2°F.

Shoot length was measured weekly and number of days for the appearance of visible flower buds and for 1st open flowers was recorded. Total number of nodes, node number at which flowering occurred and fresh and dry wt were determined at the termination of the experiment.

Cross gradient, "European" clones: Two wk rooted terminal cuttings from "European" and "Wisconsin" clones were placed in 4-inch pots, and pinched 2 wk later. Two wk after pinching, 1/2 the number of the plants received 8 ppm ancymidol (30 ml per pot). The remaining 1/2 were untreated and served as controls. The photoperiod and light intensity treatments used are enumerated in the previous experiment. Cultural treatments were similar to those of other experiments.

Temperature: Cuttings of the "European" clone were taken at

the same time of the year and given the same treatments as the "Wisconsin" clone except only unpinched and pinched terminal cuttings were used. Both clones received 30 ml of 8 ppm ancymidol solution per 4-inch pot and their responses were compared.

Plants were placed in controlled environmental chambers at temp of 68, 75, and 82°F and light was provided by fluorescent tubes (cool white) supplemented with incandescent lamps. Total light intensity was approximately 1900 ft-c. The humidity level was maintained at a water vapor deficit of approximately 5 mm Hg.

Light Quality and Duration: Pinched young plants (4 months) and older stock plants (1 1/2 years) were transplanted to 4 and 10-inch plastic pots, respectively, and natural day-length supplemented with incandescent light (16, 20, and 24 hr) and natural daylight (12 and 16 hr) under greenhouse conditions. Two wk later. 1/2 the number of plants for each 4 and 10-inch pot were treated with 30 ml/pot of 30 ppm ancymidol. The medium and nutrient regimes were similar to those mentioned earlier. Shoot length was measured every 2 wk and the total number of flowers was recorded.

Plants grown in the peat-perlite (standard medium) were placed in the greenhouse. After 2 wk the plants were treated with 8 ppm ancymidol as a drench using 30 ml per 4-inch pot grown under a 15 hr photoperiod. Six wk later 18 plants were placed in 2 controlled environmental chambers with both

incandescent and cool white fluorescent light with 82°F day and night temp, 75% relative humidity and 9 and 16-hr photoperiod. Seven of the plants in the 9-hr chamber were transferred to the 16 hr chamber and exposed to 1 hr of incandescent light before the dark period. Likewise, 7 plants in the 16-hr chamber were moved to the 9-hr chamber and exposed to 1 hr of fluorescent light before the dark period. Both groups were then returned to their original chamber at the start of the dark period. Seven plants remained continuously in each chamber and were covered 1 hr before the dark period which served as controls. The R (1.11 $\mu W\ cm^{-2},\ nm^{-1}$ at 650 nm) was obtained with the filtering of radiation from cool white fluorescent lamps through a single layer of red and yellow cellulose acetate. The FR (0.69 $\mu W\ cm^{-2},\ nm^{-1}$ at 750 nm) radiation was obtained from an incandescent lamp filtered through 1 layer each of red and blue cellulose acetate. Spectrocomposition of the sources were checked with an ISCO Model SR Spectroradiometer. There was no increase of air temp in the chambers during the 1-hr treatment.

Throughout the experiment all plants were kept in the same controlled environmental chambers except during the daily radiation with R or FR light. Plants were moved randomly within the chamber to minimize possible positional effects.

Statistical significance among means for individual treatments was calculated using the Fisher protected LSD method as outlined by Swanson (17). This method was used in all experiments.

RESULTS AND DISCUSSION

Cross gradient: Stem elongation of the "Wisconsin" clone was about 5 times greater in untreated than in treated plants (Table 1). The responses using terminal and node cuttings were similar. The magnitude of elongation within both the untreated and treated plants was independent of light intensitity and photoperiod. The small differences within the treatments may be attributed to plant growth variation. Plants receiving 20% light intensity in the interrupted night treatment were shorter than those under the other light intensities.

Number of Nodes: Untreated plants differentiated more nodes (Table 1); from observation, however, both untreated and treated plants flowered at essentially the same nodes.

Total Number of Flowers: Floriferousness was dramatically increased by the ancymidol (Table 3) regardless of light intensity and daylength (Fig. 1). With 20% light intensity, there were fewer flowers in short and long days compared to the higher light intensities. Untreated plants grown under interrupted night had the fewest flowers at all 3 intensity levels. There was no significant difference between terminal and node cuttings (Table 2).

There was a slight but significant increase in number of flowers in short days compared to long day treatment for

Table 1. Growth and development of Clerodendrum, "Wisconsin" clone from stem-bud and terminal cuttings in response to 30 ppm ancymidol, light intensity and photoperiod.

	Elongation (cm)[z]									Nodes (number)								
	Short day			Long day			Interrupted night			Short day			Long day			Interrupted night		
Plant Variable	100%	60%	20%	100%	60%	20%	100%	60%	20%	100%	60%	20%	100%	60%	20%	100%	60%	20%
Untreated[+]																		
Terminal cuttings	24.2[y]	31.3	29.6	25.3	24.6	27.6	34.4	22.9	20.5	12	14	14	11	12	16	12	14	19
Node cuttings	27.3	27.4	28.3	24.5	19.7	27.0	28.9	22.2	20.2	11	10	12	9	10	17	14	13	18
Average	25.8	29.4	28.9	24.9	22.5	27.3	31.7	22.6	20.4	11.5	12	13	10	11	16.5	13	13.5	18.5
Average day length		28.0	(2.4)[++]		24.9			24.9			12.2	(.71)		12.5			15	
Treated[+]																		
Terminal cuttings	5.8	6.0	5.9	5.5	8.0	4.3	6.7	4.2	3.1	9	10	10	9	10	9	9	9	8
Node cuttings	5.0	5.0	6.9	3.9	5.1	4.2	5.0	4.2	3.9	9	12	9	9	9	8	16	10	10
Average	5.4	5.5	6.4	5.3	6.6	4.3	5.2	4.2	3.5	9	11	9.5	9	9.5	8.5	12.5	9.5	9
Average day length		5.9	(NS)		5.4			4.3			9.8	(.71)		9			10.3	

[z]From time of treatment to termination.

[y]Mean (7 plants per treatment).

[+]No significant difference between node or terminal cutting with any treatment combination.

[++]Values in parenthesis are LSD at 5% for average photoperiod values within treatment and within variable.

Table 2. F Values for various treatments.

Source of Variant	Elongation (cm)	Days to Flowering	Flowers (number)	Number of Nodes	Dry Weight	Fresh Weight
Photoperiod	3.56^{++}	11.74^{+++}	24.39^{+++}	24.84^{+++}	.93	11.77^{++}
Light Intensity	1.64	7.50^{+++}	20.17^{+++}	20.95^{+++}	10.70^{++}	5.98^{+}
Type of Cutting	2.49	1.55	.09	1.60	1.96	.73
Untreated vs. Ancymidol Treated	839.35^{+++}	.00	96.29^{+++}	320.22^{+++}	136.19^{+++}	55.06^{+++}

Significant at $5\%^{+}$, $1\%^{++}$, and $.1\%^{+++}$ levels.

Fig. 1. Profusely flowering plant treated with 30 ppm ancymidol grown in natural days and 40% light reduction.

both untreated and ancymidol treated plants. Floriferousness under 100% and 60% light intensity levels was not significantly different.

Untreated and treated plants grown under interrupted night had fewer flowers than those grown in either short or long days. Clerodendrum grown in short days with interrupted light in this experiment indicates that it may be a short-day plant. Incandescent light in the interrupted night would be expected to decrease flowering as a result of conversion of P_r to P_{fr} which is inhibitory to flowering (9).

A short day plant ordinarily does not flower under long day conditions, but the fact that flowering does occur under long days suggests a dependence in the ratio of P_r to P_{fr} on light quality. Under natural light the P_{fr}/P_r ratio may be lower than with incandescent light necessitating a shorter period for reversion of P_{fr} to P_r. The dark period (8 hr) in long days is similar to the dark period before and after illumination in the interrupted night (6 and 7 hr), but the incandescent light used in interrupted night may increase the P_{fr}/P_r ratio and thereby makes a longer reversion period necessary.

Hackett and Sachs (6) showed that Bougainvillea produced fewer flowers with incandescent light in the controlled environmental chambers than with natural light illumination of the same intensity in greenhouses.

Days to Flowering: There was little difference in the number

of days to flowering for untreated and treated plants. Flower bud development, however, was delayed under long day and interrupted night conditions (Table 3).

In short and long days, days to flowering was essentially independent of light intensity, except at 20% light and interrupted nights, flowering was delayed. Some plants produced flowers in which anthesis occurred in 7 wk while other flowers on the same plant did not obtain anthesis for an additional 3 wk. This is of extreme importance in that consumers are interested in pot plants that flower for an extended period of time.

Fresh and Dry Wt: The fresh wt of untreated plants was approximately 20% greater than treated ones (Table 4). The 20% light intensity under interrupted nights for both treated and untreated plants, in most comparisons to short day and long day treatments, produced plants which had lowest dry wt, flowered latest, produced the least flowers, and elongated less. This may be due to low levels of photosynthates as a result of inadequate illumination.

Environmental Studies on the "European" clone: Treated plants of the "European" clone were approximately 1/4 the height of untreated ones (Table 5). Short and long day conditions had little influence on elongation. The elongation of untreated plants grown in short day and 20% light

Table 3. The effects of untreated and 30 ppm ancymidol on stem-bud and terminal cuttings of Clerodendrum on flowering, light intensity and photoperiod.

	Flowers (number)									Days to Flowering (number)								
	Short day			Long day			Interrupted night			Short day			Long day			Interrupted night		
Plant Variable	100%	60%	20%	100%	60%	20%	100%	60%	20%	100%	60%	20%	100%	60%	20%	100%	60%	20%
Untreated																		
Terminal cuttings	24.0[z]	16.4	14.4	11.2	11.4	8.0	.8	1.6	1.8	52.6	62.8	68.8	80.3	74.0	70.5	67.0	67.0	85.0
Node cuttings	20.8	22.2	5.0	18.8	23.0	12.8	10.4	1.4	.6	64.2	74.0	67.0	86.0	77.8	86.8	82.4	67.0	109.0
Average	22.4	19.3	9.1	15 0	17.2	10.4	5.6	1.5	1.2	58.4	68.4	67.9	83.2	75.9	78.7	74.7	67.0	97.0
Average day length		16.9	(1.56)[+]		14.2			2.8			64.9	(2.69)		79.3			79.6	
Treated																		
Terminal cuttings	76.5	78.2	36.2	63.3	84.2	39.0	51.0	47.4	6.0	67.0	67.0	68.4	76.3	73.0	74.8	69.2	68.0	91.5
Node cuttings	80.2	73.4	45.8	51.0	86.2	24.6	31.0	35.4	19.9	68.4	66.4	81.8	78.5	79.0	78.8	80.4	72.8	79.0
Average	78.4	75.8	41.0	57.2	85.2	31.8	41.0	41.4	12.9	67.4	66.5	75.1	77.4	76.0	76.8	74.8	70.4	85.3
Average day length		65.1	(6.32)		58.1			31.8			69.7	(1.76)		76.7			76.8	

[z]Mean (7 plants per treatment).

[+]Values in parenthesis are LSD at 5% for average photoperiod values within treatment and within variable.

Table 4. Effects of 30 ppm ancymidol using stem-bud and terminal cuttings of Clerodendrum on fresh wt and dry wt under different light intensities and photoperiods.

	Fresh Wt									Dry Wt								
	Short day			Long day			Interrupted night			Short day			Long day			Interrupted night		
Plant Variable	100%	60%	20%	100%	60%	20%	100%	60%	20%	100%	60%	20%	100%	60%	20%	100%	60%	20%
Untreated																		
Terminal cuttings	31.6[z]	27.0	30.0	29.7	26.6	26.9	30.1	23.9	24.2	6.8	5.8	5.9	5.5	5.8	6.2	6.0	6.3	4.8
Node cuttings	29.2	32.6	28.6	30.4	32.4	31.5	25.6	22.8	24.1	6.1	6.5	6.1	4.5	6.7	6.0	6.0	5.5	4.3
Average	30.4	29.8	29.3	30.1	29.5	29.2	27.9	23.4	24.2	6.5	6.2	6.0	5.0	6.3	6.1	6.0	5.9	4.6
Average day length		29.8 (2.69)[++]		29.6			25.2				6.2 (.67)			5.8			5.5	
Treated[+]																		
Terminal cuttings	20.3	26.1	23.7	26.6	26.1	18.3	23.3	16.8	16.0	2.4	4.0	3.0	4.2	4.0	2.1	4.7	2.6	1.7
Node cuttings	24.5	24.8	23.0	24.9	29.4	13.4	20.2	23.1	19.1	3.6	3.4	3.5	3.9	4.4	2.0	4.2	4.3	2.7
Average	22.4	25.6	23.4	25.8	27.8	15.9	21.8	20.0	17.6	3.0	3.7	3.3	4.1	4.2	2.1	4.5	3.5	2.2
Average day length		23.8 (2.43)		23.2			19.8				3.3 (NS)			3.5			3.4	

[z]Mean (7 plants per treatment).

[+]No significant difference between node or terminal cutting with any treatment combination.

[++]Values in parenthesis are LSD at 5% for average photoperiod values within treatment and within variable.

Table 5. Untreated and 30 ppm ancymidol treated plants of "Wisconsin" and "European" clones showing elongation, number of flowers and days to flowering under different light intensities and photoperiods.

	Elongation (cm)[z]						Flowers (number)						Days to Flowering (number)					
	Short day			Long day			Short day			Long day			Short day			Long day		
Plant Variable	100%	60%	20%	100%	60%	20%	100%	60%	20%	100%	60%	20%	100%	60%	20%	100%	60%	20%
Untreated																		
European clone																		
Unpinched	21.1	22.3	51.3	18.5	25.3	32.9	60.7	67.9	32.9	89.5	68.3	44.1	57	58	63	63	66	75
Pinched	17.8	22.4	49.6	31.7	24.7	24.3	61.8	55.4	30.4	72.0	59.8	30.3	69	66	81	73	72	89
Average	---	22.4	50.5	25.1	25.0	28.6	61.3	61.6	31.7	80.8	64.1	37.2	63	62	72	68	69	82
Average day length		29.3	(NS)[+]		26.2			51.1	(6.3)		60.7			65.7	(3.7)		73.0	
Treated																		
European clone																		
Unpinched	4.7	3.0	3.0	5.3	4.6	5.7	91.0	94.7	56.6	114.2	127.0	66.3	65	67	72	73	74	85
Pinched	12.3	11.0	6.8	8.0	12.4	8.9	81.4	73.7	38.6	78.5	78.3	43.0	74	76	86	82	78	97
Average	9.0	7.0	4.9	6.7	8.5	7.3	86.2	84.2	47.6	96.4	107.6	54.7	69.5	71.5	79	77.5	76	91
Average day length		6.9	(NS)		7.5			72.6	(9.1)		84.6			73.3	(4.1)		81.5	
Wisconsin clone virus infected																		
Pinched	10.8	11.5	9.1	11.6	12.9	12.3	80.1	78.7	30.9	84.2	86.2	35.3	66	68	70	72	75	77
Average		10.4			12.2			63.2			68.6			68			74	

[z]From time of treatment to termination.

[+]Values in parenthesis are LSD at 5% for average photoperiod values within treatment and within variable.

intensity was more than doubled those grown under 60% illumination. Similar results have been reported with other plants under low light intensities (12).

Treated plants produced more flowers than untreated ones under all treatment conditions (Table 5). Unpinched, treated plants had more flowers than pinched ones. The unpinched plants produced less vegetative growth, therefore, it is possible that the terminal apices had a higher concn of ancymidol which stimulated increased flower initiation. Our studies have shown that abrupt termination of vegetative growth can cause rapid escalation of flower bud initiation. Unpinched and pinched untreated plants showed no significant difference in number of flowers.

Short days significantly reduced flower numbers produced by both untreated and treated plants as compared to long day plants. Results within 100% and 60% intensity levels were very similar, but flowering was measurably less at the 20% level.

Flowering of pinched treated plants was delayed as much as 10 days as compared to untreated plants. Untreated and treated unpinched plants flowered as much as 18 and 14 days, respectively, before pinched ones. Plants in short days flowered sooner than those grown under long days. Only the 20% light intensity treatment delayed flowering, yielding greater values than those for the 100% and 60% intensity levels.

Elongation, number of flowers, and days to flowering in the "Wisconsin" clone was similar to the "European" clone

under all environmental conditions. Although the "Wisconsin" clone contained the virus, growth and flowering was similar to the "European" clone.

Temperature: Regardless of temp the "Wisconsin" clone produced very few flowers (Table 6). Elongation was not controlled at 82°F and the number of days to flowering was twice as many as in previous experiments (Fig. 2). The reason for these conflicting results are explained in the conclusion. In the "European" clone there was an increase in number of flowers as the temp increased above 68°F for both untreated and treated plants with more flowers on the latter.

Light Quality and Duration: Under incandescent light, ancymicol treated plants had less vegetative growth. There was a gradual increase in elongation with increased light duration (Table 7) for plants not treated with ancymidol.

Incandescent light virtually terminated flowering at all light exposure levels in untreated plants. Treated plants flowered and stock plants produced 2 to 4 times more flowers than young plants. In both young and stock plants, fewer flowers were differentiated as light duration was increased.

In only natural day, treated plants again showed less vegetative growth. Elongation was slightly less in all treatments as light was increased from 12 to 16 hr.

Table 6. The effect of temp on growth and flowering on untreated and 8 ppm ancymidol treated plants of the "Wisconsin" and "European" clone grown in controlled environment chambers.

Temperature (F)		"Wisconsin" clone			"European" clone		
		Elongation (cm)[z]	Ave. No. of Flowers	Average No. of Days to Flowering	Elongation (cm)[z]	Ave. No. of Flowers	Average No. of Days to Flowering
68°F	untreated	38.0	5.3	90.0	22.4	57.0	58.1
	treated	11.6	30.0	95.5	15.3	85.0	57.0
75°F	untreated	26.9	0.0	0.0	27.3	69.1	75.9
	treated	16.8	8.3	86.5	16.3	91.3	71.5
82°F	untreated	40.5	0.0	0.0	27.4	93.8	60.0
	treated	34.9	6.0	86.3	24.0	93.0	58.4
	LSD 0.05	15.3	10.6	9.9	8.7	26.0	NS

[z]Growth in cm of untreated and treated plants from time of treatment to flowering.

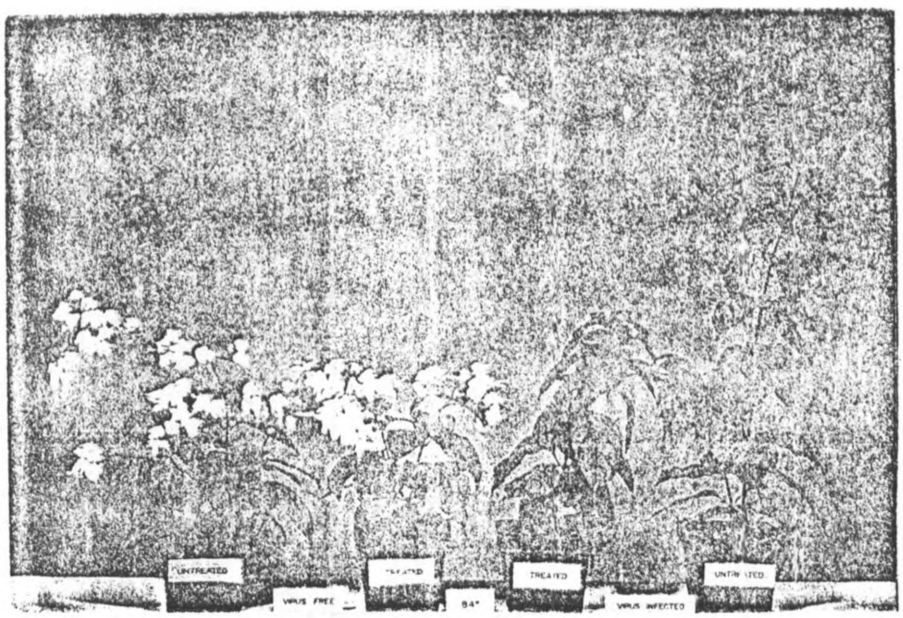

Fig. 2. Plants grown at 84°F. Two plants at left "European" clone. Plant at far left, virus-free untreated and adjacent plant to left treated with 30 ppm ancymidol. Vegetative plant at the right, "Wisconsin" clone, virus infected, treated and adjacent one, untreated.

Table 7. Pinched untreated and 30 ppm ancymidol treated 4 month (young) and 1½ year (stock) plants grown under natural days and natural days supplemented with incandescent light.

	Natural Light and Incandescent Light						Natural Daylength			
	Elongation (cm)[z]			Flowers (number)			Elongation (cm)		Flowers (number)	
Treatment	16 hr	20 hr	24 hr	16 hr	20 hr	24 hr	12 hr	16 hr	12 hr	16 hr
Untreated young plants	37.6[y]	40.3	47.5	0	0	0	34.3	26.4	2.0	3.0
Untreated stock plants	48.6	54.2	60.6	0	0	2	28.6	24.2	16.3	15.6
Treated young plants	6.2	5.1	5.0	43.4	27.6	14.0	5.7	5.2	59.3	46.5
Treated stock plants	5.3	5.6	4.0	98.5	84.6	68.0	8.5	7.6	143.5	97.6

[z]Time of treatment to termination.

[y]Mean (7 plants per treatment).

With natural day, floriferousness of treated plants increased. Untreated and treated stock plants were at least 100% more floriferous than young plants. Treated plants differentiated fewer flowers as the exposure increased under natural day length and natural daylength plus supplementary incandescent light.

Most untreated and treated plants grown under continuous greenhouse conditions remained vegetative. Some plants were transferred to controlled environmental chambers with long and short day photoperiods. Ancymidol treated plants exposed to 1 hr of R had almost 5 times the number of flowers as the untreated ones (Table 8). Photoperiod had little effect on number of flowers initiated, but R light may have been the stimulus for flowering.

CONCLUSIONS

The thrust of these experiments is to determine what triggers flowering in Clerodendrum "Wisconsin" clone. In its natural habitat, flowering occurs in late spring and early fall. When taken from its native surroundings, the questions arise concerning what factors, such as environmental conditions, are necessary to produce flowers. Clerodendrum flowers in both short and long days, but under interrupted

Table 8. The effect of 1 hr end-of-day red, far-red, or no light treatment on untreated and 8 ppm ancymidol treated plants.

	Flowers (number)			
	Long Day		Short Day	
Treatment	Untreated	Treated	Untreated	Treated
Greenhouse				
Control	0[z]	.6	---	---
Controlled Environment Chamber				
Control	0	.5	0	.8
Red	11.3	55.8	11.5	48.5
Far Red	0	3.8	0	2.8

[z]6 plants per treatment.

short days flowering was inhibited. Similar experiments showed that sometimes flowering was more prolific in either short or long days.

In all experiments where low light intensities (20% of a sunny day), or 1200 ft-c were used, very few flowers were produced, explaining why this plant does not flower in the winter under normal Wisconsin conditions. Flowering tended to lessen under a light intensity below the 60% level. The reason for this is probably 2-fold: firstly, the R, FR ratio (which will be discussed later) and secondly, the light intensity was less than 1200 ft-c and below this level the lack of photosynthates limit flowering. This may explain why the plant flowers in only late spring and early fall where the light intensity in nature is comparable to the 60% used in these experiments.

Temp apparently has little effect on flower induction but does have some effect on flower development. At 68^{o}F, the "European" clone was very floriferous, which is opposite to the findings of Hildrum (8). At the low temp, (68^{o}F) there was very little growth and flowering was minimal in the "Wisconsin" clone. This possibly explains why the "Wisconsin" clone does not flower in the greenhouse in the winter where the mean temp usually is at this level. According to Tuskamoto et al. (18) marigolds produced flowers at 30^{o}C in short days but not under long days. However, at 20^{o}C plants flowered similarly in both short and long days. This is in contrast to Clerodendrum where flowering occurred only at a

temp higher than 70°F in both daylengths, which suggests that photoperiod and temp are not interrelated in this species. At a mean temp of 88°F with some days as high as 120°F, flowering continued and the number of flowers produced remained similar.

Natural daylength together with incandescent light and in controlled environmental chambers with incandescent and fluorescent light inhibited flowering. Our observations agree with Hildrum (8) that flowers are not produced in midsummer, which he reports may be the result of high temp. It has been shown in our experiments that Clerodendrum plants exposed to 1 hr of R light before the dark period induced flowering, whereas, plants subjected to 1 hr of FR light remain vegetative. The differential responses to the 2 different wavelengths of light agree with similar differential responses on other plants (2, 4, 12, 19) and were attributed to the relative ratios of R and FR radiant energies emitted by the 2 light sources. Fluorescent light emits a very small amount of FR compared with R, whereas, the incandescent light emits a high proportion of FR radiant energy (3). The R radiation is the most efficient part of the spectrum for flower induction for many plants (16). It has been established elsewhere (12) that FR radiant energy induces stem elongation. The incandescent source supplies sufficient FR to prevent flowering. This agrees with our findings that under supplementary incandescent light in the summer, long vegetative shoots are produced which agrees with Meijer (11)

that a high R to FR ratio probably causes flower induction. Because of the higher ratio of R to FR and increased light intensity, the combination of these 2 factors stimulated flowering in the spring and fall while in the summer the low ratio of R to FR radiation (15) inhibited flowering, therefore, Clerodendrum is of the indeterminate type, and flowering is stimulated by a large R to FR ratio. A light intensity above 1200 ft-c is essential.

In earlier experiments, ancymidol was thought to be the causal agent for flower induction; from more recent and thorough investigations on the mechanism of this growth regulator, it now seems that it enhances flowering only when plants have entered the inductive phase. This was supported by the results of the 1 hr exposure to R and FR light. Although some growth regulators have been reported to delay flowering, ancymidol, in all experiments, did not alter the days to flowering. Although as many as 300 flowers were formed on an ancymidol treated plant, the size of the flowers was the same as on untreated plants with as few as 4 flowers.

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CHAPTER III

EFFECTS OF GROWTH REGULATORS, NITROGEN LEVELS AND pH ON GROWTH AND FLOWERING OF *CLOERDENDRUM THOMSONAE* BALF.

ABSTRACT

Encapsulated cycocel and ancymidol were the only growth regulators that affected both growth and flowering of Clerodendrum. The use of cycocel was discontinued because it induced chlorosis and, furthermore, it is very costly. Of the growth regulators tested, only morphactin affected axillary shoot development. However, its use is not practical because of the extensive leaf distortion, thickening of the stem, rosetting at the growing point and curvature of the central axis.

Elongation was controlled in both the "Wisconsin" clone and the "European" clone by a single treatment with ancymidol. There were 5 to 12 times as many flowers in the ancymidol treated "Wisconsin" clone compared to the control; the "European" clone produced only a few more flowers than plants not treated with ancymidol. The number of days to flowering was essentially the same for untreated and ancymidol treated plants. Dry wt of leaves and stem decreased with increased ancymidol concn which correlates with the decrease in root growth.

Plants receiving as low as 80 ppm of N and as high as 320 ppm N showed little significant difference in elongation. In calcined clay elongation was retarded and a greater number of flowers was produced; in peat-perlite mixture elongation was not controlled and fewer flowers were produced. Plants grown at pH 6.5 produced almost 3 times more flowers in both media than pH 4.5.

INTRODUCTION

Without exogenous growth regulators, the "Wisconsin" clone produces few flowers and, therefore, commercial production will be dependent on the use of retardants. Hildrum (12), working with the "European" clone, found that ancymidol (A-Rest) treated plants were shorter and flowering was slightly increased compared to untreated plants.

Other growth regulators also were tested to determine their effect on growth and flowering. Larson (21) treated poinsettias with encapsulated cycocel (2-chloroethyl)trimethylammonium chloride) (CCC) and obtained good height control. Succinic acid, 2,2-dimethyl hydrazide (SADH), applied as a foliar spray, was an effective growth retardant on over 50 species of plants (3, 6, 7, 16, 17, 19, 24, 25). Internodes were shorter, terminal growth suppressed, and foliage usually was darker green (1, 4, 5, 14, 23).

Clerodendrum produces very few axillary shoots; therefore, it is necessary to develop a method to induce prolific axillary shoot development which not only increases the number of flowers, thereby enhancing the attractiveness of this plant, but also increases shoot production for propagation. Schneider (27) and Tjia (33) reported that morphactins inhibited elongation of the main shoots and induced the development of lateral buds in chrysanthemum which resulted

in dwarf, compact, bushy plants. Williams (35) and Shanks (28) demonstrated that some cytokinins and ethephon increased the number of axillary shoots. Shanks (29) has reported similar responses with SADH.

Since only small amounts of ancymidol are required to retard growth, the lowest rate of application at which most favorable growth and flowering occurs should be determined. Many investigators (9, 10, 11, 15, 18, 20, 22, 26, 30, 32) have reported that ancymidol applied to the growing media severely arrested leaf formation and internode elongation.

Shoub and De Hertogh (31) reported that ancymidol lessens rooting in chrysanthemums. Knowledge of the top-root ratio of untreated and treated plants is important because of possible cultural and production considerations associated with water relations and nutrition.

MATERIALS AND METHODS

Growth regulators: Six experiments were conducted using different growth regulators, each replicated 2 to 4 times. Rooted cuttings were potted in 4-inch pots and pinched 2 wk later. The plants were fertilized with 1/2 strength Hoagland's nutrient solution (13) for 3 consecutive irrigations and flushed every 4th irrigation with distilled water. Encapsulated CCC was incorporated into the medium at 20, 24, and 30 g per 4-inch pot. Foliar applications of ancymidol were applied at 10, 20, 50, 100 and 200 ppm. In subsequent

experiments, ancymidol was applied as a drench. Alar (SADH) was applied either once or twice as a foliar application. Elongation of shoots and number of flowers were recorded at the termination of the experiment.

The response of axillary buds to growth regulators: Uniform stock plants, approximately 6 and 18 months old, of the "Wisconsin" clone were selected and grown in 6-inch clay pots. Sixteen plants were used for each treatment. Half of the number of plants in each age group were soft pinched and were given a foliar application of benzoadenine at either 20 or 40 ppm, ethephon (2-chloroethyl)phosphonic acid) at 500 and 1000 ppm, morphactin (methyl-2-chloro-9-hydroxyfluorene-9-carboxylate) at 5, 10 and 15 ppm, cytokinin (dimethylalyl-adenosine) at 20 and 40 ppm or alar at 4.5 ppm. A 2% poly-oxyethylene, sorbitan monolaurate (Tween 20) was used as a surfactant. Elongation and increase in number of axillary shoots were recorded at the termination of the experiment.

Different concn of ancymidol: Cuttings from both "Wisconsin" and "European" clones were selected for uniform height and were rooted and potted in 4-inch pots using the standard medium. Five plants were used per treatment and placed in a greenhouse with 80°F day and 72°F night temp. The nutritional regime included 3 consecutive irrigations of 1/2 strength Hoagland's solution followed by a distilled water flush. The treatments included 1 through 10 ppm and 30 ppm

ancymidol applied as a soil drench (30 ml per pot). Untreated plants served as controls. Final elongation, number of days to flowering, and number of flowers were recorded.

Root development with ancymidol: Plants were treated with 1, 4 and 10 ppm of ancymidol. The plants were removed from the pots, roots severed from the tops and washed with distilled water to remove the medium and then placed in beakers of water. Each of the 7 plant replicates were placed in a separate beaker containing a measured volume of 0.5 ml/l methylene blue dye solution sufficient to cover the roots (8). The roots remained in the dye solution for 15 min with occasional swirling and were then transferred to a beaker containing a measured volume of acidified isopropyl alcohol (25% solution with 5 ml HCl/1) sufficient to cover the roots. After 10 min, all roots were removed from the alcohol solution and the light transmittance of the solution was read at a wave length of 620 nm on a Beckman Model B Colorimeter. The colorimeter was standardized at 100 by using the isopropyl alcohol displacement solution. The percent transmittance of the alcohol dye solution was recorded which indicates the total surface area of roots; dry wt of stems, leaves and roots also was determined.

Different concn of N: The causes of sporadic flower production was investigated. Rooted cuttings were potted in 4-inch pots in calcined clay or equal amounts of peat-perlite and

treated with 30 ppm ancymidol (30 ml per pot). All plants were transferred to controlled environmental chambers at 75°F with fluorescent tubes (cool white) supplemented with incandescent lamps. The light intensity was approximately 1750 ft-c. The humidity level was maintained at a water vapor pressure deficit of approximately 5 mm Hg.

Full strength Hoagland's solution to provide 40 ppm N and increased levels at 40 ppm increments to 320 ppm were used. Initial plant height and pH of the medium were recorded at the start and elongation, days to flowering, total number of flowers and final pH were recorded at the termination.

pH, ancymidol and flowering: One-half of the rooted cuttings were potted in 4-inch pots with peat-perlite and the other 1/2 in calcined clay and grown in the greenhouse. The nutritional regime consisted of 1/2 strength Hoagland's solution every 3rd irrigation. After 2 wk, plants were pinched. Plants with axillary shoots, 3, 6, and 9 cm, were treated with ancymidol. pH of the peat-perlite and the calcined clay was 4.5 and 6.5, respectively. The pH of peat and perlite was raised from 4.5 to 6.5 by adding $CaCO_3$ (2) and the pH of the calcined clay was lowered from 6.5 to 4.5 by soaking 700 g of calcined clay in distilled water with 5 ml HCl added. After 24 hr the medium was drained and rinsed several times with distilled water. The plants at both pH levels and media were treated with 8 and 30 ppm ancymidol (30 ml per pot).

Eight ppm ancymidol was applied twice at 2 wk intervals. Initial plant height and pH of the medium was recorded at the outset; elongation, days to flowering, total number of flowers and final pH were recorded at termination.

RESULTS AND DISCUSSION

Effect of different growth regulators on growth and flowering: Plants grown in a medium containing encapsulated cycocel elongated less and produced more flowers with 20 and 24 g than did the control plants; however, foliar chlorosis was induced at 30 g per 4-inch pot (Table 1).

One foliar application of ancymidol had no significant effect on inhibiting elongation and stimulating flower production at 10, 20 and 50 ppm; 2 applications at 50 ppm and a single application at 100 and 200 ppm retarded elongation and produced some flowers. However, a foliar application of ancymidol is much less effective than a drench in controlling elongation and inducing flower initiation to the extent that foliar applications may not be commercially practical for the "Wisconsin" clone. One reason which could account for greater activity of a drench, as compared to foliar applications is that the Clerodendrum root system may absorb ancymidol more rapidly than the leaves. Alar used as a foliar treatment had little effect on elongation and flower number regardless of concn or number of applications.

Of the numerous growth regulators tested, only ancymidol and CCC increased flower number. Ancymidol and CCC may

Table 1. Single and multiple applications of several growth regulators on the elongation and growth of Clerodendrum.

Treatment	Rate concn.	Elongation (cm)	Flowers (number)
Control		26.5[z]	0
Encapsulated cycocel	30g/4" pot	14.0	0[y]
	25g	14.5	30.5
	20g	13.2	17.0
Alar 1250 ppm[x]		28.0	0
Alar 1250 ppm[xw]		19.8	0
Ancymidol[x]	10 (ppm)	33.6	0
	20	35.6	0
	50	27.8	0
	50[w]	20.4	11.5
	100	14.5	22.3[v]
	200	13.9	23.5
LSD 0.05		7.9	5.9

[z]Mean (10 plants per treatment).

[y]Leaves distorted and necrotic.

[x]Foliar application.

[w]2 applications.

[v]Many developing axillary buds.

decrease the endogenous level of GA (22) which could account for the increased flowering.

Development of axillary shoots with growth regulators: Considerable research has been conducted on the use of growth regulators to stimulate axillary shoot development. Inconsistent responses to the growth regulators currently limit their use (29).

The only growth regulator which was effective for both elongation and axillary shoot development was morphactin (Table 2). The age of the morphactin treated plants were incidental; however, unpinched plants produced approximately twice as many shoots as pinched ones. Striking increases in axillary shoot growth were observed with morphactins at 5, 10 and 15 ppm. Tjia et al. (33) reported that morphactins produce 1 to 10% more axillary shoots in several species; nearly all of the nodes produced axillary shoots in the "Wisconsin" clone. However, continued growth of the shoot apices was inhibited and extensive leaf distortion occurred (Fig. 1). Other visual morphological abnormalities included brittle leaves, modified leaf shape, thickening of the stem near the growing point and curvature of the normally straight central axis. These adverse effects makes the use of morphactin on Clerodendrum commercially impractical.

Ethephon, the cytokinins, alar and cycocel had little or no effect on increasing the number of axillary shoots at the concn used.

Table 2. Elongation and axillary shoot production by morphactin treated *Clerodendrum*.

Treatments ppm	Elongation (cm)[z]				Average no. of axillary shoots			
	YP[y]	YPN[x]	OP[w]	ONP[v]	YP	YPN	OP	ONP
Morphactin[u] 5 ppm	13.2[t]	44.0	12.3	64.5	20.0	44.0	20.0	41.3
Morphactin 10 ppm	11.0	41.1	11.3	39.5	25.0	42.0	25.3	45.7
Morphactin 15 ppm	8.2	17.0	8.0	34.6	25.3	49.0	27.3	48.7
LSD 0.05					NS	NS	NS	NS

[z]Growth of the central axis in cm of untreated and treated plants from time of treatment to flowering.

[y]Young pinched plant.

[x]Young unpinched plant.

[w]Old pinched plant.

[v]Old unpinched plant.

[u]Methyl-2-chloro-9-hydroxyfluorene-9-carboxylate.

[t]8 plants per treatment.

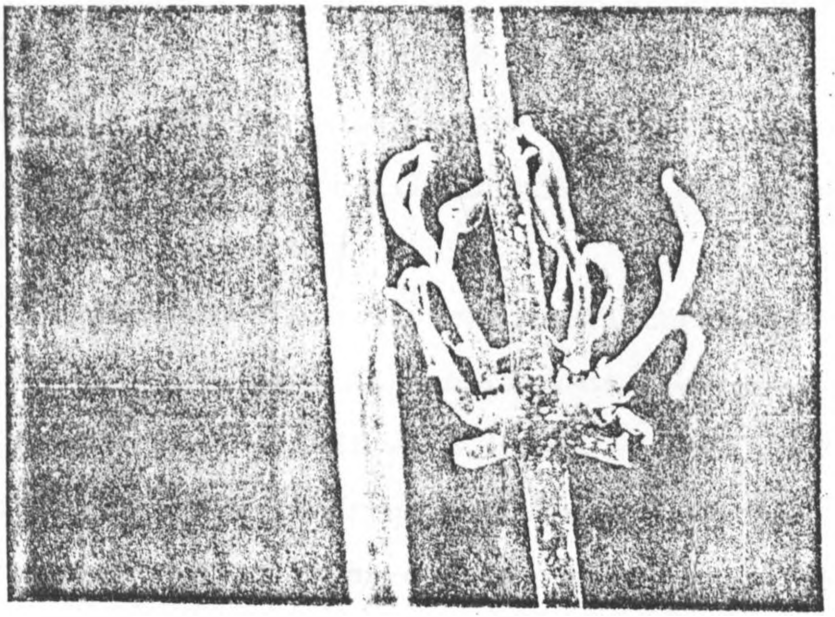

Fig. 1. Lower portion of Clerodendrum stem with node showing axillary shoot development in response to 15 ppm morphactin. Note distorted and malformed leaves.

The effect of different concn of ancymidol on "Wisconsin" and "European" clones: Elongation was controlled in both the "Wisconsin" and "European" clones by treatment with ancymidol (Table 3). Generally, elongation increased as the concn of ancymidol decreased from 9 ppm to 1 ppm. Ancymidol treatment increased flowering 5 to 12 times that of the control in "Wisconsin" but showed little effect on flowering in the "European" clone. Although at 1 ppm, ancymidol failed to control elongation, 7 times more flowers were produced than the controls in the "Wisconsin" clone. Days to flowering were essentially unaffected by ancymidol and were very similar in both clones.

The number of flowers varied with the concn of ancymidol; the shortest axillaries at the time of treatment produced the greatest number of flowers in the "Wisconsin" clone. At a given concn the shorter axillaries may have received more of the ancymidol than did the taller ones on a per g of fresh wt basis. In both clones, the variation in number of flowers may also be related to nodal position as described in Chapter I.

Root development with ancymidol: Transmittance values increased as the concn of ancymidol increased (Fig. 2) indicating a reduction in the amount of roots as the ancymidol concn was increased. Dry wt of leaves and stems also decreased with increasing ancymidol concn which correlates with the decrease in root growth (Fig. 3).

Table 3. The effect of ancymidol on pinched Clerodendrum, "Wisconsin" and "European."

Treatment	"Wisconsin" clone			"European" clone		
	Elongation (cm)[z]	Flowers (number)	Days to flowering	Elongation (cm)	Flowers (number)	Days to flowering
Control	33.7[y]	10.2	54	14.0	84.0	67
30 ppm	5.7	82.2	69	3.3	88.2	65
10	6.1	53.9	52	4.0	76.2	58
9	5.1	99.2	65	3.8	65.0	54
8	5.4	87.0	56	3.0	99.0	65
7	8.0	129.3	67	5.6	79.0	57
6	8.4	88.6	60	4.5	79.6	56
5	9.7	77.5	54	7.0	92.0	63
4	10.6	101.0	59	8.5	81.2	59
3	13.3	110.5	62	12.3	67.5	54
2	18.2	81.7	56	12.6	55.3	52
1	39.6	75.3	54	12.3	60.4	53
LSD 0.05	6.3	41.1	NS	5.0	NS	12.2

[z]Elongation from treatment to termination.

[y]Mean (7 plants per treatment).

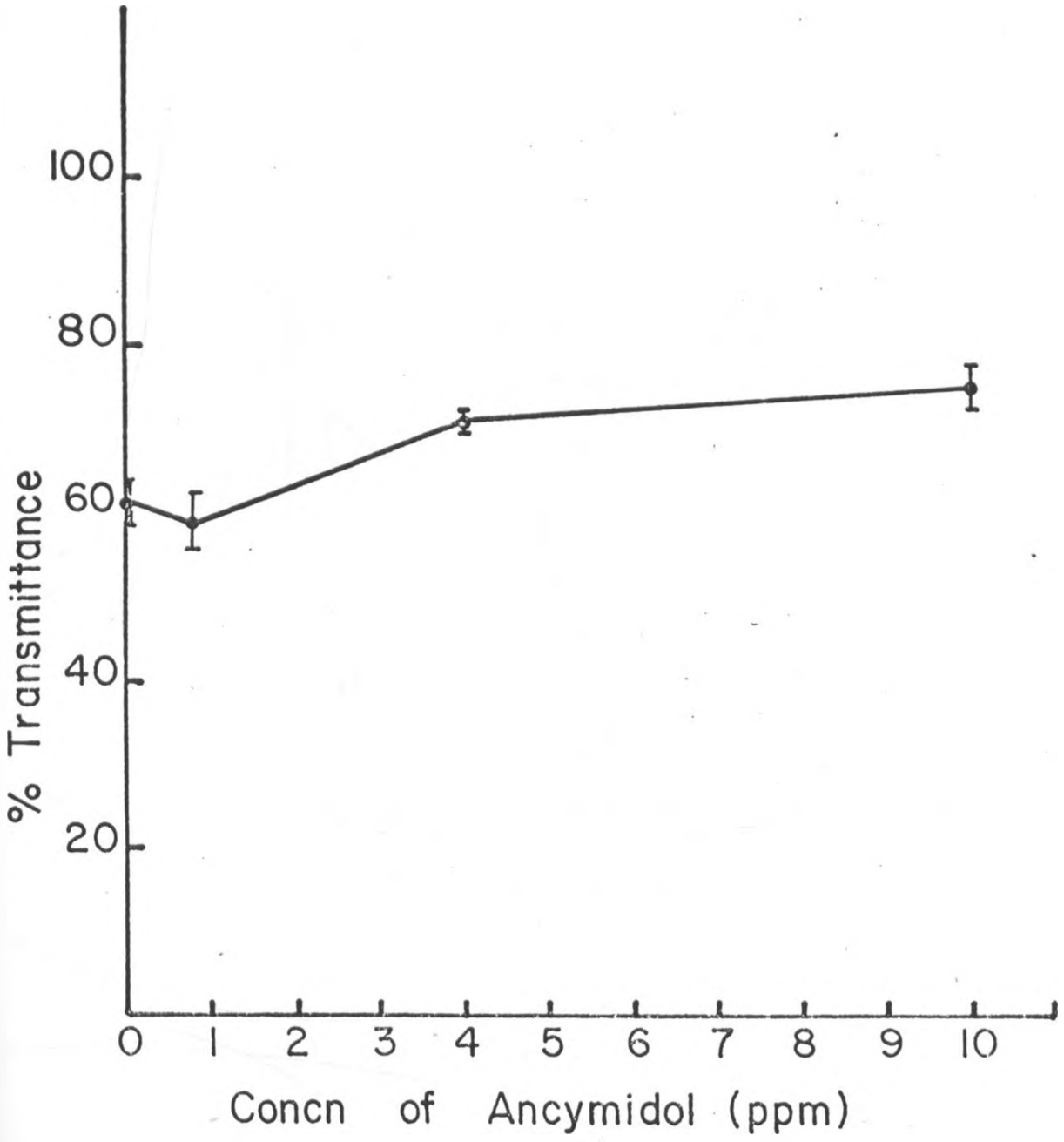

Fig. 2. The effect of 3 concn of ancymidol on the absorption surface of the roots of Clerodendrum. Vertical bars indicate the standard error of the mean.

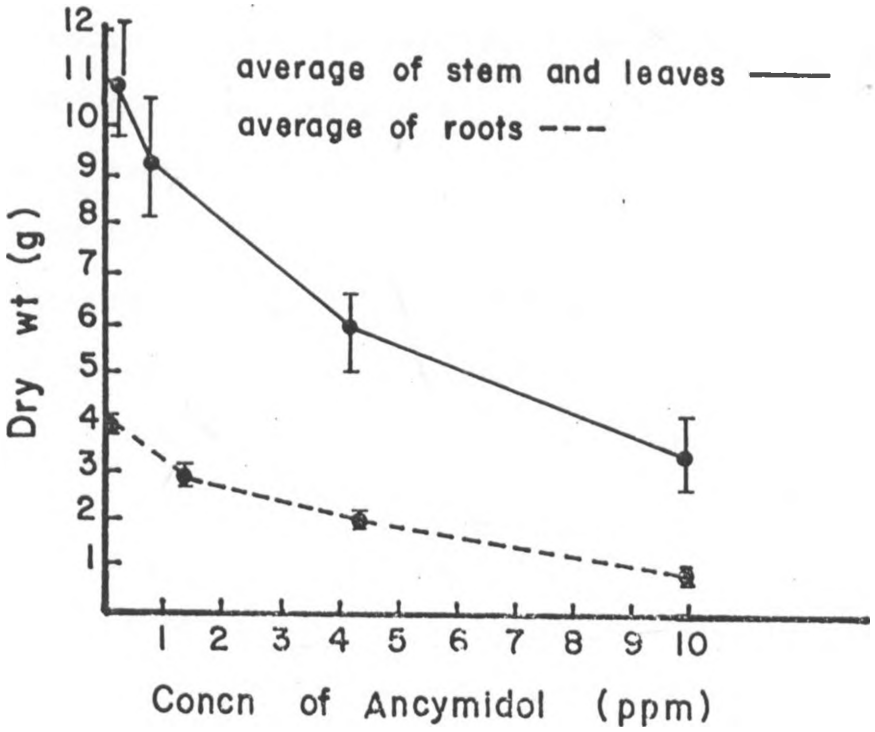

Fig. 3. Dry Wt of stems, leaves, and roots untreated and treated with 3 concn of ancymidol. Vertical bars indicate the standard error of the mean.

Since both the above and below ground portions of the plants were affected to a similar degree, cultural practices for ancymidol treated Clerodendrum will probably not have to be altered.

Different concn of N on growth and flowering: Some variable responses observed in ancymidol treated plants might be the result of N content. Plants receiving as low as 80 ppm and as high as 320 ppm N showed almost no significant difference in elongation, number of days to flowering, number of flowers and pH in the peat and perlite mixture (Table 4). Generally, nitrogen did not adversely effect growth and flowering except at 40 ppm, which is a lower concn than is commonly used for commercial production of greenhouse flower crops (Fig. 4).

In the calcined clay, elongation was retarded and greater number of flowers were produced; whereas in peat-perlite mixture, elongation was not controlled and fewer flowers were produced. Although numerous flowers were produced in calcined clay and few flowers in peat-perlite, the number of days to flowering were similar.

pH in the peat-perlite was 4.0 compared to 5.9 in the calcined clay. When the pH is at 5.9 or lower, protonation of the hydroxyl group on ancymidol may occur and subsequently complexing may take place with the negatively stabilized

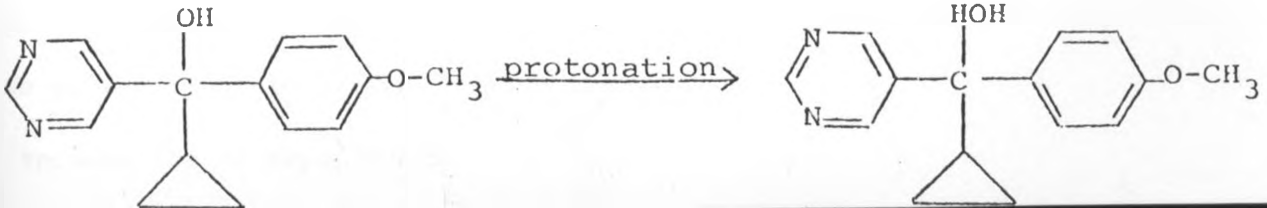

Table 4. The effect of different concn of N applied to peat-perlite or calcined clay on elongation, days to flowering and number of flowers on Clerodendrum.

Peat-Perlite	LSD 0.05	Nitrogen (ppm) 40	80	120	160	200	240	280	320
Height (cm)[z]	4.6	7.0[y]	8.8	12.4	9.4	12.4	11.0	10.6	10.0
Elongation (cm)[x]	16.3	16.6	44.8	42.7	35.9	41.2	57.0	32.2	41.8
Days to flowering	14.2	63.3	54.0	55.2	66.8	52.0	54.0	57.5	55.8
Flowers (number)	NS	11.0	8.2	18.4	30.4	14.4	12.0	11.2	39.0
pH[w]	.2	3.8	3.9	4.0	4.0	4.1	4.1	4.1	4.1

Calcined clay		Nitrogen (ppm) 40	80	120	160	200	240	280	320
Height (cm)[z]	3.9	7.8	15.3	8.5	9.4	13.4	10.8	7.4	9.2
Elongation (cm)[x]	NS	15.8	14.5	12.8	14.4	18.2	16.6	13.0	13.6
Days to flowering	NS	54.0	63.3	50.6	59.5	54.0	57.5	57.5	75.0
Flowers (number)	NS	51.0	37.0	47.6	41.4	72.3	51.0	39.5	50.8
pH[w]	NS	5.7	5.9	6.0	6.0	5.7	6.0	6.2	6.0

[z]At time of treatment.

[y]Mean (7 plants per treatment).

[x]Time of treatment to termination.

[w]At termination of experiment.

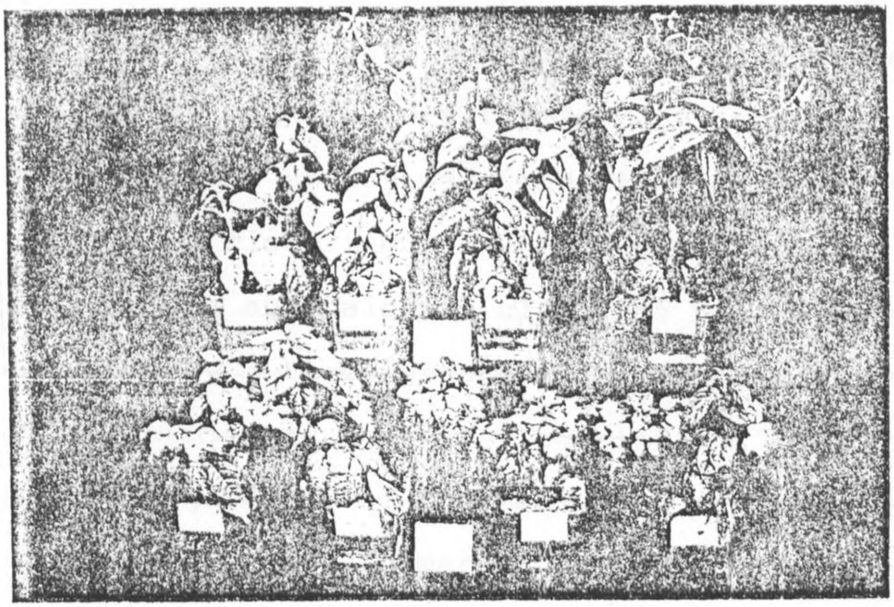

Fig. 4. Ancymidol treated Clerodendrum at different ppm of N (showing 40, 80, 120, 160). Plants in top row grown in peat-perlite, had few flowers. Plants in the bottom row grown in calcined clay, flowered.

colloid in peat. Whether or not this is the actual mechanism has not been proven, but the effect of pH on the activity of ancymidol has been established in this study.

Effect of pH on ancymidol treated plants: In earlier experiments with ancymidol treated plants, the pH of the medium changed from 4.5 to 6.5 because of the hard water applied daily and fertilizer solution applied once per week. At the higher pH more flowers were produced. With daily fertilization, only a few flowers were formed. An experiment using media at a constant pH 4.5 was conducted to verify that pH has an effect on flowering. When the pH of 6.5 was used in both peat-perlite and calcined clay, the number of flowers was approximately 3 times greater than at pH 4.5 (Table 5). However, the number of days to flowering and elongation were very similar regardless of the media and pH used. Tschabold et al. (34) working with numerous flowering plants reported that using both a bark and silt loam media fertilized with Hoagland's standard nutrient solution and given a drench of ancymidol showed greater growth retardation in the silt loam medium. Their and our experiments agree in that media type influences the effectiveness of ancymidol on plants.

Table 5. The effect of pH 6.5 and 4.5 in 2 different media on number of Clerodendrum flowers, days to flowering and elongation.

	Peat-perlite		Calcined clay	
	pH 6.5	pH 4.5	pH 6.5	pH 4.5
Flowers (number)	62.2[z]	18.3	50.0	15.0
Days to Flowering	62.0	58.0	65.0	59.0
Elongation (cm)[y]	6.3	5.8	5.5	5.0

[z]6 plants per treatment.

[y]Time of treatment to termination.

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CHAPTER IV

THE INFLUENCE OF GROWTH REGULATORS ON THE FLOWER DEVELOPMENT AND PHYSIOLOGY OF *CLERODENDRUM THOMSONAE* BALF.

ABSTRACT

A comparison of the effects of 3 growth regulators on growth and flower development of Clerodendrum, revealed that ancymidol retarded growth of plants, whereas GA_3 alone, and GA_3 plus ancymidol promoted stem elongation.

Untreated and 30 ppm ancymidol treated plants grown under a 15 hr photoperiod remained vegetative; under a 12 hr photoperiod all plants flowered. Treated plants grown under a 12 hr photoperiod initiated and differentiated floral parts sooner than untreated plants. Ancymidol enhanced the development of floral parts; however, untreated plants flowered at nearly the same time as treated ones. Plants treated with GA only and GA plus ancymidol remained vegetative in both 12 and 15 hr photoperiods. Terminal apices treated with GA were broader and more elevated than ancymidol treated plants. Stem-tips treated with both GA and ancymidol after 8 days had apical meristems that were smaller than GA treated ones.

There was very little difference in the amount of gibberellic acid extracted from plants grown under the 2 photoperiods except the induced ancymidol plants produced almost twice the amount of α amylase. There was a striking increase in α amylase units produced by plants that differentiated floral parts compared to those that remained vegetative. The greatest difference occurred at 2 days, with an abrupt decrease to 8 days which then leveled off

for the remaining 12 days. The effectiveness of ancymidol in the stimulation of GA activity which induces plants to flower strengthens the hypothesis that this chemical action does not interfere with inhibition of GA biosynthesis.

INTRODUCTION

Clerodendrum "Wisconsin" clone flowers sparsely, but treatment with ancymidol induces the plant to flower profusely. The mode of action of ancymidol has been investigated (15). This growth regulator not only provides possibilities for controlling plant growth, but also the opportunity for analyzing the endogenous control of plant growth. Ancymidol causes retardation of growth, but in Clerodendrum it also induces flowering under certain environmental conditions. This provides an opportunity to compare the development of the stem-tip from untreated and ancymidol treated vegetative and flower induced plants. Comparison also can be made of vegetative and induced plants as related to the amount of gibberellin.

Evans (8) concluded that the presence of gibberellins was a probable prerequisite for flower induction in all plants and that the gibberellin level was often limiting in long-day plants. Chouard (3) demonstrated that many long-day plants have been induced to flower with gibberellins. However, Nitsch (12) found that in many short-day plants, gibberellin inhibited flowering. Baldev and Lang (1) suggested that the growth retardant effects of synthetic compounds that also stimulate flowering may decrease gibberellin synthesis.

Weisser and Sachs (19) showed that in chrysanthemum the retardation site of action of ancymidol was in the shoot system and was independent of the effects of ancymidol upon

the root system. Leopold (11) found that ancymidol was active in growing plant systems, but was antagonistic to gibberellin action. Therefore, the determination of the amount of gibberellin in ancymidol treated flowering plants would help to elucidate the flower physiology of Clerodendrum.

MATERIALS AND METHODS

Stem-tips: Plants were grown in 4-inch pots, to a height of 9 cm in the greenhouse with 78°F day and 72°F night temp under 12 and 15-hr photoperiods. Untreated stem tips (controls) were sampled at 4, 8, 12 and 16 days after the plants reached 9 cm. Plants of the same height were given a single drench treatment with 30 ppm ancymidol. At 4, 8, 12, 16 and 20 days after treatment, stem apices were sampled to determine whether they were vegetative or initiating floral parts. The tips were fixed in FAA (formalin-aceto-alcohol), aspirated, dehydrated with tertiary butyl alcohol and infiltrated and embedded in Paraplast. Six stem tips were cut 10 μ in thickness on a rotary microtome. The sections were stained with safranin O, crystal violet and light green SF yellowish. After anatomical examination, representative photomicrographs were taken. Camera lucida drawings also were made of a treated axillary tip showing the development of floral parts.

Growth regulators: Comparisons of 7 unpinched stem tips were

made of 1) untreated plants; 2) plants treated with a single drench of 30 ppm ancymidol; 3) plants treated with 25 µl/l of GA_3 added weekly to the stem tip with a medicinal syringe and 4) plants treated simultaneously with 2) and 3). These experiments were repeated 3 times in a photoperiod of 12 hr in late April and 15 hr in late June and at 78°F day and 72°F night temp. Samples of stem tips and leaves were taken of untreated and the 3 growth regulator treatments at the 2 photoperiods at 1, 2, 4, 8, 12, 16 and 20-day intervals. The stem tips were processed for anatomical examinations as previously described.

Barley endosperm bioassay: An experiment was designed to determine if there was any change in the GA level between untreated, ancymidol treated, GA treated, and ancymidol plus GA_3 treated plants. This experiment was conducted in late April and late June. It had been previously observed that plants treated with ancymidol flower in late April, but not in the summer. Therefore a comparison of the GA levels at vegetative and flowering stages was made. The anatomy of the stem tip from 4 to 20 days was examined to compare the stage of development of the stem-tip with the levels of ancymidol and GA at comparable times during late June. The method used to extract GA from the terminal apex and leaves from the top, center and base of the plant was a modification of the techniques used by Chrispeels and Varner, and others (4, 14, 18). Five gm of tissue were used for each extraction. We found it

extremely important to macerate the plant tissue thoroughly. The entire procedure was accomplished in a 40°F room to minimize any oxidation-reduction reactions which might occur. Fifty ml of 70% ethanol was added to the tissue in an 8-inch diameter mortar and thoroughly macerated for 30 min, and further macerated to obtain finely ground and pulverized samples. The extract was filtered through miracloth to remove the solids and the supernatant centrifuged for 30 min at 10,000 g. The precipitate from this was discarded and the supernatant evaporated in an Erlenmeyer flask using a flash evaporator and frozen provided the entire procedure could not be completed. One hundred ml of distilled water was added to the flask and the pH adjusted to 3.0. Approximately 1 hr was necessary to dissolve the residue.

The supernatant obtained from above was partitioned twice with 60 ml of ethyl acetate. The top yellowish layer (ethyl acetate) was removed and evaporated to dryness with a flash evaporator. The residue from the evaporated ethyl acetate (plant extract) was dissolved in 1.8 ml of distilled water and 0.2 ml of .01 molar calcium chloride, and sodium acetate buffer solution. This supernatant was added to 25 ml flasks which completely covered 8 barley seeds.

The barley seeds were sterilized in 95% ethanol for 30 sec and 20 min in 10% Clorox. The seeds were cut in transverse halves and the embryoless halves transferred to sterile petri dishes containing sterile wetted filter paper. The petri dishes were then refrigerated for 24 hrs. The following day,

10 embryoless half seeds were transferred to 25 ml flasks containing the buffer and the plant extract. The flasks were incubated for 24 hr in a shaking water bath maintained at 25°C. The incubation temp is very critical, as we found temp deviating from 25°C gave inconsistent results. Twenty-four hr later the supernatant containing any enzymes liberated from the seeds was poured into graduate cylinders. The seeds were rinsed with 3 ml of distilled water which was added to the supernatant. The volume was adjusted to 5 ml. The α amylase was assayed as follows: zero, .02, .05, .10 and .20 ml of the supernatant was pipetted into each tube. Distilled water was added to bring the volume of each tube to exactly 1.0 ml. The reaction was started by adding 1.0 ml of a starch solution (4). Five min was allowed for the reaction, and then stopped by adding 1.0 ml of iodine solution (4) to each tube. The blue starch iodine complex was diluted by adding 5.0 ml of distilled water. The entire 8 ml was poured into a spectrophotometer cuvette and the transmittance at 620 nm was read on an Acta III double beam Spectrophotometer zeroed against a solution containing 1 ml of iodine solution plus 7 ml of water. The ΔA_{620} was calculated for each assay tube by changing the transmittance to absorbance and subtracting the absorbance of the solution containing no supernatant from the observed transmittance value for each of the other tubes. Then the amylase units of activity were calculated as follows:

$$\text{amylase units} = A(\text{absorbance})_{620}/(\text{volume of enzyme soln})(\text{reaction-time})$$

A standard curve was plotted from the amylase activity obtained by using concn of GA, 10^{-5} to 10^{-9} which was repeated 6 times.

The solution containing the iodine complex was read immediately and the temp for this procedure was maintained at a constant 72°F. It was observed that at higher and lower temp, inconsistent results were obtained because the iodine complex faded.

RESULTS AND DISCUSSION

The flower of Clerodendrum "Wisconsin" clone has 5 sepals and 5 petals, 4 stamens and a superior ovary with 4 ovules (Fig. 1). Flowers arise from axillary buds in the bractlets on the pedicles. Untreated and treated plants grown under a 15-hr photoperiod remained vegetative. The vegetative apex had 4-5 layers of tunica overlying the corpus. The rib meristem cells were tabular shaped and somewhat vacuolate. The procambium was differentiating into the leaf primordium (Fig. 4A). Untreated and treated plants grown under a 12-hr photoperiod initiated flowers some days after the plants were 9 cm tall. The untreated terminal apex 4 days after the start of the treatment showed a discrete tunica and corpus in the vegetative apex (Fig. 2A). After 8 days the untreated tip remained vegetative with a primordium in the axil of the leaf (Fig. 2B). At 12 days the terminal apex was vegetative and similar to the 8 day terminals (Fig. 2C). However, in the same tip, the lower axillaries were

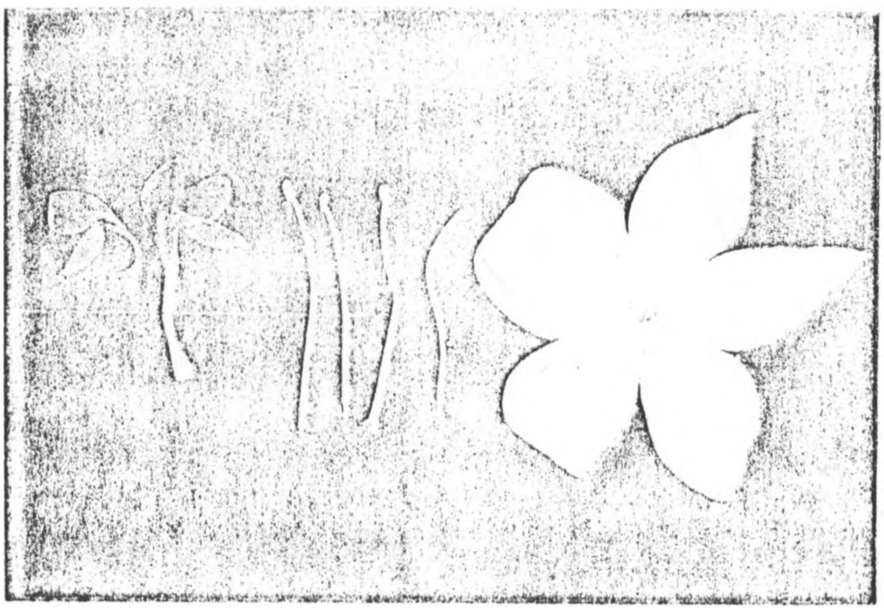
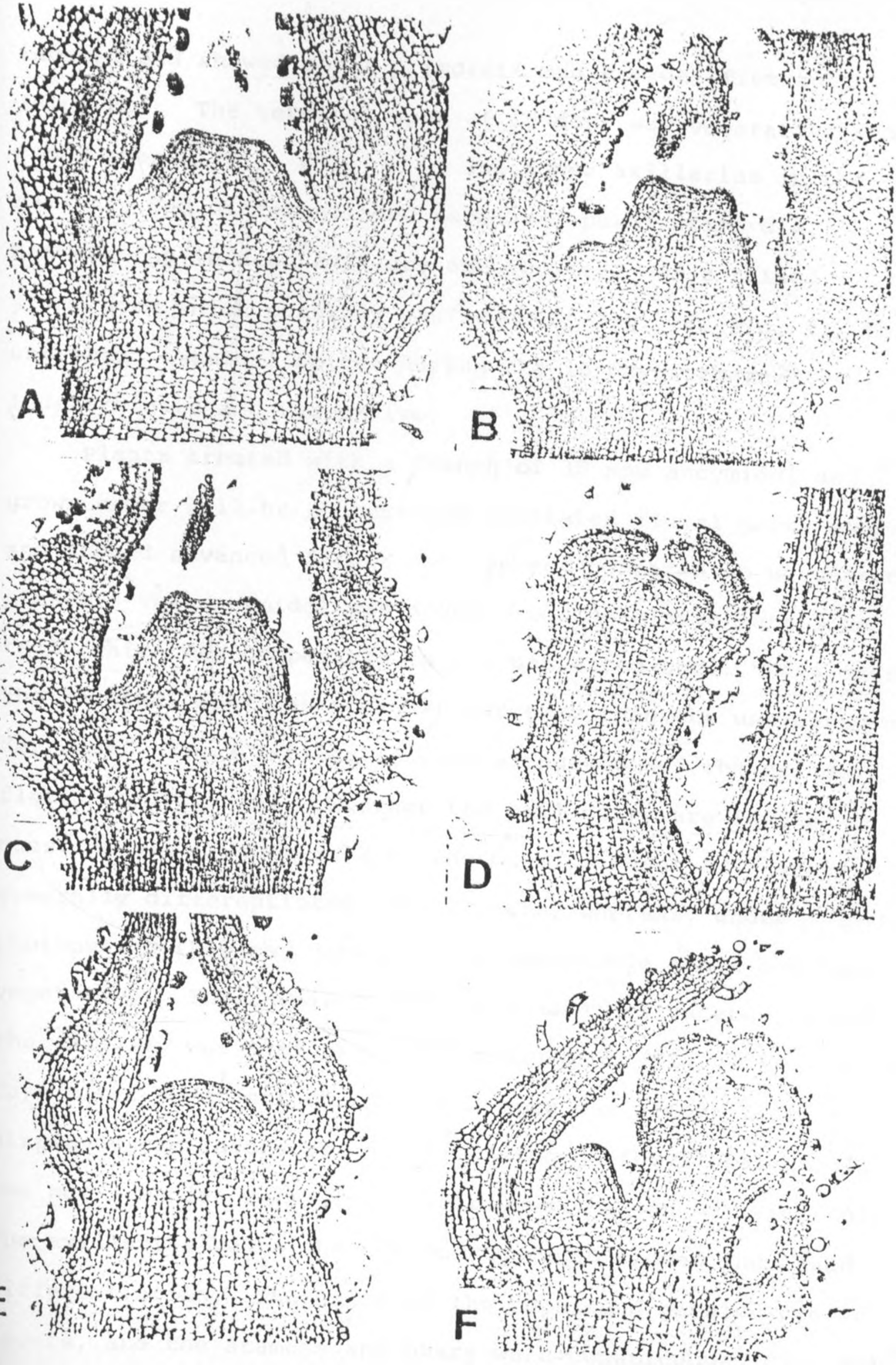


Fig. 1. Floral parts of Clerodendrum thomsonae showing irregular shaped united petals, 4 stamens, and united sepals.

apices of Clerodendrum taken at intervals starting when all plants were 9 cm tall. Untreated apices represent the controls for plants treated with growth regulators. A. Vegetative apex at 4 days. B. Vegetative apex at 8 days. C. Vegetative apex at 12 days. D. Axillary apex from same plant as C at 12 days showing differentiation of sepal primordia. E. Vegetative apex at 16 days. F. Axillary apex from same plant as E at 16 days showing differentiation of sepal and petal primordia. X 101.


A
B
C
D
E
F

induced and showed sepal primordia and a wide, flat apex (Fig. 2D). The terminal apex at 16 days was vegetative and domed-shaped (Fig. 2E). The lower axillaries in the same tip had differentiated sepal and petal primordia. Between the induced axillary and the leaf was a raised primordium which possibly was induced (Fig. 2F, Fig. 3). The untreated terminal apices during the 16 days remained indeterminate and vegetative.

Plants treated with a drench of 30 ppm ancymidol and grown under a 12-hr photoperiod initiated floral parts sooner and showed advanced flower development compared to untreated plants. The ancymidol was added when the plants were 9 cm tall, which was comparable to the untreated ones. At 4 days the terminal apex was somewhat narrower than the untreated 4 day apex. The 2 axillaries were elongated and the apices flattened (Fig. 4B). Whether the axillaries are induced is questionable. Under a 12-hr photoperiod these axillaries generally differentiated into flowers, whereas, under a 15-hr photoperiod they may develop to a comparable stage but remain vegetative. The terminal apex at 8 days was vegetative and the axillary was similar to the 4 day ones (Fig. 4C). Twelve days after treatment the terminal apex was almost flat and displayed the layers of tunica. Although the floral axillary was not a median cut, it was fairly well advanced (Fig. 4D). The axillaries below the one in Figure 4D showed increased differentiation. The cells of the sepalsand petals were in layers, and the stamens and ovary were conspicuous (Fig. 4E).

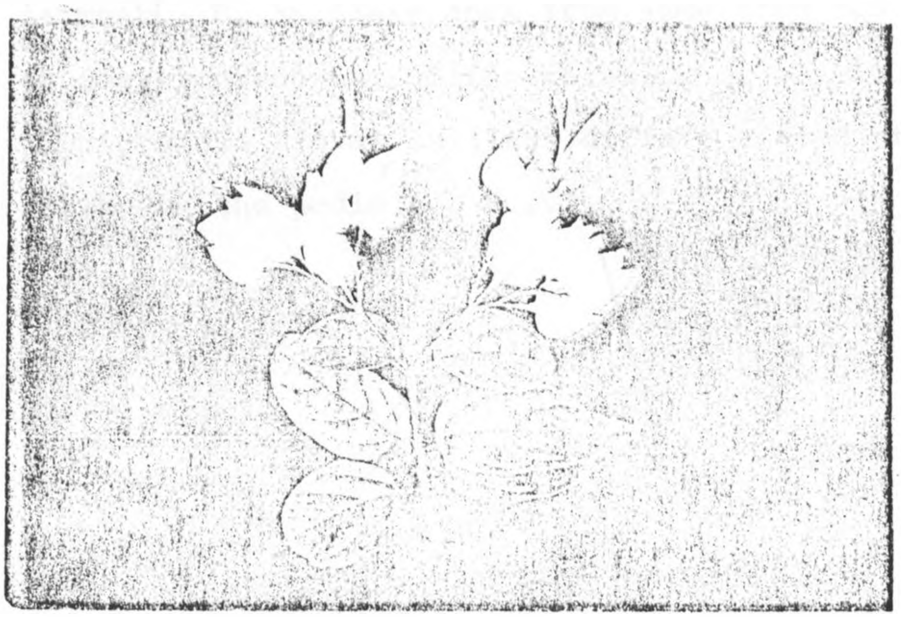
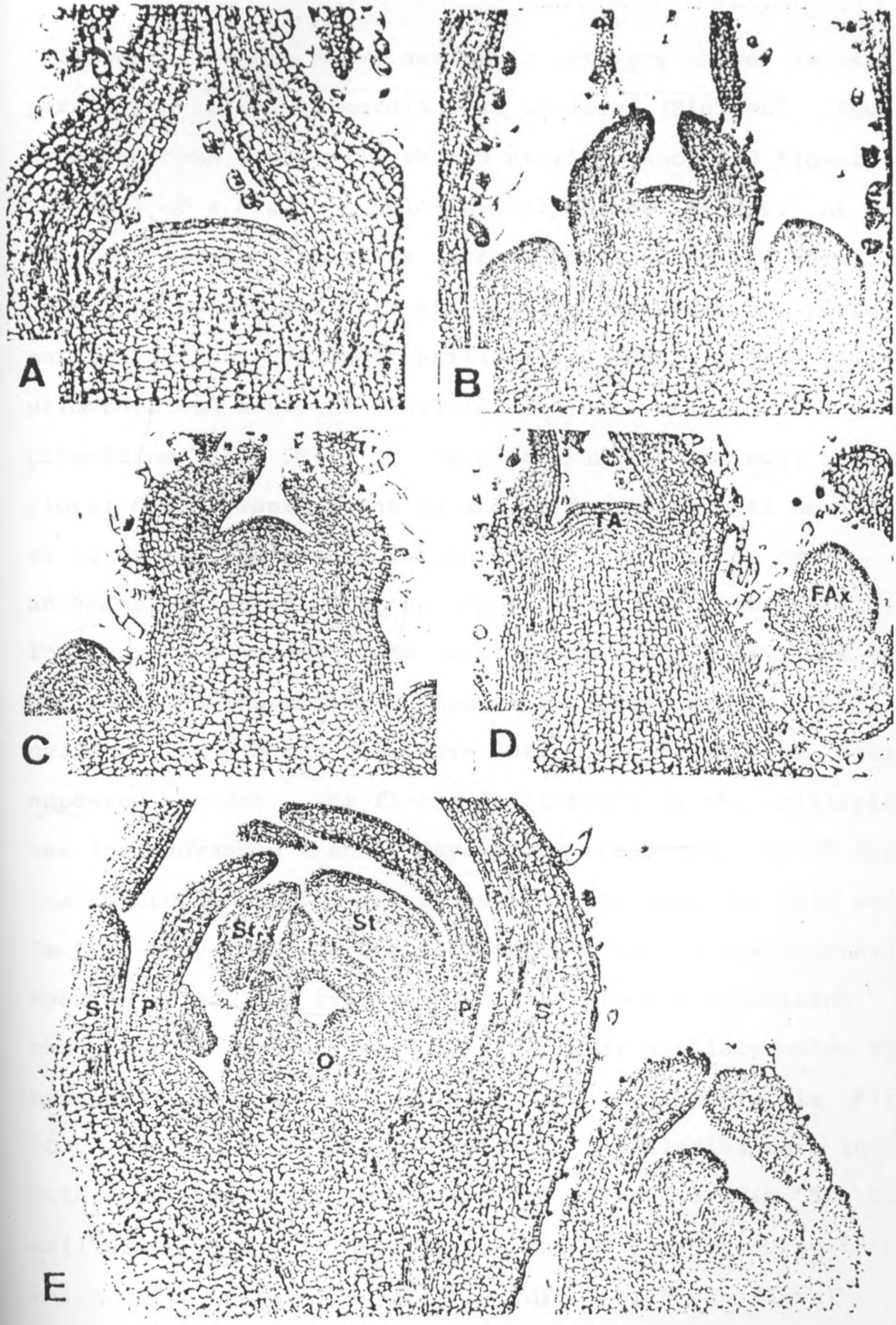


Fig. 3. A portion of the main shoot of Clerodendrum thomsonae showing inhibition of the terminal apex and flowering of the 2 axillaries.

apices of Clerodendrum taken at intervals starting when all plants were 9 cm tall. The plants were given a drench treatment with 30 ppm ancymidol. Terminal apex, TA; floral axillary, FAx; sepal, S; petal, P; stamen, St; ovary, O. A. Vegetative apex 4 days after treatment showing the corpus beneath the 4 layered tunica. B. Vegetative terminal apex and 2 elongated axillaries which may possibly be induced 4 days after treatment. C. Vegetative terminal apex and elongated axillary (left) as in B, 8 days after treatment. D. Vegetative terminal apex and floral axillary apex (right) 12 days after treatment. E. Axillary apex from same stem tip as D at a lower position showing differentiation of all floral parts. Right, small floral axillary differentiated at a lower bractlet on the pedicle. X 101.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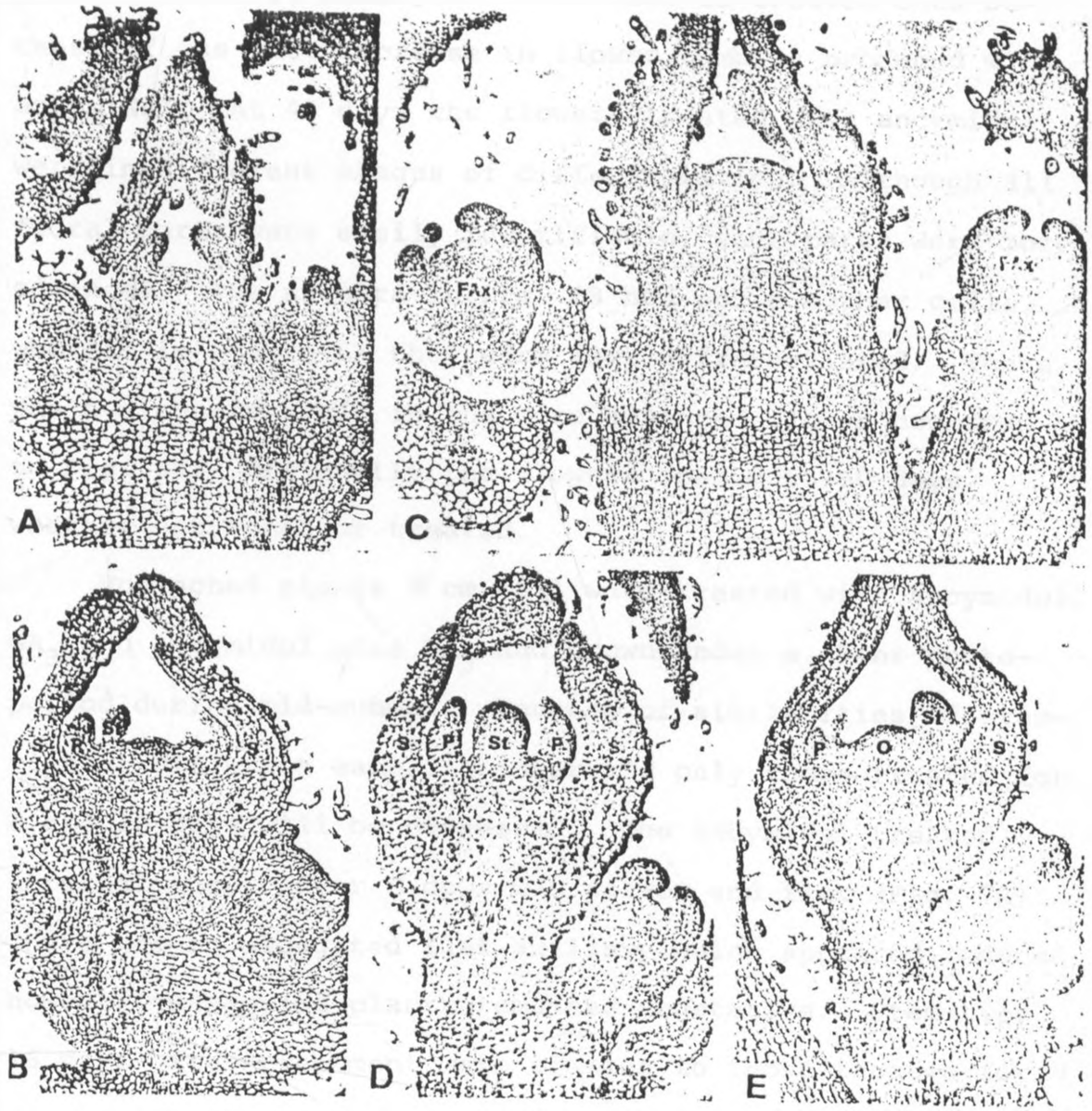
A flower below the above described axillary showed sepal, petal, and stamen primordia less advanced (Fig. 4E). The expanded cyme frequently showed smaller, unopened flowers in the axil of a bractlet below a fully opened flower. At 16 days the terminal apex was narrow and flat and the axillaries were induced and possibly starting to differentiate floral parts (Fig. 5A). A lower axillary had sepal, petal, stamen primordia and a slight elevation indicative of the ovary primordium (Fig. 5B). Of the tips examined the most advanced floral development in the 12 and 16 day treatments occurred at 12 days. The reason for this may be the concn of ancymidol absorbed into the tip and the slight variability in plant development. The terminal apex at 20 days was broad and flat but showed the tunica-corpus organization. The cells in the rib meristem were less vacuolate and the stem appeared broader. The floral development of the axillaries was less advanced than 12 days after treatment. At 20 days the internode length was greater. The reason for this may be that ancymidol was not reacting as fast in the terminal apex as in earlier treatments, therefore the inhibiting characteristics were subdued. The first axillary below the terminal apex had sepal and beginning petal primordia (Fig. 5C). Two and 3 nodes below the apex, axillaries were induced with sepal, petal, stamen and ovary primordia (Fig. 5D, E). Axillaries also were apparent in the axil of the bractlets which differentiated sepal primordia. Although floral initiation in the untreated plants was much slower than in ancymidol treated ones, the time red petals and stamens

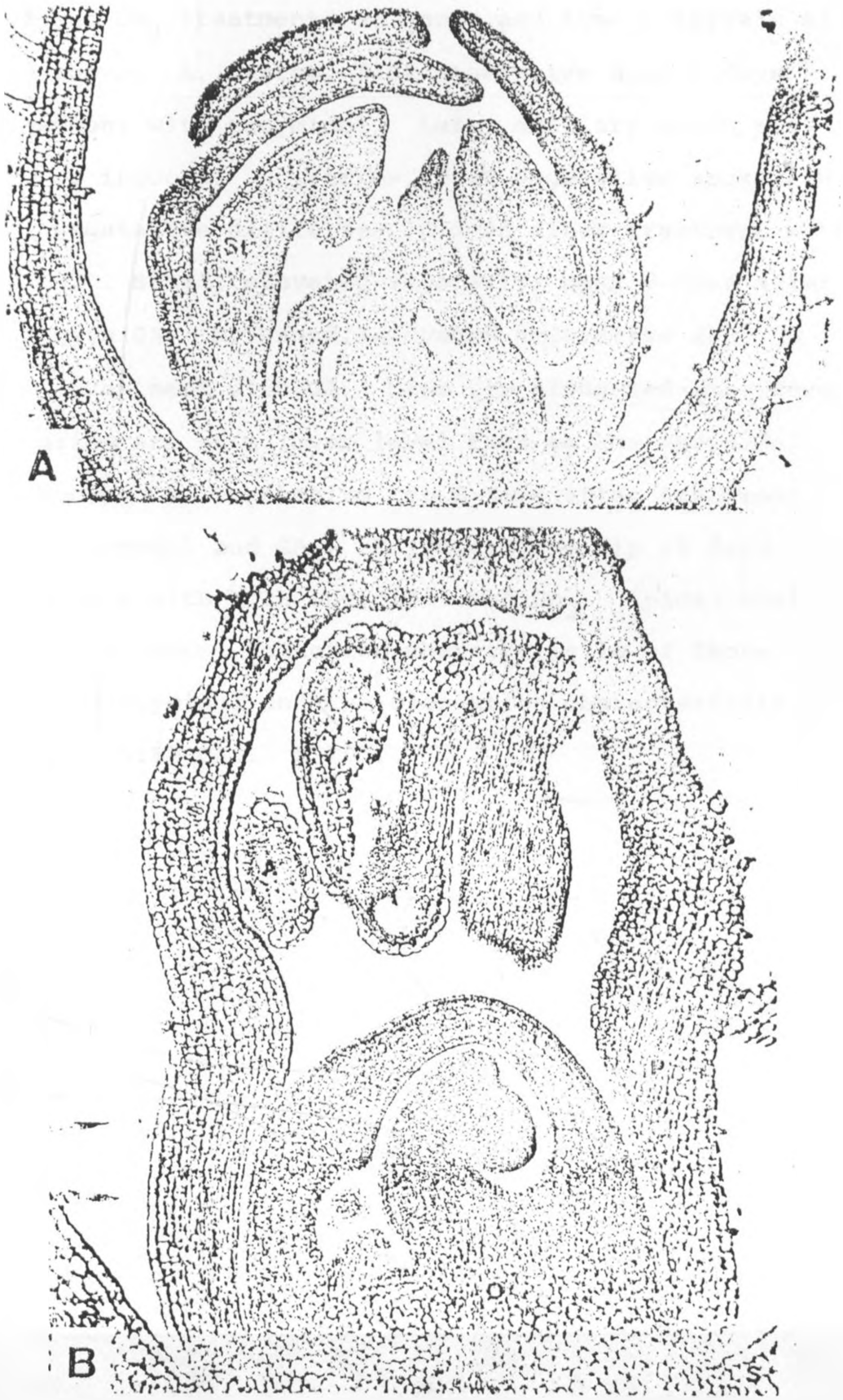
apices of Clerodendrum taken at intervals when all plants were 9 cm tall. The plants were given a drench treatment with 30 ppm ancymidol. Terminal apex, TA; floral axillary, FAx; sepal, S; petal, P; stamen, St; ovary, O. A. Vegetative apex and 2 axillaries which are possibly induced 16 days after treatment. B. Floral apex from same plant as A showing differentiation of floral parts. Right, induced apex with sepal primordia in axil of bractlet. C. Vegetative terminal apex and 2 lateral floral apices with sepal primordia. D. Floral apex from same tip as C showing differentiation of floral parts. Right, induced axillary with sepal primordia in the axil of the bractlet. E. Floral axillary from same tip as C showing differentiation of floral parts. Right, induced axillary in axil of bractlet. X 101.


A
C
FAx
FAx
B
P
St
S
D
S
P
St
P
S
E
St
S
P
O
S

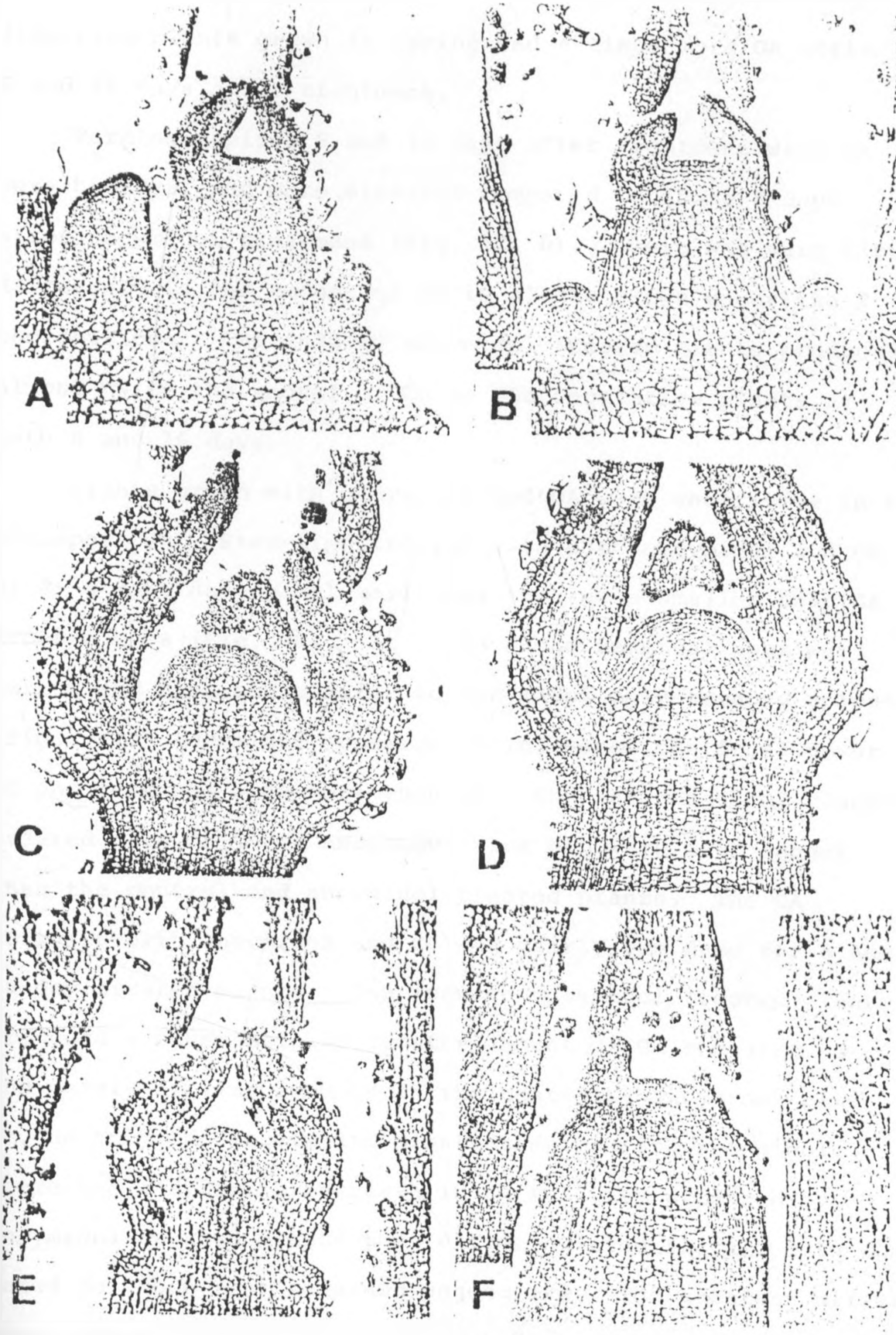
emerged from the white sepals were similar. Ancymidol enhances the development of floral parts, but the untreated plants flower at nearly the same time as treated ones because of the vast increase in flower numbers produced with ancymidol. At 45 days the flowers treated with ancymidol were in different stages of differentiation. Although all floral parts were easily identifiable, some parts were more advanced. The anthers in Fig. 6B had spore mother cells, whereas in Figure 6A they were less differentiated. There was a discrete ovule in the more advanced flower compared to Fig. 6A. Ordinarily the flowers opened 55-60 days whether untreated or treated.

Unpinched plants 9 cm tall were treated with ancymidol, GA_3 and ancymidol plus GA_3 and grown under a 15-hr photoperiod during mid-summer. Because of similarities of stem-tips at different sampling intervals only those treated for 8 and 16 days will be discussed. The ancymidol treated terminal apex, after 8 days was narrow and flat (Fig. 7A). There was an elongated flat axillary which appeared induced, however, unsampled plants remained vegetative. Stem-tips 16 days after treatment were similar to those treated for 8 days (Fig. 7B). Ordinarily plants grown in early spring flowered 7 weeks after treatment. The GA activity as determined by the barley endosperm test 8 and 16 days after treatment with ancymidol was similar for vegetative plants grown in mid-summer. However, tissue from ancymidol treated

treatment with ancymidol showing an early and late stage of flower differentiation. Sepal, S; petal, P; stamen, St; anther, A; ovary, O. A. Axillary differentiating flower parts. B. Axillary showing advanced differentiation of flower parts. Spore mother cells apparent in anther and ovule in ovary. X 101.


St
A
A
A
P
P
B
S

after a single drench treatment with 30 ppm ancymidol per 4-inch pot, 25 cc of 25 ppm gibberellic acid (GA_3) applied to the terminal apex at 3 weekly intervals, and both ancymidol and GA_3 treatments at concn and time intervals as mentioned above. A. Narrow, flat vegetative apex 8 days after treatment with ancymidol. Left, axillary which possibly may be induced. B. Narrow, flat vegetative apex with 2 lateral vegetative axillaries 16 days after treatment with ancymidol. C. Broad, elevated vegetative apex 8 days after treatment with GA. D. Broad, elevated vegetative apex 16 days after treatment with GA. Stem tip elongated therefore the axillaries are at a lower level than on the ancymidol treated shoots. E. Vegetative tip 8 days after treatment with both ancymidol and GA_3. F. Vegetative tip 16 days after treatment with both ancymidol and GA_3. Apical meristems have the floral induction characteristic of those treated with ancymidol and the elongation characteristic of those treated with GA_3. X 101.


A
B
C
D
E
F

flowering plants grown in spring had 4 times more GA activity 8 and 16 days after treatment.

Terminal apices 8 and 16 days after treatment with GA were broader, and more elevated compared to those 8 days after ancymidol treatment (Fig. 7C, D). There was very little difference in the amount of GA in plants grown under the 2 photoperiods. The induced ancymidol treated plants produced almost twice the amount of GA as the vegetative plants at both 8 and 16 days.

Plants grown with ancymidol and GA were vegetative in both photoperiods. Stem-tips treated with both ancymidol and GA after 8 days had apical meristems that were smaller than GA treated ones (Fig. 7E). At 16 days the terminal apex was narrow and flat and similar to ancymidol only treated apices (Fig. 7F). Ancymidol appeared to influence the development of the terminal apex more than GA. The internodes of plants treated with GA_3, and ancymidol plus GA_3 were much longer than the control and ancymidol treated plants. The GA activity with ancymidol and GA was nearly the same for 8 and 16-day treated plants. The induced ancymidol treated plants produced a substantially greater amount of GA compared to the nearly similar amounts in the controls, ancymidol plus GA, GA only and vegetative plants treated with ancymidol. There was a striking increase in GA activity produced by ancymidol treated plants that differentiated floral parts compared to those that remained vegetative. The greatest difference occurred at 2 days, with an abrupt decrease to 8 days which then leveled

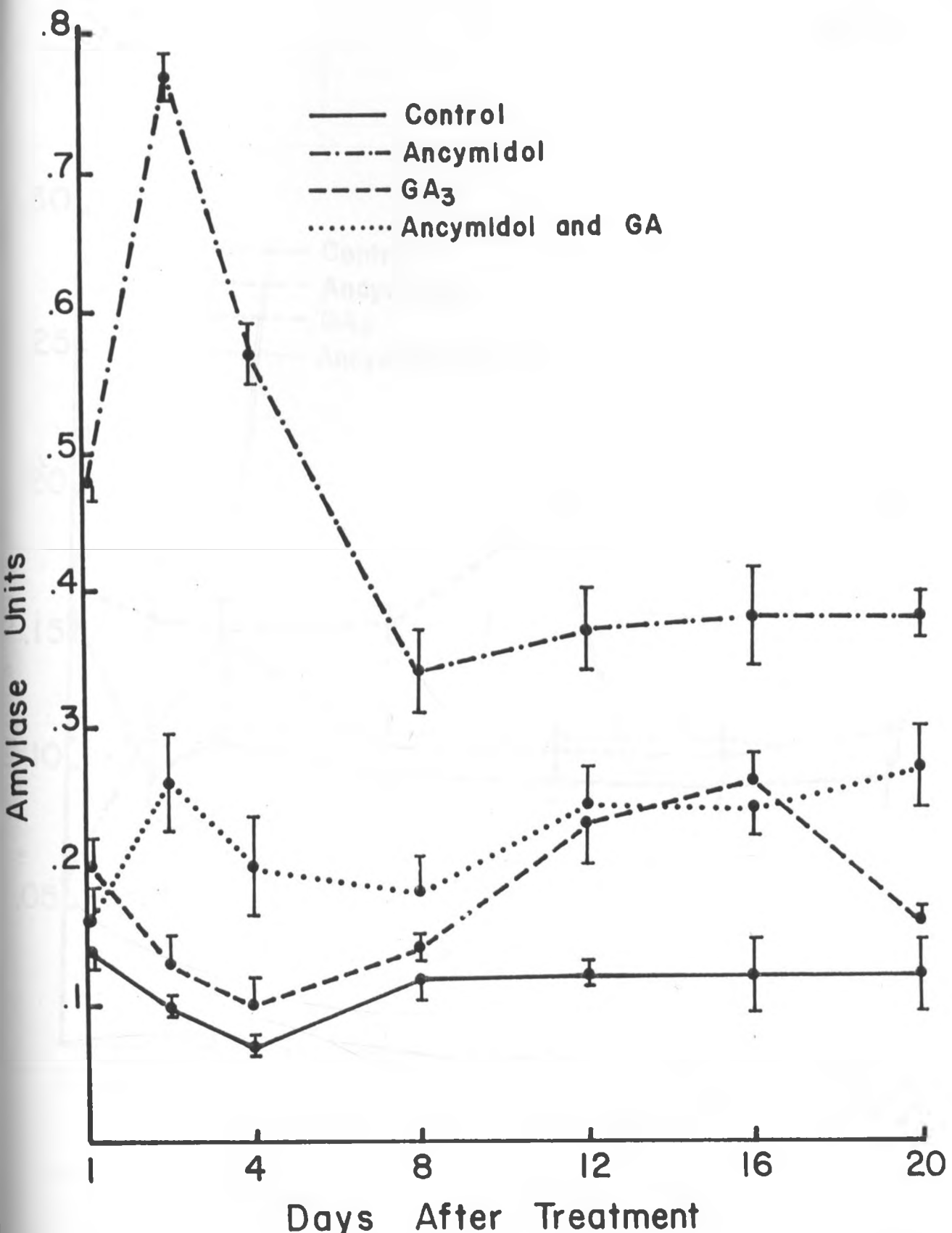


Fig. 8. α amylase produced at different intervals using the barley endosperm assay on untreated and ancymidol and GA treated plants grown under 12 hr photoperiod in late spring. Amylase units = A_{620}/(volume of enzyme soln) (reaction time). Vertical bars indicate the standard error of the mean.

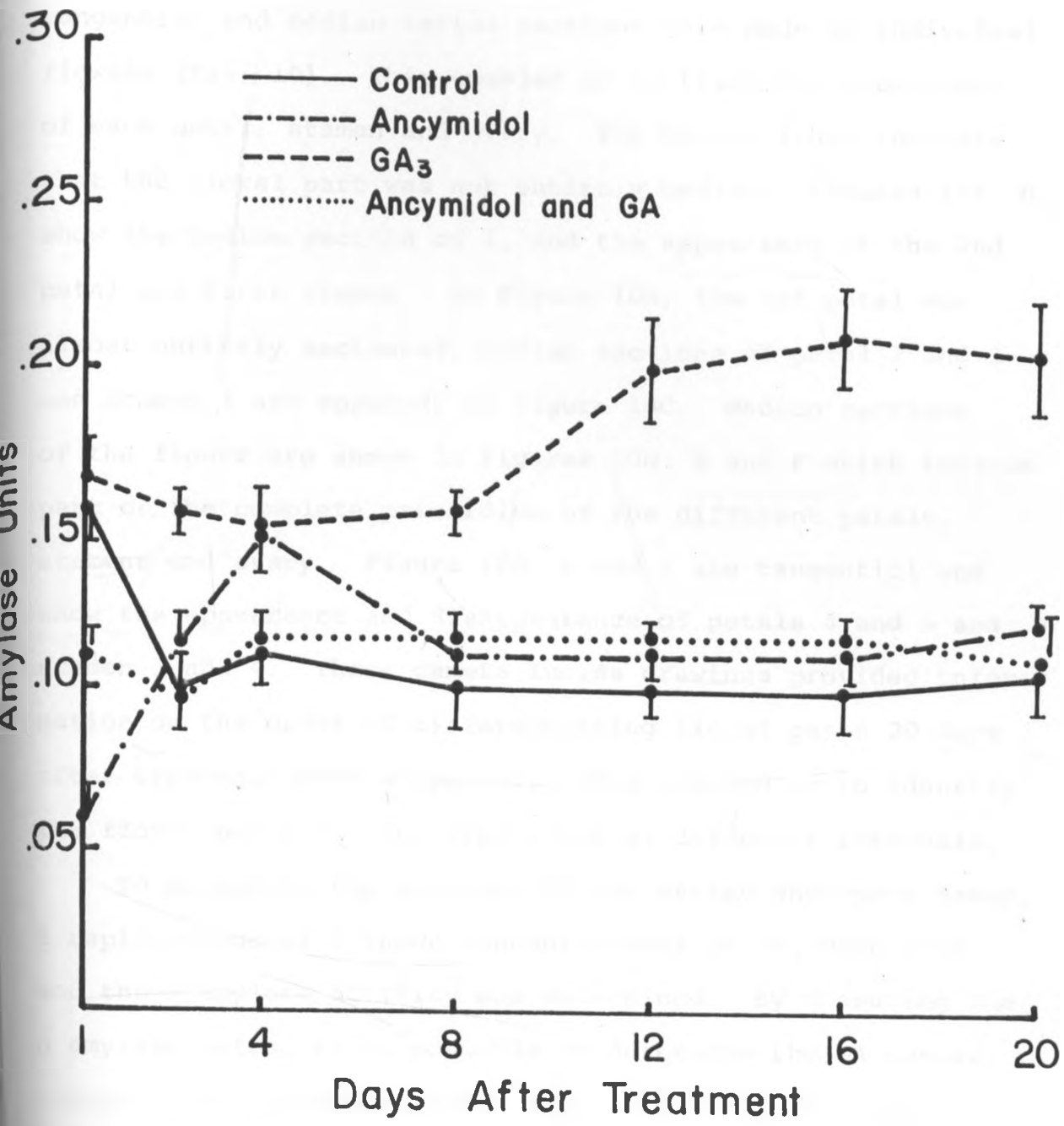


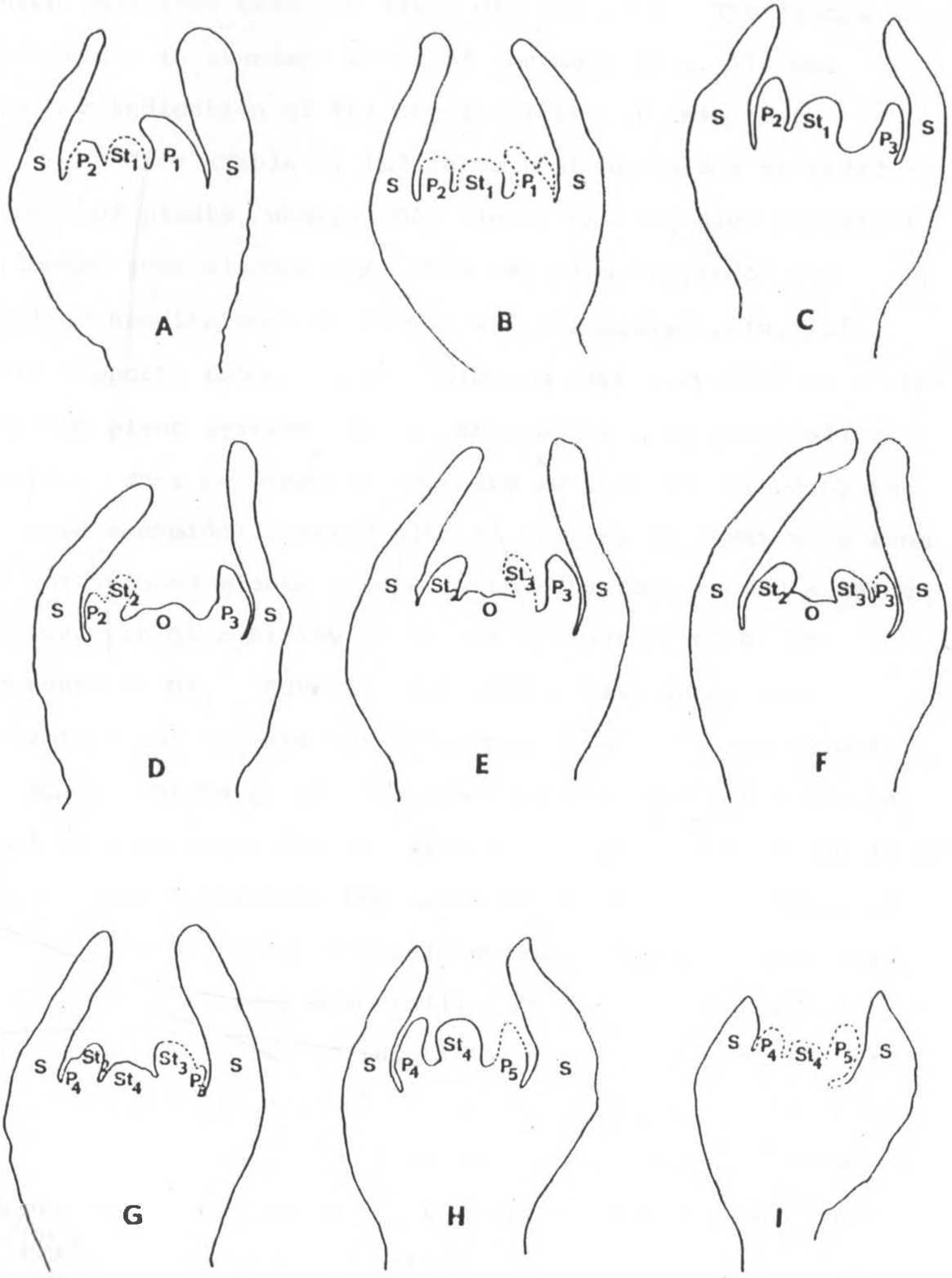
Fig. 9. α amylase produced at different intervals using the barley endosperm assay on untreated and ancymidol and GA treated plants grown under 15 hr photoperiod in late spring. Amylase units = A_{620}/(volume of enzyme soln)(reaction time). Vertical bars indicate the standard error of the mean.

off for the remaining days.

To understand the development of all parts of a flower, tangential and median serial sections were made of individual flowers (Fig. 10). This enabled us to trace the appearance of each petal, stamen and ovary. The broken lines indicate that the floral part was not entirely median. Figures 10A, B show the medium section of 1, and the appearance of the 2nd petal and first stamen. In Figure 10B, the 1st petal was almost entirely sectioned, median sections of petal 2 and 3 and stamen 1 are apparent in Figure 10C. Median sections of the flower are shown in Figures 10D, E and F which include part or the complete primordium of the different petals, stamens and ovary. Figure 10G, H and I are tangential and show the appearance and disappearance of petals 4 and 5 and stamen 3 and 4. These camera lucida drawings provided information on the order of differentiating floral parts 20 days after treatment with ancymidol. This enabled us to identify the floral parts in stem tips taken at different intervals.

To determine the accuracy of the barley endosperm assay, 6 replications of 5 known concentrations of GA_3 were used and the α amylase activity was determined. By measuring the α amylase units, it is possible to determine the GA concentration from a standard curve (Fig. 11). The standard error of the mean was determined and only slight variation was found

again tangential sections through a single flower treated 20 days with 30 ppm ancymidol. Broken line indicates the presence of a floral part which is not median. Clerodendrum has 5 sepals, 5 petals, 4 stamens and a superior ovary. Sepals, S; petals, P; stamens, St; and ovary, O. A. Sepals, petal 1 median, petal 2 non-median, and 1 stamen non-median. B. Sepals, petal 1, and 2 non-median; stamen 1 almost median. C. Sepals, past petal 1, petal 2 and 3, and stamen 1 median. D. Sepals, petal 2 non-median, petal 3 median, stamen 2 non-median, and first evidence of ovary. E. Sepals, petal 3 and stamen 2 median and 3 non-median, evidence of ovary. F. Sepals, petal 3 non-median, stamen 2 and 3 median, evidence of ovary. G. Sepals, petal 3 and 4 non-median, stamen 2 median, and stamens 3 and 4 non-median. Tangential sections, passed ovary. H. Sepals, petal 4 median, petal 5 non-median, and stamen 4 median. I. Sepals, petal 4 and 5, and stamen 4 non-median. This figure shows representative diagrams from serial sections of the same differentiating flower. Approximately X 50.

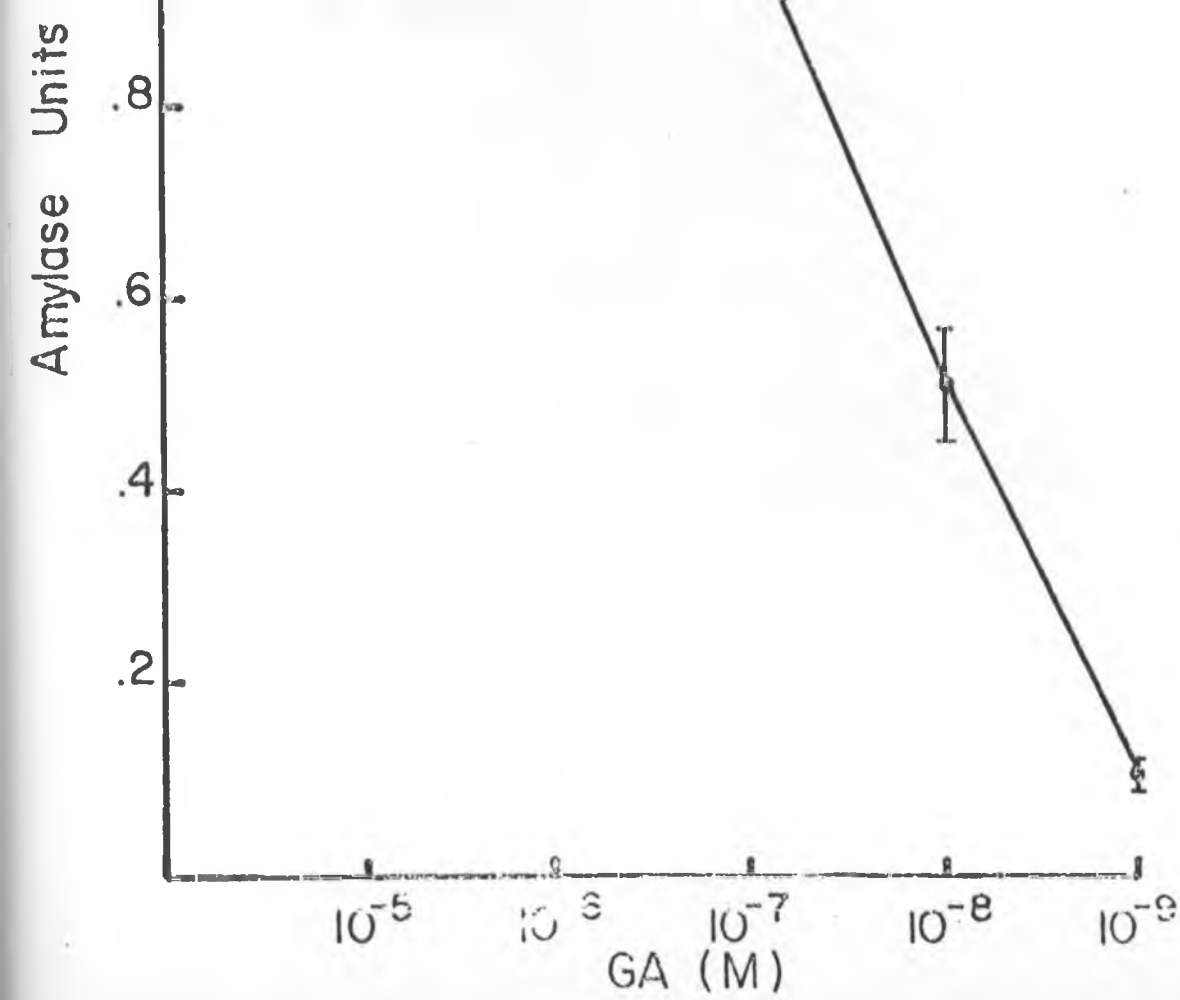

S
P2
St1
P1
S
A
S
P2
St1
P1
S
B
S
P2
St1
P3
S
C
S
St2
P2
O
P3
S
D
S
St2
St3
P3
S
E
S
St2
O
St3
P3
S
F
S
St2
St3
P4
St4
P5
S
G
S
P4
St4
P5
S
H
S
P4
St4
P5
S
I

which indicates that the assay was reliable. The little difference in standard error of the mean (Fig. 11) was another indication of the repeatability of this test.

The data (Table 1) indicates that ancymidol retarded growth of plants, whereas GA_3 alone, and GA_3 plus ancymidol promoted stem elongation. When GA_3 plus ancymidol was applied simultaneously, growth was stimulated (Fig. 12). This supports Leopold's (11) findings that ancymidol is active in growing plant systems, but is antagonistic to gibberellin action. This response is contrary to that exhibited by the induced ancymidol treated plants (Fig. 8, 9). Extracts from flower induced plants treated with ancymidol showed a large increase in GA activity which was an indication of an increase in GA_3. Several experiments have shown that ancymidol and GA_3 are not antagonistic which supports our findings. Dicks et al. (7) reported that ancymidol and GA_3 applied separately did not affect pedicel length of the first flower; but increasing the level of GA_3 in the presence of ancymidol promoted pedicel elongation. There is some evidence that different gibberellins cause different plant responses. The limited information indicates that ancymidol does not inhibit GA synthesis (6, 11). Syndor and Larson (16) found that GA_7 is active in overcoming azalea flower bud dormancy and that GA_3-activity might possibly be ascribed to cell elongation prior to anthesis.

The mode of ancymidol action may be specific for different gibberellins in that it may be inhibitory to the

of GA using the barley endosperm assay. Amylase units = A_{620}/volume of enzyme soln)(reaction time). Vertical bars indicate the standard error of the mean.


Amylase Units
.8
.6
.4
.2
10^{-5}
10^{-6}
10^{-7}
10^{-8}
10^{-9}
GA (M)

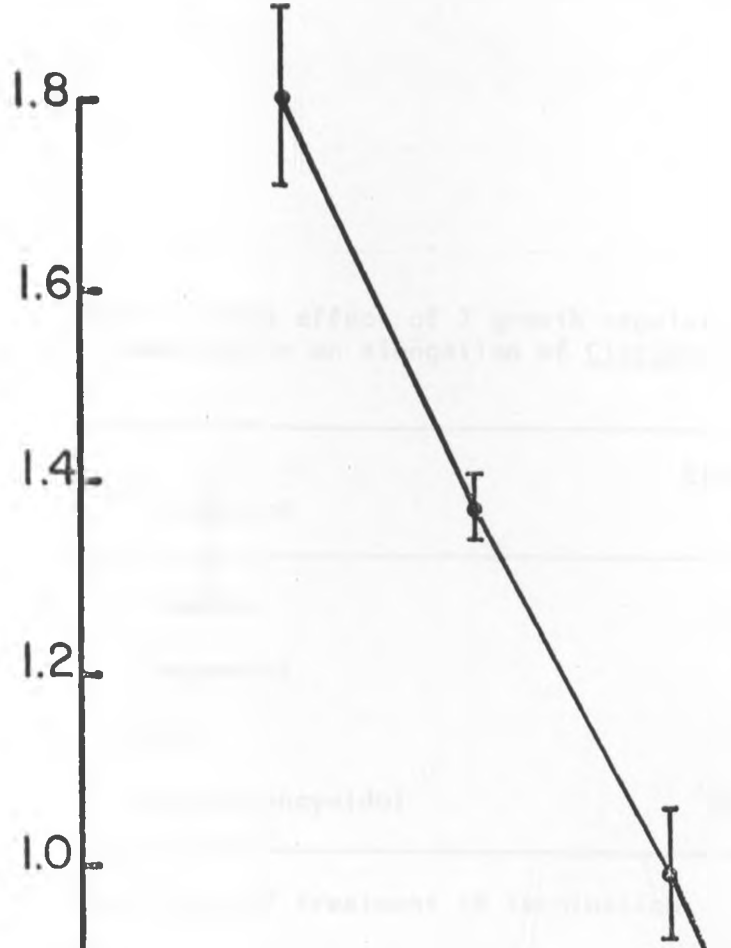

1.8
1.6
1.4
1.2
1.0

Table 1. The effect of 2 growth regulators alone and in combination on elongation of Clerodendrum.

Treatment	Elongation (cm)[z]
Control	9.1[y]
Ancymidol	1.9
GA	25.5
GA and ancymidol	22.5

[z]From time of treatment to termination.

[y]6 plants per treatment.

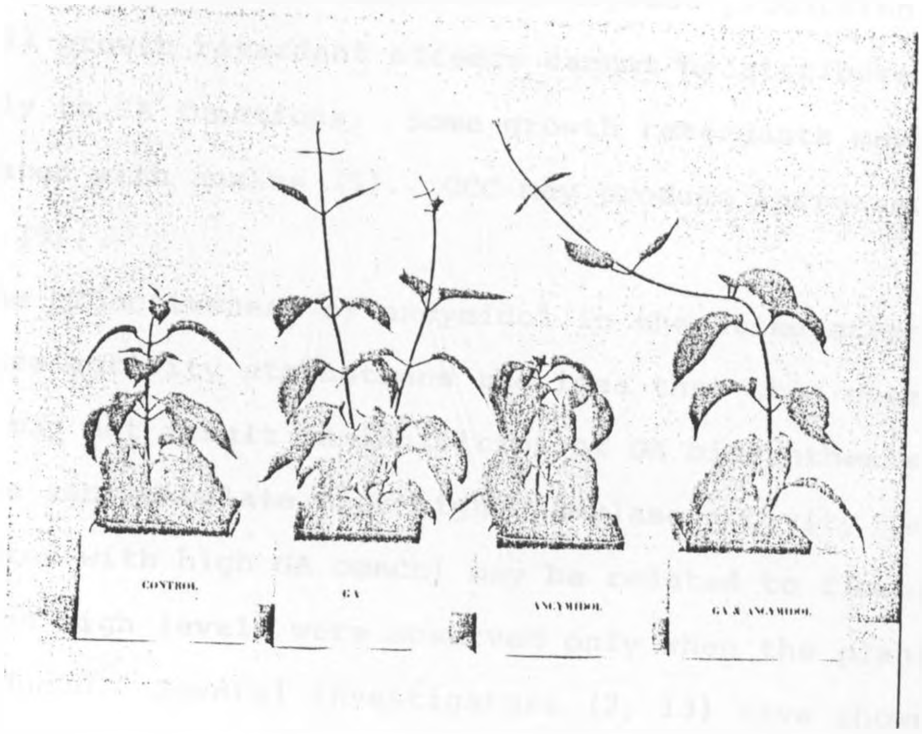


Fig. 12. Untreated and treated plants showing the relief of dwarfing effects of ancymidol on Clerodendrum by GA_3 and GA_3 plus ancymidol.

action of some and stimulatory to others. In our experiments, ancymidol stimulated GA_3 activity which is shown by the large increase of α amylase units. However, there is no supporting evidence from the barley endosperm experiments that ancymidol was antagonistic to GA_3, because if it was, one would expect lower levels of α amylase production.

All growth retardant effects cannot be attributed directly to GA functions. Some growth retardants may be associated with auxins (5). CCC may produce increased growth (9).

The effectiveness of ancymidol in the stimulation of α amylase activity strengthens the idea that this chemical action may not result in inhibition of GA biosynthesis.

One can speculate that high α amylase activity (which correlates with high GA concn) may be related to flowering since the high levels were observed only when the plants were induced. Several investigators (2, 13) have shown that flowering occurs with the addition of exogenous GA. Lang (10) found an increase in GA after induction of Hyoscyamus and a small increase in winter wheat. Tompsett and Schwake (17) reported that GA intensified flowering of Chrysanthemum morifolium.

Our evidence for the effect of GA on flowering remains incomplete; however, GA may be a causal factor in flower induction.

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APPENDIX

RECOMMENDATIONS TO COMMERCIAL GROWERS RELATIVE TO THE CULTURAL PRACTICES REQUIRED FOR GROWING *CLERODENDRUM THOMSONAE* BALF.

Recommendations for culture of Clerodendrum thomsonae Balf., "Wisconsin" clone

STOCK PLANTS

Clerodendrum thomsonae grows and develops best in full sun at greenhouse temperatures of 70°F or above. To date, lighting the stock plants, for 4 hrs during the middle of the dark period (during anytime of the year), has been our most effective treatment for producing vegetative growth. The plant in nature is a shrubby vine, therefore, the shoots develop rapidly.

We have studied the effect of stock plant age and node position on the stock plants by growing daughter plants from single node cuttings taken from 5 and from 2½ month old stock plants. Plants grown from the cuttings taken from the older stock plants were taller and more juvenile or vining. Basal nodes produced taller plants than did nodes from the upper portion of the stock plant shoot. The nodes producing the most flowers are those from the young stock plants and are located at the top one-third of the stem.

Our best terminal cuttings were produced on young stock plants. We take cuttings from 2 to 4 surges of growth, the stock then is 6-8 months old, a year old is the very maximum. Too much pruning then is necessary to remove the abundance of weak shoots which must be eliminated to produce the vigorous, good diameter growth used for propagation. The most uniform cuttings were the active eye, but it takes 2 wk longer for

flowers to develop compared to terminal cuttings.

Some lower leaf drop may occur from late December to mid February. We suspect low light intensity and/or semi-dormancy.

PROPAGATION

We remove the shoots from the stock plants leaving only 1 or 2 nodes at the base of the shoot remaining on the stock plant. A 2 1/2 to 3-inch terminal cutting is removed from the tip of the shoot, the leaves are then removed from the remainder of the shoot which is then cut just above each node to produce several single node cuttings from each shoot. The base of the cuttings is dusted with a rooting compound containing 0.4% NAA or 0.3% IBA before sticking. Defoliation of the single node cuttings before sticking gives the most uniform breaking of the axillary buds.

The terminal cuttings are pinched as soon as they are 2 to 3 inches in height.

The most satisfactory propagation medium, in our experience, is a Canadian peatmoss--coarse horticulture perlite mix, basically 50:50 but in winter more perlite, in summer less.

The cuttings are rooted under intermittent mist with just enough moisture to keep the terminal cuttings turgid. The misting frequency is gradually reduced so that at 10 days after the cuttings were stuck the mist is off. Hard water causes some necrosis of the stem tips on the terminal

cuttings; with distilled water there is no problem.

A temperature of 72-74°F at the base of the cuttings is critical in our experience; our propagation greenhouse temperature is 72°F.

With this regime: callus 3-5 days
roots 5-10 days
pot 10-14 days

PRODUCTION

We have experimented with numerous media; most were satisfactory; least satisfactory, however, was vermiculite instead of perlite. We use 1/2 coarse horticulture perlite and 1/2 Canadian peatmoss; in summer a little more peatmoss, in winter less. This medium is very light; the addition of some sand would make the pots less subject to tipping. The hard water problem in our area makes it essential for us to start with a pH of 5.0 to 5.5. We use Fritted Trace Elements in the mix. Iron chlorosis has been a problem when the pH reaches 6.3 and when this occurs the application of iron sulfate at 1 to 3 oz. per gallon of water has given very good results.

We usually grow 1 cutting in a 4-inch pot but 2 or 3 cuttings in 5-inch pots have been grown very satisfactorily. We've also grown some hanging baskets in which there has been grower interest. The baskets are planted just as the Clerodendrum thomsonae begin to flower. They've lasted very well for us, 3 months.

We constant feed the "Wisconsin" clone for the first 3 wk after potting with a complete fertilizer at 200 ppm N, then reduce the N to 100 ppm. We've had less experience with the "European" clone but to date, 200 ppm N constant feed seems satisfactory.

Full sun gives best growth and development. Although short days enhance flowering, the effect has been minimal. High light intensity is more critical for the initiation and development of flowers.

With an April potting the production time is 12 wk; from a late November potting the minimum time is 16 wk. In the middle of the summer the plants remain vegetative even if treated with ancymidol. Our experimental results indicate that when the ratio of red to far red is reduced, as occurs in summer, flowering is greatly inhibited for both ancymidol treated and control plants. Although we have conducted minimal experimentation on this problem, flowering may be enhanced by using several layers of cheesecloth above greenhouse grown plants in the summer to reduce the amount of far red light.

When shoots are 3 to 5 inches tall we apply 1 application of ancymidol (A-Rest) at 8 ppm, 30 ml per 4-inch pot. We get a better, more effective response consistently with the drench. When ancymidol is used, the pH of the media should be approximately 6.0 to obtain the best results in retarding growth and enhancing flower production.