INFLUENCE OF ETHEPHON ON GROWTH AND FLOWERING OF FLUE-CURED TOBACCO

by

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(ABSTRACT)

Tobacco (Nicotiana tabacum L.) leaf initiation stops when floral induction is completed. Floral induction (an internal biochemical change which signals development of flowers) can occur prematurely, during the pre- and/or post-transplant environment. Plants which flower prematurely have few leaves and low yields. Removal of the influorescence on these plants will break apical dominance and allow production of additional leaves from an axillary bud. This practice requires additional labor and increases production costs. Objectives of this study were to 1) determine how application timing, rate, localization, and on-plant duration of ethephon (2-chloroethylphosphonic acid) influences growth and flowering of flue-cured 'NC 82' tobacco; 2) examine the relationship between temperatures in the seedling environment and premature flowering and determine how time of plant bed cover removal affects premature flowering; and 3) quantify the ethylene released from buds, leaves, stems, and roots of tobacco seedlings at various days after application of ethephon.

Ethephon applied to flue-cured tobacco seedlings before the completion of floral induction significantly reduced premature flowering and increased days to flower, number of leaf nodes per plant, and yield.

Multiple applications and increased rates of ethephon did not increase the number of leaf nodes beyond the level obtained from a single application of 960 mg L^{-1} ethephon solution applied at the rate of 44 mL m^{-2} of plant bed. In wash-off studies, maximum benefit was obtained when ethephon remained on the seedlings one to two hours after application. In localization studies, increases in number of leaf nodes per plant were not different when 0.09 and 0.51 mg of ethephon was applied to the bud and largest leaf, respectively. Ethylene released from ethephon-treated greenhouse seedlings remained detectable four weeks after treatment. On-farm test locations with the two highest percentages of premature flowering had the lowest average daily minimum temperatures during the pretransplant period. Premature flowering was significantly increased at two of seven locations by removal of the perforated plastic plant bed covers two weeks compared to one week before transplanting. Floral induction of tobacco seedlings in controlled pretransplant environments was obtained by continuous 15°C temperature and 8 h photoperiods for 3 weeks.

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INTRODUCTION

Tobacco (Nicotiana tabacum L.) is a high value cash crop whose leaves are the marketed product. Initiation of leaves stops when flower development begins. Flowers normally emerge approximately 50 to 60 days after transplanting. However, the switch from leaf to flower initiation can occur prematurely, while the tobacco plant is in the seedling stage. Premature flowering can reduce yields, unless the influorescence is removed from the plant to break apical dominance and allow an axillary bud to produce additional leaves. This practice requires additional labor and increases production costs. Crop management is further complicated by the nonuniformity of such a crop, since the axillary shoots generally develop and mature more slowly than plants that did not flower prematurely.

Flue-cured tobacco producers have encountered problems with premature flowering in recent years. Producers should plant cultivars which yield high quality cured leaf to consistently market a desirable product. However, cultivars such as 'NC 82', 'K-326', and 'Coker 319', which typically yield high quality leaf, also tend to be susceptible to premature flowering.

Ambient environmental conditions which induce tobacco plants to flower prematurely have not been precisely characterized. Studies in controlled-environment growth chambers have related premature flowering to seedling exposure to cool temperatures, especially when combined with low light levels and/or short days (42).

Tobacco seeds are too small for direct field seeding, so seedlings

are produced in densely seeded areas, referred to as "plant beds" (generally 84 or 167 m^2), from which seedlings are transplanted to the field. Plant bed seedlings are grown under perforated plastic, polyester, nylon, or cotton covers which protect the seedlings from cool temperatures to some extent. The covers are removed from the plant bed, usually one to three weeks before transplanting. Mean temperatures in the Southeastern USA rise as spring progresses, and therefore the date of cover removal could potentially influence the level of premature flowering.

Ethephon (2-chloroethylphosphonic acid), a plant growth regulator, has been shown to increase the numbers of leaves per plant and days from transplant to flower in burley tobacco plants grown under controlled florally inductive environments but not in plants grown under controlled noninductive and natural environments (43). Ethephon also temporarily suppressed seedling stem elongation in that study. Ethephon decomposes upon absorption within the plant to release ethylene, an endogenous plant hormone or growth regulator which is produced in minute quantities in most plant tissue. Physiological effects of ethephon are attributed to ethylene.

An ethephon formulation (tradename "Florel") was registered for pretransplant use on tobacco to temporarily suppress seedling growth and inhibit premature flowering. Instructions on the label applied primarily to the use as a growth suppressor. Important questions concerning the use of ethephon on tobacco seedlings were not addressed. These questions were, therefore, the basis for the development of the following research

objectives. The objectives of this study were:

(1) To determine how application timing, rate, localization, and on-plant duration of ethephon influence flowering, leaf production, growth, yield and quality of flue-cured tobacco.

(2) To determine how pretransplant and early posttransplant field temperatures and timing of plant bed cover removal are related to premature flowering.

(3) To study the influence of ethephon application on ethylene evolved from buds, leaves, stems, and roots of tobacco seedlings at various days after application.

LITERATURE REVIEW

Flowering of Tobacco

Flue-cured tobacco is a high value crop whose leaves are the marketed product (37). Leaf initiation stops when floral induction is completed (39). Floral induction normally occurs after the initiation of 20 to 25 leaves, and the developing influorescence generally emerges approximately 50 to 60 days after transplanting. However, floral induction can occur while the tobacco plant is in the seedling stage, whether before (40) or after transplanting (68). Flowering as a result of pre- and post-transplant induction has been referred to as "premature" (40) and "early" (68) flowering, respectively. Plants induced in the seedling stage flower within three to six weeks after transplanting and develop low numbers of leaves. Pretransplant induction (40) results in earlier flowering with development of fewer leaves than does posttransplant induction (68). Actual leaf numbers and times to flowering will depend on pre- and post-transplant growing conditions (29,68,61). Induction could occur partially in the pretransplant environment and be completed in the field soon after transplanting (40). Whether flowering is a result of pre- or post-transplant induction is not as critical to this study as whether flowering occurs earlier and with development of fewer leaves than flowering of control plants.

The term "premature" involves the dimension of time; however, time of flowering, when defined solely by the number of days from transplanting to flower, may not be a precise indicator of floral induction. Floral induction precedes floral differentiation (46,26), and

the inflorescence may not emerge until three to four weeks after induction. The lag between time of floral induction and emergence is greater under environmental conditions unfavorable for rapid growth and development. Number of leaf nodes or leaves at flowering are more precise indicators of time of floral induction (46,9), and parameters such as percent premature flowering and days to flower must be interpreted with reference to leaf node data.

A constant number of days from transplanting to flowering may not appropriately define premature flowering for all growing conditions. Flowering within 45 days after transplanting may be premature in one season yet not in another, for pre- and post-induction environments can vary from season to season and even within a season. Kasperbauer has therefore used a critical point of 35 (40) and sometimes 45 (42) days to define premature flowering, based on observations (40) that pretransplant florally induced burley tobacco plants usually flowered a minimum of four to five weeks after transplanting. Favorable or unfavorable post-induction growing conditions can shorten or lengthen this time period, respectively. Data for days to flower for a given experiment should be critically examined and the critical point for premature flowering adjusted as necessary. In most seasons, flowering patterns are such that selecting the critical point is not problematic. The term premature flowering is applied in this study to plants developing a visible (to the unaided eye) floral bud within 35 or, in some experiments, 45 days after transplanting.

Flowering involves complex interactions between physiological,

biochemical, and genetic processes in response to environmental stimuli and time (58). Photoperiod and temperature are the primary environmental stimuli directly affecting floral induction (9). Extensive reviews of the literature on flowering have been compiled (9,10,87,46,26). Tobacco has been classified as a day-neutral plant (DNP) (31,16), inferring that flowering occurs after a period of vegetative growth, irrespective of daylength (9). Certain cultivars of flue-cured and burley tobacco have displayed characteristics typical of short-day plants (SDP) (40,68), which are induced to flower when nights or dark periods exceed a critical minimum duration (9).

In controlled environment studies, days to flower were not different for burley plants in night temperatures of 20 or 30°C when grown in 18 h photoperiods, but plants flowered earlier in 20 than in 30°C night temperatures when the photoperiod was 8 h (38). Night temperatures influenced flowering of plants of certain cigar and Turkish tobacco cultivars (16) and of a *Nicotiana rustica* L. X *N. tabacum* L. hybrid (64). Burley plants grown in day/night temperatures of 30/20°C flowered earlier in 8 than in 12 h photoperiods, and plants grown in 8 h photoperiods flowered earlier and with fewer leaves in constant 20 than in 30°C temperatures (39). Plants of three tobacco types (burley, flue-cured, and dark-fired) grown at 18 compared to 28°C flowered earlier and with reduced numbers of leaves when the photoperiod was 8 h; but in 16 h photoperiods, decreasing the temperature from 28 to 18°C decreased days to flower for burley plants only (41). Corresponding leaf numbers decreased for all three types, although to a greater extent in burley.

Flue-cured and burley tobacco seedlings exposed for 14 days to a florally inductive environment, consisting of an 8 h photoperiod and 18°C, flowered earlier and with reduced number of leaves compared to noninduced control plants when transferred to a 16 h and 28°C environment. Increased duration of exposure, 28 days, did not further influence these flowering characteristics. Except for a decreased number of leaves in the dark-fired cultivar, days to flower and number of leaves at flowering did not change significantly in flue-cured, burley, or dark-fired tobacco plants exposed in the seedling stage to a florally inductive environment (8 h photoperiod and 18°C) for 30 compared to 15 days. However, when exposed to an environment consisting of 18°C and 16 h photoperiod with a 50% reduction in light level, and both flowering parameters decreased in all three types when duration of exposure was increased from 15 to 30 days (41). Days to flower did not differ in the flue-cured cultivar. Interruption of the long inductive nights with low level light from white incandescent lamps, a treatment which typically inhibits flowering of SDP (9), increased the days to flower and number of leaves at flowering in cultivars of all three tobacco types, whether exposed to the inductive environment for 15 or 30 days (41). The increases due to night interruption were greater in the burley cultivar and were not different for the two exposure treatments. The increases in these flowering parameters for the flue-cured and dark-fired cultivars were greater in plants exposed to inductive conditions for 30 days.

A two-week exposure of burley tobacco seedlings to a controlled florally inductive environment at various times after seeding indicated

that readiness for floral induction (ripeness-to-flower) was not attained until some point between 22 and 29 days after seeding (40). Following transplanting, the level of premature flowering, defined as flowering which occurred within 35 days after transplanting, increased from 33 to 100% as time from seeding to the start of exposure was increased from 29 to 43 days. Duration of exposure to the florally inductive environment also influenced flowering. Exposure at 29 days after seeding for three compared to two weeks duration increased the level of premature flowering from 33 to 100%. Interaction of light and temperature in seedling floral induction was demonstrated when premature flowering of plants exposed to 8 h photoperiods and 18°C for 28 days immediately prior to transplanting was 100% but decreased to 0% when photoperiod was 16 h or when temperature was 28°C. Subsequent premature flowering was 100% for seedlings exposed immediately before transplanting for 10 days to an environment consisting of 18°C and 8 h photoperiods with light level reported as 2200 ft-c (2200 ft-c approximates 22% full sunlight and is therefore roughly equivalent to 550 μmol $m^{-2}s^{-1}$ photosynthetically active radiation). Premature flowering was 33% when the photoperiod was 13.5 h and light level 400 ft-c (roughly equivalent to 100 μmol $m^{-2}s^{-1}$), suggesting that low light level partially substituted for short photoperiods in floral induction of burley tobacco seedlings (40).

Days to flower for flue-cured tobacco 'NC 2326' were not significantly altered (ranging from 37 to 49 days) when plants, after developing 10 leaves, were grown in 9 h photoperiods in photoperiod/night temperatures ranging from 18/14 to 30/26°C, but averaged 68 days in the

temperature treatment of 34/30°C (29). At two and three weeks after simulated transplanting, plants of flue-cured tobacco 'Coker 319' were in a more advanced stage of floral development when grown in short (9 h) compared to long (9 h + 3 h night interruption) photoperiods, and after two weeks were in a more advanced floral stage when grown in 22/18 and 26/22 compared to 18/14 and 30/26°C posttransplant temperatures (68). In a related experiment, plants grown in 26/22°C were in a more advanced stage of floral development at two weeks after simulated transplanting when posttransplant photoperiods were 9 compared to 12 and 15 h. At three weeks after simulated transplanting, the numbers of leaves per plant averaged 24.3, 27.9, and 29.3 for transplants grown in 18/14, 22/18, and 26/22°C, respectively when summed over short and long photoperiods. Leaf number at the time of floral initiation in flue-cured tobacco cultivar NC 2326 increased with photoperiod/night temperatures from 24 leaves at 18/14 to 45 leaves at 34/30°C (61). Time of floral initiation and final number of leaves of flue-cured cultivar NC 2326 grown in 22/18°C and long days were only slightly influenced by daily accumulated photosynthetically active radiation (PAR) when light was experimentally varied from 10.5 to 40.5 mol m^{-2} day^{-1} (69). However, floral initiation was delayed and final number of leaves increased for plants grown in 26/22°C as PAR decreased.

Plant bed seedlings of a flue-cured breeding line, when allowed to attain an advanced stage of development before transplanting, flowered earlier and with fewer leaf nodes than plants transplanted at less advanced stages, except when seedlings received restricted watering (33).

Temperatures at the plant bed site were not reported. However, in a related experiment using controlled environments, earliest flowering occurred when seedlings were grown for at least three weeks in 16 h photoperiods and 30/25°C and then transferred to 8 h photoperiods and 20/15°C day/night temperatures for at least 10 days, with subsequent high temperatures and long days hastening floral development. The ripeness-to-flower stage was interpolated to be approximately 25 cm^2 total leaf area with a total leaf number of approximately 10, and this stage of development was attained in a minimum of 25 and 32 days after seeding when plants were grown in 30/25 and 20/15°C, respectively, in 16 h photoperiod controlled environments (33). In related experiments, seedlings of a flue-cured breeding line and of a flue-cured SDP mutant grown in controlled florally inductive environments were reported to attain ripeness-to-flower only after the unfolding (expansion) of the third leaf, with the cotyledons and first two leaves alone being unable to facilitate induction (34). The ability to facilitate induction by the third and successive leaves was apparently lost rapidly with age but was not influenced by previous water stress. Experiments involving grafting techniques indicated that the shoot apex of small tobacco seedlings (having 6 unfolded leaves and approximately 35 cm^2 total leaf area) was responsive to a floral stimulus received from a florally induced graft stock. In another experiment, defoliation treatments applied to seedlings starting the day after transplanting indicated that removal of the young, rapidly expanding leaves in the bud region of the plant prevented flowering, whereas removal of older, expanded leaves

delayed but did not prevent flowering.

In experiments using dissecting microscopy techniques, the shoot apices of four-to-five-month-old plants of SDP Nicotiana tabacum L. 'Maryland Mammoth' were observed to become completely committed to flowering after a period of between 7 and 14 consecutive inductive long nights (75).

Ethephon and Ethylene

The compound 2-chloroethylphosphonic acid ($ClC_2H_4PO_3=$, "ethephon"), upon absorption by plant tissue, readily decomposes to ethylene (C_2H_4) plus phosphate and chloride ion via a base-catalyzed elimination reaction (82,52,11,22). The effects of ethephon on plants are attributed to the released ethylene (72,73,82,81,50). Ethylene, a gaseous growth regulator effective in trace amounts, is involved in many plant processes, occurs naturally in most plant tissue (13,85) and is considered a plant hormone (60,47).

Ethylene production, usually at low levels, is a natural process in the life of plants, especially during such developmental stages as germination, ripening of fruits, abscission of leaves, and senescence of flowers and leaves. However, mechanical wounding, environmental stresses such as chilling, drought, and flooding, and certain chemical substances including the plant hormone auxin can induce ethylene production. Current understanding of the pathway and regulation of internally and externally induced ethylene biosynthesis in vivo in higher plants has been elucidated in recent reviews (85,86,83,84,4,47). The primary steps in the biosynthetic pathway involve reactions of

methionine to S-Adenosylmethionine (SAM) to 1-Aminocyclopropane-1-carboxylic acid (ACC) to ethylene (85). Ethylene biosynthesis is known to be regulated in various ways (85). Ethylene can be autocatalytic or autoinhibitory, depending on the type and stage of development of the plant tissue. Ethylene has been shown to to inhibit ACC synthase activity in tobacco leaf discs. This enzyme catalyzes the conversion of SAM to ACC. Inhibition of ethylene production by light is a function of decreased internal CO_2 concentrations, and in adequate levels of CO_2, light stimulates ethylene production in many green tissues. The mode of action by which ethylene initiates its effects on plants, is not fully known, but appears to involve a dissociable ethylene-receptor complex in which ethylene binds reversibly and specifically to a receptor site (14,47,86). Ethylene binding as a specific requirement for ethylene action has not been determined (86).

Experiments utilizing ^{14}C-ethephon indicated that ethephon was translocated from fruit tissue (81) and leaves (24,81,76,51), generally to rapidly growing leaves or fruits. Labeled ethephon, applied to mature tobacco leaves on plants with the apical bud removed, was translocated within the treated leaves, mostly acropetally, but ^{14}C activity was not detected in stem sections below or above the treated leaves (23). Radioactivity ethephon which may have been translocated and subsequently converted to ethylene, which may have then been released from the plant tissue, would be undetected in tissue samples. Furthermore, if ethephon is transported in a source to sink pattern (76), little transport would be expected from mature leaves on tobacco plants with the apical bud

removed and with chemical inhibition of axillary bud growth. Greater-than-control levels of ethylene, evolved from the shoot apex region of squash plants (Cucurbita pepo L.) when a lower leaf was treated with ethephon, were postulated to result from transport not of ethephon but of an ethylene precursor, although ethephon transport was not specifically investigated (35). Xylem transport of an ethylene precursor has been determined (12). Transport of ethylene itself, though water soluble (3), is not considered to occur over long distances (88), although ethylene diffusion over short distances within plant tissue has been reported (8).

Studies of endogenous ethylene levels and of effects of exogenous applications of ethylene (and ethylene-releasing compounds, such as ethephon) and ethylene inhibitors have demonstrated the critical involvement of ethylene in numerous metabolic and physiological processes involved in the growth and development of plants (13,60,2,15,3,47,83). Such effects include the promotion of maturation and ripening of fruit, promotion of senescence and abscission, inhibition of cell division and expansion, inhibition of stem elongation with promotion of stem lateral expansion, inhibition of polar auxin transport, inhibition of DNA synthesis, chlorophyll loss, promotion of femaleness (in cucurbits), promotion of flower fading, promotion or inhibition of flowering, and also induction of xylem differentiation (54,55,56), improved frost tolerance (49), and promotion of polysome prevalance and new gene expression (70). The effect of ethylene on plants is influenced by the environment, species, stage of development, cell and tissue type, ethylene concentration, and interactions with other plant hormones (47).

Discovery of the involvement in senescence by ethylene prompted the testing of ethephon on mature flue-cured tobacco for effects on leaf senescence and ripening and on subsequent chemistry of the leaves. Ethephon applied to greenhouse-grown tobacco plants yellowed the leaves within five days after treatment and did not adversely affect total N, total alkaloids, or reducing sugars in the dried leaf (19). In field experiments, ethephon applications to cultivar Hicks yellowed all but the immature upper leaves, slightly reduced price index and yield, reduced total N and total alkaloids in the cured leaf, elevated starch levels but did not affect reducing sugars in the cured leaf (53). Applications of ethephon to cultivar Coker 254 yellowed the more mature leaves within three days and remaining leaves within 10 to 14 days, increased starch levels in green leaves treated at 180 mg plant^{-1}, increased reducing sugars in the cured leaf, and apparently lowered nitrate reductase activity (50). Applied to cultivar Virginia Gold, ethephon yellowed the leaves on the lower half of the plants within four days, increased price index when all leaves of both untreated and treated plants were harvested during a single day, increased reducing sugars and decreased total N in the cured leaf (21). When mature tobacco leaves were exposed to ethylene, chlorophyll content and endogenous ethylene production decreased (63). Leaf discs, from mature tobacco plants, treated with ^{14}C- ethephon evolved ^{14}C- ethylene continuously during a 96 h period, with rapid evolution during the first 24 h followed by a decline in rate over the subsequent 72 h (22). The rate decline was attributed to decreased availability of hydroxyl ions needed for ethephon degradation.

Ethephon was applied to burley tobacco 'Ky 14' seedlings at rates of 500 and 1000 mg L^{-1}, with subsequent transplanting at 1, 3, 7 and 10 days after treatment (48). Early growth was suppressed, flowering was delayed, and yield and value were reduced for ethephon treated plants transplanted one day after treatment compared to the later transplantings. Plant responses to the two rates were not significantly different except for a greater delay in flowering at the first transplant date with the higher rate.

Pretransplant applications of a 1000 mg L^{-1} ethephon solution to burley tobacco seedlings 11 days after transfer to a controlled florally inductive environment increased days from transplanting to flowering and number of leaves at flowering in two of three years (43). Ethephon applied to seedlings growing in a florally noninductive environment did not significantly affect days to flower or leaf number, although flowering was delayed two to three days by ethephon treatment. When floral induction was completed before the application of ethephon, the ethephon treatment did not reverse the induction and so did not increase the number of leaves at subsequent flowering. Pretransplant ethephon treatment increased yield (when applied before completion of floral induction) of plants exposed to the inductive environment, but did not affect yield of plants exposed to the noninductive environment. Total alkaloids in the cured leaf were not affected by ethephon treatment. In a related experiment, seedling stem length did not increase during the period of two to nine days after ethephon treatment when ethephon concentrations were > 1000 mg L^{-1}. At ethephon concentrations of 250 and

500 mg L^{-1}, stem lengths were likewise less than those of untreated seedlings by four days after application. The effect of ethephon concentration on stem elongation remained obvious 21 days after treatment.

Ethylene-induced inhibition of flowering has also been observed in cockleburs (Xanthium pensylvanicum Wallin.) (1) and Japanese morning glory (Pharbitis nil L.) (65). Ethylene-induced promotion of flowering has been reported in pineapple (Ananassa sativa L.) (18) and Plumbago indica L. (59).

MATERIALS AND METHODS

On-Farm Field Test

An on-farm test to evaluate the influence of ethephon and plant bed cover management on flowering, leaf production, yield, and quality of flue-cured tobacco was conducted in Virginia at four locations in 1983 and three locations in 1984. Cultivar NC 82, reported to be susceptible to premature flowering (36,37), was grown at each location. Seedlings were grown under perforated plastic covers in 84 m^2 plant beds (except that the 1984 May location plant bed was 167 m^2) partitioned into four equal-sized experimental units. Cover management treatments involved removal of the cover at an estimated 14 days before transplanting or at seven days after the first cover removal date. Ethephon treatments, applied at the latter cover removal date, included not sprayed and sprayed. A stock solution of 960 mg L^{-1} ethephon was prepared by mixing 237 mL of "Florel" (donated by Union Carbide) in 9.47 L of water, and this solution was applied at the rate of 136 mL m^{-2} of plant bed using a CO_2 pressurized sprayer equipped with three nozzles on a 1.83 m boom and operated at 2.8 kg cm^{-2} pressure. Ambient high and low temperatures were monitored daily starting about 40 days after seeding using maximum-minimum thermometers stationed at each location. Temperature sensors were shaded naturally or with white cloth and were positioned 30 to 40 cm above ground.

Seedlings were transplanted approximately seven days after ethephon treatment in a randomized complete block design with three replicates. Conventional cultural practices were used after transplanting. Plot size

was 337.5 m^2, and data were collected from the center two rows of four-row plots (four locations) and from the center row of three-row plots (three locations). Parameters measured were days from transplanting to flower, number of leaves per plant at flowering, yield, and quality. Plants were considered "in flower" on the date that the floral bud first became visible to the unaided eye. Data for flowering were collected weekly. Plants in flower within 45 days after transplanting were considered to be flowering prematurely. The lowest leaves on the transplanted seedling, which do not develop to normal harvestable size, and leaves less than one cm wide were not included in the leaf counts.

Stem heights (measured from soil level to the base of the bud) and total leaf area of representative seedlings were measured at each cover removal date. Leaf areas were obtained by multiplying weights of leaf tracings on paper by the average weight of one square cm of the same paper. These measurements provided characterization of seedling development stage at cover removal and at the time of ethephon application. Cured leaves from each plot were weighed and assigned an official U.S. Government grade (by a U.S. Government Inspector), and yields and grade indices (77) were computed. Increased quality index indicates increased quality of the cured leaves.

The data were analyzed for individual locations, with factorial arrangement of the exposure and ethephon treatments. Analysis of arc-sin-transformed data for percentage premature flowering yielded identical results as analysis of nontransformed data, and therefore, nontransformed data are reported. Treatment means presented are averages of all plants

(approximately 175 to 250 plants) from data row(s) of each plot.

On-Station Field Tests

General Procedures. Plant beds were seeded to cultivar 'NC 82' tobacco on 2 March 1984 for all field experiments (plant bed ethephon applications, stage of development, and ethephon volume tests) conducted at the Southern Piedmont Center. Plant beds were divided into 4.2 m^2 experimental units following removal of the cotton covers on 7 May. Ambient temperatures at the plant bed were monitored daily using a maximum-minimum thermometer. Ethephon treatments were replicated once. Ethephon was applied as a 960 mg L^{-1} solution at the rate of 136 mL m^{-2} using a CO_2 pressurized backpack sprayer equipped with a 1.83 m boom with three nozzles and operated at 2.8 kg cm^{-2} pressure. Apart from experimental treatments, plant bed seedlings were grown according to conventional cultural practices. Seedlings were transplanted in a randomized complete block design with four replications (except for three replications in the ethephon volume study). Plots consisted of one row of 22 plants spaced 51 cm apart with 1.22 m between rows. Field plots were managed according to conventional cultural practices, except that the influorescence of plants flowering prematurely was removed and a formulation of fatty alcohols was applied to control axillary bud growth and enable evaluation of the influence of premature flowering on yield. Under conventional production practices, prematurely flowering plants would be allowed to produce an axillary bud for additional leaf production. Data were collected to obtain days to flower and total

number of leaf nodes per plant at flowering. Plants were considered to be in flower on the date the floral bud became visible to the unaided eye. Data were collected for flowering on Monday, Wednesday, and Friday of each week. Plants flowering within 35 days after transplanting were considered to be flowering prematurely. All visible nodes, including those with senesced leaves and small leaves partially enveloping the emerging floral bud were included in the node counts.

Cured leaf yields and grade indices were determined as described in the on-farm test. A representative, whole-plant sample of cured leaf was obtained from each plot and analyzed for nicotine (30) and reducing sugars (20). Sulfanilic acid was substituted for buffered analine solution in the nicotine analysis.

The data were analyzed by analysis of variance and the Least Significant Difference (LSD) was used at the 0.05 level of probability to separate means of statistically significant effects. Analysis of arc-sin-transformed percentage premature flowering data yielded identical results as analysis of nontransformed data, and therefore, nontransformed data are reported. Treatment means presented are averages from 18 to 22 plants.

Experiments

Plant Bed Ethephon Applications. This experiment was conducted to compare performance of tobacco plants treated with single and multiple applications of ethephon. Ethephon treatments consisted of an untreated control, one application in the plant bed (18 May), two applications in the plant bed (18 and 24 May), and one application in the plant bed with

another application in the field 10 days after transplanting (18 May and 4 June). Seedlings were transplanted on 25 May.

Stage of Development. This experiment was conducted to obtain information about the appropriate time to apply ethephon to plant bed seedlings for control of premature flowering. Ethephon was applied to seedlings at three different stages of development: 1.5, 2.5, and 5.0 cm stem heights (averages of 6 representative plants) measured from soil level to the base of the bud. Total leaf areas at the time of ethephon application averaged 82, 192, and 202 cm^2 for seedlings treated at stem heights of 1.5, 2.5, and 5.0 cm, respectively. Leaf areas were obtained as described in the on-farm test. Ethephon was applied to the 1.5 cm seedlings on 9 May and to the 2.5 and 5.0 cm seedlings on 15 May, and the seedlings were transplanted on 21 May.

Ethephon Volume. This study was conducted to investigate a potential interaction between ethephon and subsequent planting date. Treatment volumes of 960 mgL^{-1} ethephon solution were 0, 41, 81, 122, or 244 $mL\ m^{-2}$ of plant bed. Seedlings were transplanted three and seven days after the 18 May ethephon applications in a randomized complete block design. Stem heights from soil level to the base of the bud of five representative plant bed seedlings were measured weekly for four weeks after treatment. Weekly stem height measurements from transplanting until flowering were taken from five consecutive plants, beginning with the second plant in each row. Data were analyzed by analysis of variance for a 2 X 5 factorial arrangement of transplant date and ethephon treatments. The ethephon volume effect was also analyzed

separately within each transplant date.

Greenhouse and Growth Chamber Tests

General Greenhouse Procedures. Greenhouse tests, conducted in 1984 at the Virginia Tech Southern Piedmont Center, were repeated for each experiment (except ethephon volume). Plants for the first series of tests were seeded on 13 Aug. and seedlings were planted on 10 Sept. in styrofoam flats containing vermiculite. Seedlings were watered as required and nutrients were applied weekly. Nutrient solution, prepared by mixing 75 g of Peters Hydro-Sol (5-11-26 analysis) and 50 g of $CaNo_3$ in 75.6 L of water, contained the following concentrations of nutrients in units of nmol L^{-1}: 10 928 N, 1 581 P, 5 473 K, 3 300 Ca, 1 259 Mg, 1 240 SO_4, 55 Fe, 9 Mn, 2 Zn, 2 Cu, 47 B, 1 Mo, 1 Cl, and 16 Na. Plants for the second tests were seeded 28 Sept. 1984, and seedlings were planted in styrofoam flats on 23 Oct. Seedlings were supplied with nutrient solution twice per week.

Treated seedlings were potted in 15-cm-diameter clay pots containing vermiculite. Pots were placed two each in 2.8 L pans to which approximately 1.4 L of nutrient solution were added weekly. Single-plant experimental units were assigned to pans in a randomized complete block design replicated five times. Pots were re-randomized one per pan and spaced 30 to 40 cm apart on the greenhouse bench to prevent light competition problems (approximately four weeks after potting). Supplemental fluorescent lighting from General Electric F40LW-R5-WMII Lite White lamps was supplied in the greenhouse during the second series

of tests to extend the short, winter daylengths to 14.5 hours. Temperatures in the greenhouse ranged from 21 to 32°C.

Data were collected for days from potting to flower and number of leaf nodes at flowering as described in the field test methods. Stem heights, measured from the potting-media to the base of the bud, were recorded for most experiments. Final stem heights, from potting-media to the uppermost leaf > 1.0 cm wide, were measured at the full flower stage (i.e., when a majority of the florets were open and pink) for the second test of these experiments. Other data collected are as described for the individual experiments. Data were analyzed by analysis of variance for a randomized complete block design with five replicates and statistically significant means were separated by the LSD method. Treatment means presented are averages of 5 plants (except 10 plants for the ethephon volume test).

General Growth Chamber Procedures. Seedlings in styrofoam flats were transferred from the greenhouse to a floral induction growth chamber for a three week period. The chamber was controlled at 15°C temperature and 8/16 h light/dark periods. Chamber lighting was provided by three Sun-Brella lamps from Environmental Growth Chambers (Chagrin Falls, OH), each equipped with a metal halide (General Electric Multivapor MVR400U) and a high pressure sodium (General Electric Lucalox LU400) lamp. Photosynthetically active radiation (PAR) averaged 263 μmol $m^{-2}s^{-1}$ at top of the plants.

Control seedlings in two experiments (ethephon growth effect and ethylene quantification) were transferred to a florally noninductive

growth chamber for three weeks. This chamber was controlled at 25°C and 13.5 hour photoperiod. Chamber lighting was as described for the induction chamber, except that PAR averaged 178 μmol $m^{-2}s^{-1}$ at top of plants. The second test of the growth chamber ethephon timing study was also conducted in this growth chamber, but temperature and photoperiod were controlled at 15°C and 8 h, respectively.

Nutrient solution and water were applied to seedlings in the growth chambers as described for the greenhouse seedlings. Ethephon treatments were applied during the period in which the seedlings were in the growth chambers. Seedlings were temporarily removed from the chambers for treatment applications. Ethephon was applied as one mL of a 960 mgL^{-1} solution per plant as a mist from an "Ethrel" test kit sprayer to the adaxial surface of the leaves. Seedling leaf areas at the time of transfer to the growth chambers were determined by multiplying the leaf length by width by an adjustment factor of 0.7 obtained from similar size plants of known leaf length, width, and area. A similar adjustment factor was reported previously (66). Stem heights were measured from the potting media to the base of the bud. Appendix Table 1 shows seedling leaf areas and stem heights when transferred to induction chambers and days after transfer to chambers when ethephon was applied.

Experiments

Growth Chamber Ethephon Timing. This experiment was conducted to determine if tobacco flowering response to ethephon was influenced by the timing of ethephon application in relation to duration of seedling exposure to a controlled florally inductive environment. Two milliliters

of a 960 mg L^{-1} ethephon solution were applied as a mist to each seedling after 0,7,14, or 21 days exposure to the inductive environment. Seedlings were removed from the induction chamber for application of ethephon to reduce the possibility of accidental application to other seedlings. Treated seedlings were immediately placed in a separate chamber (15°C and PAR averaging 149 µmol $m^{-2}s^{-1}$ at top of plants) for two to seven days to minimize the possibility of contamination of other seedlings by ethylene released from treated seedlings. Treatments included an untreated control, and in test one a "late" control was also included. Late control seedlings were not transferred to the floral induction chamber until nine days of the 21 day induction period had elapsed. Control and late control seedlings were therefore exposed to the florally inductive environment for 21 and 12 days, respectively. After the induction period, seedlings were transferred to a lath house for conditioning to the ambient environment for seven days prior to transplanting. Treatments were assigned to plots in a randomized complete block design with four replications. Plots consisted of one row of 18 plants 51 cm apart with 1.22 m between rows. Plants were grown in the field according to conventional cultural practices. Plants flowering within 45 days after transplanting were considered to have flowered prematurely. Other data were collected as described for the on-station field tests. Appendix Table 2 shows dates when relevant procedures were conducted and characterizations of seedlings stage of development when transferred to floral induction chambers.

Stage of Development. This greenhouse and growth chamber experiment

was conducted to determine the influence of seedling stage of development on efficacy of ethephon in delaying flowering. Ethephon was applied when seedlings attained a stem height of 1.5 or 2.5 cm. Seedlings were removed from the floral induction chamber for ethephon applications and were returned to the chamber after air drying for 45 to 60 minutes at approximately 15°C.

Ethephon Volume. The influence of ethephon rates on stem elongation and flowering of plants not exposed to a florally inductive environment was studied in the greenhouse. A 960 mgL^{-1} ethephon solution was applied to seedlings at rates of 0,41,81,122, or 244 $mL\ m^{-2}$. Treatments were applied on 25 Sept. 1984 (28 and 15 days after seeding and potting, respectively) and were replicated 10 times. Potted seedlings were placed in an area having the same dimensions as the plant bed experimental units of the previously described field test of this experiment, and ethephon was applied to that entire area. Stem heights were measured weekly and days to flower were recorded.

Ethephon Wash-off. This experiment was conducted to study the relationship between vegetative/floral development and the elapsed time between ethephon application and washing of the seedlings with a 23-second simulated 2.5 cm rainfall. Seedlings were washed at 15,30, 60, 120, or 240 minutes after application of ethephon. A not washed control treatment was included in test two. Seedlings were removed from the floral induction chamber for treatment. Seedlings washed at 15, 30, and 60 minutes were kept outside the chamber (in temperatures of approximately 15 to 20°C) until washed; the remaining seedlings,

including controls, were returned to the chamber after 60 to 80 minutes. All seedlings were returned to the induction chamber immediately after washing.

Ethephon Localization. This greenhouse and growth chamber experiment was conducted to determine the influence of on-plant localization and amount of applied ethephon on growth and flowering of tobacco. Localized plant parts treated were yellow leaf, leaf 1, leaf 2, leaf 3, leaf 4, all leaves, bud, bud (3X concentration of ethephon), all leaves plus bud, and roots. Ethephon from a 1.0 mL syringe was applied to the leaves as droplets spaced five cm apart. The larger leaves, therefore, received greater amounts of ethephon. Actual amounts applied are shown in the results. The yellow leaf was the lowest leaf and had senesced. Leaves one through four were the successive leaves above the yellow leaf. Seedlings treated in the bud received one droplet of ethephon solution. A second bud treatment consisted of a 3X concentration of ethephon (2880 instead of 960 mg L^{-1}). Ethephon was applied to the roots as 20 mL of a 480 mg L^{-1} ethephon solution poured onto the vermiculite potting media. Seedlings were returned to the chamber immediately after treatment. Untreated seedlings were used as controls. The data were analyzed by analysis of covariance, with the amount of ethephon as the covariant.

Ethephon Growth Effect. Plant response to ethephon applied to seedlings exposed to florally noninductive and inductive environments was examined in a greenhouse/growth chamber experiment. Seedlings were transferred to a florally noninductive (25°C and 13.5 photoperiods) or

inductive (15°C and 8 h photoperiods) environment for three weeks. One half of the seedlings in both environments were treated with ethephon during this period. After three weeks in the growth chambers, seedlings were returned to the greenhouse. Leaf lengths and widths and stem heights were measured at various times after treatment. Days to flower and number of leaf nodes at flowering were also recorded. The data were analyzed by analysis of variance for a 2 x 2 factorial arrangement of the environment and ethephon treatments.

Ethylene Quantification. The relationship between ethephon application to tobacco seedlings and corresponding levels of ethylene released from different plant parts at various times after ethephon application was investigated in a greenhouse/growth chamber experiment. Seedlings exposed to a florally inductive environment were treated with ethephon. Noninduced and induced plants were included as controls. Six plants from each of the three treatments were harvested at indicated days after ethephon treatment and transferred to the laboratory. Vermiculite was gently washed from the roots and leaves were gently rinsed with running tap water. Seedlings were dissected into four parts: apices (the bud, including the stem segment down to first node below bud), stems (segment from first node below bud to crown), leaves (cut into 1-cm-diameter discs before weighing), and roots (including the below-soil-line segment of the stem). Plant parts were either air dried or blotted dry, and after being weighed were incubated for three h in 20 mL distilled water in petri dishes placed under fluorescent lighting to allow wound ethylene to subside (5). Separate parts from three plants (one

replication) were transferred to 25 mL Erlenmyer flasks containing one mL distilled water. Flasks were sealed with rubber serum stoppers and placed in darkness at 28°C for 24 h. A one mL sample of air was then withdrawn from the flask head space with a gas-tight syringe and injected into a Hewlett-Packard HP 5750 gas chromatagraph (GC) for ethylene analysis (71,62). The GC was equipped with an alumina F1 column operated at 110°C oven temperature and a flame ionization detector operated at 175°C. Flow rates for oxygen, hydrogen, and nitrogen (carrier gas) averaged 320, 40, and 50 mL min^{-1}, respectively. Calibration points were obtained by measuring peak areas obtained from known amounts of ethylene standard, and a cubic regression curve was fitted to these data points. The equation obtained from this regression model was used to quantify the ethylene in the samples. Rates of ethylene evolved from plant parts within a treatment were analyzed by analysis of variance for each sampling date.

RESULTS AND DISCUSSION

On-Farm Field Tests

Differences in the level of premature flowering among locations contributed to a significant location X ethephon interaction, and the magnitude of differences in error variances also precluded the combining of data across locations. Therefore, the results for individual locations are presented.

Excluding the Crews location, ethephon applied to pretransplant flue-cured tobacco seedlings significantly reduced premature flowering and increased the number of leaves per plant at flowering (excluding the Scarce location) at all five locations having at least 7% premature flowering in 2-weeks-exposed control plants (Tables 1 & 2). Ethephon also increased the number of days from transplanting to flower at these locations. The three locations which had a significant pretransplant duration of exposure effect also had a significant exposure X ethephon interaction (Appendix Tables 3 & 4). The influence of ethephon on premature flowering at the 1983 May and Scarce locations, on number of leaves at the 1983 Hawthorne location, and on days to flower at the 1983 May location was greater when the ethephon was applied to seedlings from which the perforated plastic covers were removed two weeks instead of one week before transplanting (Table 1). This interaction appears to be the result of a greater level of floral induction in the control seedlings exposed to the ambient environment for two weeks. Increased induction could have resulted from increased duration of exposure to a florally inductive environment. Also, at one location (1983 May), lower

Table 1. Influence of exposure to ambient temperatures and pre-transplant ethephon on five agronomic characteristics of flue-cured tobacco grown at four locations, 1983.

Weeks of exposure	Ethephon	Days to flower	Leaves per plant	Premature flowering	Yield	Quality index
				%	kg ha^{-1}	
		Hawthorne location				
1	No	45.2	19.7	23.5	3434	72.7
	Yes	47.7	20.2	6.9	3241	74.0
2	No	44.6	18.1	29.5	3213	72.7
	Yes	47.6	20.5	9.5	3453	73.0
$LSD_{0.05}$		1.6	1.0	21.6	NS	NS
CV (%)		1.8	2.7	62.3	5.4	2.7
		May location				
1	No	51.8	21.9	6.4	4417	48.7
	Yes	52.5	24.2	2.4	3997	48.7
2	No	48.3	20.8	23.1	4190	51.7
	Yes	52.6	24.9	2.4	4010	54.7
$LSD_{0.05}$		2.5	2.0	11.8	NS	NS
CV (%)		2.4	4.3	69.0	13.7	7.1
		Crews location				
1	No	53.1	19.7	7.0	2259	57.7
	Yes	53.3	18.9	3.8	2396	59.3
2	No	51.9	17.1	10.9	2194	57.3
	Yes	52.8	19.4	10.1	2475	54.3
$LSD_{0.05}$		NS	NS	NS	NS	NS
CV (%)		2.2	12.1	59.1	8.9	3.9
		Scarce location				
1	No	64.7	22.6	1.4	2227	45.7
	Yes	67.5	22.7	1.1	2204	42.3
2	No	62.8	21.7	7.1	2237	43.7
	Yes	67.1	23.0	1.2	2242	42.7
$LSD_{0.05}$		4.1	NS	2.4	NS	NS
CV (%)		3.1	3.3	43.6	9.4	9.9

Table 2. Influence of exposure to ambient temperatures and pre-transplant ethephon on five agronomic characteristics of flue-cured tobacco at three locations, 1984.

Weeks of exposure	Ethephon	Days to flower	Leaves per plant	Premature flowering	Yield	Quality index
				%	kg ha^{-1}	
		Hawthorne location				
1	No	51.8	19.8	20.3	2946	65.3
	Yes	53.8	21.7	7.6	3162	66.3
2	No	49.1	18.9	37.5	2829	68.7
	Yes	53.4	22.0	11.3	3198	68.0
$LSD_{0.05}$		2.9	1.9	16.8	223	NS
CV (%)		2.8	4.5	43.8	3.7	6.4
		May location				
1	No	70.4	25.1	0.1	3293	57.7
	Yes	71.3	24.7	0.0	3638	53.3
2	No	70.0	24.7	1.3	3109	55.0
	Yes	71.9	24.5	0.1	3415	56.7
$LSD_{0.05}$		NS	NS	NS	NS	NS
CV (%)		1.4	2.2	260.0	11.8	6.6
		Guthrie location				
1	No	55.4	23.2	5.0	1919	48.0
	Yes	56.0	23.7	1.4	2026	46.7
2	No	55.1	23.7	4.8	1919	49.0
	Yes	55.8	23.2	2.9	2016	46.0
$LSD_{0.05}$		NS	NS	NS	NS	NS
CV (%)		2.7	1.8	79.3	8.1	4.1

temperatures during the initial seven days exposure were observed (Table 3). Increased duration of exposure of burley and flue-cured tobacco seedlings to a controlled florally inductive environment was reported by Kasperbauer (41) to decrease days to flower and leaves per plant at flowering.

Average ethephon-induced decreases in premature flowering for plants exposed to the ambient environment for one and two weeks, respectively, were 4.0 and 20.7 percentage points at the 1983 May location and 0.3 and 5.9 percentage points at the Scarce location (Table 1). Average increases in days to flower as a result of ethephon applications at the 1983 May location were 0.7 and 4.3 days for plants exposed for one and two weeks, respectively. Average ethephon-induced increases in leaves per plant were 0.5 and 2.4 leaves at the 1983 Hawthorne location for plants exposed for one and two weeks, respectively. For locations with a significant ethephon effect but without a significant exposure X ethephon interaction, the average ethephon-induced increases in days to flower and leaves per plant and decreases in premature flowering were 2.7 days, 2.9 leaves, and 18.9 percentage points, respectively, when summed over the exposure treatments.

The two highest percentages of premature flowering occurred at the two locations with the lowest average daily minimum temperatures during the four weeks immediately prior to transplanting (Table 4). This relationship is consistent with previous reports of work with burley (40) and flue-cured tobacco (33) in controlled environments, in which earliness of flowering was related to decreased temperatures in the

Table 3. Ambient temperatures and premature flowering at on-farm tests with exposure duration x ethephon interactions for certain parameters, 1983.

Location	Weeks of exposure	Mean daily minimum temp. from cover removal until transplanting	Premature flowering
		°C	%
May	1	15.6	4
	2	13.7	13
Hawthorne	1	4.3	15
	2	5.9	20
Scarce	1	11.2	1
	2	12.3	4

Table 4. Mean daily minimum temperatures at plant bed locations immediately before and after transplanting, and percentages of premature flowering (summed over treatments).

Location	4 Weeks preplant	1 Week postplant	Premature flowering
	°C	°C	%
1983 Hawthorne	4.4	11.1	17
May	11.7	13.9	9
Crews	8.9	11.1	8
Scarce	8.9	15.6	3
1984 Hawthorne	6.1	7.2	19
May	7.8	10.6	0
Guthrie	7.2	12.2	4

seedling environment. In this study, however, the relationship between pretransplant ambient temperature and premature flowering was not consistent across locations (Table 4). This indicates that temperature is not the only factor involved in floral induction of tobacco seedlings.

Stage of development of tobacco seedlings has been reported to affect response to temperature (40,33,34). Seedling stage of development was characterized at the times of cover removal and ethephon application for the 1984 tests. Seedlings exposed for two weeks and one week at the Hawthorne location, the only one with florally inductive conditions in 1984, averaged 110 and 173 cm^2 in leaf area and 2.2 and 5.9 cm in stem height at the two respective times when the plant bed covers were removed (Table 5). Average leaf area and stem height was 171 cm^2 and 6.1 cm, respectively, when ethephon was applied. Lack of seedling-stage floral induction at the other 1984 locations prevents conclusions from these data, except that seedlings of these developmental stages can obviously be florally induced. Dates when relevant management tasks were performed and treatments were applied at each location are shown in Appendix Table 5.

Ethephon increased the number of days to flower for plants from both one- and two-week exposure durations at the 1983 Hawthorne location. Ethephon-induced increases in number of days to flower at the 1983 May and Scarce and 1984 Hawthorne locations were obtained only for plants exposed to the ambient environment for two weeks (Tables 1 and 2). Despite a significant ethephon effect on days to flower at the 1984 May location (Appendix Table 4), differences in exposure X ethephon

Table 5. Characterization of seedlings at time of plant bed cover removal for on-farm test locations, 1984.

Location	Weeks of exposure	Avg. total leaf area	Avg. stem ht.
		cm^2	cm
Hawthorne	1	173	5.9
	2	110	2.2
May	1	200	9.5
	2	126	5.3
Guthrie	1	252	9.0
	2	81	2.5

interaction means were not significant (Table 2).

Since the tobacco influorescence is terminal, floral induction results in termination of leaf initiation. Delayed floral induction may permit initiation of additional leaves. Applications of ethephon have been shown to temporarily retard growth and development of tobacco seedlings (48,43). Ethephon-induced increases in days to flower could result from the initiation of additional leaves and/or delayed development. Involvement of both factors would seem likely when ethephon was applied to seedlings growing in a florally inductive environment, whereas delayed development would appear to explain an ethephon-induced increase in days to flower under florally noninductive conditions.

Premature flowering is related to days to flower, and reduced premature flowering can also be an indication of initiation of additional leaves and/or delayed growth and development. At each of four locations with a significant ethephon effect on premature flowering (Appendix Tables 3 & 4), ethephon reduced premature flowering, most dramatically in the two-weeks exposed plants (Tables 1 and 2). At three of the four locations, the effect of ethephon on days to flower and leaves per plant was also significant. At the 1983 Scarce location, reduced premature flowering apparently was a result of ethephon-induced delayed development since the ethephon effect was significant for days to flower but not for number of leaves per plant.

The leaves are the marketed product in commercial tobacco production, and increased leaf production would be a major benefit from application of ethephon to tobacco seedlings. Ethephon applications

significantly increased the number of leaves per plant at flowering for the two-weeks exposed plants at the 1983 Hawthorne and May and 1984 Hawthorne locations (Tables 1 & 2). The exposure effect on number of leaves was not significant at any location (Table 2). Leaf numbers per plant were not significantly different for ethephon-treated seedlings exposed to the ambient environment for one week or two weeks before transplanting (Tables 1 & 2). The ethephon-induced increase in number of leaves for plants exposed for two weeks to a florally inductive ambient environment indicates that pretransplant ethephon applications delayed floral induction.

An increase in yield of ethephon-treated plants at the 1984 Hawthorne location was the only significant effect of exposure or ethephon on either yield or quality (Table 2). To avoid potential yield reductions from premature flowering, the tops of plants flowering prematurely were removed at all locations to break apical dominance and permit production of additional leaves from an axillary bud. Conventional removal of tops ("topping") of nonprematurely flowering plants was inadvertently implemented at a less than normal leaf number in control plots at the 1984 Hawthorne location, and the reduced yields of the untreated plots were the result of these topping procedures. Yield decreases would be expected for prematurely flowering plants not treated with ethephon if production of an axillary bud was not permitted.

Despite premature floral induction at the 1983 Crews location, ethephon did not significantly influence flowering (Table 1). Floral induction may have been completed before the ethephon treatments were

applied. Delayed flowering as a result of ethephon-induced suppression of growth and development was not observed at this location. Kasperbauer reported that ethephon applied after floral induction did not affect leaf number or days to flower (43).

Increased days to flower and leaves per plant at flowering as a result of pretransplant ethephon applications to flue-cured tobacco seedlings are in agreement with results of similar studies with burley tobacco seedlings grown in a controlled florally inductive environment (43). Premature flowering can be a serious problem for tobacco producers, especially those growing susceptible cultivars such as NC 82, since premature flowering increases labor requirements and production costs and can decrease yield. Results of these tests indicate conclusively that ethephon applied to flue-cured tobacco NC 82 seedlings grown in florally inductive ambient environments in on-farm plant beds can significantly decrease premature flowering and increase the average numbers of leaves per plant at flowering and days from transplanting to flowering. Timing of removal of perforated plastic plant bed covers can influence the flowering of and also the effect of pretransplant ethephon on the flowering of flue-cured tobacco.

Plant Bed Ethephon Applications

In earlier studies (48,43), ethephon was applied to tobacco seedlings as a single pretransplant treatment. In this study, the ethephon was applied as single and double pretransplant applications and as a single pretransplant combined with a second application 10 days

after transplanting.

Single and multiple applications of ethephon significantly reduced premature flowering and increased the number of days to flower and leaf nodes per plant (Table 6). Multiple applications, whether pre- or post-transplant, did not add to the effect of a single pretransplant application.

Pretransplant single and double applications of ethephon, compared to controls, significantly increased yield (Table 6). However, yield of plants treated with the combination of pre- and post-transplant ethephon applications was not significantly different from yield of control plants. The additional application 10 days after transplanting severely suppressed early-season plant growth, but no differences were observed in heights at flowering among plants receiving the different ethephon treatments. Delayed early development probably contributed to the reduced yield of plants receiving the combination pre- and post-transplant treatment compared to controls. Increases in leaf weight normal occur after removal of the influorescence (78,79), and delayed development apparently delayed these weight increases. Delayed growth and development of plants receiving the combination pre- and post-transplant ethephon treatment apparently decreased cured- leaf quality compared to that of controls (Table 6). Quality has been associated with a decline in nitrogen utilization and an increase in starch accumulation in tobacco leaves at the time of flowering (78,79), and a delay in the timing of these events could reduce quality. In contrast, cured-leaf quality of plants treated with single and double pretransplant

Table 6. Influence of number and timing of ethephon applications on agronomic characteristics of flue-cured tobacco.

No. of ethephon applications		Premature flowering	Days to flower	Nodes per plant	Yield	Quality index	Nicotine	Reducing sugars
pre-transplant	post-transplant							
		%			kg ha^{-1}		%	%
0	0	21.9	40.7	25.3	3467	59.7	1.74	23.5
1	0	3.2	45.1	28.3	3767	60.8	1.61	21.8
2	0	1.1	46.9	28.4	3780	56.8	1.64	21.1
1	1	2.1	48.8	29.0	3649	54.9	1.85	22.6
$LSD_{0.05}$		14.2	3.8	2.1	222	3.1	NS	NS
CV (%)		125.1	5.3	4.7	4.2	3.4	12.44	7.9

applications of ethephon was not different from quality of control plants. However, quality of plants treated with pre- and post-transplant multiple applications was significantly lower than that of plants treated with only a single application of ethephon.

Percentages of nicotine and reducing sugars in the cured leaves were not significantly different among treatments (Table 6). Similar results have been reported from experiments with burley tobacco (43).

Results of this experiment indicate no benefit from more than one application of ethephon to tobacco seedlings for control of premature flowering. Cured-leaf quality was actually reduced by multiple applications, and yield was lowered when the single pretransplant application was supplemented with a second ethephon application 10 days after transplanting.

Stage of Development

Tobacco seedling stage of development affects the duration of exposure to an inductive environment required for floral induction. Larger seedlings can be induced to flower more quickly than smaller seedlings exposed to the same florally inductive environment (40,41).

Although the environmental conditions and duration of exposure to such conditions necessary for floral induction of tobacco seedlings under ambient conditions have not been precisely characterized, the label on the formulation of ethephon used in this study ("Florel") only states that ethephon should be applied three to four days before transplanting.

Field test. Percentage premature flowering of untreated and treated

plants was not significantly different because of the low level (4.2%) of premature flowering in control plants (Table 7). Even with a low level of premature flowering, number of leaf nodes per plant at flowering was significantly increased by application of ethephon to seedlings at each treatment height. Differences in numbers of leaf nodes among treatments were not large and may not have been agronomically significant, but were sufficiently consistent (the coefficient of variation was 1.7%) to be statistically significant. The average number of days from transplanting to flower was increased by ethephon application to seedlings with stem heights averaging 1.5 and 2.5 cm. Days to flower for plants treated at the 5.0 cm stage were not significantly different from days to flower of control plants. These results suggest that the generally observed delay of growth and development from ethephon was greater when ethephon was applied to small seedlings.

Yield, quality indices, and percentages of nicotine and reducing sugars in the cured leaves were not different among treatments (Table 7). Pretransplant ethephon application did not reduce yield or quality of plants not exposed to a florally inductive environment, regardless of seedling stem height when treated. Yield increases from ethephon application may have been observed if the level of premature floral induction had been higher. Seedlings in this experiment were small relative to those in the other field experiments at the Southern Piedmont Center, and this may account for the low level of floral induction.

Greenhouse tests. Ethephon applied to seedlings of 1.5 cm stem height dramatically increased days to flower and number of leaf nodes per

Table 7. Influence of flue-cured tobacco seedling stem height on agronomic and chemical responses to ethephon application.

Stem ht. (cm) when treated with ethephon	Premature flowering	Days to flower	Nodes per plant	Yield	Quality index	Nicotine	Reducing sugars
	%			kg ha^{-1}		%	%
Control	4.2	46.4	27.0	3983	58.5	2.05	22.0
1.5	0.0	48.5	28.0	4081	59.9	1.78	20.5
2.5	0.0	48.8	28.0	4084	60.5	1.74	20.8
5.0	0.0	47.3	28.5	4119	61.8	1.99	22.9
$LSD_{0.05}$	NS	2.1	1.0	NS	NS	NS	NS
CV (%)	199.2	2.2	1.7	2.7	4.2	9.0	8.3

plant (Table 8). This treatment was applied seven days after transfer of seedlings to the floral induction chamber and indicates that floral induction had not been completed at that time.

In test one, days to flower for controls and seedlings treated at the 2.5 cm stem height were not different (Table 8). Although the numbers of leaf nodes at flowering for plants receiving these two treatments were statistically different, the numerical difference was small and not considered agronomically important. Completion of floral induction in the 2.5 cm seedlings prior to attainment of treatment size caused the lack of meaningful differences among plants receiving these two treatments. When transferred from the greenhouse to the floral induction chambers, seedlings in test one averaged 8.7 mm in stem height and, in the 15^{o}C environment, did not reach 2.5 cm until after the three-week induction period.

In test two, seedlings averaged 16.0 mm in stem height when placed in the induction chambers, and had attained the 2.5 cm stem height nine days later. Therefore, significant increases in days to flower, leaf nodes and stem heights at flowering were observed in plants treated at this stage of development (Table 8). The increase in nodes per plant was significantly greater when the ethephon was applied to seedlings averaging 2.5 compared to 1.5 cm in stem height. Corresponding increases in days to flower and stem height at flowering followed the same trend but were not statistically significant. The reason for the increased leaf node response to ethephon by the seedlings averaging 2.5 compared to 1.5 cm in stem height is not known. The conclusion is nonetheless valid

Table 8. Influence of ethephon applied to greenhouse-grown flue-cured tobacco seedlings at two stages of development.

Stem ht. (cm) when treated with ethephon	Test 1		Test 2		
	Days to flower	Leaf nodes per plant	Days to flower	Leaf nodes per plant	Stem ht. (cm) at flower
Control	9.6	11.0	11.6	12.0	51.9
1.5	32.4	25.6	44.2	24.2	99.9
2.5	10.8	11.8	59.0	33.8	125.3
$LSD_{0.05}$	2.7	0.7	16.6	8.5	31.1
CV (%)	10.7	2.8	29.8	24.9	23.1

that ethephon applied to flue-cured tobacco seedlings prior to the completion of controlled floral induction significantly increased days to flower and number of leaf nodes at flowering.

Ethephon Volume

Field test. Evaluation of pretransplant ethephon rates for control of premature flowering and for seedling growth suppression in flue-cured tobacco has not been reported. In this study, on the sixth day after treatment, the lowest one or two leaves of seedlings treated at the 244 mL m^{-2} rate were slightly yellowed and the bud and upper leaves were slightly crinkled compared to seedlings receiving the other treatments. Four days later, these effects were accentuated and the plants had developed a rosette appearance. Buds of these plants, in contrast to other plants, were shorter and appeared to be positioned lower in relation to leaves basipetal to the bud. Buds of control seedlings were light green to yellowish, which is a characteristic of rapid growth. In contrast, buds of the 244 mL m^{-2}-treated plants were dark green. No differences in plant habit were observed for seedlings receiving ethephon at the rates of 0, 41, 81, or 122 mL m^{-2}, except that on day 20 after treatment the upper leaves of seedlings treated at 41, 81, and 122 mL m^{-2} appeared slightly more pointed at the leaf tip. Ethephon treatments were replicated only once in the plant bed, and as a result of the small number of statistical degrees of freedom, weekly increases in stem height of plant bed seedlings were not significantly different among treatments (Table 9). However, the general trend was that an increased ethephon rate suppressed stem elongation. Significant differences in stem

Table 9. Weekly stem elongation for flue-cured tobacco plant bed seedlings following treatment with ethephon at 5 rates.

Ethephon rate	Stem elongation after treatment -----Week----- 1	2	3	4
mL m^{-2}	cm/week			
0	3.6	13.1	16.7	9.4
41	2.2	12.7	13.7	5.4
81	3.0	10.5	13.8	4.9
122	1.9	11.2	16.4	6.6
244	1.5	9.0	12.9	3.5
$LSD_{0.05}$	NS	NS	NS	NS
CV (%)	41.8	20.4	11.0	36.9

elongation were obtained for the third week after transplanting (Appendix Table 6), with elongation significantly reduced in plants receiving ethephon at the 244 mL m^{-2} rate compared to controls (Table 10). In general, stem elongation was suppressed as ethephon rate was increased. Differences in stem elongation were not significant for weeks one and two after transplanting. Transplant stress may have slowed growth of all seedlings and made treatment effects undetectable. Four weeks after transplanting, differences in stem elongation were not significant, suggesting that the plants had recovered from suppression of growth. Ethephon-induced suppression of stem growth in burley tobacco has previously been reported (43).

Percentage premature flowering of plants transplanted three days after treatment (3-day transplants) was not affected by ethephon rate (Table 11) and was significantly less than percentage premature flowering for the 7-day transplants (Appendix Table 7). Ethephon application significantly reduced premature flowering in the 7-day transplants. The greatest reduction was obtained from the 244 mL m^{-2} rate (Table 11). Numbers of leaf nodes per plant were not different among ethephon rates for plants transplanted three days after treatment, but for the 7-day transplants, the number of nodes for control plants was significantly less than those for ethephon treated plants (Table 11). Differences in node counts among the different rates were not significant. This suggests that the differences in premature flowering among ethephon rates for the 7-day transplants resulted from delayed growth and development and not from initiation of additional leaves. Differences in premature

Table 10. Posttransplant weekly stem elongation summed over two transplant dates of flue-cured tobacco treated pretransplant with 5 rates of ethephon.

Ethephon rate	Stem elongation after treatment --Week-- 1	2	3	4	5
mL m^{-2}			cm/week		
0	2.5	4.0	9.5	20.9	30.3
41	1.7	3.2	7.9	18.5	28.8
81	2.0	3.6	9.5	21.1	28.0
122	1.7	3.1	7.5	18.2	27.2
244	1.5	2.6	7.0	16.9	27.4
$LSD_{0.05}$	NS	NS	2.3	NS	NS
CV (%)	36.4	24.4	15.8	13.5	13.8

Table 11. Flowering characteristics of flue-cured tobacco transplanted at 2 dates following application of ethephon at 5 rates.

Ethephon rate	Premature flowering		Days to flower		Nodes per plant	
	Transplant day					
	3	7	3	7	3	7
mL m^{-2}	%					
0	1.4	51.1	48.2	36.3	27.5	22.2
41	2.8	16.7	48.4	43.0	27.6	26.1
81	1.4	24.0	48.7	41.1	27.7	25.8
122	1.4	19.0	49.4	43.0	26.9	26.0
244	0.0	3.0	53.0	46.6	27.2	26.8
$LSD_{0.05}$	NS	10.6	1.8	4.9	NS	1.8
CV (%)	168.4	48.0	1.9	6.2	2.5	3.8

flowering of control and treated plants, when transplanted seven days after treatment, are apparently due both to the initiation of additional leaves and to delayed development.

Average number of days from transplanting to flower for the 3-day transplants was not different among treatments, except for a significant increase for plants receiving the high rate of ethephon (Table 11). This indicates that the high rate of ethephon delayed plant development to a greater extent than the lower rates. Days to flower for the 7-day transplants were significantly higher for treated compared to control plants, except that days to flower for plants treated at the 81 mL m^{-2} rate and for control plants were not statistically different. Data for premature flowering and leaf nodes suggest that the lack of difference in time of flowering between plants receiving these two treatments may be more statistical than biological.

Yield of the 7-day transplants followed the pattern for nodes per plant, with ethephon applications resulting in significant increases in yield, and with no significant differences in yield among plants receiving the four pretransplant ethephon rates (Table 12). When seedlings were transplanted three days after treatment, ethephon applications did not significantly affect yield at the 0.05 probability level. Yield data (Table 12) suggest an apparent ethephon-induced reduction in yield of 3-day transplants, but lack of differences in number of leaf nodes (Table 11) support the lack of significant differences in yield.

Quality index is derived from the official U.S. Government grades

Table 12. Yield, quality index, and chemical content of flue-cured tobacco transplanted at 2 dates following application of ethephon at 5 rates.

Ethephon rate	Yield		Quality index		Nicotine		Reducing sugars	
	transplant day		transplant day		transplant day		transplant day	
	3	7	3	7	3	7	3	7
$mL\ m^{-2}$	$kg\ ha^{-1}$				%		%	
0	4162	2726	67.0	68.7	1.65	2.35	24.2	22.8
41	3823	3560	62.0	68.3	1.64	1.89	23.9	22.9
81	3816	3517	67.7	69.3	1.87	2.00	22.4	22.7
122	3870	3480	70.7	69.7	1.73	1.76	21.7	23.5
244	3974	3517	66.3	76.0	1.72	1.84	19.0	21.9
$LSD_{0.05}$	NS	498	NS	NS	NS	NS	NS	NS
CV (%)	3.6	7.9	6.8	7.8	9.6	11.8	10.2	5.8

applied to flue-cured tobacco (77) and is an indicator of cured-leaf quality. Quality indices of the cured leaves of control and ethephon treated plants were not significantly different within transplant dates (Table 12). Percentages of nicotine and reducing sugars in the cured leaf are important criteria in the desirability of flue-cured tobacco to purchasers (79). In this study, differences in percentages of nicotine and reducing sugars between cured leaves of control and ethephon treated plants were not significant (Table 12).

Significant differences were observed in percentage premature flowering, days to flower, number of leaf nodes per plant at flowering, yield, quality and percent nicotine for 3-day and 7-day transplants (Appendix Table 7). Increased premature flowering and decreased days to flower (Table 11) and yield (Table 12) for plants from seedlings transplanted seven compared to three days following ethephon treatment were observed at all rates of ethephon. Decreased number of leaf nodes for the 7-day compared to 3-day transplants appears to result mainly from the reduced number of nodes in 7-day control plants (Table 11). Higher quality index of the 7-day transplants was observed for ethephon rates of 41 and 244 mL m^{-2} (Table 12).

Increased nicotine level of the 7-day transplants could be due to the reduced yield of these plants (Table 12). Nicotine is synthesized in the roots and transported to the above ground parts of the tobacco plant (28). Reduced yield could result in a higher concentration of nicotine in the leaves if the root systems of the high and low yielding plants synthesized similar amounts of nicotine.

Explanation of the effect of posttreatment transplant date on premature flowering and quality is more difficult. Link (48) reported delayed flowering and reduced yield of burley tobacco plants when transplanted one day following ethephon treatment but not when transplanted 3, 7, or 10 days posttreatment. No explanation was given for this effect. Differences in performance among transplant dates of flue-cured tobacco were reported by Miner (57) to be the result of different environmental conditions between dates. In this study, the transplant dates were 21 and 25 May, and 1.3 cm of rainfall, ending a two-week drought, were measured at the Southern Piedmont Center on 23 May. Variations in mean daily temperature during this period were minimal, and the rainfall on 23 May would not appear to explain the effect of transplant date in this study. Plants under stress from drought and other environmental causes synthesize ethylene at increased rates (85). Transplant shock may therefore have induced ethylene biosynthesis. Detection of ethylene from ethephon-treated mature flue-cured tobacco plants was reported to decline rapidly after 24 h and to have apparently stopped after approximately 96 h (22). Therefore, the amount of ethylene from ethephon decomposition and perhaps also from ethephon-induced biosynthesis of ethylene in the tobacco seedlings may have been greater at three than at seven days after ethephon treatment. However, results of a related experiment (ethylene quantification) indicated minimal differences in ethylene evolved from tobacco seedlings four and eight days after ethephon treatment (see Table 23). These factors may have resulted in higher ethylene concentrations in the 3-day

transplants. A florally inductive posttransplant environment would have been necessary for this possibility to have caused the observed effects of transplant date. Temperatures at the Southern Piedmont Center during 21 May to 3 June may have been florally inductive (Appendix Table 8).

Another interpretation of the effect of transplant date can be made. The 7-day transplants were exposed to the pretransplant environment for an additional four days, and this increased duration of exposure may have resulted in the increased level of floral induction. Percentage premature flowering was approximately 1 and 51% in the controls transplanted 21 and 25 May, respectively (Table 11). The average daily minimum temperature was 17.3°C for 21 through 25 May and 9.8°C during the pretransplant period 23 April through 20 May. These temperatures were apparently florally inductive. Furthermore, the larger seedlings probably intercepted most of the ethephon spray, and therefore the smaller seedlings underneath received significantly less volume of ethephon. Three days later, the larger plants were removed from the plant beds and transplanted, and the majority of the remaining seedlings, from which the 7-day transplants were selected, were ones which had received less ethephon. The 7-day transplants, therefore, flowered earlier and with fewer leaves, and yield was lowered (Table 11). Also, average quality index of the 7-day transplants was higher than that of the 3-day transplants (Table 12). Weybrew and Woltz have associated quality improvement in flue-cured tobacco with depletion of available soil nitrogen at approximately the time of flowering (78,79). Twenty centimeters of rain fell in July and probably leached much of the soil

nitrogen from the root zone. After adjusting for the four day differences in transplant date, the 7-day transplants flowered an average of almost 3.5 days earlier than the 3-day transplants. Depletion of available soil nitrogen probably occurred at or near the time of flowering in the 7-day transplants and may account for the higher quality of these plants.

Greenhouse test. The influence of ethephon on days to flower, stem height at flowering, and stem elongation for greenhouse-grown plants are shown in Tables 13 and 14. Except for during the third week after treatment, ethephon applications significantly influenced stem elongation from the time of treatment until at least six weeks after treatment (Table 13). The general pattern was for an increased rate of ethephon to decrease stem elongation. This same pattern was observed in the field test. Lack of significant differences in stem elongation during week three cannot be explained. Days to flower were increased by application of ethephon at rates of 81, 122, and 244 mL m^{-2} (Table 14). Since the seedlings were not exposed to a florally inductive environment, the effect of ethephon on days to flower for these plants is apparently due to suppression of growth and development. Lack of significant differences in stem heights at the time of flowering (Table 19) indicates that growth suppression was temporary.

Appearance of the greenhouse-grown seedlings following ethephon treatment was generally similar to that of the plant bed seedlings. By day 6 after treatment, the lower one or two leaves of seedlings treated at the 244 mL m^{-2} rate were yellow or yellowish orange. The small leaves

Table 13. Weekly posttreatment stem elongation of greenhouse-grown flue-cured tobacco treated with 5 rates of ethephon.

Ethephon rate	Stem elongation					
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
mL m^{-2}	cm/week					
0	5.5	9.0	10.2	19.7	29.0	27.9
41	4.5	8.3	11.3	19.8	28.3	29.8
81	3.6	7.8	10.0	17.7	23.2	28.2
122	3.5	6.4	9.4	15.9	21.9	26.4
244	2.0	4.8	10.0	14.1	19.0	20.9
$LSD_{0.05}$	1.3	1.8	NS	2.7	4.3	4.2
CV (%)	38.5	26.2	21.9	17.3	19.7	17.3

Table 14. Stem height at flowering and days to flower for greenhouse-grown treated with 5 rates of ethephon.

Ethephon rate	Stem ht. at flowering	Days from potting to flower
mL m^{-2}	cm	
0	83.6	36.4
41	86.1	37.8
81	89.7	41.3
122	86.7	42.0
244	90.9	47.6
$LSD_{0.05}$	NS	3.2
CV (%)	14.6	8.7

in the bud were mottled and the leaf tips and margins were light green. The extreme tip of these leaves was brown and the upper leaves crinkled. On day 17 after treatment, seedlings treated at the 244 mL m^{-2} rate were distinctly shorter than the other seedlings and had developed a rosette appearance as a result of shorter internode spacing and suppressed growth of the upper leaves. Seedlings treated at the 41 mL m^{-2} rate and control seedlings were similar in appearance. Buds of seedlings treated at the 81 and 122 mL m^{-2} rates appeared slightly rosette-like and the upper leaves were slightly retarded in growth. In general, green color of the seedlings increased in intensity as the ethephon rate was increased. This was attributed to higher concentrations of nitrogen due to suppressed growth of these seedlings. Leaves to which ethephon was applied at the 244 mL m^{-2} rate remained suppressed in size throughout the duration of this experiment.

Growth Chamber Ethephon Timing

The results of this experiment indicate that the timing of the ethephon application was not critical during the first two weeks of a three week exposure to a florally inductive environment. Percentage premature flowering and days to flower were significantly reduced by ethephon applied to seedlings after 0, 7, or 14 days exposure, and were not different among these treatments (Table 15). In test one, ethephon applied after 0, 7, or 14 days reduced premature flowering from 47 to 0% and increased the average days to flower from 44 to approximately 70. Also, numbers of leaf nodes per plant were increased from 18 to approximately 26 and yield was increased from 2578 to approximately 3156

Table 15. Influence of ethephon, applied after various days of pretransplant exposure to a controlled florally inductive environment, on subsequent agronomic and chemical characteristics of flue-cured tobacco.

Day of ethephon treatment	Days to flower	Premature flowering	Leaf nodes per plant	Yield	Quality index	Nicotine	Reducing sugars
		%		kg ha^{-1}		%	%
			Test 1				
Control	43.8	47.4	18.0	2578	58.0	1.9	24.3
Late Control	58.5	1.6	25.5	3388	55.5	2.0	24.4
0	71.1	0.0	26.8	3118	63.5	1.7	22.6
7	69.7	0.0	26.9	3293	62.3	1.8	21.8
14	71.3	0.0	27.1	3056	59.0	1.9	22.8
21	53.7	40.3	20.7	2737	62.5	1.8	22.3
$LSD_{0.05}$	4.5	9.4	2.1	390	NS	NS	NS
CV (%)	4.9	41.9	5.8	8.5	6.5	11.7	6.3
			Test 2				
Control	43.0	18.1	24.3	3506	62.3	2.2	21.1
0	48.7	0.0	25.1	3414	63.3	2.2	23.1
7	48.5	1.5	25.0	3389	61.3	2.0	22.4
14	47.9	0.0	25.7	3484	64.3	2.1	21.1
21	43.8	12.7	23.7	3176	60.0	1.9	22.0
$LSD_{0.05}$	2.1	10.1	1.0	NS	NS	NS	NS
CV (%)	2.9	101.3	2.6	7	7.7	16.1	10.9

kg ha^{-1} by ethephon applied at any of these times.

Percentage premature flowering and number of leaf nodes per plant for plants with ethephon applied on days 0, 7, or 14 and for noninduced, late control plants were not different (Table 15). Therefore, application of ethephon to seedlings prior to completion of floral induction completely negated floral induction. However, ethephon treatment after 0, 7, or 14 days exposure, compared to late control plants, delayed days to flower. This appears to be the result of delayed growth and development resulting from ethephon treatment.

The level of floral induction was lower in test two, and only 18% premature flowering was observed (Table 15). Consequently, yield was not reduced by premature flowering, and number of leaf nodes was not increased by ethephon applied after 0, 7, or 21 days exposure to the controlled florally inductive environment. However, leaf nodes for plants treated after 14 days exposure were statistically greater than those of control plants, but were not different from those of plants treated after zero or seven days. The difference in nodes between controls and plants treated after 14 days is not likely of agronomic significance since premature flowering, days to flower, and yield were not different for plants treated after 0, 7, or 14 days exposure. Warmer temperatures during the one week conditioning period in the lath house and also following transplanting (Appendix Table 9) may explain the reduced level of induction in test two. However, this interpretation would require the assumption that floral induction had not been completed in some plants during the three-week period in the growth chambers.

Thomas et al. (68) reported reduced leaf numbers of flue-cured tobacco as a result of lower posttransplant temperatures.

Seedling stage of development can also affect floral induction. When transferred to the floral induction chambers, seedlings in this study averaged 131 cm^2 in leaf area and 4.0 cm stem height in test one and 73 cm^2 and 1.6 cm, respectively, in test two. Average leaf area of seedlings in both tests exceeded the reported 25 cm^2 minimum required for floral induction of a N. tabacum L. breeding line (33). However, Kasperbauer (40) reported that floral induction of burley tobacco occurred after fewer inductive cycles as plant size or age increased. Floral induction of tobacco seedlings has also been associated with rapid growth of leaves (34). In this study, the average total increases in stem height from day 0 to day 21 were 7.4 and 7.0 mm for control seedlings in tests one and two, respectively. Corresponding increases in total leaf area per plant from day 0 to day 21 averaged 15.4 and 41.8 cm^2 for control seedlings in tests one and two, respectively. Senesced lower leaves were not included in the day-21 leaf area measurements. The difference in stem height increases of seedlings in tests one and two appears to be insignificant, and the reduced level of floral induction in test two cannot be explained on the basis of a decreased rate of leaf growth.

Despite the reduced level of floral induction in test two, the responses of premature flowering and days to flower followed the same pattern in both tests. Ethephon applied on day 0, 7, or 14 reduced premature flowering to almost zero and increased days to flower (Table

15). Premature flowering was not significantly reduced when ethephon application was delayed until the end of the three week induction period. Days to flower were not increased in test two by ethephon applied after 21 days exposure, possibly due to a warmer posttransplant environment in test two (Appendix Table 9). Growth suppression would be expected to be less severe in more favorable growing conditions.

Ethephon, which releases ethylene upon absorption by plant tissue, had been reported by Kasperbauer (43) to increase days to flower and number of leaves at first flower in two of three tests when applied to pretransplant burley tobacco seedlings 11 days after exposure to a 21-day controlled florally inductive environment. Influence of ethephon when applied to seedlings of flue-cured tobacco and at times prior or subsequent to 11 days exposure to a florally inductive environment had not been previously reported. Although evidence (43) suggested that ethephon application would not negate an already completed floral induction, whether the timing of the application was otherwise critical was not known.

The delay in flowering of the day-21 ethephon treated plants could be expected as a result of ethephon-induced suppression of growth and development. However, even the small increase in leaf nodes observed in test one was not expected since ethephon was applied after the floral induction period. Kasperbauer reported that ethephon applied after floral induction did not increase leaf numbers of burley tobacco (43). He postulated that burley tobacco seedlings treated with ethephon were unresponsive to a florally inductive environment during the period of

ethephon-induced temporary growth suppression (43). If floral induction in some plants was only partially but nearly completed after 21 days, a subsequent florally inductive environment during the one week period of conditioning in the lath house and/or after transplanting (Appendix Table 9) could have completed induction of the control seedlings. In that situation, sensitivity to the inductive environment of the day-21 treated seedlings could have been delayed by ethephon treatment. After the ethephon effect subsided, the plants treated after 21 days exposure could be florally induced more quickly than those exposed 0, 7, or 14 days before treatment, assuming an additive response to exposure. However, a more probable explanation for the increased number of leaf nodes for day-21 treated compared to control plants is that the magnitude of difference in percentage premature flowering (7 percentage points less in the day-21 treated plant) may have been sufficient to account for a slight increase in average number of leaf nodes. Also, number of leaves per plant at flowering (data not presented) was determined concurrently with number of leaf nodes, but did not include lower leaves which had senesced or upper leaves less than 1.0 cm wide. The average number of leaves were 14 and 13 for the control and day-21 ethephon treated plants, respectively, and were not statistically different. This was the only situation in which statistical differences for leaf nodes and number of leaves did not follow the same pattern among treatments.

Premature flowering was less and days to flower and number of leaf nodes were greater for late control (included only in test one) compared to control plants (Table 15), indicating that the late control seedlings

were not florally induced. The additional nine inductive cycles to which the controls were exposed resulted in a large increase in premature flowering. Floral induction under these conditions was apparently completed at some point between 12 and 21 days exposure to the inductive environment. In earlier reports, a minimum of 10 and approximately 14 days, respectively, were required for floral induction of a flue-cured breeding line (33) and burley tobacco (40,41) seedlings.

Yield followed the same pattern as number of leaf nodes per plant, with no significant difference in yields of control and day-21 ethephon treated plants, and no difference in yields of late controls, included in test one, and plants treated with ethephon on days 0, 7, or 14 (Table 15). These results show that premature flowering can seriously reduce yields. With 47% premature flowering in control plants, pretransplant ethephon application increased average yields by as much as 715 kg ha^{-1}. Yield increases from ethephon applied to burley tobacco seedlings exposed to a florally inductive environment were also reported by Kasperbauer (43). Ethephon did not affect the quality index or percentages of nicotine and reducing sugars in the cured leaves (Table 15). In contrast, ethephon applied to posttransplant, maturing tobacco plants has been reported to significantly influence cured leaf quality, nicotine, and reducing sugars (19,21,50,53). Potential influences of ethephon on cured leaves are apparently lost if ethephon is applied to plants in the seedling stage of development.

Within aproximately one week after treatment, the lower leaves of treated seedlings (each seedling received approximately two mL of 960 mg

L^{-1} ethephon, which is approximately a 4 to 5X label rate) began to yellow more rapidly than those of control seedlings. These leaves of treated seedlings developed small necrotic spots and were easily abscissed during handling. Ethephon-induced yellowing and senescence of mature leaves of tobacco is well documented (19,21,50,53). The younger leaves near the apical bud did not yellow, and were darker green than corresponding leaves of control seedlings. At transplanting, most leaves had abscissed and only a few very small bud leaves remained. Initial growth of these transplants was extremely slow, but at flowering, the plants had attained normal size. This severe stunting made transplanting and early cultivation difficult, and extreme care was taken to avoid covering the seedlings with soil. However, this was a minor problem considering the subsequent benefits in yield and crop uniformity resulting from ethephon-induced reductions in premature flowering. Severity of stunting may be reduced by application of lower volumes of ethephon per plant. Comparable rates of ethephon had a greater influence on appearance of tobacco seedlings grown in the greenhouse and growth chamber than on seedlings grown in an outdoor plant bed. Greenhouse-grown seedlings appeared more succulent and may have absorbed ethephon more readily than plant bed seedlings. Yellowing of lower leaves and growth suppression was more obvious for seedlings treated in the growth chamber than for seedlings treated in outdoor plant beds.

Ethephon Wash-off

In conducting research with ethephon on tobacco seedlings in the

ambient environment, questions were raised concerning a critical posttreatment time period before which a rainfall would significantly reduce the effect of ethephon by washing off the material. Literature searches yielded no reports of relevant experiments with tobacco seedlings.

Significant differences were not obtained for days to flower and number of leaf nodes for controls and plants washed 15 and 30 minutes after treatment, except that leaf nodes of plants washed 30 minutes after treatment were greater than controls in test one (Table 16). When washing was delayed until 60 minutes after treatment, significant increases in both parameters over controls were observed for days to flower and leaf nodes.

In test one, plants washed 120 minutes after treatment flowered later and had more leaf nodes than those washed at 60 minutes (Table 16). Days to flower and leaf nodes were not additionally increased by delaying washing until 240 minutes after treatment.

In test two, no differences were observed in days to flower and leaf nodes for plants washed at 60, 120, and 240 minutes after treatment (Table 16). Ethephon treated plants which were not washed flowered significantly later and with increased number of leaf nodes than plants washed 120 minutes after treatment. Days to flower and leaf nodes were not significantly different for plants washed after 240 mintues and those not washed.

Stem heights at flowering, measured only in test two, responded to ethephon in similar ways as days to flower and leaf nodes. At flowering,

Table 16. Influence of pre-wash, on-plant duration of ethephon applied to greenhouse-grown flue-cured tobacco seedlings exposed to a florally inductive environment and washed with a simulated 2.5 cm rainfall.

Pre-wash duration of ethephon (minutes)	Test 1		Test 2		
	Days to flower	Leaf nodes per plant	Days to flower	Leaf nodes per plant	Stem ht. at flower
					cm
Control	10.8	12.0	14.6	11.6	46.7
15	11.2	13.0	17.2	13.2	53.6
30	17.0	17.4	16.6	13.8	48.2
60	21.2	19.6	53.6	28.0	110.9
120	30.4	25.4	45.8	25.8	100.0
240	30.8	25.0	55.4	30.0	116.6
Not Washed	-	-	-	-	-
$LSD_{0.05}$	7.6	4.6	14.4	7.1	28.5
CV (%)	28.3	18.7	28.5	24.1	25.1

stem heights were not different for controls and plants washed at 15 and 30 minutes, but these heights were significantly lower than those of plants receiving the other treatments (Table 16). Stem heights at flowering were not significantly different for plants washed at 60, 120, and 240 minutes after ethephon treatment. Not-washed plants were taller than plants washed at 120 minutes but were not different in height from plants washed at 60 and 240 minutes.

Although the differences were not statistically significant, plants washed at 120 minutes in test two tended to flower earlier, had fewer leaf nodes, and were shorter than plants washed at 60 or 240 minutes after ethephon treatment (Table 16). Experimental units were individual plants, and plant to plant variation could greatly influence treatment means. In test two, two of the five plants washed at 120 minutes after treatment flowered considerably earlier than the remaining three plants. Floral induction had apparently been completed in these plants before ethephon was applied. Earlier flowering of these two plants would therefore explain the variation observed in the flowering of plants washed at 120 compared to 60 and 240 minutes after treatment.

These results indicate that a 2.5 cm rainfall within between 30 to 60 minutes after application of ethephon can negate the reduction in premature flowering obtained from ethephon. These results also suggest that for maximum benefit, the ethephon should not be washed off the seedlings within a period of approximately one to two hours after application.

Stem heights were measured at weekly intervals after treatment.

Lack of differences in stem elongation of seedlings in the growth chamber are attributed to slow seedling growth in the 15°C environment, and perhaps also to a lag in response to ethephon. A similar lag in response to ethephon applied to burley tobacco was obtained by Kasperbauer (43), who reported no difference in stem heights of ethephon treated and control plants two days after treatment, whereas differences were observed at subsequent times.

After subsequent transfer of seedlings to the greenhouse, stem elongation of seedlings in test one were not different among treatments during the first week (posttransplant days 7 to 14) (Table 17). During the second week, stem elongation for seedlings washed at 240 minutes after treatment was less than for control seedlings and seedlings washed at 30 minutes. In test two, stem elongation during the first eight days after transfer from the growth chamber to the greenhouse did not follow a logical pattern. Stem elongation for not washed seedlings and those washed at 60 minutes was less than that for seedlings washed at 15 and 120 minutes (Table 17). This treatment grouping for seedling stem elongation is not readily explained. During the subsequent seven days (posttreatment days 20 to 27), stem elongation for seedlings washed at 240 minutes and those not washed was significantly less than that for control seedlings and treated seedlings washed at 15 and 30 minutes after treatment. Furthermore, stem elongation for not washed seedlings during this period was significantly less than that for seedlings washed at 240 minutes. In general during these periods, differences in stem elongation among seedlings receiving the different treatments followed a less

Table 17. Influence, on stem elongation, of pre-wash, on-plant duration of ethephon applied to greenhouse-grown flue-cured tobacco seedlings exposed to a controlled florally inductive environment and washed with a simulated 2.5 cm rainfall.

Pre-wash duration of ethephon (minutes)	Stem elongation in greenhouse			
	Test 1		Test 2	
	7-14*	14-21	12-20	20-27
	cm/week			
Control	0.9	3.9	1.0	4.8
15	1.1	3.6	1.2	4.5
30	1.2	3.9	1.0	5.2
60	0.8	3.4	0.7	2.9
120	0.8	2.6	1.2	4.1
240	0.7	2.4	1.0	3.7
Not washed	-	-	-	-
$LSD_{0.05}$	NS	1.4	0.4	1.1
CV (%)	63.7	32.3	29.7	20.9

*Posttreatment days

logical pattern than the parameters associated with flowering (Tables 16 and 17). Stem elongation may respond to ethephon less directly than does flowering.

Ethephon Localization

Application of ethephon to localized sites on flue-cured tobacco seedlings was studied to determine the site or sites on the plant to which ethephon must be applied in order to delay induction. The statistical probability of a greater F value for the effect of ethephon amount on flowering parameters ranged from 0.42 to 0.98, indicating that the changes in the amount of ethephon applied did not cause significant variations in seedling response within a given treatment.

In test one, days to flower and leaf nodes per plant at flowering were not significantly different for controls and seedlings to which ethephon was applied to the yellow leaf or the fourth leaf (which was very small) above the yellow leaf (Table 18). Senesced and immature leaves export little if any photosynthate (45,76,51) and, assuming ethephon transport in the phloem in a source to sink relationship (76), these leaves may have exported insignificant amounts of ethephon to the shoot apex. Plants treated at leaf two flowered later and had more leaf nodes than plants treated at leaf one, but were not significantly greater than those for plants treated at leaf three. Leaf three received less ethephon than leaf one, but the difference was not critical or perhaps leaf three exported more ethephon to the shoot apex than did leaf one. Leaf two was generally the largest leaf on each seedling and therefore

Table 18. Influence, on flowering, of localization and amount of ethephon applied to greenhouse-grown flue-cured tobacco during 3-week exposure to a controlled florally inductive environment.

Ethephon application			Test 1		Test 2		
	Avg. amt.		Days	Nodes	Days	Nodes	Stem ht.
Site	Test 1	Test 2	to flower	per plant	to flower	per plant	at flowering
	mg						cm
Control	0.00	0.00	8.8	11.6	15.0	12.6	46.9
Yellow leaf	0.21	0.32	16.6	16.6	30.2	18.8	70.7
Leaf 1	0.44	0.44	22.4	20.2	56.0	29.2	117.0
Leaf 2	0.52	0.51	32.4	26.0	80.3	37.3	141.8
Leaf 3	0.27	0.26	26.0	22.8	65.6	34.2	137.7
Leaf 4	0.08	0.02	9.2	12.4	11.6	12.6	47.9
All leaves	1.19	1.16	30.2	25.8	105.6	42.2	151.5
Bud	0.03	0.03	20.8	21.0	54.4	30.0	122.4
Bud (3 X conc.)	0.09	0.09	25.8	23.6	67.6	34.8	131.6
All leaves + bud	1.53	1.35	38.2	31.0	89.4	39.8	149.9
Roots	10.00	10.00	22.2	19.4	78.2	34.4	137.4
$LSD_{0.05}$			9.0	5.4	14.2	5.6	22.7
CV (%)			30.7	20.2	18.8	14.8	15.6

received more ethephon (Table 18). Application of ethephon to all four leaves did not significantly increase days to flower or number of leaf nodes when compared to seedlings to which ethephon was applied only to leaf one, two, or three. Application to all four leaves plus the bud resulted in the greatest numbers of days to flower and leaf nodes per plant at flowering. Numbers of days to flower and leaf nodes for plants receiving this treatment were significantly higher than those for all other treatments except applications to all the leaves and to leaf two. Seedlings to which these three treatments were applied received greater amounts of ethephon than seedlings receiving the other treatments, excluding the roots treatment. Days to flower and leaf nodes were not significantly different among plants to which the ethephon was applied to the bud at concentrations of 960 and 2880 mg L^{-1}, although plants which received the higher concentration flowered approximately five days later and had approximately four more leaf nodes. Days and nodes for seedlings treated in the bud with an average of 0.09 mg ethephon were not significantly less than those of seedlings to which an average of 1.19 mg ethephon was applied to all the leaves. This indicates that not only the amount, but also the localization of ethephon application is important. This observation is supported further by comparison of ethephon applications to leaf four and to the bud. Significant increases in days to flower and leaf nodes were obtained by application of 0.03 mg ethephon to the bud but not by application of 0.02 to 0.08 mg to leaf four. Leaf four was located very near the bud, but being an immature leaf, probably did not export ethephon to the shoot apex, where floral initiation

occurs. Application of 10 mg of ethephon to the roots via the potting media resulted in significant increases in days to flower and leaf nodes compared to controls and to seedlings treated at leaf four (Table 18). This suggests that ethephon taken up by the roots [or perhaps an ethylene precursor (35)] was subsequently translocated to the shoot apex.

Results of test two were similar to those of test one, with the following exceptions. Application of ethephon to the yellow leaf resulted in days to flower and leaf nodes which were significantly higher than those of control seedlings (Table 18). The yellow leaves in test two were slightly less yellow than those in test one, and perhaps were still capable of exporting materials to acropetal plant parts. Also, seedlings to which ethephon was applied to all four leaves flowered the latest and with the most leaf nodes. Furthermore, days to flower for seedlings receiving this treatment were statistically greater than those of seedlings to which ethephon was applied to all four leaves plus the bud. Days to flower and leaf nodes for seedlings receiving ethephon via the roots were not significantly less than those of seedlings to which ethephon was applied to all the leaves plus the bud or to leaf two. Increased numbers of days to flower and leaf nodes per plant in test two were attributed to the extended daylength with supplemental lamps in the greenhouse. In both tests of this and the other greenhouse experiments, days to flower and leaf nodes were positively and highly correlated, having correlation coefficients averaging approximately 0.97 (data not presented). This correlation is not surprising since a delay in floral initiation permits initiation of additional leaves. Stem heights at

flowering, measured only in test two, closely paralleled the grouping of treatment means for days to flower and number of leaf nodes at flowering (Table 18).

These results indicate that both the amount and the localization are important in the application of ethephon to flue-cured tobacco seedlings for influence on flowering. Although these results cannot be used as evidence for a specific mechanism of ethylene action in the delay of floral induction of tobacco seedlings, a basis for general speculation is provided. Assuming that a flowering stimulus or signal is synthesized in the leaves and transported to the shoot apex (9,45), at least two possibilities exist for the ethylene-induced delay of floral induction. One, the signal is not sent, either by inhibition of synthesis or by inhibition of transport. Ethylene has been reported to restrict transport of auxin (13,14,80). Second, ethylene prevents the processing of the signal by the shoot apex, i.e., the apex is rendered temporarily unresponsive to the floral signal. Application of ethephon to tobacco leaves clearly delayed floral induction. Whether this was the result of transport, presumably of ethephon (76,81), to the shoot apex with subsequent reduction of apex responsiveness to the floral signal, or of inhibition in the leaves of the synthesis and/or transport of the floral signal cannot be determined from this study. However, application of small quantities of ethephon to the bud region delayed floral induction, suggesting that the ethylene may have caused the shoot apex to become temporarily unresponsive to the floral signal. This assumes that the ethephon was not transported basipetally from the bud in significant

quantities. The possibility cannot be excluded that basipetally transported ethephon inhibited the synthesis and/or transport of the flowering stimulus. However, since ethephon applied to leaf four did not affect flowering, basipetal transport of ethephon would necessarily extend below leaf four in order to influence flowering.

If ethylene inhibited responsiveness of the shoot apex to the floral signal, ethephon applied to the leaves or roots of seedlings could be transported to the shoot apex and subsequent degradation of ethephon to ethylene could cause the inhibition of responsiveness to the signal. A flowering signal would require processing at the shoot apex, which would require synthesis of new enzymes. Ethylene has been reported to inhibit DNA synthesis by reducing the activity of DNA polymerase (16). Synthesis of new enzymes would require DNA templates (28). A shoot apex to base gradient was reported in the capacity of stem segments of a cigar tobacco cultivar to produce flower primordia *in vitro*, and the segments that formed 100% flower buds contained 10 times more DNA than the segments that formed a lower percentage of flower buds (74).

Weekly changes in stem elongation were also measured. Stem elongation for controls and seedlings treated at leaf four were the highest and were not significantly different (Table 19). Also, stem elongation of seedlings treated at leaf two, all the leaves, and all leaves plus the bud was not significantly different and was generally less than stem elongation of seedlings which received other treatments. Stem elongation was generally not different among seedlings treated in the bud at the two concentrations of ethephon. These data, grouped by

Table 19. Influence, on stem elongation, of localization and amount of ethephon applied to greenhouse-grown flue-cured tobacco during 3-week exposure to a florally inductive environment.

Ethephon application			Stem elongation						
			Test 1				Test 2		
	Avg. amt.		In GC*	In GC	In GH	In GH	In GC	In GH	In GH
Site	Test 1	Test 2	0-5**	5-13	13-20	20-27	0-13	13-20	20-27
	mg		cm/week						
Control	0.00	0.00	0.2	1.9	3.5	12.9	0.2	1.3	5.0
Yellow leaf	0.21	0.32	0.1	1.1	1.4	8.7	0.3	1.0	4.4
Leaf 1	0.44	0.44	0.0	0.9	1.5	7.1	0.3	1.0	4.0
Leaf 2	0.52	0.51	0.1	0.8	1.1	3.9	0.1	0.7	1.3
Leaf 3	0.27	0.26	0.1	0.8	1.6	7.4	0.1	0.9	3.3
Leaf 4	0.08	0.02	0.1	1.5	3.0	12.3	0.2	1.5	5.8
All leaves	1.19	1.16	0.1	0.9	0.5	2.7	0.1	0.3	0.3
Bud	0.03	0.03	0.1	1.3	2.2	9.6	0.4	1.1	4.7
Bud (3 X conc.)	0.09	0.09	0.0	1.1	2.1	9.6	0.0	1.0	3.1
All leaves + bud	1.53	1.35	0.1	0.7	0.6	2.5	0.0	0.5	0.7
Roots	10.00	10.00	0.1	0.8	1.0	7.0	0.1	0.7	2.5
$LSD_{0.05}$			NS	0.3	1.0	4.0	0.2	0.32	1.44
CV (%)			143.8	19.7	47.9	41.2	98.9	27.5	35.1

*GC = growth chamber, GH = greenhouse

**days posttreatment in respective locations.

treatment, followed a similar pattern as the data for flowering parameters (Tables 18 and 19).

Ethephon Growth Effect

In these tests, ethephon was applied to flue-cured tobacco seedlings exposed for three weeks to controlled florally noninductive or inductive environments. Days to flower and number of leaf nodes per plant at flowering were affected by the floral-response environment and ethephon (Appendix Table 10). An environment X ethephon interaction was significant in test two of the experiment.

In test one, ethephon applied to seedlings exposed to inductive and to noninductive environments significantly increased the number of leaf nodes per plant (Table 20). Numbers of leaf nodes of control seedlings in the noninductive environment were greater than those of controls in the inductive environment. However, the ethephon-induced increase in number of nodes was of similar magnitude in each environment. The ethephon-induced increases in days to flower were not statistically significant, but average days to flower for plants in the florally inductive environment were significantly less than those for plants in the noninductive environment.

In test two, days to flower and leaf nodes were significantly greater for control plants exposed to a florally noninductive compared to inductive environment (Table 20). However, days to flower and leaf nodes of ethephon treated plants exposed to a florally inductive environment were not significantly different from those of treated plants exposed to a noninductive environment. Ethephon not only negated the effect of

Table 20. Influence of ethephon applied to flue-cured tobacco seedlings during 3-week exposure to controlled florally noninductive and inductive environments.

Florally inductive environment	Ethephon	Test 1		Test 2		
		Days to flower	Nodes per plant	Days to flower	Nodes per plant	Stem ht. at flowering
						cm
No	No	27.8	25.6	59.2	32.4	145.1
No	Yes	31.2	30.4	66.7	34.5	141.5
Yes	No	11.0	15.0	14.6	12.8	42.9
Yes	Yes	17.4	20.0	72.0	35.0	131.6
$LSD_{0.05}$		7.1	4.4	9.4	2.0	62.2
CV (%)		23.5	14.0	12.9	5.0	39.1

floral induction, but actually increased days to flower and nodes of treated plants exposed to an inductive environment compared to untreated plants not exposed to a florally inductive environment. Stem heights at flowering were not significantly different for control and treated plants exposed to a noninductive environment and for treated plants exposed to a florally inductive environment. However, stem height at flowering for untreated plants exposed to an inductive environment were significantly less than those of plants receiving the other three treatments.

Ethephon did not decrease days to flower or number of leaf nodes of plants in either environment (Table 20). Similar results were reported by Kasperbauer in work with burley tobacco (43). In test two of this study, days to flower and leaf nodes of treated plants were not different when exposed to either environment and were significantly greater than days and nodes of control plants exposed to a noninductive environment. In contrast, days to flower and leaf nodes of treated plants exposed to the florally inductive environment in test one were significantly less than those of treated plants exposed to a noninductive environment. In this test, ethephon treatments were applied after 13 days exposure to the respective environments, while treatments in test two were applied eight days after exposure to each environment. Floral induction in some of the plants exposed to the florally inductive environment in test one had apparently been completed before application of the ethephon treatments. Therefore, the ethephon-induced increase in leaf nodes was no greater for plants exposed to the inductive compared to noninductive environment. In test two, the ethephon treatments were applied before the completion of

floral induction and therefore the ethephon-induced increase in number of leaf nodes per plant was of greater magnitude for plants exposed to the inductive compared to noninductive environment. This explains the absence and presence of a significant environment x ethephon interaction in tests one and two, respectively (Appendix Table 10).

In general, the environment had a greater effect on stem elongation during the three-week period than did ethephon (Appendix Tables 11 and 12). After subsequent transfer to the greenhouse, the ethephon treatment generally had a greater effect on stem elongation than did the environment to which the plants were previously exposed.

When stem elongation was measured in the growth chambers for the first three and 13 days after treatment in tests one and two, respectively, the ethephon-induced decreases in elongation were not significant, but elongation of seedlings in the inductive environment was significantly less than that of seedlings in the noninductive environment (Tables 21 and 22). In test one, with seedlings still in the growth chambers, stem elongation during the next five days for treated seedlings exposed to the inductive environment was significantly less than that of control seedlings in the same environment (Table 21). Stem elongation of untreated and treated seedlings in the noninductive environment was not different. This indicates that after an initial lag in seedling response to ethephon, the ethephon-induced reduction in stem elongation was greater in seedlings growing under conditions unfavorable for rapid growth.

After transfer of seedlings from the growth chambers to the

Table 21. Influence, on stem elongation, of ethephon applied to flue-cured tobacco seedlings during 3-week exposure to controlled florally noninductive and inductive environments, Test 1.

Florally inductive environment	Ethephon	Stem elongation					
		In growth chambers		In greenhouse			
		0-3*	3-8	8-11	11-15	15-18	18-22
		mm/period					
No	No	8.1	5.5	5.1	8.0	8.1	15.6
No	Yes	6.6	6.0	3.3	7.6	6.6	16.4
Yes	No	2.7	4.0	5.7	10.4	10.5	31.2
Yes	Yes	2.1	0.5	2.1	6.0	4.5	17.6
$LSD_{0.05}$		4.0	2.8	NS	NS	3.7	8.7
CV (%)		60.4	50.8	69.8	40.3	35.5	31.6

*posttreatment days in respective locations.

Table 22. Influence, on stem elongation, of ethephon applied to flue-cured tobacco seedlings during 3-week exposure to controlled florally noninductive and inductive environments, Test 2.

Florally inductive environment	Ethephon	Stem elongation			
		In growth chambers	In greenhouse		
		0-13*	13-17	17-24	24-26
		mm/period			
No	No	13.8	7.2	22.6	10.0
No	Yes	10.7	5.2	22.0	8.8
Yes	No	3.1	5.8	25.6	14.4
Yes	Yes	1.8	1.8	10.4	3.0
$LSD_{0.05}$		3.2	3.5	6.4	5.3
CV (%)		32.0	49.9	22.9	42.9

*posttreatment days in respective locations.

greenhouse, elongation during the first week was not significantly different among treatments (Table 21). During the next week, stem elongation of ethephon treated seedlings exposed to the florally inductive environment was significantly less than that of corresponding control plants. Seedlings treated with ethephon in this environment were stressed, first from low temperature and then from ethylene, causing the recovery of vegetative growth to be delayed. In test two, differences in stem elongation of control and treated seedlings exposed to an inductive environment was significant at all three measurement periods during the first two weeks after transfer to the greenhouse (Table 22). Differences in stem elongation of control and treated seedlings exposed to the noninductive environment were not significant during this period.

Ethylene-induced inhibition of stem elongation in pea seedlings has been attributed to a changed orientation in the direction of deposition of newly formed cellulose microbibrils (14,25,67). This could also explain the inhibition of stem elongation of ethephon treated tobacco seedlings. Elongation of cucumber hypocotyl sections appears to depend on the cooperative actions of gibberellin and auxin (44), and since ethylene has been reported to interfere with auxin transport (14,80), hormone imbalance may also account for the ethephon-induced inhibition of stem elongation in tobacco seedlings.

Changes in total plant leaf areas following ethephon applications were not statistically significant for untreated compared to treated plants exposed to noninductive or inductive environments (data not presented). This suggests that stem elongation may be more directly

affected by ethylene than are leaf length and/or width.

Kasperbauer (43) studied the flowering of burley tobacco treated with ethephon, and hypothesized that delayed floral induction coincided with suppressed growth. In this study, differences in stem elongation and number of leaf nodes were significant for control compared to treated plants exposed to an inductive environment (Tables 20, 21, and 22). However, seedling stem elongation was not significantly different for control and treated plants exposed to a florally noninductive environment (Tables 21 and 22), but the differences in number of leaf nodes at flowering were significant for these same plants (Table 20). This suggests that statistical differences in stem elongation responses of tobacco seedlings to ethephon may not necessarily coincide with statistical differences in number of leaf nodes at flowering. Growth suppression apparently is not required for an ethephon-delayed floral induction of tobacco seedlings.

Ethylene Quantification

Flue-cured tobacco seedlings exposed to a controlled florally inductive environment were treated with ethephon, and ethylene levels from air samples of the headspace of sealed flasks containing dissected plant parts were analyzed by gas chromatography at different times after treatment. Since samples from untreated seedlings exposed to noninductive or inductive environments contained undetectable levels of ethylene, only the data from the treated seedlings is presented.

Two days after ethephon treatment in test one, amounts of ethylene

evolved per gram of tissue fresh weight during the 24 h dark incubation period in sealed flasks in 28°C temperature were significantly higher from the buds and stems than from the roots, with an intermediate level from the leaf discs (Table 23). Rates of ethylene evolved from the plant parts were not significantly different at subsequent measurements, except for the measurements made on days 22 and 29 after treatment. Differences were obtained at these times because the ethylene evolved from the buds and leaf discs had decreased to undetectable levels as a result of low weights of these sample parts and of the time course decline in ethylene evolved from all plant parts. Rates of ethylene released from ethephon-treated tobacco tissue were similar to rates observed in previous work with ethylene treated tobacco leaf discs (6). Ethylene has also been detected from leaf discs of tobacco plants not treated with ethephon or ethylene (5,6,7).

In test two, samples were taken on days 11 and 18 after ethephon treatment. Differences in rate of ethylene evolution were not significant among plant parts on either sampling day (Table 24). The rates were higher than those from the same plant parts at similar times after treatment in test one (Tables 23 and 24). The reason for the higher rates in the second test is not apparent. Seedlings in both tests received approximately the same amount of ethephon, and differences in seedling size at the time of treatment were not great. Treatments were applied 14 and seven days after transfer to the induction chamber in tests one and two, respectively.

The general information from the two tests is consistent. Ethylene

Table 23. Ethylene evolved from green tissues of different parts of flue-cured tobacco seedlings at different times after treatment with ethephon, Test 1.

Plant parts	Ethylene (nL $g^{-1}h^{-1}$) measured on indicated posttreatment day*							
	2	4	6	8	11	15	22	29
Buds	1.5	0.9	1.1	0.5	0.5	0.1	0.0	0.0
Stems	1.2	0.6	0.5	0.5	0.7	0.3	0.2	0.1
Leaf discs	1.0	1.2	1.2	1.2	0.6	0.1	0.0	0.0
Roots	0.4	0.5	0.5	0.7	0.8	0.4	0.4	0.1
$LSD_{0.05}$	0.7	NS	NS	NS	NS	NS	0.1	0.1
CV (%)	21.3	84.9	75.9	31.9	13.6	38.3	21.6	44.3

*Seedlings were in floral induction chambers through day 6 and in greenhouse at subsequent measurements.

Table 24. Ethylene evolved from green tissues of different parts of flue-cured tobacco seedlings at different times after treatment with ethephon, Test 2.

Plant parts	Ethylene (nL $g^{-1}h^{-1}$) measured on indicated posttreatment day*	
	11	18
Buds	3.1	0.8
Stems	2.1	1.2
Leaf discs	1.2	0.4
Roots	3.0	1.8
$LSD_{0.05}$	NS	NS
CV (%)	26.3	44.0

*Seedlings were in the growth chamber and greenhouse on days 11 and 18 respectively.

was detected from ethephon-treated flue-cured tobacco seedlings but not from untreated seedlings, and ethylene evolved from treated seedlings was still detectable in stems and roots 18 and 29 days after treatment (Table 23). Since ethephon was applied only to above-ground parts but was also detected from the roots, ethephon was apparently transported to the roots. The apparent transport of ethephon from the fruit to the roots of tomato plants has been reported (81).

The possibility cannot be excluded that a compound other than ethephon was the transported entity (35). When radioactive ethephon was applied to leaves of mature flue-cured tobacco plants, radioactivity was not detected elsewhere in the plant (23). However, application of ethephon to a single leaf of squash plants caused increased production of ethylene from other parts of the plant (35).

Decomposition to ethylene of the ethephon applied to mature tobacco plants was reported to be apparently completed by approximately four days after treatment (22). If this time course of ethephon breakdown is applicable for ethephon applied to tobacco seedlings, detection of ethylene many days beyond day four after treatment (Tables 23 and 24) suggests that ethylene from ethephon decomposition had induced the biosynthesis of additional ethylene. Ethylene-induced biosynthesis of ethylene has been reported for other plants (86), although not for mature tobacco plants (63).

Ethylene detected at above-control levels in tobacco seedlings four weeks after ethephon application (Table 23) helps explain the prolonged inhibition of stem elongation (see Tables 21 and 22) and the ability of

ethephon to delay floral induction even when applied immediately prior to the beginning of a three-week floral induction period (Table 15). This study suggests that ethylene continues to exert effects on tobacco plants for an extended period of time after application of ethephon.

SUMMARY

Tobacco growers must plant cultivars yielding high quality cured leaf in order to consistently market a desirable product. Flue-cured 'NC 82' tobacco, the cultivar used in this study, produces leaves which are generally high in quality (39,40). However, 'NC 82' is also susceptible to premature flowering (39,40). Premature flowering results in increased labor and production costs, a nonuniform crop, and potential yield reductions.

Results of this study show that ethephon applied to flue-cured tobacco 'NC 82' seedlings prior to the completion of floral induction significantly decreased premature flowering and increased days to flower, number of leaf nodes per plant at flowering, and yield. Magnitude of the ethephon-induced reduction in premature flowering was increased at two of seven on-farm test locations when the protective plant bed covers were removed two weeks compared to one week before transplanting. This interaction was observed for number of leaf nodes at one of seven locations. Highest levels of premature flowering were related to lowest average daily minimum temperatures during the pretransplant period in on-farm tests. In controlled environments, floral induction in seedlings was obtained from a three-week exposure to 15°C temperature and 8 h daylength. Under field conditions, daylength will not vary significantly during a three to four week pretransplant period. Photosynthetically active radiation can vary considerably, and low light levels were reported to partially substitute for short days in floral induction of burley tobacco seedlings (40). Under ambient conditions, reduced light

levels are often associated with periods of low temperatures.

Determination of the precise number of inductive cycles required for floral induction of 'NC 82' seedlings was not an objective of this study. However, a general indication of the number of inductive cycles required for floral induction is necessary in order to know when to apply ethephon for control of premature flowering.

In controlled environments, induction was apparently completed at a point between 14 and 21 inductive cycles. Ethephon applied after 0, 7, or 14 inductive cycles significantly decreased premature flowering and increased the average numbers of days to flower and leaf nodes per plant at flowering and yield. However, application of ethephon after 21 inductive cycles did not significantly decrease premature flowering or increase number of leaf nodes per plant or yield. Under field conditions, higher levels of premature flowering (51 compared to 1%) in control plants transplanted seven compared to three days after application of ethephon were attributed to increased floral induction as a result of the additional four days exposure to the ambient environment. Timing of the ethephon application is therefore critical when the time of completion of floral induction is approached. The time at which induction is completed under ambient environmental conditions has not been precisely characterized. In the ethephon/transplant date experiment, the cotton covers had been removed from the plant bed 14 and 18 days before the 3- and 7-day transplants, respectively, were transplanted. Average daily minimum temperatures during the period from cover removal until transplanting of the 3-day transplants was 8.6^{o}C.

Average daily minimum temperature during the subsequent three-day interval between transplant dates was 17.3°C.

Timing of ethephon application was also investigated on the basis of tobacco seedling stage of development. Under a three-week controlled florally inductive environment, numbers of days to flower and leaf nodes per plant were increased significantly by application of ethephon to seedlings averaging 1.5 cm stem height. Time of attainment of the 2.5 cm treatment height in the florally inductive environment was dependent on size of the seedlings when transferred to the controlled environment. When the 2.5 cm stem height was reached before floral induction had been completed, response to ethephon application was similar to that for the 1.5 cm plants. In controlled environment experiments, total seedling leaf area was characterized at the time of transfer to floral induction chambers. Seedlings averaging 39 cm^2 total leaf area when transferred to controlled florally inductive environment for three weeks were florally induced to a similar degree as seedlings averaging 74 cm^2 total leaf area.

In the ethephon localization experiment, both the on-plant site and amount of ethephon application influenced flowering. Ethephon applied to the largest leaves significantly increased numbers of days to flower and leaf nodes per plant. Applications of ethephon to senesced and newly emerged (non-bud) leaves generally did not increase these parameters for flowering. Small amounts (0.03 to 0.09 mg) of ethephon applied to the bud also increased numbers of days to flower and leaf nodes per plant at flowering. Furthermore, application of ethephon to the roots also

increased these flowering parameters. Transport of ethephon in a source to sink pattern in the phloem is postulated from these results. Similar conclusions have been reported from studies using ^{14}C-ethephon to investigate ethephon transport (24, 51, 76). Delayed flowering as a result of ethephon applied to the bud suggests that ethylene, the physiologically active product of ethephon decomposition, causes a temporary inhibition of responsiveness of the shoot apex to the floral stimulus [which is presumably synthesized in the leaves and transported to the shoot apex (9, 10)]. A general mode of action for ethylene-induced delay of floral induction of tobacco seedlings can be postulated. A flowering signal would require processing at the shoot apex, which would require synthesis of new enzymes. Ethylene has been reported to inhibit DNA synthesis by reducing DNA polymerase activity (16). Synthesis of new enzymes would require DNA templates (28). A shoot apex to base gradient was reported for the capacity of stem segments of a cultivar of cigar tobacco to produce flower primordia *in vitro* (74); segments that formed 100% flower buds contained 10 times more DNA than segments that formed a lower percentage of flower buds.

In other experiments, ethephon-induced reductions in premature flowering were not increased by a second pretransplant application of ethephon. Cured-leaf quality was reduced by multiple applications, and yield was reduced when the single pretransplant application was supplemented with a second application of ethephon 10 days after transplanting. Furthermore, application of ethephon at rates of 81, 122, or 244 mL m^{-2} of plant bed did not increase the number of leaf nodes per

plant beyond the level obtained from 44 mL m^{-2} of plant bed.

Additional experiments, conducted in the greenhouse and growth chamber, indicated that a 2.5 cm rain, which washed ethephon from tobacco seedlings, within one to two hours after ethephon application decreased the ethephon-induced increases in numbers of days to flower and leaf nodes per plant. Rain within 30 to 60 minutes after ethephon application completely prevented ethephon from influencing flowering.

Influence of ethephon on stem elongation of tobacco was also examined. Ethephon applied to plant bed seedlings at the rate of 244 mL m^{-2} of plant bed temporarily suppressed stem elongation. Stem elongation of plant bed seedlings treated at rates of 44, 81, or 122 mL m^{-2} was not significantly less than that of controls. However, for seedlings grown in the greenhouse, ethephon applied at rates of 122 and 244 mL m^{-2} reduced stem elongation. Succulent, greenhouse-grown tobacco seedlings may absorb ethephon more readily than do plant bed seedlings. Reductions in stem elongation were greater for seedlings grown in controlled environments of 15°C temperature and 8 h daylength than in 25°C and 13.5 h daylengths. The ethephon-induced suppression of stem elongation was compounded by an environment which did not promote rapid growth. Application of approximately 0.5 to 1.5 mg ethephon to the largest leaf, all four leaves, and all four leaves plus the bud suppressed stem elongation to a greater extent than 0.03 to 0.09 mg ethephon applied to the bud or 0.08 mg applied to the small leaf nearest the bud. This apparently suggests that amount of ethephon applied influences stem elongation of tobacco. Additional experiments, in which different

amounts of ethephon are applied to the plant parts in a factorial arrangement, are needed in order to reach conclusions on the influence of on-plant application site on stem elongation. In all experiments, suppression of stem elongation was temporary. Although differences in stem elongation remained significant up to three and four weeks after treatment (in the ethephon volume and growth effect experiments), stem heights at flowering were not different among treated plants. Delayed floral induction of burley tobacco seedlings by ethephon has been postulated by Kasperbauer (43) to coincide with ethephon-induced suppression of growth. In the ethephon growth effect study, differences in both stem elongation and number of leaf nodes at flowering for control compared to ethephon-treated plants were significant for plants exposed to a controlled florally inductive environment. In contrast, differences in seedling stem elongation of control and ethephon-treated plants were not significant for seedlings exposed to a noninductive environment. However, differences in number of leaf nodes at flowering were significant for these plants. Statistical differences in stem elongation of tobacco seedlings treated with ethephon do not necessarily coincide with statistical differences in number of leaf nodes at flowering. Growth suppression apparently is not required for an ethephon-induced delay of floral induction of tobacco seedlings.

In a related experiment, flue-cured tobacco seedlings were treated with ethephon during a three-week exposure to a controlled florally inductive environment, and ethylene levels from air samples of the headspace of sealed flasks containing dissected seedling parts were

analyzed by gas chromatography at different days after treatment. Levels of ethylene from control seedlings were undetectable. However, ethylene from ethephon treated plants remained detectable, although in decreasing quantities over time, at 18 and 29 days after treatment in the two tests of this experiment. This indicates that ethylene continued to act within the plant for an extended period of time. This supports results from other experiments in this study in which ethephon applied after 0, 7, or 14 days exposure to a controlled florally inductive environment was equally effective in reducing premature flowering and increasing numbers of days to flower and leaf nodes at flowering, and yield. Detection of ethylene for an extended period of time in ethephon-treated plants also supports the suppression of stem elongation observed three to four weeks after treatment.

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Appendix Table 1. Dates of relevant procedures and seedling stage of development when seedlings for growth chamber ethephon timing experiment were transferred to floral induction chambers.

Experiment No.	Seeded (in greenhouse)	Planted into flats	Transferred to induction chamber	Transplanted to field	When transferred to chamber	
					Total leaf area*	Stem ht.
					cm^2	cm
1	18 Feb.	20 March	4 April	2 May	131	4.0
2	16 March	9 April	2 May	31 May	73	1.6

*Leaf area and stem ht. are averages of 25 representative seedlings.

Appendix Table 2. Characterizations of tobacco seedling stage of development when transferred to floral induction growth chamber for 21 days and number of days after transfer when ethephon was applied in greenhouse/growth chamber experiments.

Experiment		Seedling characterization when transferred to chamber		Days after transfer to chamber when treated
Name	No.	Stem ht.*	Leaf area	
		cm	cm^2	
Stage of development	1	0.9	39	7 & 23 Oct.**
	2	1.6	74	19 & 24 Nov.**
Ethephon wash-off	1	1.3	50	11
	2	1.6	74	9
Ethephon localization	1	1.3	50	12
	2	1.6	74	9
Ethephon growth effect	1	1.9	39	13
	2	2.0	43	8

*stem ht. and leaf area are averages of 9 representative seedlings.

**times when ethephon applied to seedlings of 1.5 and 2.5 cm stem ht., respectively.

Appendix Table 3. Analysis of variance for five agronomic characteristics of flue-cured tobacco grown at seven on-farm test locations, 1983.

Variance		Days to flower	Leaves per plant	Premature flowering	Yield	Quality index
Source	df			% Mean squares	kg ha^{-1}	
			Hawthorne location			
Rep	2	0.3	0.1	18.8	139 687	4.1
Exposure	1	0.3	1.3	55.2	52	0.8
Ethephon	1	23.1÷	6.2**	998.6*	1 344	2.1
Exp x Eth	1	0.2	2.9*	8.9	111 554	0.8
Error	6	0.7	0.3	116.7	25 855	3.9
			May location			
Rep	2	2.6	0.1	76.6	64 178	43.6
Exposure	1	8.8*	0.1	212.4*	27 648	60.8
Ethephon	1	18.7**	31.1÷	457.1**	215 472	6.8
Exp x Eth	1	8.9*	2.1	208.3*	33 920	6.8
Error	6	1.5	1.0	35.1	258 761	13.3
			Crews location			
Rep	2	0.9	2.5	19.6	132 708	8.1
Exposure	1	3.8	3.2	77.3	127	21.3
Ethephon	1	0.1	1.5	12.4	104 347	1.3
Exp x Eth	1	0.1	7.0	4.2	12 352	16.3
Error	6	1.3	5.2	22.1	34 021	5.1
			Scarce location			
Rep	2	33.5*	0.6	0.7	93 182	17.6
Exposure	1	4.4	0.2	25.6**	1 323	2.1
Ethephon	1	38.3*	1.6	28.6**	161	14.2
Exp x Eth	1	1.7	1.1	24.1	481	4.1
Error	6	4.1	0.5	1.4	34 827	18.6

*, **, ÷ Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

Appendix Table 4. Analysis of variance for five agronomic characteristics of flue-cured tobacco grown at seven on-farm test locations, 1984.

Variance		Days to flower	Leaves per plant	Premature flowering	Yield	Quality index
Source	df			% Mean squares	kg ha^{-1}	
			Hawthorne location			
Rep	2	4.9	2.0	36.2	1 057	26.1
Exposure	1	7.2	0.2	328.7	3 924	18.8
Ethephon	1	29.1**	18.5**	1140.8**	203 581**	0.1
Exp x Eth	1	4.0	1.3	137.4	13 804	2.1
Error	6	2.1	0.9	70.6	9 890	18.6
			May location			
Rep	2	1.9	2.6*	0.8	206 479	8.1
Exposure	1	0.1	0.4	1.3	98 827	0.3
Ethephon	1	5.6*	0.3	1.3	252 590	5.3
Exp x Eth	1	0.9	0.1	0.9	954	27.0
Error	6	1.0	0.3	1.1	125 140	13.3
			Guthrie location			
Rep	2	0.2	2.1	9.2	1 161	14.1
Exposure	1	0.1	0.1	1.4	61	0.1
Ethephon	1	1.4	0.1	23.2	25 117	14.1
Exp x Eth	1	0.1	0.8	2.2	61	2.1
Error	6	2.2	0.2	7.8	20 394	3.8

*, ** Significant at the 0.05 and 0.01 probability levels, respectively.

Appendix Table 5. Dates when indicated management tasks were performed at on-farm test locations, 1983-84.

Location	Seeding date	Cover removal dates[1]	Transplant date
Hawthorne '83	1 March	1 & 8 May	18 May
May '83	10 March	16 & 23 May	30 May
Crews '83	4 March	4 & 11 May	26 May
Scarce '83	23 March	19 & 25 May	1 June
Hawthorne '84	8 March	2 & 9 May	15 May
May '84	18 February	23 & 30 April	5 May
Guthrie '84	23 March	8 & 15 May	23 May[2]

[1]Ethephon applied on second cover removal date.

[2]Actual dates were May 22 and 25; May 23 is the average.

Appendix Table 6. Analysis of variance for weekly rates of stem elongation of posttransplant flue-cured tobacco NC 82 plants transplanted 3 and 7 days after treatment with ethephon at 5 rates.

Variance Source	df	Posttransplant period of stem elongation				
		Week 1	Week 2	Week 3	Week 4	Week 5
		Mean squares				
Reps	2	0.66	11.96**	51.38†	88.60**	92.93
Transplant day	1	10.19**	0.87	3.49	16.64	6.86
Ethephon rate	4	1.89	3.62	16.16**	40.26*	19.32
TD X ER	4	0.38	0.80	2.74	18.75	21.09
Error	18	0.96	1.32	3.52	13.69	31.50

*, **, † Significant at the 0.05, 0.01, and 0.001 probability levels respectively.

Appendix Table 7. Analysis of variance for agronomic and chemical characteristics of flue-cured tobacco transplanted 3 and 7 days after treatment with ethephon at 5 rates.

Variance		Premature flowering	Days to flower	Nodes per plant	Yield	Quality index	Nicotine	Reducing sugars
		%			kg ha^{-1}		%	%
Source	df	Mean squares						
Reps	2	0.002	2.9	6.5	146 062*	17.7	1.06†	36.7**
Transplant day	1	0.342†	425.6†	29.6†	1 933 449†	100.8*	0.52**	1.4
Ethephon rate	4	0.048**	44.9†	4.6	64 552	32.1	0.09	9.6
TD X ER	4	0.045**	10.0	5.6**	287 991†	27.3	0.09	5.3
Error	18	0.008	4.6	0.9	38 564	24.0	0.06	4.6

*, **, † Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

Appendix Table 8. Mean daily minimum temperatures recorded at the Virginia Tech Southern Piedmont Center in 1984 during certain weeks in relation to on-station field tests.*

Week	Temperature
	°C
7-13 May	12.2
14-20 May	9.9
21-27 May	17.8
28 May - 3 June	13.6
4-10 June	19.4
11-17 June	20.3
18-24 June	20.2

*Plant bed covers removed 7 May; transplant dates were 21 and 25 May.

Appendix Table 9. Average daily minimum temperatures immediately following three weeks exposure of tobacco seedlings to a controlled florally inductive environment in growth chamber ethephon timing experiment.

Experiment no.	Pretransplant	Posttransplant environment			
	In lath house (1 week)	Week 1	Week 2	Week 3	Week 4
	°C		°C		
1	13.7	13.6	10.5	12.5	17.6
2	16.2	13.6	21.4	20.8	17.9

Appendix Table 10. Analysis of variance for effects of ethephon applied to flue-cured tobacco seedlings during exposure to controlled florally noninductive and inductive environments.

Variance Source	df	Test 1 Days to flower	Test 1 Nodes per plant	Test 2 Days to flower	Test 2 Nodes per plant
		Mean squares			
Reps	4	18.8	15.1	115.6	6.3
Environment (Env)	1	1170.5†	551.3†	2655.5†	585.8†
Ethephon (Eth)	1	120.1*	120.1**	5088.1†	732.1†
Env x Eth	1	11.3	0.1	2385.7†	375.2†
Error	12	26.5	10.2	46.4	2.0

*, **, † Significant at the 0.05, 0.01, and 0.0001 probability levels, respectively.

Appendix Table 11. Analysis of variance for weekly stem elongation of flue-cured tobacco seedlings treated with ethephon during exposure to controlled florally noninductive and inductive environments, Test 1.

Variance		Posttreatment days					
		In growth chamber		In greenhouse			
Source	df	0-3	3-8	8-11	11-15	15-18	18-22
		Mean squares					
Reps	4	1.0	0.4	1.2	0.8	3.8**	0.9
Environment (Env)	1	13.9**	2.2**	0.1	0.1	0.1	21.5**
Ethephon (Eth)	1	0.6	0.6	4.1	1.5	8.0**	13.2*
Env x Eth	1	0.1	0.7	0.5	1.3	2.7	15.8*
Error	12	0.9	0.2	0.9	0.6	0.8	2.5

*, ** Significant at the 0.05 and 0.01 probability levels, respectively.

Appendix Table 12. Analysis of variance for weekly stem elongation of flue-cured tobacco seedlings treated with ethephon during exposure to controlled florally noninductive and inductive environments, Test 2.

Variance Source	df	Posttreatment days: In growth chambers 0-13	In greenhouse 13-17	In greenhouse 17-24	In greenhouse 24-26
		Mean squares			
Reps	4	0.1	0.7	0.6	14.2
Environment (Env)	1	2.8†	1.8*	1.9	0.6
Ethephon (Eth)	1	0.2*	2.8*	6.4**	49.6**
Env X Eth	1	0.1	0.3	5.4**	23.5**
Error	12	0.1	0.4	0.4	3.8

*, **, † Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

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